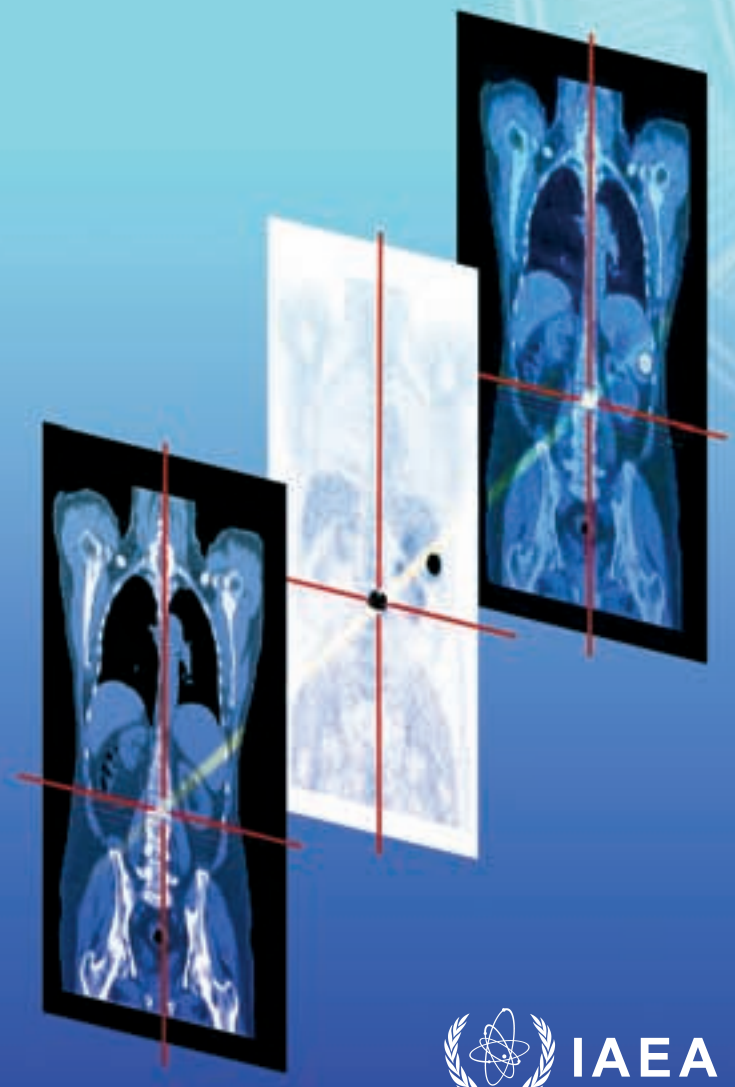
Nuclear Medicine Resources Manual



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NUCLEAR MEDICINE
RESOURCES MANUAL

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The Agency's Statute was approved on 23 October 1956 by the Conference on the Statute of the IAEA held at United Nations Headquarters, New York; it entered into force on 29 July 1957. The Headquarters of the Agency are situated in Vienna. Its principal objective is "to accelerate and enlarge the contribution of atomic energy to peace, health and prosperity throughout the world".

NUCLEAR MEDICINE RESOURCES MANUAL

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FOREWORD

Over the past decade many IAEA programmes have significantly enhanced the capabilities of numerous Member States in the field of nuclear medicine. Functional imaging using nuclear medicine procedures has become an indispensable tool for the diagnosis, treatment planning and management of patients. However, due to the heterogeneous growth and development of nuclear medicine in the IAEA's Member States, the operating standards of practice vary considerably from country to country and region to region. This publication is the result of the work of over 30 international professionals who have assisted the IAEA in the process of standardization and harmonization.

This manual sets out the prerequisites for the establishment of a nuclear medicine service, including basic infrastructure, suitable premises, reliable supply of electricity, maintenance of a steady temperature, dust exclusion for gamma cameras and radiopharmacy dispensaries. It offers clear guidance on human resources and training needs for medical doctors, technologists, radiopharmaceutical scientists, physicists and specialist nurses in the practice of nuclear medicine. The manual describes the requirements for safe preparation and quality control of radiopharmaceuticals. In addition, it contains essential requirements for maintenance of facilities and instruments, for radiation hygiene and for optimization of nuclear medicine operational performance with the use of working clinical protocols. The result is a comprehensive guide at an international level that contains practical suggestions based on the experience of professionals around the globe.

This publication will be of interest to nuclear medicine physicians, radiologists, medical educationalists, diagnostic centre managers, medical physicists, medical technologists, radiopharmacists, specialist nurses, clinical scientists and those engaged in quality assurance and control systems in public health in both developed and developing countries.

The IAEA is grateful to all those who have contributed to and reviewed this manual.

The IAEA officers responsible for this publication were A.K. Padhy and K.K. Solanki of the Division of Human Health.

EDITORIAL NOTE

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Chapter 1

GENERAL INTRODUCTION

The key to the successful development of nuclear medicine is that it must be appropriate to the culture of the country. The present Nuclear Medicine Resources Manual offers guidance on human resources and training needs in the practice of nuclear medicine for medical doctors, physicists, technologists, technicians and nurses. Nuclear medicine physicians must be able to interpret the wishes of their clinical colleagues and demonstrate how clinical practice can be improved by the use of nuclear medicine techniques. It is, of course, imperative to achieve a certain standard of clinical practice before it can benefit from nuclear medicine. The introduction of complex nuclear medicine techniques for imaging or treating cancer with radiolabelled antibodies and peptides is only useful where there is an existing cancer service with qualified nuclear medicine staff at all levels. The International Atomic Energy Agency (IAEA) has a longstanding tradition of conducting regional training courses and arranging further training abroad for individuals to ensure the safe practice of nuclear medicine in its client countries.

The present manual sets out the prerequisites for the establishment of a nuclear medicine service. Basic infrastructure should include suitable premises, a reliable supply of electricity, air-conditioning, temperature control and dust exclusion for gamma cameras and other equipment. Local government and customs officials must be familiar with the properties of radiopharmaceuticals and be prepared to expedite customs clearance procedures since radiopharmaceuticals decay if they are delayed in customs. The manual also contains details of the required instrumentation as well as instructions on maintenance and optimization of performance.

There is also a section on practical clinical protocols and, unlike traditional textbooks where the emphasis is on outlining *why* protocols should be followed, this manual describes *how* they should be followed. It also stresses the importance of an accurate interpretation of results and describes pitfalls likely to be encountered. There are five parts in a nuclear medicine report:

(1) The patient and demographic data;
(2) The details of the test undertaken and the patient's response;
(3) A description of the findings;
(4) A conclusion based on these findings;
(5) The clinical data and request, and clinical advice as a result of the study.

In all cases, practicality is emphasized rather than perfection.

Both in vivo and in vitro methods are described, highlighting the growth of cardiac and cancer imaging techniques in vivo and of molecular biology and radioimmunoassay (RIA) techniques in vitro. Differential management (i.e. results directly affecting patient management) is required over and above the traditional differential diagnosis. Nuclear medicine permits:

(a) Investigations that establish a specific diagnosis, as in thyroid disease, pulmonary embolism or exercise induced stress fracture;
(b) Investigations that aim to exclude a particular diagnosis, such as myocardial perfusion imaging (presence of significant ischaemic heart disease) or renography (presence of functionally significant renovascular disorder);
(c) Follow-up investigations such as myocardial perfusion imaging after angioplasty or coronary bypass surgery, and the identification of tumour recurrence or metastasis using increasingly specific imaging agents.

The benefits of ^{131}I therapy for thyroid cancer are well known. The range of applications and the clinical efficacy of internally targeted radionuclide therapy are growing. This manual describes its safe use and appropriate indications. Conditions that are being successfully treated at present include neural crest, neuroendocrine tumours and non-Hodgkin's lymphoma, as well as the effective palliation of the pain from bone metastases.

Radiopharmaceuticals are the mainstay of nuclear medicine, permitting an increasingly specific yet sensitive demonstration of clinical pathophysiology. This manual describes the requirements for the safe handling, quality assurance and quality control of radiopharmaceuticals, as well as protocols for general radiation safety and radiation protection in nuclear medicine practices.

Nuclear medicine is neither an ornamental nor a status technology. High technology nuclear imaging and therapy is an investment in health. It reduces the pain of investigation by showing from the outside what is inside. It measures from the outside how the inside works. It enables objective outcome analysis. It characterizes tissue, for example, as cancerous or not, but, at the same time, relies on quality assurance at all levels for hardware and software, as well as competence in technology, physics and medicine.

The manual endeavours to demonstrate the universality of nuclear medicine, its uniformity and harmony. Other benefits of nuclear medicine include safety, non-invasiveness and cost effectiveness.

In the future, there will be increased emphasis on distance learning and on 'hub and spoke' type systems, so that local data acquisition can be transferred to a centre for data analysis and for second, or specialist, reporting.

The accreditation of staff and their departments, with full documentation of procedures to international standards, will become a requirement.

The IAEA continues to support nuclear medicine throughout the developing world and will continue to play a leading role in setting and maintaining standards of practice. This manual should be regularly updated to help meet this obligation.

Chapter 2

HUMAN RESOURCE DEVELOPMENT

Today's world is fast changing. As the pendulum of change swings towards free enterprise and market oriented economies, health care and medical services are also moving into the realm of business and industry. Efficient management is essential to the success of any undertaking, and nuclear medicine is no exception. It should be regarded as an enterprise that requires efficient organization and management if it is to adapt successfully to the pressure of change brought by the new market order. Human resources act as the hub that drives all the other resources in an enterprise, whether material or financial, and their strategic importance cannot be ignored.

Human resources can be defined as the total knowledge, skills, creative abilities, talents and aptitudes of the workforce in a given organization, including the values and attitudes of the individuals making up the organization.

No development is possible without proper planning, and human resource planning is a prerequisite to human resource development. Human resource planning in nuclear medicine must provide for the implementation of ongoing activities, meeting the demands of changing technologies and expansion programmes, replacing a workforce dwindling as a result of retirement or separation, and deploying staff to take care of any excess or shortage as the case may be. To summarize, the objective of human resource planning in nuclear medicine should be to optimize the human resource contribution to its growth and development, and to prepare nuclear medicine to meet the inevitable challenge of change.

Strategic thinking plays a vital role. It is imperative to define the objectives of a nuclear medicine enterprise in order to forecast future needs. A comparison of current human resources with future needs will reveal deficiencies or gaps in the competence of the workforce and provide a framework for remedial action. Proper job analysis will lead to a clear division of responsibilities and avoid unnecessary duplication and overlap. These steps represent the groundwork for realistic and, above all, practical human resource planning. While doing all these, it is good to keep in mind that practicality should be given preference over perfection.

In developing countries, the objectives of nuclear medicine can vary from country to country. From a purely scientific point of view, H.N. Wagner would include the following objectives:

(a) Detection of focal organ disease before it becomes global;

(b) Diagnosis based on altered function or biochemistry;
(c) Detection of altered function before it changes structure, and definition of the molecular basis of such changes;
(d) Characterization and portrayal of dynamic states of body constituents in the form of molecular or biochemical images;
(e) Development of stereoscopic radiotracer molecules.

The above goals reflect an academic and research oriented viewpoint. They are borne out of the inherent strengths of nuclear medicine, namely its tracer principle and the capability to exploit newly emerging technologies to its advantage.

The same goals can, however, also be defined from the more pragmatic point of view of medical imperatives. This is of particular relevance to developing countries, where there is a sense of urgency arising from the external challenges facing the practice of nuclear medicine today. These challenges include competing medical technologies for diagnosis, ever shrinking health care budgets in comparison with the demands, and an increasing awareness on the part of the consumers of their right to high quality services and products. The objectives are both short and long term. In the short term, the goals in nuclear medicine are to:

(a) Demonstrate the appropriateness of procedures for diagnosis and/or treatment of a given disease or disorder;
(b) Provide total quality assurance;
(c) Reduce the cost of procedures.

Once these objectives have been met, long term goals will also be achieved, namely the integration of nuclear medicine into national health care programmes on a par with other disciplines such as radiology, clinical pathology and biochemistry. At this point, nuclear medicine will have found its proper place among contemporary health care technologies and its future will have been secured.

It is relatively easy to forecast human resource needs once the objectives of nuclear medicine are clear, provided a reliable database is available showing the breadth and depth of nuclear medicine practice, the range of nuclear medicine products and services, and the profile of the nuclear medicine workforce. It will be possible to extrapolate future needs from this database in terms of the size of the workforce, staff in each category (physicians, physicists, technologists, radiopharmacists, nurses and other support staff), and qualifications and experience. It is important to note the age structure of the workforce in order to plan for replacements as a result of retirement and separation.

At this stage it is useful to develop profiles of the current and future workforces, analysing present strengths as well as the new competences that will be required in order to meet the required nuclear medicine objectives. It is then a matter of harnessing the old and new competences that will ensure the success of nuclear medicine and the personal fulfilment of the workforce.

The ultimate aim of human resource development is to place the right people at the right time in the right position so as to tap the full potential of the workforce for the benefit of the organization and its staff. There is a current shift in paradigm towards individual centred human resource management. An employee is not merely allocated work and treated simply as another resource, but the self-respect and dignity of the individual are protected and respected. Human resource development (HRD) builds a work culture where each of its members is happy and satisfied with work and life. HRD is not a means to an end, but an end in itself. Development of the individual is the ultimate and single goal of HRD, working on the principle that if an organization takes care of its staff, the staff in turn will take care of the organization.

Most nuclear medicine practitioners are involved, consciously or subconsciously, in HRD, whether at the unit, division or department level, although their impact may be quite limited. Knowing the complexities of human resource development, it is an almost impossible task at the regional or interregional level, although, at the country level, impact will be high and the effort cost effective.

At the country level, the development of human resources for nuclear medicine involves partnerships with the government (ministries of health and education at the centre and at the regional level), professional bodies (e.g. societies of nuclear medicine and their branches, and associations of medical physicists) and academic bodies (national boards and colleges of nuclear medicine). Sincerity of purpose, commitment to the cause, and close co-operation and collaboration among partners are essential for effective HRD in nuclear medicine.

HRD entails the effective management and development of staff to match present and future needs. At the country level this is a complex task and requires a prodigious amount of data collection, processing, analysis, interpretation and implementation. The conventional tools of HRD include recruitment, induction, mentoring, training, development, teamwork, performance appraisal, feedback and counselling, and rewards and disincentives. Depending on the exigencies of the situation, some of these functions may have to be centralized while others should be decentralized.

Although each of these tools is important, this chapter will focus on recruitment, on training and on performance appraisal, feedback and counselling for personal development, all of which require a great deal of

thought, innovation and attention to detail. It appears that there are no clear recruitment standards for posts in nuclear medicine. A minimum recruitment standard should be defined for each substantive post in every category of job in nuclear medicine. These standards should be binding on all hospitals, institutions and clinics that provide nuclear medicine services for patient care. A task force comprising representatives from each party in HRD should take responsibility for preparing the minimum recruitment standards. It should be mandatory to involve a suitable member from each job family to help prepare the minimum standards, thus ensuring confidence in, and adherence to, the requirements of the recruitment process. Over and above these minimum standards, the employing authority concerned should prepare detailed job analyses for each post in nuclear medicine, including a clear and concise job description, job specification and job design. They should also define standards of performance, develop models for personal competence and link these for each job. These standards and models will serve as benchmarks for comparing actual performance of individuals, a crucial step in the implementation of performance appraisal, feedback and counselling for personal development. Collection and codification of all these data on recruitment at the national level should lead to guidelines for the recruitment of a national nuclear medicine workforce that will serve as a reference for all those engaged in the practice of nuclear medicine in a particular country.

The recruitment process should reflect the values of the organization and its goals. Professional expertise and personal integrity are of crucial importance in the selection process, since without the right people for the right job there is little chance of success.

Training is fundamental to HRD since it ensures a viable and knowledgeable workforce. By measuring the actual performance of each person of the workforce with the agreed standards of performance, it will be possible to identify training needs. Training should only be conducted with the full consent of the future trainee, whose individual aptitudes and capabilities should first be considered. Training should be seen as a competence building and personal development function rather than as either a perk or a disciplinary exercise. A training programme should lead to concrete plans of action and new directions to meet the challenges of the future. It should serve the purpose of the establishment as well as the needs of the employee. In this respect, constructive trainee participation in the formulation of the training programme is necessary.

Training programmes for the different job categories in nuclear medicine range from those for a Diploma in Radiation Medicine (DRM), a Diploma of the National Board (DNB) or a Doctor of Medicine (MD) degree in nuclear medicine for physicians to a one year diploma course in medical physics or a one year diploma course (Diploma in Medical Radioisotope Techniques (DMRIT))

for nuclear medicine technologists. Basic training should be supplemented by specialized training that is dependent on the needs of the establishment and the individual. Training in the field of nuclear medicine is a continuous process. With good planning and organization, it should not be difficult to provide continuous education and training to all categories of professionals, using, where necessary, the services of existing training centres. What needs to be specified clearly is the standard of the end product of training. Personal competence models can be developed and linked to standards of performance upon the completion of training. This will help in the monitoring, evaluation and improvement of the training programme. Periodic accreditation of professionals in nuclear medicine through an acceptable evaluation process should be part of continuing education and training programmes for the nuclear medicine workforce. This will not only ensure that the workforce has up-to-date knowledge and skills to provide the best service to customers, but will also serve to boost morale and confidence.

Performance appraisal, feedback and counselling are essential ingredients of HRD. The implementation of these tools requires a high degree of sensitivity, objectivity and firmness on the part of higher management. Performance appraisal should be approached positively. It is a highly developmental mechanism and not a tool for dispensing discipline or perks. The whole process should be aimed at rating individuals on a scale. For the purpose of measurement, competence has been broken down into knowledge, skills and attitude, and incorporated into the performance appraisal mechanism. An appraisal exercise should be carefully planned and the assessment based on mutually agreed targets. Appraisals should be carried out periodically so that the organization can track the growth and development of a person over a period of time. The appraisal should also help in the planning of further training needs. Positive feedback and counselling will reveal any deficiencies or negative attitudes. Feedback and counselling should be considered as an aid to learning and development.

Whereas HRD was originally conceived as a management tool to increase productivity and profit in business and industry, it has now become an important part of many organized endeavours. It is an integral part of a system known as Enterprise Resource Planner (ERP) that is currently the object of keen interest in the business world. Strategic planning, and the use of computers and information technology (IT), should all make HRD in nuclear medicine easier than before. Software application programmes are provided by IT experts such as PeopleSoft to make the HRD process considerably less daunting than it otherwise might appear.

It is important to see the whole picture and not to be distracted by the day-to-day needs and pressures of running a nuclear medicine service. To

summarize, HRD can provide a sense of direction to a nuclear medicine group, by providing definite goals and the means of achieving these goals, as well a sense of fulfilment to those involved.

2.1. TRAINING OF MEDICAL DOCTORS

2.1.1. Introduction

Training in nuclear medicine requires a combination of general medical professional training and specific nuclear medicine training.

Within a nuclear medicine service, the medical doctor, who is also referred to as a 'nuclear physician', plays an important role. Nuclear medicine is a multidisciplinary practice and the training of medical doctors is critical to the performance of a nuclear medicine department. However, in most countries there is no dedicated academic facility responsible for the education that nuclear medicine doctors require.

The responsibility of the nuclear medicine physician is to:

— Define the patient's and clinician's reasons for the request or referral;
— Determine and organize the appropriate tests and protocols;
— Tailor the protocols to the needs and condition of the patient;
— Assess and carry out interventions (physiological, pharmacological or mental stress related);
— Adjust the study analysis and interpretation according to the clinical information;
— Interpret the results and their clinical, biological and pathological implications;
— Hold follow-up consultations with the patient;
— Ensure the safety of both the patient and staff;
— Provide training (and education) for technical and junior medical staff.

A practitioner in the field of nuclear medicine must possess a fundamental knowledge and a training in medicine. In addition they should preferably have a postgraduate qualification in nuclear medicine.

Most countries in the world at present, especially developing countries, have no postgraduate training programme for medical doctors in nuclear medicine.

In order to ensure an adequate nuclear medicine service, those responsible must recognize the need for well trained and specialized nuclear physicians. The

authorities in most countries agree that nuclear medicine practice without qualified and certified medical doctors is ethically and legally questionable.

Training requires the following components:

(a) Trained teachers who are professional nuclear medicine practitioners;
(b) Doctors hoping to pursue a career in nuclear medicine;
(c) An established syllabus;
(d) Mechanisms for the supervision of trainers;
(e) Mechanisms for the supervision and assessment of trainees.

In addition, while some countries may set entry requirements for training, others may adopt a system of continuous assessment throughout the training course and/or a final assessment.

Successful trainees are awarded with a final certificate, degree or diploma that is recognized by the government, local health authority and hospital as an assurance of specialist competence in nuclear medicine.

2.1.2. General professional training

Nuclear medicine specialists must have a sound understanding of general and emergency medicine, including resuscitation, surgery, gynaecology, paediatrics and psychiatry. Nuclear medicine could be regarded as the last refuge of the physician in a hospital since all hospital departments seek nuclear medicine services to a greater or lesser extent.

A general professional training in nuclear medicine is offered to doctors who have obtained their qualifications and completed a requisite period, usually of a year, as a medical or surgical house officer before obtaining registration as a medical practitioner. This requires a minimum of two years in clinical posts approved by the national training authority. During this time, the doctor should be directly involved in patient care and gain broad experience in a variety of clinical fields. Ideally, at least three quarters of the time spent in such clinical posts should include experience in the admission and follow-up of acute clinical emergencies. A minimum of six months of this time should include experience in ‘unselected emergency care’, i.e. acute medical care covering the breadth of emergency medicine with an on-call commitment of at least four on-call days per month. A further six month assignment to a department of radiology is recommended for nuclear medicine trainees who are not following a career in radiology.

2.1.2.1. Entry requirements

A desire to pursue a career in nuclear medicine is essential. Unfortunately, there is an increasing tendency among national authorities to set very specific, narrow and even discriminatory requirements for entry into particular specialties. It is recommended that there should be a final examination to ensure that candidates have adequate knowledge and skills to practice nuclear medicine.

2.1.2.2. Training paths

The training period for postgraduate nuclear medicine starts four years after the completion of general medical training, either from an internal medicine background or following training in diagnostic radiology. Training in diagnostic radiology usually takes four years, with a fifth year devoted to specialty training in areas such as magnetic resonance imaging (MRI), ultrasound, X ray, computed tomography (CT) or nuclear medicine. There should be a general training in radiology of at least eight weeks during the four year period. If a radiologist wishes to undertake only imaging in nuclear medicine, then the fifth year of specialist training in nuclear medicine may be sufficient, providing a subspecialty training in radionuclide radiology, following the United Kingdom (UK) example. However, if a radiologist who has completed a four year general radiology training to certification level wishes to undertake further training in nuclear medicine to certification level, then a two year period of specialist nuclear medicine training that must include radionuclide therapy is recommended.

2.1.2.3. Requirements for training

The national health authority (NHA) in each country is responsible for the training of medical specialists. The NHA may devolve this responsibility to specialist societies such as a recognized College of Physicians or Radiologists and/or to a university. The responsible training body is required to set standards both for training and for the supervision of trainees. As a minimum requirement, officials from the training body should visit centres of specialist training in nuclear medicine to ensure the availability of trained professionals in nuclear medicine. These visits should also determine whether the resources and equipment satisfy the requirements of the training programme, and that trainees have sufficient space in which to work as well as access to information on web sites and in libraries. It is also important to ensure the availability of funding for accommodation, subsistence and the purchase of books, to be provided by the

earnings from a part-time or full-time clinical post or on the basis of a governmental grant. Where the above conditions cannot be met by a national authority, consideration should be given to sending the trainees to a centre outside the country, for example by studying for an academic degree in nuclear medicine at a recognized institute abroad. Upon a trainee's return from a period of specialized study abroad, it is the responsibility of the NHA to ensure that the trainee is employed in a field that makes best use of his or her newly acquired knowledge.

2.1.2.4. Assessment of trainees

Each training programme should contain a standard against which the progress of the trainee can be assessed for each element of the syllabus. The assessor should preferably be external to the department that is providing the training, such as a postgraduate dean, a consultant in nuclear medicine from another hospital or other senior person. The assessment may take the form of an interview, a written paper, an essay, a set of multiple choice questions, or an oral examination of displayed images of various nuclear medicine techniques in clinical practice. Continuous assessment is another alternative. Each end of year assessment should carry a score that indicates how the candidate has progressed against the set target.

2.1.2.5. Syllabus

This section provides an indication of training for each of the four years:

Year 1

(a) Scientific principles:

— Basic physics and mathematics;
— Instrumentation;
— Principles of computing;
— Basic radiation biology and radiation protection;
— Basic radiopharmacy and radiochemistry;
— Principles of tracer technology.

(b) Clinical nuclear medicine:

— *Diagnostic*: normal and abnormal appearances of images, mode of pharmaceutical uptake; normal variants and common artefacts in bone, heart, lung, kidney, brain, thyroid, tumour and infection images.
— *Therapeutic*: basic principles of radionuclide therapy; treatment of hyperthyroidism, thyroid cancer and metastatic bone pain.
— *Principles of radiation protection*: ALARA (as low as reasonably achievable), ALARP (as low as reasonably practicable).

Year 2

(a) Requirements of Year 1 in greater depth:

— Tracer kinetics;
— Computing and image processing;
— Radiobiology including the biological effects of high and low levels of radiation;
— Linear hypothesis and the threshold hypothesis of the biological response to low level radiation;
— The effective dose equivalent and the calculation of radiation dose from radiopharmaceuticals.

(b) Radiopharmacy:

— Properties of commonly used diagnostic and therapeutic radiopharmaceuticals;
— Production of radionuclides by reactors, cyclotrons and radionuclide generators;
— Quality assurance and quality control of radiopharmaceuticals.

Year 3

(a) Requirements of Year 2 in greater depth:

— Principles of radiology including dual energy X ray absorption (DEXA), ultrasound, CT and MRI imaging;
— Co-location of nuclear medicine images and those from other imaging techniques;

— Special diagnostic investigations in cardiology, lung disease, gastro-enterology, hepatobiliary diseases, nephro-urology, neurology and psychiatry, endocrinology, haematology, oncology and infection.

(b) Therapeutic applications:

— Treatment of bone metastases, neural crest tumours, polycythaemia and solid malignancies;
— Use of radionuclide monoclonal antibodies and radionuclide labelled peptides for tumour therapy.

Year 4

Further practice and experience of techniques learned in years 1–3:

— Legal and regulatory requirements;
— Audit;
— Departmental and hospital management;
— Research techniques and evaluation;
— Teaching and training.

2.1.2.6. Practical training

Postgraduate trainees are obliged to play an active in-service role in the practice of nuclear medicine in order to familiarize themselves with all the techniques required of a nuclear medicine practitioner, such as:

(a) Protocols of in vivo and therapeutic procedures;
(b) Data acquisition and processing with various types of equipment, quality control of instruments and labelled agents;
(c) Interventional procedures, including physiological, pharmacological and mental stress related for diagnostic applications, and all therapeutic interventions;
(d) In vitro protocols and procedures, if appropriate.

2.1.2.7. Arrangements

The aim of postgraduate training is for trainees to attain a sufficiently high standard of theoretical and practical learning to qualify as a nuclear physician.

The minimum time requirement for postgraduate training is highly dependent on regulations in force in each country. Generally a course length of four years is desired. The teaching schedule given in Table 2.1 is recommended.

TABLE 2.1. SUGGESTED SCHEDULE FOR POSTGRADUATE TRAINING

Subject	Duration (h)	Suggested content of teaching	Recommended practice and time period
Nuclear physics	40	Decay features, spectra, radiation hygiene, dosimetry	Understanding spectra, units of radiation and monitoring devices. Internal distribution of isotopes
Reactor cyclotron generators	20	Radioisotope production and detection	Radioisotope identification (5–7 d)
Radiochemistry	40	Labelling, technical design and quality control, interaction and kinetics	Synthesis, labelling, quality control and animal tests (3–4 weeks)
Radiobiology	60	Dosimetry, bio-modelling, tracer technology and radiation protection	Dose effect, molecular biology and radiation injury (4 weeks)
Instrumentation	50	Scintillating cameras, SPECT[a], imaging procedures and computers	Daily operation and quality control, troubleshooting (4 weeks)
Related fields	100	Medical imaging modalities, epidemiology and statistics	Short round (6 weeks)
Clinical use	240–300	Cardiology, neurology, gastrointestinal (GI) tract, respiratory system, endocrine system, bones, haematology, tumours and infections	Clinical practice, image interpretation, etc. (12–18 months)
In vitro use	10	RIA[b] and autoradiography	RIA practice (2 weeks)
Therapy	60	RIT[c], palliation and brachytherapy	Ward duties (2–3 months)

[a] SPECT, single photon emission computed tomography.
[b] RIA, radioimmunoassay.
[c] RIT, radioimmunotherapy.

Since trainees will take on the responsibilities of a nuclear physician, they must pass a qualifying test that covers both theoretical knowledge and practical abilities in the daily practice of nuclear medicine. A board or similar form of authority will award a certificate to successful trainees.

2.1.2.8. Training in research and information retrieval

The following specific elements should be emphasized in the training of research techniques:

— Research project design;
— Understanding the elements of research that can lead to bias;
— Design of single centre and multicentre trials;
— Analysis of results;
— Statistics for analysing results;
— Parametric and non-parametric methods;
— Requirements for the publication of research;
— Legal and ethical requirements: the local Research Ethics Committee;
— Radioactive material licensing requirements for clinical practice and research;
— Translation of laboratory work into clinical practice;
— Obtaining information about, and contributing to, evidence based nuclear medicine;
— The Cochrane library (Update Software, Oxford).

2.1.2.9. Teacher training

The following specific elements should be emphasized:

— General teaching techniques;
— Preparation of teaching materials;
— Use of teaching aids;
— Teaching by example;
— Assessment of trainees;
— Setting of exam questions, particularly of multiple choice questions.

2.1.3. Undergraduate training

Undergraduate training refers to teaching and training that is provided by, and takes place within, a medical college. While some colleges offer nuclear

medicine as a subject, most medical schools now provide short courses on nuclear medicine.

The primary goal of training undergraduates is to introduce them to various radionuclide diagnostic and therapeutic methods and to give them an overview of the basic concepts, principles and major clinical applications of the specialty and its place in medical practice. Undergraduate training provides a basic understanding of nuclear medicine for all medical staff but is not sufficient for a qualified practitioner who is going to pursue a career in nuclear medicine.

It is essential that all undergraduate medical students be taught about radiation. With the increasing use of X rays, CT and MRI in the teaching of anatomy, there is general recognition of the power of these radiological techniques. Unfortunately the potential of nuclear medicine in demonstrating physiology is usually not recognized in the teaching of physiology. Since physics has been dropped from the curriculum of pre-medical studies in many countries, an appreciation of the physical properties and biological effects of radiation is often lacking. This deficiency must be remedied.

Regulations concerning the protection of the patient from ionizing radiation means that qualified doctors must undergo some form of radiation protection instruction or course in order to practise in their particular field: cardiologists, in the screening of pacemaker wire; orthopaedic surgeons, in X raying the hip during the introduction of a prosthesis; ward medical staff, in injecting radiopharmaceuticals. Ideally, this certification of competence should be obtained during undergraduate training rather than during the period of clinical experience. In any event, the theoretical part should be made mandatory even if the practical instruction is at postgraduate level.

2.1.3.1. Syllabus

The scope of undergraduate training should cover:

(a) An introduction to nuclear physics:

- Basic physics (alpha, beta and gamma rays; photons, electrons and positrons);
- Basic units, such as μCi, MBq, rad, gray and sievert.

(b) Natural radiation:

Radiation is a natural phenomenon and like every natural phenomenon can be beneficial or detrimental. All physical processes have advantages and disadvantages: in the case of electricity one can turn on a light or be

struck by lightning. Water can be drunk or one can drown in a flood. Gravity enables one to walk or fall off a cliff. Radiation is the source of chest X rays and of nuclear fallout.

(c) How to react in an accidental exposure and in a nuclear accident.

(d) Radiochemistry and tracer technology.

(e) Nuclear medicine and radiological instruments.

— Gamma cameras;
— X ray machines;
— CT;
— MRI.

(f) Clinical applications and radionuclide therapy:

— The use of nuclear medicine in physiology, clinical practice, endocrinology and oncology;
— Imaging techniques and image interpretation;
— In vitro studies and sample counting of radioactive specimens;
— Subspecialties (e.g. cardiology).

(g) Radiobiology, radiation dosimetry, safety and protection:

— The biological effects of very low level radiation;
— The linear hypothesis and the threshold hypothesis;
— Understanding the risks of radiation relative to other risks.

The goals and content of training determine the corresponding learning arrangements. Normally, theoretical teaching should be no less than 30–36 class hours, plus 10–14 hours of practical training (Table 2.2).

2.1.3.2. Practical training

In order to provide a good training, a medical teaching facility must fulfil certain basic requirements. For example, it should comprise a full scale nuclear medicine practice, with qualified, highly experienced medical personnel, including a medical doctor, radiochemist, medical physicist and a group of technologists or technicians. The facility should also have available a gamma counter, calibrator, gamma camera and SPECT, and preferably other

TABLE 2.2. SCHEDULE FOR UNDERGRADUATE MEDICAL STUDENTS

Subject	Classes per week (h)	Suggested content of teaching	Recommended practice and duration of session (h)
Nuclear physics	4	Atomic configurations and decay detection	Counting (0.5) and assessment (1)
Radiochemistry	2	Labelling, kit, generator, quality control, and examples of Tc, I and FDG[a]	Elution of a generator (0.5)
Radiobiology	2	Dosimetry, protection and tracer technology	Dose calibration (0.5) and radiation protection
Instruments	2	Scintillation cameras, SPECT, imaging procedures and computers	Departmental tour (1)
Clinical use	14–20	Cardiology, neurology, GI tract, respiratory system, endocrine system, bones, haematology, tumours and infection	Clinical practice (4–8)
In vitro use	2	RIA, autoradiography and samples	RIA practice (2)
Therapy	4	RIT, palliation and brachytherapy	Ward tour (1)

[a] FDG, ^{18}F fluoro-deoxy-glucose.

equipment. The department of nuclear medicine must have a sufficient variety and quantity of work and services to offer trainees meaningful work experience. The person(s) in charge of the training should have adequate academic knowledge as well as teaching experience in nuclear medicine. The facility must be spacious enough to accommodate all trainees at one time.

It is important to test all trainees before the completion of training. The test should comprise a series of simple questions on basic concepts, key features and principles of nuclear medicine, attempting to test the understanding and knowledge of what students have learnt during their training period.

2.1.4. Sub-specialty training

2.1.4.1. Nuclear cardiology

Nuclear cardiology has been shown to be a cost effective technique for evaluating patients with suspected coronary artery disease.

The concept

Nuclear cardiology is a super-specialty in which various techniques of nuclear medicine are utilized for diagnostic and therapeutic purposes in cardiology. Only doctors with certification in nuclear medicine are qualified to enrol for such courses. Training focuses on the mandatory, optional and preferential techniques and methods in nuclear cardiology, as well as related aspects of quality assurance.

Scope of training

(a) Theoretical learning includes:

— General anatomy, physiology and pathology;
— Clinical categorization of heart diseases;
— Epidemiology, diagnosis and treatment;
— Fundamental aspects of cardiac nuclear medicine (indications, contra-indications and limitations).

(b) Practical learning includes:

— Technical considerations;
— Patho-physiological concerns;
— Bias;
— Physical examination;
— Monitoring and managing of medication for patients with heart disease;
— Cardiac resuscitation techniques.

(c) Related fields:

— Electrophysiology, serum markers and imaging modalities (CT, MRI, digital subtraction angiography (DSA) and ultrasound);
— Cardiac intervention (surgical, radiological and pharmacological);
— Cardiology, cardiac surgery and paediatric cardiology.

(d) Technical aspects:

— Selection and handling of radiopharmaceuticals and instruments;
— Protocols of acquisition, gating, tomography and stress;
— Data analysis, image interpretation, factors of influence and quality control;

— Diagnosis, differentiation and decision making.

A problem arises in reconciling the views of cardiologists who wish to practice nuclear medicine solely in the form of nuclear cardiology and those of nuclear medicine specialists who feel that unless a cardiologist has received full training in nuclear medicine, he or she should not be permitted to practise nuclear cardiology. Conversely, cardiologists regard the nuclear medicine practitioner undertaking cardiological investigations, particularly stress testing of patients at risk, as having neither adequate cardiological training nor the understanding necessary to perform such studies safely and interpret their results appropriately in the light of echo cardiographic and angiographic findings.

The nuclear medicine community is keen that cardiologists learn nuclear medicine techniques, understand their benefits for patients with cardiac disease and increase the application of these techniques among the population at risk. On the other hand, the nuclear cardiologist can be regarded as a serious competitor to the nuclear medicine physician. There is no simple solution to this problem. In the United States of America (USA) a cardiologist can practise nuclear cardiology after a three month training period in nuclear medicine. In Europe, a cardiologist can receive certification to practise nuclear cardiology only after a full four years of training in nuclear medicine.

Qualifications

A special committee should be responsible for issuing certificates to those who complete the training and pass the examination. Practical aspects should play an important role in the examination.

2.1.4.2. Endocrinology

The concept

Nuclear endocrinology is a specialization within nuclear medicine in which nuclear medicine techniques are utilized for diagnostic and therapeutic purposes for patients with abnormal hormone secretion. Only doctors with certification in nuclear medicine are qualified to enrol for such courses. Trainees will focus on the mandatory, optional and preferential techniques and methods in nuclear endocrinology, as well as their related quality assurance aspects.

The most traditional, and one of the most rewarding, aspects of nuclear medicine is the comprehensive management of the patient with thyroid overactivity and the patient with thyroid cancer.

The thyroid clinic is the key to thyroid management. Patients with a diagnosis of probable hyperthyroidism require clinical assessment, a full examination, blood tests and therapy. Patients should receive counselling on the effects of the treatment on their spouse, children, relatives and friends. The management of the administration of radioiodine on an outpatient or inpatient basis must be conducted in a safe and responsible manner. The continued follow-up of the patient completes this process. Practice and treatment in a thyroid clinic can include:

(a) Thyroid cancer:

— Diagnosis;
— Referral for primary surgery;
— Nature of the histology;
— Requirement for lymph node removal;
— Imaging;
— Ablation dose after thyroidectomy and post-ablation imaging;
— Follow-up — the protocol of follow-up with ^{131}I tracer scans, thyroglobulin assays and of thyroid hormone medication within this monitoring process needs to be learned and incorporated into the practice guidelines.

(b) Adrenal medulla and related neural crest tumours:

Paraganglioma, malignant paraganglioma, ganglioblastoma and neuroblastoma may all be detected with ^{123}I or ^{131}I meta-iodo-benzyl-guanidine (MIBG). The indications for diagnosis, patient management, the use of ^{131}I MIBG in therapy and the follow-up of patients are part of specialist nuclear endocrinology.

(c) The adrenal cortex:

Imaging with radiolabelled cholesterol now has few indications in Conn's and Cushing's syndromes and is infrequently used; this type of imaging is mainly used to help determine the nature of adrenal masses found incidentally on X ray, CT or MRI images, and thus called 'incidentaloma'.

(d) Parathyroid imaging:

There is a specialized test with specific indication, technique and interpretation.

(e) Diabetes mellitus:

Nuclear medicine techniques are used to identify the complications of diabetes involving the heart, kidneys, brain and peripheral circulation.

(f) Sexual function:

Both impotence and infertility can be investigated by specialist nuclear medicine techniques.

Scope of training

(a) Theoretical learning includes:

— Anatomy, physiology and pathology of the endocrine glands;
— Clinical categorization of hormone secretion diseases;
— Epidemiology;
— Diagnosis and treatment;
— Fundamental aspects of scanning each abnormal gland (indications, contraindications and limitations).

(b) Practical learning includes:

— Technical considerations;
— Patho-physiological concerns;
— Physical examinations;
— Monitoring and management of heart disease and patient's medication;
— Intervention techniques used in endocrine nuclear medicine.

(c) Related fields:

— Hormone detection and laboratory measurements;
— Modes of imaging (CT, MRI, DSA and ultrasound);
— Medical intervention and interaction in endocrinology;
— Cardiology, gynaecology, paediatrics and nutrition.

(d) Technical aspects:

— Selection and handling of radiopharmaceuticals and instruments;
— Protocols of acquisition, tomography, pharmacology and hormonal stress;

— Data analysis, image interpretation, factors of influence, quality assurance and quality control;
— Diagnosis, differentiation and decision making.

Qualifications

A special committee should be responsible for issuing certificates to those who complete the training and pass the examination. Practical aspects should play an important role in the examination.

2.1.4.3. Nuclear oncology

The concept

Nuclear oncology is a specialization within oncology in which nuclear medicine techniques are utilized for diagnostic and therapeutic purposes. Only doctors with certification in nuclear medicine are qualified to enrol for such a course. The trainees focus on the mandatory, optional and preferential techniques and methods in nuclear oncology, as well as their related quality assurance aspects.

Scope of training

(a) Theoretical learning includes:

— General anatomy, physiology and pathology of the body;
— Pathological categorization;
— Clinical stages of tumours;
— Epidemiology;
— Diagnosis and treatment;
— Fundamentals of nuclear medicine in oncology (indications, contraindications and limitations).

(b) Practical learning includes:

— Technical considerations;
— Patho-physiological concerns;
— Bias;
— Physical examinations;
— Monitoring and managing of patients with tumours;
— Histological and cytological techniques used in oncology.

(c) Related fields:

— Histology, cytology, serum markers and imaging modalities (CT, MRI, DSA and ultrasound);
— Tumour treatment (surgical, radiological and pharmacological);
— Biology, molecular biology, general surgery, chemotherapy and radiotherapy.

(d) Technical aspects:

— Selection and handling of radiopharmaceuticals and instruments;
— Protocols of targeting, acquisition and tomography;
— Data analysis, image interpretation and factors of influence;
— Quality assurance and quality control;
— Diagnosis, differentiation and decision making.

Specific elements

Training in nuclear oncology requires an understanding of the following factors:

(a) The nature of cancer, including receptor binding, signal transduction, oncogenes and anti-oncogenes, apoptosis and the effect of radiation on normal and on cancer cells.
(b) The detection of cancer by imaging techniques, ranging from the non-specific to the cancer specific.
(c) Techniques that are designed to image a lesion with high sensitivity, but low or context dependent specificity. An example is the 'catch all' approach of bone scans, ^{67}Ga citrate scans and positron emission tomography (PET) with ^{18}F fluoro-deoxy-glucose (FDG).
(d) Imaging techniques with good sensitivity and moderate specificity, such as those using ^{201}Tl, ^{99m}Tc methoxy-isobutyl-isonitrile (MIBI) and tetrofosmin.
(e) The use of peptides, such as ^{111}In octreotide and its derivatives, in diagnosis.
(f) The use of radiolabelled monoclonal antibodies in imaging.
(g) The advantages and disadvantages of PET.
(h) The use of radiolabelled peptides, antibodies and chemical agents.
(i) The rules for interpretation of radiopeptide scintigraphy and radioimmuno-scintigraphy.

These techniques can all be applied to the detection, staging and evaluation of recurrent diseases. They can contribute to their prognosis, and help provide information regarding the response to treatment.

Qualifications

A special committee should be responsible for issuing certificates to those who complete the training course and pass the examination. Practical aspects should play an important role in the examination.

2.1.4.4. Unsealed radionuclide therapy

The concept

Therapeutic nuclear medicine is a specialization within cancer therapy in which specific nuclear medicine techniques and significant amounts of radiopharmaceuticals are utilized to treat benign and malignant diseases. Only doctors with certification in nuclear medicine are qualified to enrol in such courses. Trainees focus on the mandatory, optional and preferential techniques and methods in nuclear medicine therapy, as well as the related quality assurance aspects.

Scope of training

(a) Theoretical learning includes:

— Theory, principles and physiological foundations of nuclide therapy;
— Radiobiology, dosimetry and radiation safety;
— Patient care;
— Handling of waste;
— Fundamental aspects of cardiac nuclear medicine (indications, contra-indications and limitations).

(b) Practical learning includes:

— Technical considerations;
— Radiation protection;
— Dosimetry concerns;
— Bias;
— Physical measurements, monitoring and managing of medication for radionuclide treated patients;

— Surgical and biological techniques used in emergency situations.

(c) Related fields:

— Other therapeutic modalities (medication, chemotherapy and intervention);
— Treatment enhancement–reduction methods (surgical, biological and pharmacological);
— Nuclear physics, radiochemistry and emergency medicine;
— Terminal medical care.

(d) Technical aspects:

— The selection and handling of radiopharmaceuticals, patients, instruments and waste;
— Protocols of patient preparation, therapy planning and administration;
— Drug dosage estimation, monitoring, quality assurance and quality control;
— Long term follow-up with treatment side effect management.

Specific elements

The understanding of the following factors is also highlighted in radionuclide training:

— The use of ^{131}I in thyroid cancer;
— The use of ^{131}I MIBG in neural crest tumours;
— The use of ^{90}Y octreotide analogues in the treatment of neuroendocrine tumours;
— The use of bone seeking, beta emitting radiopharmaceuticals in the treatment of bone metastases;
— The use of radiolabelled lipiodol, particles and colloids in the treatment of liver cancer;
— The use of direct injection of radionuclide therapy agents into brain tumours and other tumour sites;
— Ethical considerations for protection in radiation treatments and patient information related to radionuclide therapy issues;
— Clinical interactions with medical and clinical oncologists, radiotherapists and radiologists using MRI, X ray, CT and ultrasound techniques for cancer diagnosis and staging, evaluation of therapy and detection of recurrences;

— Ward supervision and interaction with nursing staff;
— The cancer therapy team.

Qualifications

A special committee should be responsible for issuing certificates to those who complete the training and pass the examination. Practical aspects should play an important role in the examination.

2.1.4.5. Nuclear neurology

The concept

Nuclear neurology is a specialization within neurology in which various nuclear medicine techniques are utilized for purposes of diagnosis and investigation. Only doctors with certification in nuclear medicine are qualified to enrol on such courses. Trainees focus on the mandatory, optional and preferential techniques and methods in nuclear neurology, as well as their related quality assurance aspects.

Scope of training

(a) Theoretical learning includes:

— Anatomy, physiology and pathology of the central nervous system (CNS);
— Categorization of neural and psychiatric disorders;
— Epidemiology, diagnosis and treatment;
— Fundamental neurological nuclear medicine (indications, contraindications and limitations).

(b) Practical learning includes:

— Technical considerations and patho-physiological concerns and bias;
— Physical examinations;
— Monitoring and management of medication of patients with neuropsychological disease;
— Intervention and surgical techniques used in neurology and neurosurgery.

(c) Related fields:

— Electrophysiology, laboratory measurements and imaging modalities (CT, MRI, DSA and ultrasound);
— Two dimensional (2-D) and three dimensional (3-D) coordination systems for the brain (e.g. that of Talairach) and image fusion;
— Functional aspects of the brain, cerebellum and spinal cord.

(d) Technical aspects:

— Selection and handling of radiopharmaceuticals and instruments;
— Protocols of acquisition, tomography and intervention;
— Data analysis, image interpretation and factors of influence;
— Quality assurance and quality control;
— Differential diagnosis and decision making.

Qualifications

A special committee should be responsible for issuing certificates to those who complete the training and pass the examination. Practical aspects should play an important role in the examination.

2.1.4.6. Nuclear nephro-urology

The concept

Nuclear nephro-urology is a specialization within genito-urinary medicine in which various nuclear medicine techniques are utilized for the purposes of diagnosis and therapy in the genital and urological systems. Only doctors with certification in nuclear medicine are qualified to enrol in such courses. Trainees focus on the mandatory, optional and preferential techniques and methods in nuclear urology, as well as their related quality assurance aspects.

Scope of training

(a) Theoretical learning includes:

— Anatomy, physiology and pathology of the genito-urinary system;
— Clinical categorization of genital and renal diseases, and epidemiology;
— Diagnosis and treatment;

— Fundamental aspects of nuclear medicine (indications, contraindications and limitations).

(b) Practical learning includes:

— Technical considerations and patho-physiological concerns;
— Bias;
— Physical examinations;
— Monitoring and management of medication and manipulation for kidney and genito-urinary tract disease, including management of dialysis and transplant patients.

(c) Related fields:

— Pathology, laboratory measurements and imaging modalities (CT, MRI, DSA and ultrasound);
— Renal intervention (surgical, radiological and pharmacological);
— Immunology, endocrinology, gynaecology and paediatrics.

(d) Technical aspects:

— Selection and handling of radiopharmaceuticals and instruments;
— Protocols of dynamic and static image acquisition, tomography and intervention;
— Data analysis, image interpretation, factors of influence, quality assurance and quality control;
— Differential diagnosis and decision making.

Qualifications

A special committee should be responsible for issuing certificates to those who complete the training and pass the examination. Practical aspects should play an important role in the examination.

2.1.4.7. Respiratory medicine

The concept

Nuclear medicine is frequently used as a specialization within respiratory medicine for diagnostic and therapeutic purposes in lung and respiratory diseases. Only doctors with certification in nuclear medicine are qualified to

enrol in such courses. Trainees will focus on the mandatory, optional and preferential nuclear technology techniques and methods used in this field, as well as their related quality assurance aspects.

Scope of training

(a) Theoretical learning includes:

— Anatomy, physiology and pathology of the lungs and the respiratory tract;
— Clinical categorization of pulmonary diseases;
— Epidemiology, diagnosis and treatment;
— Fundamental aspects of nuclear medicine (indications, contraindications and limitations).

(b) Practical learning includes:

— Technical considerations and patho-physiological concerns;
— Bias;
— Physical examination, monitoring and management of medication for patients with lung disease;
— Techniques used in lung–cardiac resuscitation.

(c) Related fields:

— Ventilation/non-ventilation functions;
— Laboratory measurements and imaging modalities (CT, MRI, DSA and ultrasound);
— Therapies for lung and respiratory diseases (surgical, radiological and pharmacological);
— Cardiology and thoracic surgery.

(d) Technical aspects:

— Selection and handling of radiopharmaceuticals and instruments;
— Protocols of acquisition, gating and tomography;
— Data analysis, image interpretation, factors of influence, quality assurance and quality control;
— Differential diagnosis and decision making.

Qualifications

A special committee should be responsible for issuing certificates to those who complete the training and pass the examination. Practical aspects should play an important role in the examination.

2.1.4.8. Gastro-intestinal nuclear medicine

The concept

The application of nuclear medicine to the digestive system involves many areas of specialization in which various nuclear medicine techniques are used for diagnostic and therapeutic purposes in the treatment of hepatobiliary, pancreatic, oesophageal, gastric, intestinal and colon disease. Only doctors with certification in nuclear medicine are qualified to enrol in such courses. Trainees will focus on the mandatory, optional and preferential techniques and methods in nuclear medicine, as well as their related quality assurance aspects.

Scope of training

(a) Theoretical learning includes:

— Anatomy, physiology and pathology of digestive organs and tracts;
— Clinical categorization of diseases;
— Epidemiology, diagnosis and treatment;
— Fundamental aspects of each subspecialty (indications, contraindications and limitations).

(b) Practical learning includes:

— Technical considerations and patho-physiological concerns;
— Bias;
— Physical examinations;
— Monitoring and management of medications;
— Surgical techniques used in these cases.

(c) Related fields:

— Laboratory measurements and imaging modalities (CT, MRI, DSA and ultrasound);
— Various forms of intervention (radiological and pharmacological);

— Endocrinology, oncology, hepatobiliary surgery and paediatrics.

(d) Technical aspects:

— Selection and handling of radiopharmaceuticals and instruments;
— Protocols of acquisition, dynamic images and tomography;
— Data analysis, image interpretation, factors of influence, quality assurance and quality control;
— Diagnosis, differentiation and decision making.

Qualifications

A special committee should be responsible for issuing certificates to those who complete the training and pass the examination. Practical aspects should play an important role in the examination.

2.1.4.9. Orthopaedic nuclear medicine

The concept

Nuclear medicine is widely used in the diagnosis and therapeutic monitoring of abnormalities in bones, joints and muscles. Only doctors with certification in nuclear medicine are qualified to enrol in such courses. Trainees will focus on the mandatory, optional and preferential techniques and methods in the skeletal and muscular systems, as well as their related quality assurance aspects.

Scope of training

(a) Theoretical learning includes:

— Anatomy, physiology and pathology of the skeletal and muscular systems;
— Clinical categorization of abnormalities and epidemiology;
— Diagnosis and treatment;
— Fundamental aspects of nuclear medicine (indications, contraindications and limitations).

(b) Practical learning includes:

— Technical considerations and patho-physiological concerns;
— Bias;

— Physical examination;
— Monitoring and management of bone, joint and muscle diseases;
— Radiation synovectomy.

(c) Related fields:

— Pathology, laboratory measurements and imaging modalities (CT, MRI, DSA and ultrasound);
— Sports and gymnastics medicine;
— Oncology.

(d) Technical aspects:

— Selection and handling of radiopharmaceuticals and instruments;
— Protocols of acquisition and tomography;
— Data analysis, image interpretation, factors of influence, quality assurance and quality control;
— Differential diagnosis and decision making.

Qualifications

A special committee should be responsible for issuing certificates to those who complete the training and pass the examination. Practical aspects should play an important role in the examination.

2.1.4.10. Nuclear haematology and infective diseases

The concept

Nuclear medicine can be used to diagnose and monitor patients with haematological and/or infective disorders. Only doctors with certification in nuclear medicine are qualified to enrol in such courses. Trainees will focus on the mandatory, optional and preferential techniques and methods used in this field, as well as their related quality assurance aspects.

Scope of training

(a) Theoretical learning includes:

— Physiology and pathology of blood production;
— Clinical categorization of blood disease and epidemiology;

— Diagnosis and treatment;
— Fundamental aspects of nuclear medicine (indications, contraindications and limitations).

(b) Practical learning includes:

— Technical considerations and patho-physiological concerns;
— Bias;
— Physical examinations;
— Monitoring and management of patients with blood diseases;
— Techniques used in blood disease testing;
— The use of P-32 in haematological and myeloproliferative disorders;
— The use of inflammation, disease and infection specific agents.

(c) Related fields:

— Biochemistry, laboratory and imaging modalities (CT, MRI, DSA and ultrasound);
— Chemotherapy and intervention (surgical, radiological and pharmacological);
— Oncology, immunology and nutrition.

(d) Technical aspects:

— Selection and handling of radiopharmaceuticals and instruments;
— Protocols of acquisition and tomography;
— Data analysis, image interpretation, factors of influence, quality assurance and quality control;
— Differential diagnosis and decision making.

Qualifications

A special committee should be responsible for issuing certificates to those who complete the training and pass the examination. Practical aspects should play an important role in the examination.

2.2. TRAINING OF NUCLEAR MEDICINE TECHNOLOGISTS

2.2.1. Introduction

The nuclear medicine technologist plays a critical role in the routine practice of nuclear medicine, since the quality of work and care taken during diagnostic studies determines the ultimate diagnostic capability of the test being performed. In many countries, the importance of training technologists has been poorly understood, and consequently the professional development of this group has lagged behind that of others. As a result, there are many technologists working in nuclear medicine who have had little or no formal training. Both the availability and the role of technologists vary considerably from country to country. Recent IAEA projects have placed greater emphasis on the training of technologists, and the development of materials that can be used for vocational training may assist in encouraging the adoption of a basic level of training for all technologists. As nuclear medicine expands, there is a greater need to formalize training programmes in each country.

2.2.2. Role of the nuclear medicine technologist

The primary role of the nuclear medicine technologist is to perform diagnostic studies. Ideally, this involves understanding the overall procedure and taking responsibility for all aspects of the study (except for clinical interpretation). The breadth of responsibility varies in different countries, with an overlap of responsibilities between different professional groups (e.g. nurses and scientists), depending on resources. Where comprehensive training is established, the tasks undertaken by a technologist are likely to include the following:

— Dose calibration;
— Radiopharmaceutical preparation and quality control (subject to local legislation);
— Patient preparation;
— Image acquisition;
— Full study analysis;
— Electronic display of data and hard copy;
— Routine instrument quality control.

Technologists are also likely to have responsibilities in management (personnel and data), teaching and research. Although, in several countries, they may have only a very specific repetitive duty to perform, the trend is for technologists to take on overall responsibility for the execution of studies.

Involvement in the whole procedure and awareness of the outcome is important, providing not only a better appreciation of appropriate quality assurance but also improved job satisfaction. There is often confusion between the terms 'technologist' and 'technician'. In this manual the term technologist will be reserved for persons who have direct contact with patients and fulfil the roles outlined above; the term technician will be reserved for individuals who undertake maintenance of instrumentation or work in laboratories.

2.2.3. General education of nuclear medicine technologists

In many countries, the lack of structured training has resulted in the employment of a broad range of individuals, from elementary school leavers to science graduates. It has recently been suggested that the minimum level of education should be at school higher certificate level (equivalent to the entry level for tertiary education and usually taken at 18 years of age). In many countries, technologists enter the field after completion of a tertiary course in a different medical specialty (e.g. radiography, nursing or medical laboratory technology), and such courses, even without any formal nuclear medicine component, provide useful background knowledge. In some cases, graduates in general science are employed. They are usually well equipped to deal with the technical component of the work, but will normally require additional courses in relevant medically oriented subjects. It should be noted that full-time academic courses in nuclear medicine technology, as now commonly offered, tend to include a range of subjects that broaden the education of students (e.g. business management and behavioural science) rather than being merely vocationally based. What needs to be recognized is that, in order to fulfil their role, technologists require a reasonable educational background.

2.2.4. Specific nuclear medicine courses

In many countries where nuclear medicine has developed to the stage of there being a continuous demand for nuclear medicine technologists, specific courses have been established. These vary from country to country and generally include the following options:

(a) Full-time certificate, diploma or degree courses specifically for nuclear medicine;
(b) Courses designed to provide training in diagnostic imaging (radiography) that contain a significant component of nuclear medicine;

(c) Bridging courses intended to provide a suitable pathway from other disciplines;
(d) Short postgraduate courses.

The establishment of these courses has usually evolved over several years, driven by continual growth in the field. Usually the development span has evolved by the introduction of part-time certificate courses that eventually become full degree courses. Accompanying this development has been the establishment of professional societies specifically for technologists as well as the growing representation of technologists in more general societies. Nevertheless, in many countries the establishment of specialized courses and development of the profession has been slow. The difficulty is that there needs to be a critical mass of persons able to teach nuclear medicine and a definite demand for new employees before courses can be justified. Most persons who are qualified to teach are already working full-time in the clinical practice of nuclear medicine, and have little time available for teaching. Furthermore, small clinical departments are often geographically remote from established centres, and it may not be practical for students to attend formal lectures. Student numbers tend to be small given a relatively slow turnover of staff in established departments. In many countries, nuclear medicine has developed without the establishment of specialized courses, with new technologists simply gaining experience on the job. As a result, a large number of working technologists have not received any formal training in nuclear medicine.

2.2.5. Vocational training

Most nuclear medicine courses include some component of hospital experience where technologists can supplement theory with practical experience. Such experience is normally considered to be an essential component of technologist training, even where full-time degree courses exist. As indicated earlier, many technologists simply train on the job, without any formal course work, and seldom with any formal approach to their training. Short courses on relevant subjects are sometimes included (e.g. on radiation safety). Most IAEA activities tend to support vocational training, either by the provision of fellowships for experience in more advanced departments or by offering short training courses or workshops, which tend to focus on specific practical areas of nuclear medicine.

One particular IAEA project that deserves mention is the Distance Assisted Training programme, intended primarily for technologists who are working full-time. The project, originally funded by the Australian government, has involved the development of teaching materials that, while outlining basic

but essential theory, still emphasize practical aspects and encourage students to find out how to apply the principles involved to their own departments. The coverage is comprehensive and involves around 500 hours of study. The project was initiated with a small group of students in Asia but now involves a sizeable number there, as well as sister projects that have been established in Africa and Latin America. The programme offers an opportunity for students living far away from teaching centres to undertake formal training, while also encouraging countries to establish their own training programmes. The material is proving useful as a general teaching resource and is being translated into several languages (including French and Spanish).

2.2.6. Accreditation and licensing

An important component of professional development has been the establishment of mechanisms for recognizing competence in nuclear medicine, usually involving the relevant professional society or licensing body. Accreditation usually involves the establishment of a specific syllabus, with the assessment of available courses, inclusion of a period of practical experience in approved departments and possibly examination. At the stage of writing, there is no international consensus on the requirements for accreditation. An important consideration in the ongoing discussion is the recognition that not all countries can realistically achieve the same standard of training at this time; a two tier system would seem appropriate.

2.2.7. Suggested syllabus for training of nuclear medicine technologists

The following syllabus provides examples of the topics that should be included in training programmes for nuclear medicine technologists. It includes topics that are covered in the IAEA distance assisted training project:

(a) Basic nuclear physics — radioactive decay and interaction of radiation with matter.
(b) Introduction to radiopharmacy — basic principles and definitions and basic quality control.
(c) Safe handling of radionuclides — hazards of radiation, safety procedures and dealing with spills.
(d) Nuclear medicine instrumentation — dose calibrators, survey meters, probes, gamma cameras and basic quality control.
(e) Computers in nuclear medicine — interfaces with gamma cameras and general processing.

(f) Introduction to anatomy and physiology — general body systems and blood supply.
(g) Introduction to human behaviour — patient communication and handling.
(h) Applications of nuclear medicine in thyroid, liver, gastro-intestinal tract, kidneys, heart, lungs, brain and bones, in tumour imaging and in infections:
  — Anatomy, physiology and typical patient presentation;
  — Radionuclides and mechanisms of uptake;
  — Procedures specific to application;
  — Protocol development.
(i) SPECT physics and applications — filtering, reconstruction, brain SPECT and cardiac perfusion SPECT.

2.2.8. Summary

The nuclear medicine technologist is an important member of the nuclear medicine team and has a crucial role to play in ensuring that studies are carefully executed, with attention given to overall quality. With appropriate training, the technologist can accept responsibility for the routine clinical work and can assist with other tasks, including departmental management, research and teaching. The adoption of formal training programmes and recognition of qualifications by relevant national bodies will encourage the professional development of the group.

2.3. TRAINING IN RADIOPHARMACY

2.3.1. Introduction

Radiopharmacy is an essential and integral part of all nuclear medicine facilities. In practice, it is apparent that the preparation of radiopharmaceuticals is performed in a wide range of disciplines. Although pharmaceutical expertise is essential, the process is not always managed or performed by a pharmacist, which, although desirable, is not necessarily achievable. Standards of practice need to be consistently high, irrespective of the background of the staff performing the process.

Training should be adapted to the background and level of expertise of the trainees in order to ensure that they have the necessary grounding in those aspects of radiopharmacy relevant to their intended role. The pharmacist or person managing the preparation of radiopharmaceuticals needs to be able to demonstrate a thorough knowledge of all areas of the specialty. In addition, training in radiopharmacy should be a separate, required section or subject for:

— The nuclear medicine physician;
— The nuclear medicine technologist;
— Other professional and technical staff.

Staff selected for training in radiopharmacy should demonstrate:

— Orderly work;
— Conscientiousness;
— Ability to function well under pressure;
— Responsibility.

Since work in the radiopharmacy commences before activities in the rest of the department, staff should be capable of working effectively at the start of the day.

Training should include, but not be limited to, aspects of:

— Radiation safety and hygiene;
— Pharmaceutical technology and aseptic techniques;
— Radiochemistry, and preparation of radionuclides and radiopharmaceutical compounds;
— The use of radiopharmaceuticals;
— Quality control and record keeping;
— Adverse reactions;
— Factors affecting biodistributions.

Training should be conducted by a competent person with access to adequate facilities to cover all the aspects required.

2.3.2. Postgraduate syllabus for radiopharmacists and radiopharmaceutical chemists

Although a consensus has not been reached on what is required to qualify as a recognized radiopharmacist or a radiopharmaceutical chemist, it is generally accepted that three years of professional experience working in a radiopharmaceutical laboratory should be part of the training requirements.

The programme should consist of four components:

(1) Courses, including practical training as provided by universities;
(2) Three years of on-the-job training in appropriate institutions;
(3) A final examination;
(4) Continuing professional development.

2.3.3. Recommended course contents

(a) An introduction to the following disciplines:

— Biochemistry;
— Physiology;
— Pharmacology and toxicology;
— Nuclear medicine.

(b) Pharmacy:

— Pharmaceutical technology: good manufacturing practice, quality assurance, sterile manufacture, aseptic manufacture, parenteral products, formulation and packaging.
— Pharmaceutical analysis: general methods, quality assurance, quality control procedures, shelf life, regulations and legal aspects, and marketing authorizations.
— Responsibilities of personnel.

(c) Radiopharmaceutical chemistry:

— History and physics of radioactivity;
— Properties of carrier-free substances, and separation techniques;
— Production of radionuclides in nuclear reactors and cyclotrons, targetry, nuclear chemistry and generators;
— Synthesis, purity and stability of labelled compounds and radionuclides, and radiochemical purity;
— Radionuclides in analytics, autoradiography and the radiotracer principle;
— Criteria for radiopharmaceuticals, legal aspects of good manufacturing practice and quality control;
— Production of radionuclides;
— Technetium-99m generators, radiopharmaceuticals and kit preparation;
— Other radiometals and radioiodination;
— Cell labelling;
— PET radiopharmaceuticals;
— Animal models and animal protection regulations;
— Radiotracer transport, pharmacokinetics and modelling.

2.4. TRAINING IN MEDICAL PHYSICS

2.4.1. Introduction

Nuclear medicine remains a highly technical field that not only uses advanced instrumentation but also applies numerical techniques. The direct use of unsealed sources of radiation calls for particular attention to radiation safety. As in the case of the radiopharmacist, the medical physicist is not necessarily required on a full time basis in small departments but should be available for consultation. Since the medical physicist's role is largely advisory and supervisory, the number of medical physicists working in the field is small. It is therefore difficult to justify the development of training courses in most countries. Where medical physics is established as an academic specialty, there are well developed postgraduate courses, suitable for general training. Enrolment is, however, expensive so that opportunities for funded attendance are limited.

2.4.2. The role of the medical physicist

As in the case of other nuclear medicine professionals, the role of the medical physicist varies from country to country, depending to some extent on the stage of development of nuclear medicine practice. There is an overlap of duties with those of other professionals, and in some countries the distinction between the medical physicist and the technologist is hard to define. The medical physicist and the technologist in any event work closely together in many areas.

The physicist is responsible for the following areas:

(a) Radiation safety

The radiation safety officer is normally a trained medical physicist, although responsibility in a small department may be delegated to another professional, provided advice can be sought from an available expert.

(b) Specification, acceptance testing and quality control of instrumentation

The medical physicist is normally directly involved in equipment procurement and takes direct responsibility for acceptance testing and establishment of routine quality control; in many cases, technologists perform routine quality control, usually under the supervision of a medical physicist.

(c) Maintenance of equipment

The medical physicist normally undertakes first line maintenance and helps to identify and resolve any problems in liaison with the supplier or service personnel.

(d) Computer system management and support

Increasingly, the medical physicist takes responsibility for overall computer system management and provides advice on computer use as well as first line support for application software; in some countries, the medical physicist is directly involved in routine computer analysis. However, in most countries this is the responsibility of technologists.

(e) Development and validation of clinical studies

Nuclear medicine is a continually evolving field and functional information is increasingly obtained from quantitative analysis; the medical physicist usually works closely with medical staff to provide technical advice relevant to the execution of studies. Frequently, software needs to be developed or adapted with the subsequent validation of newly developed procedures.

(f) Supervision

The medical physicist supervises measurement, dispensing and administration of radiopharmaceuticals for therapeutic purposes and is also involved in radiation safety related to this procedure.

(g) Administration

Most of the above duties involve administrative tasks such as the preparation of guidelines, record keeping and communication with other professionals and suppliers; the physicist is usually directly involved in the planning of facilities, equipment used and procedures.

(h) Teaching and research

Most medical physicists are involved in teaching other professionals (e.g. in radiation safety and instrument principles); many are actively engaged in development work or undertake phantom experiments as part of validation

procedures (applied research) or are involved in clinical research projects (e.g. data analysis and statistical advice).

2.4.3. General education of medical physicists

A good general education is possibly the most important aspect of a medical physicist's training and is a factor that is often underestimated. Most medical physicists enter the field having completed a degree in physics or a similar discipline such as engineering or occasionally computer science. The ability to tackle technical or numerical problems and to apply lateral thinking to their solution requires an education that includes mathematics and a broad understanding of technical and scientific principles. The physicist should be comfortable with advanced mathematical concepts, have experience in experimental design and scientific methods, and be conversant with applied statistics, electronic troubleshooting, computer programming and instrument design. These topics are not normally covered in sufficient depth in the vocational degrees intended for health professionals such as technologists or radiographers.

2.4.4. Postgraduate courses

Most specific courses in medical physics are offered at the master's level and are intended for individuals who already have a degree in physics. The content is usually intended to provide an overview of the applications of physics to medicine and recognizes the fact that most graduates in physics have little or no background in medicine. Courses therefore usually cover anatomy and physiology and provide an introduction to other areas of medical science. The medical physics coverage is often quite broad and includes applications in therapy and general diagnostic imaging. Bridging the gap between pure physics and medicine is achievable, whereas providing the necessary mathematical and scientific background to a non-physics graduate with a background in medical science would necessitate further undergraduate study in the relevant field. Most master's programmes include some component of project work that aims to develop relevant research skills, while some programmes involve full-time research only. Few programmes, if any, provide a sufficient amount of practical experience relevant to the workplace.

2.4.5. Vocational training

The relatively small number of physicists in many countries makes it very difficult to establish and maintain postgraduate teaching programmes, with the

result that physicists in most countries train on-the-job. The turnover of physicists is far lower than that of technologists so that the number of vacancies cannot even justify broad courses that encompass radiotherapy. This makes it difficult for a physicist who may be working alone in an institution to gain the necessary experience by working alongside nuclear medicine technologists. There are no established guidelines for training in nuclear medicine physics. In the case of other professionals, the IAEA provides mechanisms for vocational training through fellowship programmes or short courses and workshops. Short, focused, courses in fields such as radiation safety can be quite effective, as can workshops on quality control or specific computer skills. However, the nature of the work, which is often advisory or developmental rather than involving routine activities, can be difficult to learn in a short attachment since the exact role of the physicist and the equipment can vary considerably between individual departments. Of paramount importance is the physicist's general education as well as his or her ability to find out and synthesize information when required, and to be aware of the existence of resources. The ability to find solutions from first principles, when faced with a question, can only develop with exposure to multiple situations and problems. This normally requires a relatively long attachment working with experienced staff.

2.4.6. Accreditation and licensing

It is widely recognized that individuals using unsealed sources should be licensed and should show an understanding of the responsibility that this involves. Radiation safety officers normally undertake a specific examination to test their knowledge and practical skills. Specific vocation based accreditation is uncommon in other areas of nuclear medicine physics. In many cases, professional societies require their members to have undertaken suitable basic education with relevant experience in nuclear medicine physics over a number of years. In some instances, examinations are set to test knowledge specific to the area of medical physics practised. However, it is the responsibility of the employing authorities and medical practitioners to assess the relevant training of medical physicists and to employ only suitably qualified individuals, or to ensure that suitable training is provided.

2.4.7. Suggested syllabus for the training of medical physicists in nuclear medicine

(a) Assumed knowledge:

— Applied physics;

— Applied mathematics and statistics;
— Computer architecture and programming;
— Electronics and instrument design.

(b) General components:

— Basic anatomy and physiology;
— Common disease processes;
— Data and image processing;
— Experimental design, including simulation and modelling.

(c) Specific nuclear medicine components:

— Radiation safety — safety procedures, regulations, radiation surveys, shielding and exposure calculations, internal dose estimation, decontamination procedures, risks of radiation, waste disposal, limits of intake and specific procedures for use of unsealed sources;
— Radiation biology — mechanisms of tissue damage and interaction of radiation with tissue;
— Types of radiation and interaction of radiation with matter — alpha, beta and gamma radiation, attenuation, scattering and shielding;
— Detection of radiation — types of detector, principles of design and performance characteristics;
— General principles of tracer studies — radionuclide production, radiopharmaceutical design and mechanism of uptake, counting statistics, tracer dilution theory and in vitro techniques;
— Imaging instrumentation — gamma camera design, collimators, photomultiplier tubes, pulse height analysis, correction circuitry, performance characteristics, acceptance testing and quality control;
— Computers in nuclear medicine — camera–computer interface, display and processing features, system architecture, networking, file formats, data transfer and the Picture Archiving and Communication System (PACS);
— Computer processing — specific numerical approaches to nuclear medicine data, including filtering, convolution and deconvolution, factor analysis, curve fitting, compartmental analysis and Monte Carlo techniques;
— Emission tomography — SPECT data acquisition, reconstruction, acceptance testing and quality control, sources of artefact and correction, quantification and basic principles of PET;

— Applications of nuclear medicine — specific goals of radionuclide studies, protocols for clinical procedures, understanding limitations in clinical interpretation, and typical artefacts and problems;
— Other imaging modalities — familiarity with X rays (including special procedures), CT, MRI, ultrasound and intermodality co-registration.

2.4.8. Summary

The medical physicist needs to be a multiskilled individual with an aptitude for general problem solving and familiarity with a wide range of the technical aspects of nuclear medicine. These skills require a strong mathematical and scientific foundation. Although postgraduate programmes are available, they normally require 1–2 years of full-time study and do not necessarily provide practical experience relevant to the workplace. Establishment of training programmes is difficult due to the small numbers involved in many countries. There is scope for improvement of the training available to medical physicists.

2.5. TRAINING IN NUCLEAR INSTRUMENTATION

In many countries, the service and repair of nuclear medicine equipment is undertaken by qualified service engineers or technicians employed by the supplier. Maintenance contracts are strongly recommended, particularly in the case of gamma cameras, for which maintenance and calibration are highly specialized procedures. Suppliers should provide specific training on their own equipment. Spare parts can only be guaranteed where the supplier or manufacturer, rather than simply a local agent, continues to be involved. In most cases, centralized electronic laboratories are equipped to deal with the repair of less specialized equipment (e.g. counting equipment) that is generally robust and does not justify dedicated maintenance staff. The local atomic energy authority can often assist. The IAEA has in the past awarded fellowships and held workshops to train technicians in some aspects of maintenance, particularly in projects where refurbished or low cost components were supplied directly by the IAEA.

In general, routine maintenance is provided by medical physicists, who can assess problems and, where possible, undertake minor repairs. The medical physicist should be familiar with the operation of the equipment and understand the principles of measurement being used in order to diagnose problems correctly. Direct repairs to electronic equipment now usually involve board replacement rather than direct circuit troubleshooting.

Familiarity with the components of personal computers is useful, as replacement components are inexpensive. Equally important is knowledge of system software, as many problems are a consequence of the software configuration rather than hardware faults. An understanding of local area networks (LANs) is becoming increasingly useful, as interconnection of equipment is often performed independently of individual suppliers.

2.6. TRAINING IN RADIATION SAFETY AND RADIATION PROTECTION

2.6.1. Introduction

Training requirements in nuclear medicine depend on whether the target group comprises technologists, medical staff, nursing staff or physicists. The training should be sufficient for, and adapted to, their particular needs. In general, the scope of knowledge required for the various categories of personnel is as follows.

(a) Technologists:

— Basics of radiation protection;
— Use of monitoring instruments;
— Safe handling of unsealed sources;
— Safe administration of radiopharmaceuticals (diagnostic and therapeutic);
— Waste management;
— Accident procedures and decontamination;
— Radiation during pregnancy.

(b) Medical staff:

— Basics of radiation protection;
— Safe administration of radiopharmaceuticals (diagnostic and therapeutic), accident procedures and decontamination;
— Radiation during pregnancy.

(c) Nursing staff:

— Basics of radiation protection (in brief);
— Safe handling of radioactive patients;
— Accident procedures and decontamination.

TABLE 2.3. IMAGING FACILITIES IN RADIATION SAFETY TRAINING

Type of centre	Techniques employed	Radiation exposure
Centres without imaging facility	In vitro	Very low
Centres with imaging facility	In vivo and in vitro	Moderate
Centres with diagnostic imaging and therapeutic facilities	Large dose therapy with beta and gamma emitters	Moderate to high

(d) Physicists:

— All of the above at a higher level;
— Patient dosimetry;
— Safety assessment;
— Local and Basic Safety Standards (BSS) regulatory requirements (see Bibliography to this section).

Irrespective of the presence of a designated radiation protection officer (RPO) in the department, physics staff must be able to perform most, if not all, of the functions of the RPO.

2.6.2. Training syllabus

The level of training in radiation safety required depends on the type of facilities available and techniques performed, and may differ considerably between institutions. The training course for trainers, however, must be of a consistently high standard.

Table 2.3 shows types of facilities and their appropriate radiation exposure levels.

Both syllabus and duration of training will depend not only on the target group (see above) but also on whether the course to be conducted is, for example, an introductory course, a specialized or customized course, or a course leading to the award of a degree, diploma or certificate.

The following syllabus outline could be modified according to the target group and level:

(a) Theoretical topics:

— Structure of matter;
— Radioactivity and radiation;
— Radiation units;
— Measurement of radiation;
— Sources of radiation, including external–internal and background radiation;
— Natural and human made radiation sources;
— Biological effects;
— Basics of radiation protection;
— BSS and International Commission on Radiological Protection (ICRP) recommendations;
— System of dose limitation;
— Radiation hazards in nuclear medicine;
— Shielding design and assessment;
— Handling of unsealed sources;
— Radioactive waste handling;
— Radiation accident procedures;
— Decontamination techniques;
— Personnel instruments, area monitoring instruments and instrumental techniques;
— Patient dosimetry;
— Dosimetry and advice for pregnant patients;
— Patient advice on breast feeding;
— Transport of radioactive material, both internal and external to the institution;
— Records required and record keeping;
— Quality assurance;
— Regulatory requirements (local);
— Responsibilities.

(b) Practical topics:

— Radiation measurement;
— Safe handling of unsealed sources;
— Radioactive waste disposal;
— Accident procedures;
— Contamination monitoring;
— Decontamination techniques.

2.6.3. Provision of training

In some countries, radiation safety is included in the training of technologists and nuclear medicine physicians. Depending on their background, physicists may or may not have had any radiation safety training. Nurses would rarely receive any.

Where the staff have had no training, there is a variety of options:

— Formal courses offered locally (e.g. by tertiary institutions or the local atomic energy agency);
— Ad hoc training courses by experienced local persons or under IAEA or similar sponsorship;
— Distance learning courses offered by various international institutions (mainly universities) or under IAEA sponsored regional cooperative agreements;
— Training placements in other, usually foreign, nuclear medicine departments.

Alternatively, some countries may wish to establish a centre of excellence with advanced facilities to work as a hub and disseminate learning to an entire region. Such centres would need support from developed countries and international organizations such as the IAEA. Experts from reputable centres could also provide training at the local site, depending on its requirements.

BIBLIOGRAPHY TO SECTION 2.6

INTERNATIONAL ATOMIC ENERGY AGENCY, International Basic Safety Standards for Protection against Ionizing Radiation and for the Safety of Radiation Sources, IAEA Safety Series No. 115, IAEA, Vienna (1996).

2.7. TRAINING IN MOLECULAR BIOLOGY USING RADIONUCLIDE METHODS

2.7.1. Introduction

The large spectrum covered by the potential use of molecular techniques, especially after the advent of the polymerase chain reaction, has led to an

increased number of requests for training. The use of radionuclides is proposed in a large variety of molecular biology protocols as they can be easily traced. The availability of practical ways of detecting the presence of a radionuclide in a specific molecule (qualitative result) and its potential to be measured (quantitative analysis) are the main reasons why radionuclides are important in molecular biology.

In the theoretical and practical training in molecular biology techniques and also in radionuclide handling, some specific points should be considered, such as the transfer of technology to scientists and technicians from other research fields (immunology, pathology and microbiology) who are not familiar with molecular biology and radionuclide techniques, and upgrading the skills of experienced scientists regarding the use of new protocols in molecular biology. Thus, training courses should be given at two levels: basic and advanced. If these aspects are not recognized, there may be a real risk of courses being either too profound to those who are not familiar with the techniques or very superficial to others who have these skills already.

2.7.2. Training courses

2.7.2.1. Selection of the participants

The best way of selecting those who will be attending the training initiative is to evaluate the previous involvement of the candidate in the course topic. Sometimes a simple curriculum vitae analysis is not sufficient to determine the suitability of candidates. Therefore, alternative criteria have to be used in addition, such as prospective participants supplying a summary of work they propose doing linked to the training theme and a list of their recent publications. The candidate should be able to specify the objectives of their project, to detail the importance of the methodology that will be learnt and how the techniques will be applied in solving specific problems.

2.7.2.2. Course content

Owing to the complexity of the protocols that are usually carried out in molecular biology training courses, the trainees should have access to the theoretical and practical programmes in advance. Distance learning packages (CDs or other electronically available formats) specifically developed for the training course and access to an Internet web site showing the guidelines of the course and the details of the programme are recommended. A list of references should also be provided.

During the course, 40% of the time available should be allocated to the theoretical background and 60% to the performance of experiments and discussion of the protocols and results. Participants should be informed beforehand about the possibility of bringing samples, when possible and allowed, to be tested in the course. Such active participation enhances the self-involvement of the students. Participants should also be asked to present ongoing relevant work they are involved with. In addition, they should be asked beforehand to bring results, if they have any, illustrating the problems they have experienced and thus be actively involved in the troubleshooting section. This should be an essential component of any training course.

Special attention should be given to the handling of radioactive material that will be mostly used in labelling probes or primers for molecular hybridization or polymerase chain reactions (PCRs). Attention should also be paid to the handling of biologically hazardous materials, such as live mycobacterium, HIV samples, ethidium bromide and phenol.

Details of a basic programme of a training course on molecular diagnosis using radiolabelled DNA probes are given below:

(a) Theoretical background:

— Advantages of using radionuclides in molecular biology.
— Molecular diagnosis — molecular probes; definition of the theoretical basis of hybridization experiments.
— PCRs.
— Good laboratory practice — handling radioisotopes and bio-safety measures.

(b) Experiments:

— DNA extraction from biopsies, blood and paraffin embedded tissues. Different methods of DNA extraction should be used:
  - column chromatography using commercial resins;
  - phenol–chloroform extraction;
  - boiling method after proteinase K digestion.
— PCRs and variations, including RT-PCR and multiplex PCR using different disease models.
— Agarose and acrylamide gel electrophoresis; Southern and dot blot experiments.
— Radiolabelling of DNA probes.
— Molecular hybridization, including reverse line assays.
— Cloning PCR products, mini-prep preparation and manual sequencing.

— PCRs, restriction enzyme digestion and restriction fragment length polymorphism (RFLP) for typing.
— Secondary structural content prediction (SSCP) and other tests for finding mutations.

One point that needs to be emphasized in the course is the handling of radioactive material and disposable waste.

Certain precautions should be taken, such as the following:

— The laboratory in which hybridizations are performed should be visibly marked with the radiation symbol and a warning of the radioactive material in use within.
— Bench-tops and surfaces where radioactive materials are to be used should be covered with absorbent paper.
— Work should be conducted in a paper lined tray to contain spills and behind an acrylic protection shield.
— Participants should wear laboratory coats, protective glasses and disposable gloves when handling radioactive materials.
— Hands and laboratory working areas should be frequently monitored using a suitable radiation survey instrument, such as a Geiger–Müller counter.
— A microcentrifuge should be exclusively dedicated to the centrifugation of radioactive materials.
— Labelled probes can be stored at –20°C in appropriate acrylic boxes.
— Solid and liquid waste from hybridization experiments and from the first washing procedure should be stored in order to allow activity to decay.
— Containers should be labelled and records should be kept to determine when the material is suitable for release as normal waste.
— The storage area should be used solely for radioactive waste.
— A decay storage period of ten half-lives will reduce the level of the initial radioactivity to one thousandth of the level of the original radioactivity.
— 'Delay to decay' is the preferred waste management option, both environmentally and economically, whenever possible.
— Low activity liquid waste, such as that from the second washing of the hybridization protocol, is either run into a particular marked sink or is stored in holding tanks, where decay is allowed to take place.
— Institutes should have a designated radiation safety office and institutional safety and waste disposal guidelines based on national guidelines.
— Valid licences allowing the use of radioactive substances should be obtained from the competent authority before projects involving radionuclides are considered.

2.7.2.3. Guest visits and fellowships

In the vast majority of cases, the simple transfer of technology during a training course is not enough to allow participants to set up the methodologies in their own setting. Difficulties are always encountered and adaptations of the protocols are necessary. It is important to emphasize during training that there are specific points related to the performance of the protocols which can be modified without compromising the perfect outcome of the assay. The possibility of making adaptations to a formal protocol is linked to a previous professional background in the area. If local expertise in molecular biology is lacking, an alternative could be an expert guest visit. This professional will take into consideration the local conditions and the availability of equipment and supplies, analysing the real situation on the spot.

Experiments will be performed, in conjunction with the group, by the expert and eventual difficulties can be solved, alternatives proposed and the best logistics worked out.

Fellowships should also be offered by the training institution to junior scientists who could spend more than one month analysing samples collected in their settings. The interesting point of this possibility is that the trainee will learn the protocols as they are performed in standard conditions and will also gain experience of the logistics of the reference laboratory.

2.8. TRAINING IN RADIOIMMUNOASSAY

2.8.1. Introduction

Since the introduction of in vitro binder ligand assays, in the later 1960s, there have been tremendous advances in the field of RIA. New and specific binders can be produced in large quantities because of the availability of the technology to generate monoclonal antibodies from hybridomas, heterohybridomas, recombinant phages, oligonucleotide arrays (for construction of antibody arrays) and, recently, synthetic binders from polymers or plastic moulds. Protein binders can also be engineered according to design by molecular modelling and point mutagenesis, then humanized by the recombinant phage technique. The availability of non-isotopic labels such as silver and gold sol has further stimulated the development of sensitive dipstick immunochromatographic assays which enable semi-quantitative point-of-care testing and self-testing to be carried out in clinics, wards and the home. The recent invention of conductive polymers and the rapid advances in optics enable the design of immunosensors. Complemented by humanized antibody

technology, this will certainly contribute to the future development of in vivo immunosensors for real time measurement of chrono-biological changes of whole blood analytes. In addition, there have also been rapid advances in solid phase design, techniques in immobilization of chemicals such as self-assembly of monolayers, and molecular visualization and micromachinery by atomic force microscopy. These will certainly be applied to immunoassays in future to increase assay sensitivity and specificity. All these new 'black box' immunoassay methodologies, mostly protected by patent and copyright, have to be calibrated against the gold standard set by RIA, which still remains the most robust and cost effective immunoassay.

In the diagnostic industry, RIA is still used to set up the first workable immunoassay methodology for new analytes before the then thoroughly evaluated method is transformed into another commercial assay format. This developmental role of RIA will be fully realized when the shift is made from the present anatomical genomic information phase with massive amounts of genetic information to the future proteomic action phase. In routine diagnostic areas, RIA will continue to be used as the reference method to solve problems generated by non-isotopic immunoassay as a result of analytical interference. With the use of modular robotic systems and improved antibody design, RIA can be automated to further reduce operational costs and is well suited to nationwide targeted screening of congenital and other disorders.

In the final analysis, RIA will continue to play a major role in most developing countries in supporting routine diagnostic services, especially for infections, tumours, coronary heart disease, diabetes and degenerative diseases. In more developed countries which are striving to achieve a comprehensive long term development of diagnostic biotechnology, the method will be used more frequently in the production processes of monoclonal antibodies.

2.8.2. Principle

Radioimmunoassay continues to maintain a favoured position among microanalytical procedures not only because of its sensitivity, acceptable precision, robustness (working best in non-optimal conditions) and wide applicability, but also because it is comparatively the least expensive of numerous methods available for the detection and measurement of substances of clinical diagnostic interest. The advantage of RIA methodology is that it is freely available in the public domain — one of the major criteria for technology transfer. It is amenable to bulk reagent based methodology, using at least some locally produced reagents, in contrast to methods totally dependent on black box type commercial kits. For this reason, RIA is seen to be the most suitable option for developing countries, where financial constraints combined with an

unreliable supply preclude the purchase of expensive commercial RIA kits. Even less accessible for developing countries are the so-called 'non-isotopic' kits, the cost of which is estimated to be at least three times that of commercial RIA kits. The principle that should underlie training in RIA, particularly in developing countries, is the provision of workers with the necessary skills and knowledge of the basic principles of RIA to enable them to use bulk reagent methodology and build up their basic troubleshooting and interpretative skills.

2.8.3. Areas of training

It is essential that all workers in an RIA laboratory should have some knowledge of the basic physics and chemistry of radionuclides, safe laboratory practice, and the laws governing the handling of radioactive materials and their disposal. They should understand that the greatest danger to an RIA worker is not from the radioactivity in the test tube but rather from infective agents that may be contained in the blood or other samples being handled and that, if precautions are taken against this microbiological hazard, the radioactive one takes care of itself. It has been computed that, while the dose from a single transatlantic flight equals 0.04 mSv and the dose from an X ray examination can be up to 10 mSv, the average annual dose for RIA using ^{125}I tracers is approximately 0.03 mSv.

It is essential for those working in RIA to have a firm understanding of the theoretical principles that underlie both limited reagent (RIA) and excess reagent immunoradiometric assay (IRMA) approaches. Academic personnel who will serve as laboratory managers need to be trained to a higher level, preferably postgraduate, than laboratory technicians, additionally learning interpretative skills according to their study approach (technical, scientific and/or medical). It should be borne in mind that technicians using bulk reagents for in-house assays need a more thorough training than those who are merely using protocols provided with commercial kits.

Thus, the main areas of training in RIA are:

— RIA methodology;
— The setting up and validation of assays;
— Quality control;
— Data processing;
— Local reagent production.

To varying degrees, the following areas are also important:

— The organization and operation of RIA laboratories;

— Basic laboratory information management;
— How to search for and access recent clinical diagnostic information on the web;
— The applications of RIA in both clinical and research areas.

2.8.4. Theory

Excess reagent IRMA methods employing labelled antibodies rather than antigens are gaining in popularity. Similarly, greater use is being made of solid phase separation methods, particularly of those not requiring centrifugation, despite a possible slight increase in cost. In these and similar areas where a range of alternative approaches is available, the technical and economic advantages and disadvantages of each should be considered so that the workers concerned may arrive at informed decisions as to the most suitable approach for their particular situation. Bulk reagent based assays cannot be set up successfully and employed without sufficient theoretical and practical training in basic aspects of assay methodology, such as:

— The characterization and evaluation of antisera;
— The linking of biomolecules to the solid phase;
— The basic principles of antibody production;
— The construction of dilution and standard curves;
— Standards and standardization;
— Assay design and optimization;
— Troubleshooting procedures.

Academics and technicians would all require training in these areas, although academics would require more in-depth knowledge of, for example, the theoretical and mathematical concepts involved in designing an assay to suit a particular need. Laboratories in countries with high socioeconomic strength should place additional emphasis on cost effective overall laboratory management, critical evaluation of reagent supplies and on making the methodology more friendly to the user.

2.8.5. Validation

The essential responsibility for detailed validation of an RIA reagent lies with the manufacturers and suppliers. Training should, however, also include the accepted standard operation procedures that manufacturers are expected to carry out before the batch release of the reagents.

Workers should be made aware that assay characteristics claimed by reagent or kit manufacturers may not always hold true because of climatic changes or the influence of, for instance, transportation or a delay in delivery. Training should include standard methods of assay validation (cross-reactivity, recovery and parallelism) and the means by which all essential features of an assay such as precision, bias, working range or sensitivity are ascertained before it is made available for clinical or research use. Workers should also be made aware that these characteristics cannot be fully determined and that they may change with fresh batches of reagents or other changes in assay conditions. In this context, knowledge of the stability and storage conditions to which different reagents may be subjected, such as stock and working solutions of standards and antisera, buffers and protein binding inhibitors, is necessary for preventing possible later problems. For senior staff with a clinical foundation, training should also include non-analytical variations and the global validation of RIA results through horizontal linkage of analytical data with other related quantitative and qualitative clinical laboratory information.

2.8.6. Quality control

The training programme should acknowledge that internal quality control (IQC) is a sine qua non of good RIA practice.

Workers should be made fully conversant with the minimum standards of day-to-day IQC practice and the checks and balances that need to be included in each assay in order to ensure reliability of analytical results. They should understand the concepts of within and between batch variability, the construction of quality control charts and curves, and of imprecision profiles and how these are used, in order to decide upon statistical acceptance or rejection of an assay result (or an entire assay on the basis of pre-set standards of precision and bias). The main causes of poor precision or unacceptable bias, especially when these are seen to occur in an assay that had previously been performing well, need to be understood so that remedial action can be taken at an early stage. Understanding the value of participation in an external quality assessment scheme (EQAS) and its role in the overall concept of quality control, what it can and cannot do, should be included as part of the training. There is, for example, a common misconception that an EQAS can serve as a substitute for an IQC.

2.8.7. Data processing

Modern computer assisted data processing of RIA and IQC data makes a significant contribution to overall quality control in RIA, enabling the storage

and retrieval of results and the monitoring of assay performance over a period of time. The ready availability of powerful desktop computers at a fraction of what they would have cost a decade or so ago makes computerized data processing a practical proposition in all RIA laboratories, even in developing countries. Suitable software packages enabling the computation of composite results from more than one hundred assays are available commercially or from non-commercial sources such as the IAEA whose product, RIA/PC, was developed a decade ago, still remains popular and is in use at IAEA supported centres. Radioimmunoassay workers should be trained to use a suitable data processing package in their day-to-day work.

2.8.8. Reagent production

The technical capabilities and range of functions of RIA centres, particularly those in developing countries, vary considerably. A laboratory attached to a small hospital may provide only a clinical service confined to analytes of common clinical importance, such as thyroid related hormones, and find it most practical and economical to meet all of its reagent requirements from outside sources, whether abroad or local. Other centres that provide an expanded service may choose to produce at least some of the required primary reagents. These may range from the simplest, such as standards and quality control material for simple analytes of unique molecular structures for the commonest assays such as thyroid hormones and cortisol, to more sophisticated materials such as solid phases, tracers and antisera. Advanced laboratories that produce and distribute reagents or operate screening programmes, such as those for neonatal hypothyroidism, may even produce their own monoclonal antibodies for IRMA procedures and use modular automation to increase the precision and efficiency of the analytical process. Consequently, appropriate training in reagent production techniques should correspond to the type of laboratory the workers concerned are employed in. Local reagent production has the potential to reduce costs. It would, however, be economically wasteful for a small centre with a workload of a few hundred samples per month to produce its own ^{125}I tracer using imported ^{125}I. In general, the larger the centre and the wider the scope of activity, the more worthwhile it is to train staff to produce their own working reagents. If a centre carrying out screening programmes for neonatal hypothyroidism or hepatitis B infection, for example, were to make its own solid phases from coating antibody solutions or labelling monoclonal antibodies, both obtainable in bulk form, costs would be reduced by a factor of 40. Training for this purpose could therefore prove very useful. Similarly, workers in reagent production and distribution centres that supply tracers or EQAS material on a national or regional scale need to receive specialized training in the relevant

techniques. They would also benefit from instruction in good manufacturing practice and the procedures of sending out packages of reagents to other users, within and eventually outside the country. The end user should be aware of the logistic difficulties of customs clearance and prompt the appropriate local authorities to avoid delays in delivery.

2.8.9. Mechanisms of training

There are a number of possible paths for training in the above areas. A course of individual instruction at a laboratory with adequate facilities and staff is the best approach, with academics trained to a higher level, preferably postgraduate, wherever possible.

2.8.9.1. Training courses

Interregional or regional training courses are organized on a regular basis by international agencies such as the IAEA or the World Health Organization (WHO) and they have proved to be very effective over the years. These usually operate at teacher training level. Participants are expected to disseminate the expertise and skills they acquire within their home countries, most commonly by means of follow-up national training courses under the aegis of local atomic energy authorities or commissions. Since courses are usually of no more than two weeks duration, a progressive series of courses should be planned in order to cover all topics. The first course could include lectures on the basic physics of radionuclides, safe handling of radioisotopes, recent advances in immunoassay, separation methods, quality control and approaches to data processing. The practical classes should provide participants with hands-on experience of a typical RIA, such as that for serum thyroxine, and a typical IRMA, for example that for thyroid stimulating hormone (TSH). A discussion and troubleshooting session should always be included. The second course could include lectures on standards and standardization, assay design and optimization, the evaluation of antisera including Scatchard analysis, iodination techniques, stability and storage of reagents, and techniques for the local preparation of simple reagents, such as standards and quality control material for selected analytes. This could also be demonstrated in practical classes and experiments carried out to validate locally produced reagents. If there has been a demonstration of an iodination technique (e.g. for thyroxine), the tracer produced can be directly compared with one imported from a commercial source. Other practical classes could be designed to demonstrate and compare different separation methods.

2.8.9.2. Quality control and data processing

The subjects of quality control and computer assisted data processing are so vital to good RIA practice that they could form one component of a national training programme. The IAEA organizes a typical national training course on these topics, typically of two weeks duration. During the first week, participants carry out standard statistical exercises and proceed to the construction of various types of calibration curves, Scatchard plots, response–error relationships and precision profiles, with no computational assistance beyond a hand-held calculator, to ensure that all underlying concepts are well understood. The second week sees a repetition of the first week, with the difference that the work is not done manually, but using a computer and a data processing software package or packages.

2.8.9.3. Advanced reagent production

A further group training activity may now be organized on advanced reagent production methods, confined to participants from centres equipped, or likely to become equipped, to undertake this activity to a significant extent. Lectures and practical sessions would cover the following:

— Preparation and characterization of solid phases (antibody coated cellulose, tubes, beads and other new solid phases);
— New methods of protein immobilization, tracers and polyclonal antisera;
— Monoclonal antibody production;
— Propagation of hybridoma cell lines in bioreactors;
— Cost effective methods for antibody purification;
— Principles of good manufacturing practice;
— ISO 9000 accreditation;
— Rules governing the storage, packaging and transport of biological–radioactive materials.

Not many laboratories, especially in developing countries, have the equipment and other facilities required for the production of monoclonal antibodies. If training in this area is required, it would be better provided on an individual basis at a suitable advanced centre.

2.8.9.4. Special training

Special events may be organized to satisfy particular needs. Participants in an external quality assurance scheme organized at the national or regional level

would benefit from a workshop on the subject that would train them to use the scheme correctly. A training course devoted to tumour marker assays would focus on the special problems involved (high dose hook, etc.).

2.8.9.5. Experts

Radioimmunoassay centres that are set up or upgraded by international agencies such as the IAEA often benefit from the services of foreign experts who may serve at the host centre for periods of up to a month or more. Such missions are both popular and effective because the same expert can train many persons and training is in a local context, taking into account circumstances in the host laboratory. An expert mission also has the advantage of establishing a relationship between a centre in a developing country, which may be working in relative isolation, and the more advanced home laboratory of the expert.

2.8.9.6. Scientific meetings

A further means of providing training in RIA is through seminars, symposia and scientific meetings organized at regular intervals by international agencies or other associations. Participants have an opportunity to update their knowledge and acquaint themselves with recent advances. Academic personnel should be encouraged to participate in such meetings.

2.8.10. Location of training

Care should be taken in deciding where training in RIA is best provided if it is to take place outside the home laboratory. The most appropriate and cost effective option for the training of technicians in developing countries is a suitable training centre within the region. In special fields, such as steroid receptor assays for example, an expert mission followed by a short period at an advanced centre outside the region may be necessary. Academics who need to be trained for longer periods and to a higher level may need to be accommodated at advanced centres in developed countries. Specially identified laboratories may be developed to become a centre of excellence for training purposes within a given country or region.

2.8.11. Follow-up

Training in RIA, as in other areas of nuclear medicine, should not be a one-off exercise but rather a continuing process. Advances are constantly taking place, and those working at laboratories, particularly in the developing world,

should be aware of opportunities to simplify methods or reduce costs and ensure that personnel are kept informed as to their suitable application to local conditions. Special efforts should be made to collect and disseminate learning resources in RIA and related areas.

2.9. TRAINING OF NURSES

The role that nurses play in patient care is just as important in nuclear medicine as in any other clinical practice. Ideally, nurses should serve in diagnostic nuclear medicine sections and be present during nuclear cardiology stress testing. A nurse is the first interface with the ward nursing of inpatients and should be able to inject ward patients with radiopharmaceuticals (e.g. for bone scans) after training in intravenous injection. The presence of nurses in the therapeutic nuclear medicine wards is essential.

Nurses in nuclear medicine are required to perform the following duties:

— General physical and mental care of patients under examination or treatment;
— Examination of vital signs;
— Administration of drugs and injections on the instruction of doctors;
— Explanation to patients of procedures and provision of support to the receptionist;
— Handling of radiopharmaceuticals and radioactive waste in cooperation with pharmacists and technologists;
— Taking appropriate radiation protection measures for patients and families, especially those comforting children and elderly people.

In order to carry out these functions correctly, nurses need a basic knowledge of radiation, radionuclides and the biological effects of radiation, and should receive training on the safe handling of radioactive materials as well as radiation protection.

Education and training should be offered both in undergraduate courses in a school of nursing and in postgraduate training courses in hospitals. Nurses should receive a final briefing before they start working in a department of nuclear medicine.

Chapter 3

ESTABLISHING NUCLEAR MEDICINE SERVICES

3.1. INTRODUCTION AND CATEGORIZATION

In the past, consideration was given to the categories of nuclear medicine ranging from simple imaging or in vitro laboratories to more complex departments, performing a full range of in vitro and in vivo procedures, that are also involved in advanced clinical services, training programmes, research and development. In developing countries, nuclear medicine has historically often been an offshoot of pathology, radiology or radiotherapy services. These origins are currently changing as fewer RIAs are performed and fully fledged independent departments of nuclear medicine are set up. The trend appears to be that all assays (radioassays or enzyme linked immunosorbent assays (ELISAs)) are done in biochemistry laboratories, whereas nuclear medicine departments are involved largely in diagnostic procedures, radionuclide therapy and non-imaging in vitro tests including RIAs.

The level of nuclear medicine services is categorized according to three levels of need:

Level 1: This level is appropriate where only one gamma camera is needed for imaging purposes. The radiopharmaceutical supply, physics and radiation protection services are contracted outside the centre. Other services, such as radiology, cover receptionist and secretarial needs. A single imaging room connected to a shared reporting room should be sufficient, with a staff of one nuclear medicine physician and one technologist, with backup. This level is appropriate for a private practice.

Level 2: This level is appropriate for a general hospital where there are multiple imaging rooms in which in vitro and other non-imaging studies would generally be performed as well as radionuclide therapy.

Level 3: This level is appropriate for an academic institution where there is a need for a comprehensive clinical nuclear medicine service, human resource development and research programmes. Radionuclide therapy for inpatients and outpatients is provided.

3.2. IN VIVO DIAGNOSTIC PROCEDURES

3.2.1. Introduction

This section deals with the establishment of a nuclear medicine service for performing diagnostic and therapeutic procedures. Recommendations related to human resources development and the procurement of equipment, specifications of imaging devices and clinical protocols are expanded on in other sections.

The first step in establishing a nuclear medicine service is to consider the space, equipment and staffing requirements.

Space requirements will vary according to the level of the service, depending on whether a simple in vitro or in vivo imaging laboratory is envisaged or whether there are plans for a full in vitro laboratory and for in vivo imaging therapeutic procedures. Space should also be allocated for an in-house radiopharmacy if unit doses are being prepared on-site from 'cold' kits and ^{99m}Tc generators.

The initial design and planning should take into account a number of factors in addition to the space needed for routine imaging and staffing needs. Radiation protection is an important issue and the following measures should be taken:

— Walls and doors of laboratories should be painted with good quality washable paint;
— Work-table tops should have a smooth laminated finish;
— Floors should be impervious to liquids;
— There should be an adequate supply of lead containers and shielding lead bricks;
— Remote handling devices are desirable;
— Ventilated fume cupboards are similarly desirable.

For diagnostic imaging, ordinary wall thickness is usually sufficient. Radioactive waste disposal must follow local radiation protection guidelines and space must be available for waste storage.

Nuclear medicine is an advanced but cost effective specialty which can solve specific clinical problems. Since it changes rapidly with the development of new technologies for imaging devices and new radiopharmaceuticals, it calls for specialized training together with specific site preparation. Integration into a total health care system requires careful planning. Nuclear medicine staff need to have sufficient administrative skills to interact with referring physicians, hospital administrators and financial supporting bodies such as

government departments, insurance companies or charitable organizations. The general public needs to be both reassured and informed (about treatment), as proper interaction with patients requires their full cooperation. The level of services, information and patient interaction varies according to region, general standard of educational and socioeconomic conditions, and the standard of health care.

Nuclear medicine services vary from one country to another, although cardiology and nuclear oncology are generally the most commonly performed studies. In certain regions, renal studies, infection localization and even liver–spleen scans are still very important. Tests such as SPECT brain perfusion imaging are usually the domain of advanced services. The planning of a nuclear medicine department should be preceded by a study of population demographics and the prevalence of diseases in the respective country. This groundwork allows for prioritization and planning of an appropriate nuclear medicine service.

Since nuclear medicine serves both inpatients and outpatients the location of the site should give easy access to both groups. At all sites it is recommended that nuclear medicine should be a separate department, or at least form an autonomous unit within other departments such as the radiology and imaging or radiation oncology services, often referred to as 'the Cancer Centre'. It is not advisable to establish a nuclear medicine service in a separate building called, for example, 'The Nuclear Medicine Institute'. This isolates nuclear medicine from the referring physicians, reduces interaction between medical staff and, furthermore, creates unnecessary fear among the public.

Nuclear medicine services can range from basic in some countries to advanced in others. The level depends on several factors:

— The socioeconomic conditions in the country;
— The standard of health care delivery, amount of government subsidy, as well as the role of the private sector, insurance companies and charitable organizations;
— The size of the country, its population and ability to run nuclear medicine technologist training programmes, nuclear medicine specialty programmes for physicians, as well as other supporting services for physicists, chemists, pharmacists, computer technicians, electronic engineers and programmers, among others.

Once the level of service has been defined, personnel training should take place before the site is prepared or equipment procured.

In some countries it is important to obtain the approval of the national atomic energy agencies before each step, although this could conceivably

create friction between physicians, institutions and the local atomic energy agency.

The IAEA's mission is to work with developing countries to overcome a lack of finance and to assist in human resource development. Each national atomic energy agency should appoint a medical representative to liaise between the IAEA and the nuclear medicine programme directors. Similarly, each country should have regulatory agencies to set the rules for licensing, radiation protection, radiation safety and radioactive waste disposal. The IAEA can play an advisory role in this regard. In some countries it is advisable to set up a planning board to supervise human resource development, oversee current services and plan future development. The planning board can also recommend guidelines to ensure continuous quality control and education.

3.2.2. The nuclear medicine service

Plans for a nuclear medicine service must address the following points:

(a) Level of service needed;
(b) Equipment specifications (Section 4);
(c) Human resource development;
(d) Site preparation;
(e) Adherence to building, fire and security codes;
(f) Delivery and testing of equipment;
(g) Procedure manuals and department policy;
(h) Service administration;
(i) Official opening ceremony;
(j) Marketing;
(k) Programmes for:
    — Physician interactions,
    — Continuous clinical evaluation,
    — Quality control,
    — Initiation of research projects;
(l) Future developments.

3.2.3. Equipment

While the capacity and quantity of individual pieces of equipment needed depend on the volume of the service, minimum requirements are as follows:

(a) A collimated scintillation probe and counting system for uptake measurements of thyroid function and other in vitro and diagnostic studies.

(b) An isotope dose calibrator.
(c) A portable contamination monitor (acoustic dose-rate meter) and/or a survey meter to monitor beta and gamma contamination.
(d) A gamma camera with computer and appropriate clinically proven software. Rectilinear scanners are no longer appropriate. If only one gamma camera is funded, it should have its own computer for static, dynamic and preferably SPECT studies with its various clinically proven acquisition and processing protocols.
(e) Provision must be made for a reasonable range of collimators (low energy general purpose, high-energy, etc.), including a pinhole collimator.

It is important that the environment in the hospital and the nuclear medicine department is suitable for the equipment as described below:

(a) A stable uninterrupted power supply is vital and it has to be secure. Prior to installation of the gamma camera and electronic instruments, and during their service lives, the equipment needs to be protected from disturbances, such as power outages, voltage fluctuations and frequency fluctuations, in the mains power supply. A power stabilizer is important.
(b) Air-conditioning is essential to maintain a clean, dust free and dry environment for electronic instruments that are sensitive to heat and moisture changes; high humidity is bad for electronic components, causing corrosion as well as current leakage.
(c) Instruments must be housed in an air-conditioned environment, and a dehumidifier may be needed to maintain humidity at about 50%.
(d) Running hot and cold water must be available.

The initial budget during the planning stage must cover maintenance of equipment as well as capital costs — this may include technician training (local maintenance and repair) or a service contract with the equipment manufacturer or the local agent to take care of maintenance.

3.2.4. Staff

The number of staff will depend on the volume of both in vitro and in vivo work.

3.2.5. Administrative functions

In planning the administrative aspects of a nuclear medicine service, the following points should be considered:

(a) The administrative duties in a nuclear medicine service including reception and secretarial support, filing and billing duties (where applicable).
(b) To be able to serve both inpatients and outpatients, the location of the reception area is important; it should be situated close to the outpatient facility.
(c) The reception should be able to accommodate scheduling demands.
(d) The inpatient waiting area should be large enough to accommodate stretchers and wheel chairs. Consideration should be given to patient privacy.
(e) Filing facilities should be easily accessible and able to store six years of files. Digital filing is fast, saves time and space, and should be encouraged.
(f) Reception staff should be authorized to request old case studies in patients' files and the files of other imaging modalities.
(g) Reception staff should be able to consult the referring physician in order to complete request forms should information be missing, and this can be supplied by having a meeting, or via fax, phone or by electronic means.
(h) All requests must be reviewed, justified and approved by a nuclear medicine physician.
(i) Nuclear medicine tests should be completed as soon as is practical.
(j) Refreshments for patients and accompanying persons should be available at a safe distance from any radioactive source.
(k) Finally, reading material, such as leaflets on nuclear medicine and leisure reading, should be provided.

3.2.6. Imaging rooms

Imaging rooms should be at least as large as given in the manufacturer's recommendations, but preferably larger, to accommodate patients on stretchers. A larger area provides a more pleasant working environment and reduces the risk of radiation to staff. In some countries, rooms should have double glazed and insulated windows to avoid the buildup of dust. Tight fitting oversize doors and efficient heating, air-conditioning and humidity control units are also required. All rooms should have their own separate power supply and stabilizers and be equipped with hand washbasins with hot and cold running water. An intercom and/or telephone are important for facilitating communication.

3.2.7. Cardiac stress laboratory for nuclear cardiology

The cardiac stress laboratory should be planned in consultation with the cardiologists and equipped for treadmills and bicycles or pharmacological stress studies. Drug and life support facilities should be available in cases of emergency.

3.2.8. Conference room

The conference room can be used primarily for interdepartmental conferences, consultations with physicians and support activities for nuclear medicine staff. While functions could be accommodated in one large room with or without a partition, two separate rooms might be preferable. Space for scan interpretation, computers and ancillary equipment such as LANs should be provided. A library, Internet access and other teaching aids should be available to the conference room(s).

3.2.9. Offices

There should be sufficient office space for physicians, radiopharmacists, physicists, chief technologists, managers and secretarial staff in addition to a staff lounge. The number of offices depends on the size of the service.

3.2.10. Other space requirements

Figure 3.1 outlines the floor plan of a typical nuclear medicine department and highlights the additional spaces that will be required for the following purposes:

— Storage of clean supplies;
— Radioactive waste disposal;
— Toilet facilities for patients;
— Staff restroom and toilet facilities;
— Showers for decontamination purposes.

3.2.11. Radiopharmaceutical laboratory

Refer to Section 3.4.

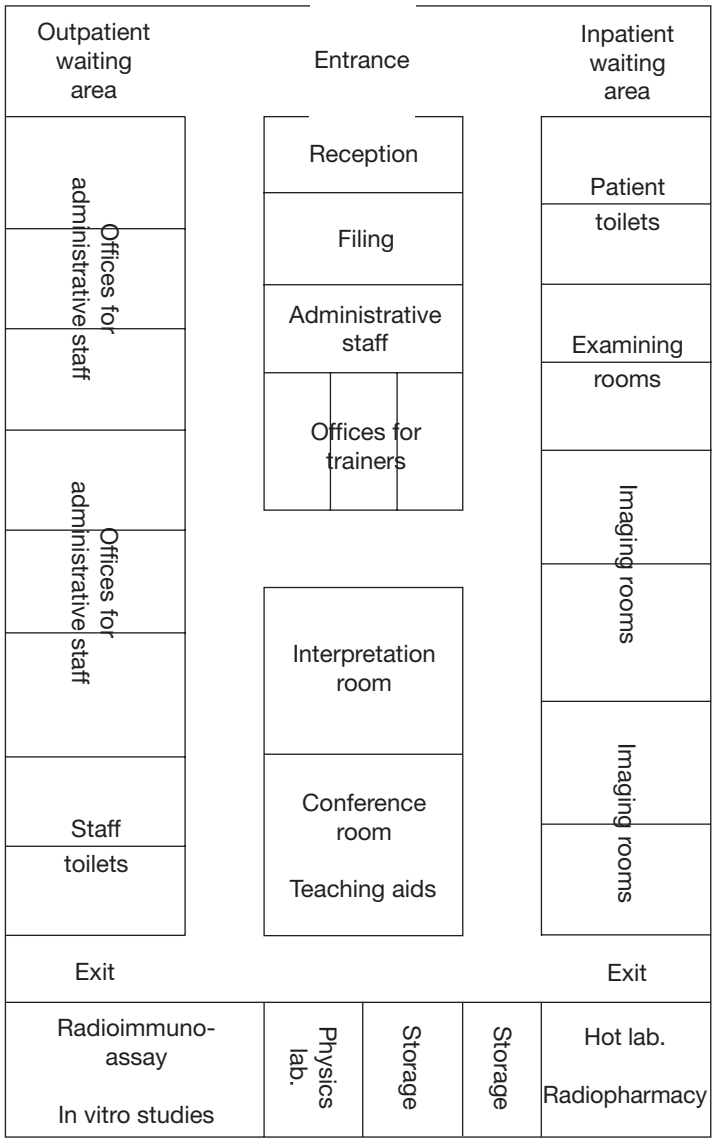


FIG. 3.1. Floor plan of a typical nuclear medicine department.

3.2.12. Operating the nuclear medicine services

The following guidelines are useful in the operation of a nuclear medicine service:

(a) Department policy should be recorded in writing and explained to staff. There should be a clear chain of management, which should be made made apparent.
(b) A copy of the Procedure Manual should be placed in all imaging rooms and technical staff briefed on procedures.
(c) Patient preparation forms should be easily accessible to the receptionist and the person who schedules studies.
(d) Nuclear medicine request forms must include information about the patient's medical profile, name, age, gender, hospital identification number, address and telephone number, name, address and telephone number of the referring physician, clinical background, and clinical data, as well as preliminary diagnosis and any tests required. The nuclear medicine physicians should consider the request for consultation, justify and approve the test before it is performed, and, if appropriate, modify it after consulting with the referring physician. Request forms should include a space to indicate approval of the test list, the radiopharmaceuticals used, as well as the dosage and route of administration. The form must be signed by the person(s) involved. Patients must sign the correct consent form (if applicable) during the interview and the signature be witnessed. The patient's records should be reviewed and the findings of other imaging modalities verified. Any special technical modification should be written on the request form for the technical staff to review.

3.2.13. Reporting studies

In general, reporting sessions should contain the following features:

(a) Physicians should review the studies before the patient leaves the floor and order further delayed scans where necessary, write a preliminary report for all inpatients and contact the referring physician with the results in the case of an emergency.
(b) Studies should be completed jointly with other staff members. Reports should be made after further consultation (if applicable), reviewed, signed and mailed or delivered within 24 hours.
(c) Follow-up for verification of accuracy of test.

(d) Joint conferences and research protocols with other specialties.
(e) Continued professional development for physicians and technical staff.

3.3. IN VITRO AND RADIOIMMUNOASSAY LABORATORIES

3.3.1. Objectives

The first point to consider in the establishment of an RIA laboratory is the purpose for which it is intended. In developed countries, RIA facilities may be created to support a specific research activity, whereas more often than not, particularly in developing countries, they are initially designed to provide a clinical diagnostic service (e.g. hormone assays). Such centres would only take on other functions, such as research and teaching, at a later stage. Within this context, the general issues that need to be considered are the location of the laboratory, building specifications, staff, training (Section 2.8) and equipment (Sections 3.3.6 and 4.5.3).

3.3.2. Location

Some of the most successful RIA laboratories, in both developed and developing countries, are attached to nuclear medicine centres that offer in vivo and in vitro diagnostic tests. An advantage to this is that the two types of tests are often complementary in the diagnostic follow-up of patients with commonly encountered disorders such as those related to the thyroid. In vitro tests, being simpler and less expensive, are often set up first and in vivo work introduced at a later stage. Provisions should, therefore, be made at the initial planning stage for future in vivo activities (with a gamma camera, etc.) at the same site as the in vitro testing facilities. On the other hand, in places where the two branches of nuclear medicine activity occupy separate premises there is little, if any, decrease in their effectiveness. The essential consideration should always be that where an RIA centre serves a cost effective clinical diagnostic function, it should be easily accessible to the end user although, in the case of in vitro tests, a high proportion of samples may be sent to the laboratory by post or other means.

Just as a clinical chemistry laboratory providing emergency services would be located in a hospital rather than within the Ministry of Health administration, an RIA centre, which serves a similar purpose, would best be located in a major provincial, district or city hospital. Other suitable locations are university medical faculties (usually associated with teaching hospitals), medical research institutes or similar institutions, provided they are oriented towards patient service.

If separate from in vivo facilities, in vitro nuclear medicine services could either be independent with their own management or form part of a clinical chemistry, biochemistry or other large department involved in analytical work. Since nuclear medicine is a distinct discipline, there are compelling reasons why an RIA centre should not be attached to the radiology or radiotherapy department. Exceptionally, large oncology, obstetrics, renal or organ transplantation units may have their own RIA laboratories engaged in clinical research, in order to measure special substances such as vasoinhibitory polypeptide or atrial natriuretic hormones.

3.3.3. The building

The design and structure of the building can affect the quality of an RIA centre. Premises should generally provide working conditions that are hygienic and spacious, and may include special features depending on the extent to which radionuclides are used. A patient reception area with a waiting room and an area for taking blood samples should be available. If the laboratory has medically qualified staff who carry out examinations or dynamic tests such as intravenous insulin stimulation, the reception area should be equipped with a couch, resuscitation trolley and other special facilities. It is essential to reserve an area for record keeping and the sorting and labelling of samples that, depending on the tests required, may be taken in the laboratory or obtained from outside. It is essential to entrust a responsible person with this duty where the consequences of error — wrong patient, wrong test — could be irremediable.

The core of the RIA unit is the area in which the assays themselves are performed. It should be spacious enough to accommodate the number of technicians employed, be well ventilated and have a constant and reliable supply of electricity and clean water. Floors and bench-tops should be smooth and of non-absorbent material to facilitate cleaning and decontamination in the event of chemical or radioactive spillage. Most RIA protocols require a decantation step following the separation procedure, and therefore sinks should be conveniently located at each workbench. A separate washbasin, labelled to this effect, should be reserved for the washing of hands of laboratory personnel, with its use prohibited for any other purpose. The washing-up area for glassware, used RIA tubes and reusable pipette tips should have one or two large sinks and a drying oven. Sensitive electronic equipment, such as counters, computers and analytical balances, needs to be stored in air-conditioned surroundings, particularly where the outside environmental conditions are hot, humid, dusty or otherwise unfavourable. If a separate room

is not available for electronic equipment the entire area needs to be air-conditioned.

A storage room for buffer chemicals, solvents, test tubes and other consumables that are often procured in bulk quantities would avoid cluttering up the main laboratory and provide greater workspace.

A more advanced laboratory preparing its own tracers using imported ^{125}I sodium iodide would need a 'hot' laboratory with sufficient space to accommodate a fume cupboard, fraction collector and/or high performance liquid chromatography (HPLC) system, as well as a refrigerator in which to store stock solutions of radioactive material. Working solutions of tracer may be stored refrigerated in the main laboratory. If reagent production activities are developed to the stage of polyclonal antisera and monoclonal antibodies, access will be required to an animal house and supportive veterinary care. This may be a control facility shared by various departments of one institution.

Where an RIA laboratory is producing ^{125}I iodinated tracers, special conditions have to be met for the storage and disposal of unused radioactive materials and waste. This is not necessary if the laboratory uses only ready-made tracers obtained elsewhere in quantities of approximately 50 μCi (1.85 MBq) at intervals between eight and ten weeks. Provided the laboratory has an efficient sewage system, the amount of ^{125}I used in a typical RIA — about 1 μCi (37 kBq) per 100 tube assay — is sufficiently low for liquid waste (supernatants) to be poured down the sink, where it is diluted by the large volume of effluent from the hospital or institution. The importance of standard radiation safety practices such as the monitoring of personnel and the work area, and the prohibition of food, drink or smoking in the laboratory, is to be highlighted. The hazards associated with the use of ^{125}I in the quantities used in RIA are sometimes exaggerated. The use of drip trays lined with absorbent paper is a wise precaution when handling radioactive solutions and minimizes the effect of accidental spillage.

In a well managed laboratory, the areas designated for assays are separated from those reserved for other activities such as patient reception, record keeping and computing. In most modern centres, seminar rooms and other general areas are located at some distance from laboratory workbenches and no one wearing a laboratory coat is allowed to enter them.

Solid waste including contaminated glassware, syringes, vials and pipette tips that are no longer usable should be stored in a marked container or bin for three half-lives before final disposal by incineration under proper conditions.

Where iodinations are being made, the laboratory will usually receive μCi amounts (usually 5–10 (185–370 MBq)) of sodium iodide ^{125}I. This should be stored refrigerated in the radiochemical laboratory (hot laboratory) where the iodination facility and tracer purification system are also located. Stock

solutions of tracer are stored in lead containers and also refrigerated in the hot laboratory. Whatever is left over or is no longer usable may be stored in a special area of the hot laboratory provided with lead shielding, for two to three half-lives, after which it may be disposed of into the sewage system. The proper recording of the receipt, dispensing and, finally, disposal of radioiodine should be a statutory requirement. This is more important than an ordinary stock book that records the receipt and issue of other consumables.

3.3.4. Staff

In order to provide the best patient service, an RIA centre should ideally be headed by a medically qualified person or include one on the staff. Clinical examination of patients will place a medically trained person in a good position to comment on test results or suggest follow-up studies in such a way as to influence patient management. In cases where the patient is not present and all that is available is a sample and a request form containing limited clinical information, physicians will be able to interpret results in an appropriate clinical context. They may also be requested to deal with patients who have been referred to the laboratory for so-called dynamic studies (e.g. insulin or arginine stimulation and thyrotropin releasing hormone (TRH) tests) from peripheral hospitals or clinics without the facilities to carry out such tests themselves. Where this type of service is being offered, the presence of a medical person is indispensable. Finally, it is not unknown that referring clinicians request the wrong tests or tests inappropriate or irrelevant to the diagnosis. In such cases, the physician can decide to confirm or exclude a particular test.

Medical personnel are not usually employed in RIA centres unless these are part of a fully fledged nuclear medicine department or larger clinical diagnostic unit offering services in several disciplines such as clinical chemistry or chemical pathology. In the majority of RIA laboratories, a manager with a background in biochemistry, pharmacology or an allied discipline takes responsibility for the analytical reliability of results. Regardless of the administrative structure, it is important that technical responsibility should be borne by a person trained in RIA as a distinct discipline and preferably to postgraduate level.

The number of technicians needed depends on the variety of assays to be performed and the workload. In the case of a basic laboratory that neither performs its own iodinations nor makes up primary reagents other than some standards and quality control material, staff should consist of a laboratory manager and at least two full-time technicians. With staff in this strength, the laboratory could offer more common tests (tri-iodothyrone (T3), T4 and TSH) approximately twice a week, with other tests (follicule stimulating hormone

(FSH), luteinizing hormone (LH), prolactin and some steroid hormones) being assayed less frequently. Additional technical staff would be required as the extent and scope of the work expands. In larger laboratories, technicians tend to specialize in particular assays, the advantage being that they develop valuable experience with particular methods and reagents.

The presence of a medical physicist is not required in an RIA laboratory on a full-time basis (he/she would normally be attached to an in vivo nuclear medicine unit), but the services of an electronic technician for routine servicing and maintenance of equipment including air-conditioners should be available as and when required. The impact of servicing and maintenance of equipment on actual assay quality is often overlooked. Instruments such as analytical balances, pH meters and even hand-held semi-automatic RIA pipettes are precision instruments, and technicians should know how to take care of them as well as how to use them.

Equipment must at all times be kept clean and properly maintained. It is good practice to maintain a 'logbook', containing the service and maintenance record of every pipette as is done in some of the better managed laboratories in developed countries. Pipettes in poor condition are often found to be a major contributing factor to imprecision (random error) in RIA, sometimes to an extent that would invalidate all results. The use of highly automated systems such as automatic pipetting stations and robotic samplers, which may add to convenience in advanced countries, is not encouraged in most routine RIA laboratories but may be appropriate in high throughput laboratories justified by economic considerations.

A person should be designated to take responsibility for radiation protection procedures, personnel and area monitoring as well as the maintenance of health records, in accordance with local regulations.

A secretary should be assigned responsibility for keeping records, managing materials and other duties. Other support staff may be required for other tasks such as washing used glassware, tubes and pipette tips, and it is essential that all staff understand the nature of the job and receive instruction on the proper procedures to be followed. Sometimes the least trained person may be unwittingly exposed to the greatest hazard.

3.3.5. In vitro diagnostic procedures

Most major items of equipment required for in vitro diagnostic procedures such as red blood cell (RBC) labelling, blood and plasma volume measurement and the Schilling test will be available at RIA laboratories, in vivo nuclear medicine units and radiopharmaceutical preparation sections. If in

vivo procedures are to be carried out, the staff concerned should receive additional special training in sterile techniques.

3.3.6. Equipment

3.3.6.1. Principle

Several factors need to be taken into account when deciding on equipment for an RIA laboratory. RIA centres may perform any or all of the functions of a clinical diagnostic service, engage in reagent production and distribution or undertake research. The extent to which RIA centres provide these services determines equipment needs. A laboratory attached to a small rural hospital with a workload of one or two 100 tube assays a day using ^{125}I does not require a 600 well automatic gamma counter or a robotic sampler. Both of these would, however, be useful in a centre carrying out a neonatal hypothyroid or similar screening programme on a national scale. Environmental issues (such as air-conditioning, cleanliness and a regular electricity supply) also play a part in the selection of equipment, but the most decisive factor, particularly in developing countries, tends to be the technical and economic ability to maintain equipment in good working order so as to ensure a reasonable lifespan.

3.3.6.2. General considerations

Solid phase methods, such as coated tubes, may obviate the need for a large capacity centrifuge, but the reagents or kits may prove more expensive than those used in a liquid phase assay. Provided good maintenance is available, a second antibody/polymer separation method may turn out to be cheaper and just as good. Magnetic separators are inexpensive and require no maintenance, but assays that use magnetizable reagents may be less accurate unless very high quality (and therefore expensive) particles are used. In the final analysis, it is a question of weighing one factor against another and deciding which combination of reagents and equipment suits the particular needs and conditions of any given laboratory.

A standby generator, preferably an uninterrupted power supply (UPS) facility, would be a useful addition in developing countries. Even more essential is air-conditioning, without which sensitive electronic equipment such as sophisticated counters and computers could soon malfunction in hot and humid climates. Even if the entire laboratory area cannot be cooled, air-conditioning should be installed in the room that houses electronic equipment. Power supplies are notoriously erratic in many parts of the developing world and

voltage fluctuations are common. This should be guarded against by the installation of power conditioners or an uninterruptible power supply. These may be included with some makes of beta or gamma counters.

3.3.6.3. Types of RIA laboratories

RIA laboratories are graded on the basis of nature and scope of activity. A Grade 1 laboratory is a basic one using reagents, whether obtained in bulk or as commercial kits, from an outside source, with minimal production of reagents confined to standards and quality control material for the simpler analytes. A Grade 2 laboratory would similarly use primary reagents obtained from elsewhere but in addition produce its own tracers, at least for selected procedures, using ^{125}I produced elsewhere in the country or obtained from abroad. A centre that, in addition to all of the above activities, also produces polyclonal antibodies falls into Grade 3. A Grade 3 laboratory could serve as a national or regional reagent production and distribution centre, or organize and operate an EQAS. Finally, monoclonal antibody production centres, if they are also engaged in RIA, are classified as Group 4.

3.3.6.4. List of equipment

For easy reference, the type of equipment needed in the various types of RIA laboratories is tabulated below. Some relevant points are worthy of mention.

(1) A beta liquid scintillation counter is not included as a regular requirement because almost all assays, even those for such analytes as steroid receptors, can now be carried out using ^{125}I.
(2) A multiple manual gamma counter is preferable to an automatic one because of the reduced possibility of mechanical, as opposed to electronic, failure.
(3) Assays for almost all common analytes are now carried out at ambient temperature and even if centrifugation is required, the instrument need not be of the refrigerated type. There may of course be exceptions, such as renin–angiotensin assays, where incubation is at low temperature, and centrifugation, if the protocol so demands, will need to be under similar conditions.

(a) Equipment for a Grade 1 laboratory

The equipment required is listed in Table 3.1.

TABLE 3.1. ADDITIONAL EQUIPMENT FOR A GRADE 1 LABORATORY

Item	Description
Gamma counter	Multiple manual, 4–20 channels, with on-board computer, and RIA and IQC software
Gamma counter	Single-well manual (as a backup instrument)
pH meter	General purpose type bench-top with standard electrolytes
Analytical balance	Sensitive to 0.1 mg
Distilled water still	2 L/h capacity
RIA pipettes and tips	Two or three sets, semi-automatic, hand-held, 20–1000 μL capacity
Stirrers	Two, with assortment of spin bars
Vortex mixers	Two
Water bath	6 L capacity
Deep freezer	Chest type, 400 L capacity, to –20°C
Refrigerator	Upright, 200 L capacity
Voltage stabilizers or UPS system	
Angled rotators	Two, to take 120 LP3 tubes each
Magnetic separators	Two
Centrifuge	Four to six place swing-out head with buckets, carriers, etc.
Laboratory glassware, test tubes, etc.	
Radiation monitor	
Drying oven	

(b) Additional requirements for a Grade 2 laboratory

The equipment required is listed in Table 3.2.

TABLE 3.2. ADDITIONAL EQUIPMENT FOR A GRADE 2 LABORATORY

Item	Description
Radioiodine fume hood or fume cupboard	Preferably built into the design of the laboratory; flow of 0.5 m²/s, open-face access
HPLC system with fraction collector, single pump	With pulse stabilizer, injection valve and guard columns
Refrigerator for hot laboratory	

(c) Additional requirements for a Grade 3 laboratory

The equipment required is listed in Table 3.3.

TABLE 3.3. ADDITIONAL EQUIPMENT FOR A GRADE 3 LABORATORY

Item	Description
Freeze dryer	6–12 L capacity with built-in shell freezer
Filtration equipment	1–2 L capacity, filter assembly and discs down to 0.2 μm
Stand-alone desktop	Pentium III processor with laser printer
Crimper for stoppering vials	Two

(d) Additional requirements for a Grade 4 laboratory

The list that follows is taken largely from the report of the Consultants' Meeting on the Production of Monoclonal Antibodies for In Vitro Immunoassay in Developing Countries, IAEA, November 1994. It applies to a small to medium scale in vitro monoclonal antibody production facility ranging from 250 to 5000 mg per month. Hollow fibre technology, home-made or commercial, may serve as a cost effective alternative. If the antibody production is to be on a large scale, i.e. of more than 10 g/month, the option would be a bioreactor with a batch size of 50–100 L. Neither of the above is included in the following list nor was considered in the aforementioned report.

— Cages, shelves, etc., for animal housing;
— Liquid nitrogen facilities;
— Biohazard cabinets of Class II;
— Inverted microscopes;
— CO_2 incubators;
— Autoclaves;
— Freezers (down to –80°C);
— Open chromatography equipment;
— Water purification units;
— Multihead peristaltic pumps;
— Sterile filters for preparation of culture media.

3.4. RADIOPHARMACIES

3.4.1. Introduction

The range of facilities required varies markedly depending on the category of the laboratory. The radiopharmacy needs the equipment necessary to provide radiopharmaceuticals of the desired quality for patient administration. The facilities should be adapted to suit the radioactive nature of the product and the fact that many radiopharmaceuticals are administered parenterally and thus need to be sterile. The radiopharmacy will also require quality control procedures, as well as areas for the receipt and storage of radioactive materials and radioactive waste prior to its disposal. Whatever functions are being performed, it is crucial that laboratories offer protection to the operator, the product and the environment.

The operator needs to be protected from radiation emitted by the products, and facilities must minimize both external radiation hazards and internal hazards arising from unintended ingestion of radioactive materials, particularly via the inhalation of volatile products. In addition, there may be chemical hazards arising from the product. In situations where blood labelling is performed, there is a potential biological hazard to the operator.

The product needs protection from unintended contamination arising during its preparation. This contamination may be chemical, radionuclidic, particulate or microbial.

The environment needs to be protected from unintentional discharges of radioactive material from the radiopharmacy. The majority of radioactivity handled will be in the form of unsealed sources with an existing potential for accidents and spillages.

3.4.2. Basic design criteria

The layout of the department should enable an orderly flow of work and avoid the unnecessary carriage of radioactive materials within the department. Attention must be given to the location of the laboratory in relation to the other facilities. While there are advantages in situating it close to the nuclear medicine department, the presence of high levels of radioactivity is a factor in considering its proximity to, for example, gamma cameras, patient waiting areas and offices. It is also important to consider whether there are working areas above or below the radiopharmacy laboratory, in order to avoid unnecessary radiation exposure to people working in those areas. Details of layout will need to be worked out locally, depending on the accommodation available. In all

cases, access to the radiopharmacy should be restricted, and for security reasons, laboratories should be lockable.

All surfaces of the radiopharmacy — walls, floors, benches, tables and seats — should be smooth, impervious and non-absorbent, to allow for easy cleaning and decontamination. Floor surfaces and benches should be continuous and coved to the wall to prevent accumulation of dirt or contamination. Such features are necessary for radiation safety and to provide a suitable environment for the handling of pharmaceutical products intended for administration to patients.

Radiation protection will require the use of shielding made from lead or other dense materials. This may be incorporated into the walls of the laboratory or can be used locally, adjacent to the source that yields the highest dose rate. This means that floors, benches and other work surfaces must be sufficiently strong to bear the weight of shielding. It is imperative that dose rates outside the laboratory, especially in areas to which the public have access, be kept below specified limits. In particular, the siting of ^{99m}Tc generators needs to be carefully considered. Although the generators contain internal shielding, additional external shielding may also be required depending on the activity of molybdenum present.

The range of products to be prepared will influence the scale and complexity of facilities required, and need to be appropriate for their intended function. They must be regularly monitored and maintained in a clean and orderly state. The general principles of good manufacturing practice (GMP) need to be applied in all cases and national requirements met.

3.4.3. Basic facilities

The simplest facility will be in departments that only prepare radiopharmaceuticals using a ^{99m}Tc generator and purchased kits. The type of generator most commonly used consists of ^{99}Mo, as molybdate, absorbed onto an alumina column. Technetium-99m is eluted from the generator by drawing sterile saline through the column. This is achieved by the use of a sterile evacuated vial supplied with the generator so that the operator does not need to be in close proximity to the generator during the process. Other, more complicated, techniques such as solvent extraction can also be used. Preparation of radiopharmaceuticals in a basic facility consists of the addition of sodium pertechnetate eluted from the generator to a sterile kit vial that contains all the ingredients necessary to produce the required radiopharmaceutical. Terminal sterilization processes are rarely carried out on the final radiopharmaceutical prepared because of time constraints. In addition, some radiopharmaceuticals cannot withstand high temperatures, rendering them unsuitable for

autoclaving, and filtration is not applicable for particulate radiopharmaceuticals. This means that the procedure has to be carried out aseptically in order to prevent microbial contamination.

3.4.4. Advanced facilities

An open fronted laminar flow workstation, which provides a stream of filtered air, is used. These safety cabinets incorporate a high efficiency particle arrestance (HEPA) filter, through which air is pumped in order to reduce particulate contamination to an acceptable level within the working zone. Such equipment is required to provide a clean environment suitable for processing pharmaceutical materials. Standards for the number of particles permissible have been published in Europe and the USA and correspond to a maximum of 3500 particles per cubic metre of a size equal to, or above, 0.5 μm and no particles equal to, or above, 5 μm. The internal surfaces of the cabinets must be made from impervious material which is readily cleanable and not affected by disinfectants or decontamination solutions.

The airflow must not be directed towards the operator and this is achieved by having a vertical stream of air that is ducted away through grilles in the base of the working zone and recirculated. This arrangement prevents air spilling out towards the operator. This requires careful balancing of the airflow, and normally a proportion of the recirculated air is released into the atmosphere. This produces a net inflow of air into the cabinet, providing a degree of protection for the operator against volatile or aerosolized radioactivity. Since this air is comparatively dirty, it must flow through grilles in the front of the base of the working zone rather than over the materials being processed.

One alternative is a totally enclosed workstation with filtered air, with the operator performing manipulations through glove ports. This system provides good operator protection from airborne radioactive contamination since the working area inside the workstation is at a lower pressure than outside. Air is ducted away to an external environment through filters which prevent the discharge of particulate radioactivity (e.g. aerosols) to the environment.

Thought must be given to the siting of workstations that are relied on to provide suitable working conditions. If the environment immediately outside the workstation contains high concentrations of particulate (including microbial) contamination, the probability of this entering the workstation increases. In certain areas of the world such as Europe, GMP requires staff to check the cleanliness of the room in which the workstation is located. This means air filtration to the room is required and access may need to be controlled. Personnel should wear protective clothing, which in addition to

protecting them from radioactive contamination will also help reduce the number of particles being shed into the environment from their skin, hair and clothing. A separate changing room, which has a step-over bench or other means of demarcation, is a useful way to control access to the room.

As little material as possible should be stored in the laboratory so as to reduce the accumulation of dirt and radioactive contamination. Materials required for the preparation of radiopharmaceuticals can be passed into the laboratory through a hatch when required.

Although it is essential to provide facilities for washing hands and the disposal of liquid radioactive waste, care must be taken in the siting of sinks, since they provide a site for accumulation of microbial contamination. The current practice is not to provide sinks in radiopharmacy laboratories, although ready access to sinks in the immediate vicinity is necessary. Showers for the decontamination of personnel are no longer provided, since they may spread any radioactive contamination present to other parts of the body, particularly the eyes, or to laboratory facilities. In situations where high levels of activity are handled, it may be desirable to have dedicated eye wash facilities available.

The radiopharmacy needs to be equipped with at least one isotope calibrator so that all activity can be measured accurately. In addition, a reference source (e.g. ^{137}Cs) will be necessary to ensure continuing reliability of the calibrator. Since radiopharmacies will be handling unsealed sources of radioactivity, contamination monitors will be required to check for any radioactivity that may have been spilt. The type of equipment available is discussed further in Section 4.5.1. The radiopharmacy needs to be equipped with suitable materials to deal with any such spillages.

Storage areas will be necessary for radioactive materials as well as for non-radioactive components used in radiopharmaceutical preparation. These areas will need suitable shielding and, depending on the type of product being prepared, a refrigerator and freezer may also be required. A store for flammable products, such as solvents used in quality control procedures, may also be required.

Figure 3.2 shows a possible layout for a basic facility.

3.4.5. More advanced facilities

Handling of volatile radiopharmaceuticals, particularly those based on ^{131}I, which are not intended for parenteral administration, should be performed within a fume cupboard, which exhausts air away from the operator. The inflow over the working aperture should not be less than 0.5 m/s, in order to provide good operator protection. The exhausted air is ducted to the atmosphere, and

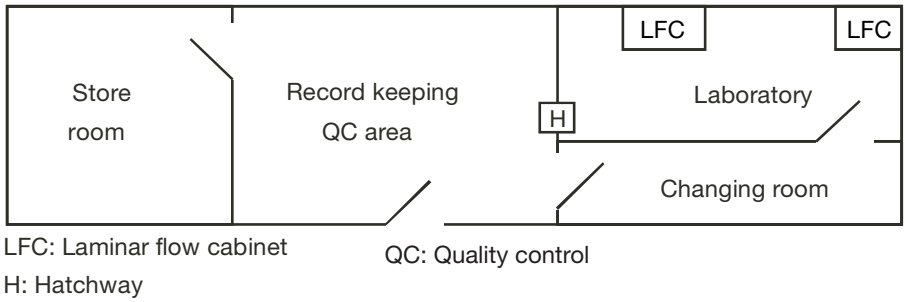


FIG. 3.2. Typical layout for an advanced radiopharmacy.

great care has to be taken when positioning the exhaust duct to ensure it effectively disperses the discharged air.

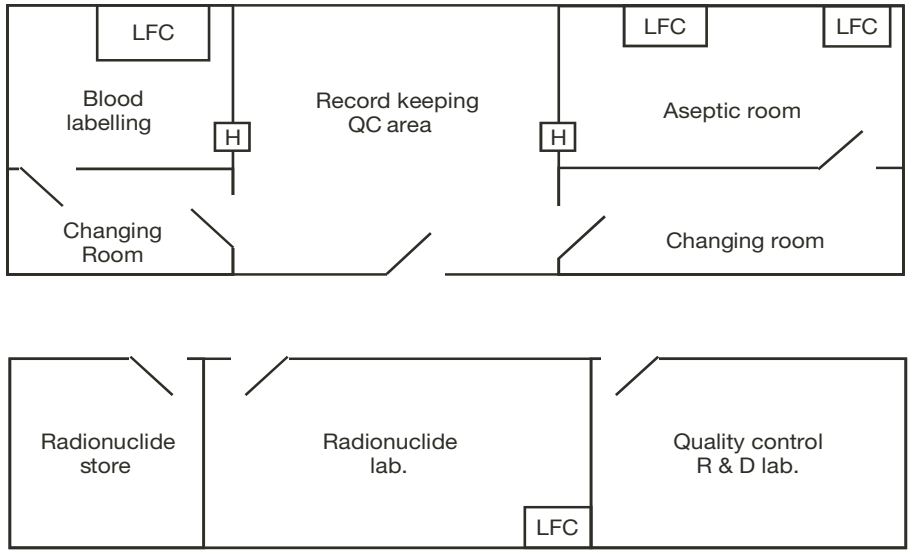
In radiopharmacies where blood labelling is performed, it is important to protect the operator and any other blood samples in the radiopharmacy from contamination with blood. It is desirable to have a separate workstation for this function, which can be readily cleaned and disinfected after each labelling procedure, thus minimizing the possibility of contaminating one blood sample with another. Totally enclosed workstations incorporating centrifuges are available, enabling the entire labelling process to be performed in a more protected environment.

A typical layout for a department preparing a wider range of radiopharmaceuticals is shown in Fig. 3.3. In the general design of a nuclear medicine department, the entry, flow and exit of patients and staff should be separated from the entry, flow and exit of radioactive materials.

3.4.6. Facilities for in-house preparation of kits

In departments where kits are prepared in-house, extra facilities are needed that are preferably distinct from those used for radioactive manipulations. For such non-radioactive, non-hazardous manipulations the most suitable solution is a laminar flow cabinet in which the flow of air is horizontal from the back of the cabinet, over the materials being processed and towards the operator. Such arrangements provide a high degree of protection against contamination of the product but are unsuitable when handling radioactive materials.

In these departments a lyophilizer will be necessary for the preparation and subsequent storage of freeze dried kits with a long shelf life. The requirements for such arrangements are beyond the scope of this manual.



LFC: Laminar flow cabinet
H: Hatchway
QC: Quality control

FIG. 3.3. Typical layout for a radiopharmacy preparing a range of radiopharmaceuticals.

3.5. MEDICAL PHYSICS

The role of the medical physicist is varied and will depend on local requirements. In most cases the physicist will not require a specific laboratory but will operate from a standard office. However, there is a need to provide for the following (even where no physicist is employed):

(a) Radiation safety:
- — Provision of a storage area for decontamination kits and radiation monitors;
- — Maintenance of records.

(b) Quality control:
- — Provision of a storage area for test phantoms (which will at times be radioactive);
- — Provision of an area for assembling and filling phantoms (allocation of a non-sterile sink in the vicinity of the hot laboratory).

(c) Equipment maintenance:
- — Provision of a workbench equipped mainly for electronic testing and repair (if direct maintenance work is performed);
- — Provision of an oscilloscope and Avometer; storage for electronic parts.

(d) Computer system management and software development:
- — Access to a workstation dedicated to these functions (or shared use, preferably on a system that is not used for routine acquisition and analysis).

(e) General administration:
- — Provision of a personal computer (preferably networked to above);
- — Provision of filing cabinets for records.

(f) Research and teaching:
- — Provision of a laboratory area for experimental work may be required, although existing facilities may be sufficient for this purpose. The exception would be a large teaching hospital with several full-time students. Computer workstations are an important feature of the training area.

The medical physics laboratory is usually a slightly expanded office and may comprise a small workbench, any necessary storage space and one or more computer terminals. The area would normally be considered 'non-active' and therefore have no specific radiation protection requirements.

3.6. POSITRON EMISSION TOMOGRAPHY

3.6.1. Introduction

3.6.1.1. Principle

PET refers to a special device or/and a study in which coincidence detection of the dual photons released from the annihilation process between positrons emitted from the nucleus of an atom and the free electrons in the surrounding environment is used, instead of the single photon detection used by a gamma camera or SPECT. Tomographic slices can be reconstructed similarly to those in SPECT. PET studies reveal the in vivo distribution and kinetics of positron emission radiopharmaceuticals.

3.6.1.2. Categorization

There are several types of PET. Usually the term indicates dedicated PET devices, which consist of 8–32 rings of detectors, either in a block design of bismuth germanate oxide (BGO), lutetium oxyorthosilicate (LSO), granium oxyorthosilicate (GSO) or sodium iodide (NaI) crystals. The detector arrays are mounted on a gantry and make a complete or part circle around the patient, enabling volume detection. The principle of coincidence detection and tomographic imaging can be extended to a recent design using a modified dual head SPECT system.

3.6.1.3. Advantages and disadvantages

PET is superior to SPECT in several aspects. It is more sensitive because it can dispense with the collimators that are mandatory in gamma cameras and SPECT studies. It also has higher spatial resolution because it provides information about the origin of the annihilation. It provides better diagnostic efficacy because most PET radiopharmaceuticals are biochemical molecules, thus enabling PET to depict diseases that are biochemical in nature.

PET is, however, more dependent on the on-site production of radionuclides, because most of them have short half-lives of less than 20 min. PET requires a highly advanced, sophisticated professional operation in a relatively developed social and technical environment. PET is also more costly than other forms of nuclear medicine service. A dedicated PET facility costs about $1–2 million, with another $2.4 million or more needed for a cyclotron and hot laboratory, in addition to the expense of construction and running costs. The initial investment is between $3 and $6 million. The production of PET radiopharmaceuticals can be shared by more than one facility.

PET imaging has become more widespread in recent years; this can be attributed to the introduction of multifunctional gamma cameras, the sharing of ^{18}F FDG production and reimbursement of the studies by government departments and insurance companies.

3.6.2. Basic concerns

At the current time, PET is the most advanced, most expensive and most sophisticated service in the field of nuclear medicine. Before establishing a PET service, it is therefore important to consider carefully the following factors:

Equipment can be chosen from a dedicated full ring PET, a dedicated incomplete ring system or a multifunctional gamma camera (SPECT with coincidence imaging).

The decision on the cyclotron depends on the clinical, academic and research demands as well as the ability to market ^{18}F FDG to nearby nuclear medicine facilities.

3.6.3. Planning for a PET facility

3.6.3.1. Space requirements

The overall size of a PET facility and the number of rooms required depends on whether it is integrated with an established nuclear medicine service or not. An average facility will include:

(a) Rooms for reception:
   — Scanner, control, waiting, injection, blood testing, reporting and administration rooms.
(b) Cyclotron specific rooms:
   — Cyclotron, control, hot laboratory, quality control, preparation, gas store and administration rooms.
(c) Other rooms:
   — Electricity, air–water cooling, ventilator–conditioner and waste control rooms.

3.6.3.2. Staff requirements

(a) Medical staff:
   — One or two doctors;
   — One or two technologists;
   — One nurse.
(b) Professionals:
   — One or two radiochemists/radiopharmacists;
   — One physicist;
   — One or two engineers and/or technologists.
(c) Other staff:
   — One receptionist.

Similarly to spatial requirements, the number of staff members depends on whether the PET facility is separate or forms part of an existing nuclear medicine service.

This list of environmental requirements is indicative of an average facility:

— Uninterrupted electricity supply;
— Clean water supply;
— Easy transportation and radiation safety;
— Humidity control.

After defining the level of service and required equipment, the director of the service should submit a specification (Section 4.4). At this stage, it is extremely important to plan the training of medical staff, technicians, physicists and engineers, arrange service contracts, and make provisions for the future updating of hardware and software.

The following can take place once installation has been completed:

— Acceptance testing;
— Phantom studies;
— Providing physicians and patients with information;
— Training of technical staff.

The following can take place after operation has commenced:

— Communication with clinicians;
— Evaluation of results;
— Follow-up;
— Joint conferences;
— Continued future technical and medical education.

3.7. CYCLOTRONS

3.7.1. Introduction

A cyclotron is a device used to produce radionuclides for PET by means of accelerating charged particles to bombard target atoms. A PET centre is a facility where at least one cyclotron is installed with a dedicated PET scanner. The cyclotron used in a PET centre is a miniature type (known as a baby cyclotron) with a lower power demand, cost and production yield than large industrial types.

A cyclotron is composed of a pair of magnets, holding a vacuum tank in which two or four D shaped electrodes are fixed. An ion source produces

charged particles (protons and neutrons) in the centre of the tank. The particles are attracted or propelled by the alternatively charged D electrodes to gain higher energy and circle in the middle of the tank under the control of the magnets. On gaining energy they move in a larger radius until they reach the desired energy. The particles are then led out to a target where special atoms are waiting. The accelerated particles bombard the target material to produce the desired new radionuclides.

Four radionuclides that are commonly produced by cyclotrons for PET are listed in Table 3.4.

3.7.2. Basic concerns

Although the principle of the cyclotron has not changed much since it was first introduced by Sir Ernest Lawrence in 1932, it is still a very expensive and complex device that requires a great deal of attention before installation can commence. In a hospital, the cyclotron is usually installed alongside the PET equipment. The main concern when preparing a PET service lies in the commissioning of the cyclotron, although there are other major considerations, such as radiochemistry.

Questions that require answers are:

(a) Is the cost of a cyclotron service, rather than the alternatives, really justified?
(b) Does the service have the proper academic environment to take full advantage of it?
(c) Is the workload in the centre or adjacent facility sufficient to keep the cyclotron running?
(d) Is there is a way to secure reimbursement of cyclotron–PET services?

TABLE 3.4. CYCLOTRON PRODUCED RADIONUCLIDES

Radionuclide	Half-life (min)
^{11}C	20
^{13}N	10
^{15}O	2
^{18}F	110

The selection of a cyclotron is a complicated process. One choice is between a cyclotron with self-shielding or a cyclotron without self-shielding. A decision also has to be made on the type of radionuclide produced and on whether a gas or a liquid target is preferable.

The extremely short half-life of cyclotron produced radionuclides means that they must be automatically synthesized into useful chemical forms for PET. This calls for the following procedures:

(a) Establishment of a hot laboratory, including hot cells for automated synthesizers and manual operation;
(b) Connection of the cyclotron to automatic and manual chemical units;
(c) Connection of the outlet of the gas from the unit to the PET room;
(d) Installation of proper devices for sterilization and quality control (e.g. thin layer chromatography (TLC), HPLC and calibrator).
(e) Satisfying special requirements, such as the legal process for production and distribution of radiopharmaceuticals.

3.7.3. Site preparation of a cyclotron facility

As stated above, since a cyclotron is almost always affiliated to a PET service, the principle of preparing a cyclotron site is to adapt it specifically to the type of cyclotron and PET radionuclides in question. Most of the preparatory measures, including the requirements for space, staff, environment and legislation, are discussed in Section 3.6.

3.7.4. Establishment of a cyclotron practice

As a first step, it is vital to define the need and scope of the service. Prior to 1990, PET was restricted to university campuses and institutes. In the last ten years, industrial and economic developments have made PET a clinical reality. Most experts agree that ^{18}F FDG alone covers over 90% of clinical needs. It is clear that not all PET facilities need an on-site cyclotron. There are commercial suppliers of FDG and several generator systems that are able to produce daughter radionuclides with positron emission. The cyclotron and affiliated hot laboratory are needed in those centres that are committed to research, either on in vivo biochemistry and physiology or on the development of radiopharmaceuticals and design of new drugs.

The evaluation of a cyclotron should take into account the following factors:

— Size, power consumption, production yield and environmental requirements;
— Human engineering design (e.g. vertical versus horizontal) and ease of servicing and maintenance;
— Running costs (target materials, volume, means of transport, cooling, etc.);
— Quality parameters and controlled methods of production.

In negotiating purchases and contracts, attention should be paid to the service, warranty and supply of special consumable goods and spare parts, as well as special tools for quality control, service and installation.

Room preparation is extremely important for cyclotrons. Special points to bear in mind include weight bearing, power, ventilation, gas and water requirements, 'clean rooms', chemical modules, control, transportation and PET centres, as well as radiation protection.

Acceptance tests include those on production yield, stability and reliability of operation.

Training in cyclotron, chemical modules, hot laboratory and quality control should be provided for operators, physicists, service engineers and radiochemists. The radiochemist and/or radiopharmacist play a vital role in radiochemistry and quality control laboratories.

3.8. ESTABLISHMENT OF A MOLECULAR BIOLOGY LABORATORY

3.8.1. Introduction

After the discovery of PCR and its application in clinical diagnosis, the design and set-up of molecular biology laboratories underwent a marked change. Today's molecular biology facilities must be in the position to perform the PCR technique coupled with molecular hybridization using radiolabelled probes. Its extreme sensitivity means that PCR tends to generate a large number of amplicons — amplified products that can be the main source of contamination in future experiments, thus providing false positive results. To circumvent this problem, it is recommended that a molecular biology laboratory be divided into three distinct areas as described below. Other recommendations concern good laboratory practice, both for the PCR method and for handling radionuclides.

The ability of PCR to produce a large number of copies of a sequence from minute quantities of DNA requires extreme care if false positives are to

be avoided. Although false positives can result from sample-to-sample contamination, a more serious source is the carry-over of DNA from a previous amplification of the same target. Because of the large numbers of copies of amplified sequences, the carry-over of the smallest quantities of a PCR sample can lead to serious contamination. It is essential that PCR reagents (primers, Taq polymerase, deoxyribonucleoside triphosphates (dNTPs), water and buffers) be stored separately from clinical samples, controls or amplicons as detailed below in order to avoid costly contamination. Strict adherence to the recommendations below will minimize the carry-over of amplified DNA.

3.8.2. PCR, contamination and good laboratory practice

Although extraneous nucleic acid from multiple sources may serve as a template for amplification, the main cause of false positive reactions appears to be PCR products from previous reactions. Caution should be taken when using numerous amplifications of the same primer pair system. The following precautions will eliminate the risk of false positives in the context of diagnostic assays.

Reactions prior to (Areas 1 and 2) and following (Area 3) amplification should be separated physically. To prevent the carry-over of amplified DNA sequences, it is important to set up reactions in a separate room or containment unit such as an ultraviolet (UV) irradiated hood or a biosafety cabinet. A further set of supplies and pipetting devices should be dedicated to the specific use of setting up PCRs. Amplified DNAs (post-PCR products) must never be brought into this area nor should reagents be taken from an area where amplicon analyses take place. Similarly, it is unwise to take devices such as pipettors into the containment area after use on amplified material.

Separate sets of automatic pipettors, disposable pipettes, a microcentrifuge, tubes and gloves should be kept in each area.

Positive displacement pipettors and plugged tips, to form an aerosol barrier, should be used in Areas 1 and 2. Positive displacement pipettes are recommended to eliminate the cross-contamination of samples by pipetting devices. In Area 3, normal unplugged tips can be used.

Reagents should be aliquoted to minimize the number of repeated samplings. All reagents used in PCRs must be prepared, aliquoted and stored in an area that is free of amplicons. It is advisable to record the reagent lots used so that if carry-over occurs it can be more easily traced.

Laboratory precautions in the handling of radioactivity should be incorporated (Area 3).

A selection of the number and types of controls should be made. Different controls should be used in each reaction:

(a) *Positive control:* In this control the target DNA will be added to the PCR mixture in order to determine if the reaction is working properly. For use as a positive control, a sample should be selected that amplifies weakly but consistently. The use of strong positives will result in the unnecessary generation of a large number of amplified sequences. Depending on the detection system used, as few as 100 copies of the target will suffice as a positive control.

(b) *Negative control:* In order to control the presence of contamination in the PCR mixture, water or the same buffer in which the extracted DNAs were resuspended (TE) is added to one of the PCR reaction tubes. Because the presence of a small number of molecules of PCR product in the reagents may lead to sporadic positive results, it is important to perform multiple reagent controls. The reagent controls should contain all the necessary components for PCR but without the addition of the template DNA. This system has proved to be extremely sensitive in detecting the presence of contaminants, as the absence of exogenous DNA enables the efficient amplification of just a few molecules of contaminating sequence.

(c) *Human DNA control:* This control will be important to address the specificity of the PCR assay. The specificity is evaluated by hybridizing. The faint bands that may be seen in this control do not imply contamination, but spurious products due to mix annealing of the primers to the human genome sequences whenever the target DNA is not present.

(d) *Inhibitor control:* All the negative PCR reactions should be repeated after being spiked with the target DNA to determine whether the negative results correspond to the absence of the target or to inhibitors. Alternatively, primers directed to human genes such as human globin can also be used.

3.8.3. Description of the various areas

3.8.3.1. Area 1: clean area

Area 1 — the most restrictive of the areas — should be limited to the preparation of solutions and the PCR master mix and should be subjected to UV irradiation overnight. Positive pressure is recommended, and the area must have access to an ice machine in order to maintain the long life of the highly sensitive reagents. Primers and dNTPs must be kept in ice and Taq polymerase at –20°C.

Attention should be given to the following points:

(a) Equipment

Ideally, the equipment should be new and custom designed for the purpose. It should never leave the room nor should it be used for a different purpose.

(b) The UV workstation

A plastic hood containing both a UV light and a fluorescent light should be installed and the UV light turned on at least 20 min before starting preparation of the master mix. The workstation should be fully decontaminated with 0.5% hypochloride followed by 70% ethanol. It should hold the following items:

— Weighing scales for preparation of reagents;

— A pH meter for preparation of reagents;

— A microcentrifuge for Eppendorf tubes;

— A UV irradiator (a UV apparatus that should irradiate (4000 J) all the pipettes, microfuge tubes, previously opened tip boxes, microcentrifuge rotor, etc.).

(c) General equipment

— A refrigerator and freezer;
— A vortex;
— A hot plate magnetic stirrer;
— A timing device;
— Laboratory coats, gloves and safety glasses;
— One set of micropipettes (20, 200 and 1000 μL) and the respective plugged tips.

Linking Areas 1 and 2, a corridor with a water purification system (rectangle) and biosafety shower (circle) should be present.

3.8.3.2. Area 2: for extraction of nucleic acids from clinical specimens

This area is dedicated to the handling of clinical samples and extraction of nucleic acids. Several pieces of apparatus and material from Area 1 will also be present in Area 2, including a UV workstation, microcentrifuge, UV irradiator,

refrigerator, freezer, vortex, timing device, laboratory coats, gloves, safety glasses, one set of micropipettes (20, 200 and 1000 μL) and the respective plugged tips. Additional equipment needed to perform the activities to be carried out in Area 2 include:

- A standard clinical centrifuge;
- A dry heat temperature block;
- A thermocycler biohazard container for biological waste.

A standard sink is also necessary in this area and is represented by an ellipse.

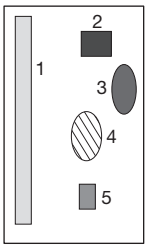
3.8.3.3. Area 3: post-amplification area — contaminated area

In Area 3, all PCR products are analysed by methods such as agarose or acrylamide gel electrophoresis and hybridization.

Gel electrophoresis is represented by:

- A power supply;
- Gel boxes.

After gel electrophoresis, the products are usually stained with ethidium bromide and visualized under UV light in a dark room, using a transilluminator and a camera. Area 3 should have dedicated sets of micropipettes (20, 200 and 1000 μL). The UV irradiator will be useful for linking DNA or RNA to nylon membranes. Other equipment needed in Area 3 includes a microcentrifuge, a pH meter, weighing scales, freezer, refrigerator, hot plate magnetic stirrer, dry heat block and microwave oven.



A radiation area, composed of an acrylic protection shield (1), a Geiger–Müller counter (2), radiation waste (3), a microcentrifuge (4) and a dry heat block (5), should be available for hybridization experiments.

A hybridization oven is important in order to optimize the experimental conditions. A biosafety shower should be available.

Other required apparatus includes a multichannel pipettor and tips, disposable reagent reservoirs, vortex mixer, timing device, refrigerator, freezer, graduated glassware, laboratory coats, gloves (protective, latex and powder free), safety glasses and a biohazard container (radiation).

3.8.3.4. General workflow

In order to achieve maximum efficiency it is essential to establish a culture of good practice in a molecular biology laboratory. Hence, there should be no contact between the pre-amplification areas and Area 3. The considerations outlined in the following paragraph should be kept in mind.

The PCR master mix is prepared in Area 1 and added to a tray containing tubes. It should be stored in the refrigerator until needed in the specimen preparation area (Area 2). Specimens and controls are processed in Area 2 and added to the tubes that are placed in the thermocycler. Products are submitted to agarose gel electrophoresis (Area 3 — with options of Southern or dot blot, radioactive labelling and hybridization) in the radiation area.

The strict observance of standard biosafety laboratory practices and good laboratory protocol PCR and molecular hybridization with radionuclides should be monitored to ensure the integrity of tests. The following rules are applicable:

(a) Personnel should always work in one direction, from pre-PCR to post-PCR areas, to avoid carry-over contamination from amplified products.
(b) Post-PCR should be kept as far as possible from pre-PCR, to avoid aerosol contamination.
(c) Working surfaces in each area must be decontaminated with 0.5% sodium hypochloride, followed by 70% ethanol before performing assay procedures in this area.
(d) Specimens must be stored separately from reagents, to avoid contamination of open reagents.
(e) When handling material containing DNA and/or RNA or amplicons, it is essential to use a pipettor with a plugged (aerosol barrier) tip or a positive displacement tip — post-PCR pipettors must never be used in pre-PCR areas.
(f) To avoid possible aerosol contamination, all centrifuges should be kept at a distance from areas where the operator is preparing the master mix and controls and adding prepared specimens to the PCR master mix.
(g) Dry baths or dry heat blocks should be used in preference to water baths, in order to avoid specimen contamination.

(h) Laboratory coats must be worn in all areas — the coat worn in post-PCR areas should never be worn in pre-PCR areas.
(i) Gloves must be worn at all times, both for operator safety and to control the spread of contamination from one area to another — they must be changed before moving to the next work area so that gloves worn in the specimen preparation area are never worn in the reagent preparation area and gloves worn in post-PCR areas are never worn in pre-PCR areas.
(j) Tubes should be spun before they are opened and care should be taken during the uncapping and closing of tubes.
(k) There should be minimum handling of samples.
(l) Non-sample components (dNTPs, primers, buffers and enzymes) must be added to the amplification reactions before the addition of sample DNA — each tube should be capped after the addition of DNA, before proceeding to the next sample.

3.9. RADIATION SAFETY

The key considerations in the planning, design and setting up of a nuclear medicine department are as follows:

(a) Allowance must be made for both diagnostic and therapeutic procedures.
(b) Unnecessary radiation exposure to staff, patients and visitors must be avoided, or kept within any limits required by the BSS or a local regulatory authority.
(c) Walls, doors and observation windows may require shielding; a calculation should be made on a case-by-case basis, depending on the distance to occupied areas, the rate of occupancy and estimated workload (e.g. GBq·h/week) for the various radionuclides to be used.
(d) The equipment used for imaging and radiation measurement is highly sensitive, and this must be taken into account when installing shielding; SPECT equipment can be even more sensitive.
(e) Moveable shielding should be used wherever possible to minimize fixed shielding, for example to shield technologists.
(f) Therapy areas must be well separated from diagnostic areas; ideally there should be separate access, i.e. corridors for patients and staff.
(g) Traffic patterns must be designed to keep movement of radioactive sources, including radioactive patients, away from imaging equipment.
(h) The ventilation of any airborne radioactive material must be considered.

(i) Surface areas (floors, work surfaces and walls) of rooms and corridors must allow for easy decontamination.

Areas where unsealed radionuclides are used are classified as low, medium or high hazard, the hazard level determining design requirements. Classification of the hazard level involves three steps:

(1) Firstly, a decision is made on the maximum activity foreseen for each radionuclide used in each room;
(2) This is multiplied by the weighting factor for the respective radionuclide (Table 3.5);
(3) And multiplied again by an operation weighting factor (Table 3.6) appropriate to the work to be performed.

The hazard category is then determined from the weighted activity by referring to Table 3.7. If more than one radionuclide is to be used, the highest hazard category determined should be applied.

The general design requirements are then taken from Table 3.7 (modified from the requirements given in a forthcoming IAEA publication on radiation protection (see Bibliography to this chapter)).

The following calculations serve as examples, when up to 5 GBq of ^{99m}Tc is to be used in the radiopharmacy for simple wet dispensing and labelling, as well as up to 4 GBq of ^{131}I to be stored for use in therapy:

(a) Technetium-99m:

Activity	= 5 GBq (5000 MBq)
Radionuclide weighting factor	= 1.0
Operation weighting factor	= 1.0
Weighted activity	= 5000 × 1 × 1 = 5000 MBq

From Table 3.7, the hazard category is medium.

Similarly, for

(b) Iodine-131:

Weighted activity = 4000 × 100 × 0.01 = 4000 MBq

From Table 3.7, the hazard category is medium. The radiation protection requirements for each hazard category are given in Table 3.8.

Radionuclide therapy areas in patient wards may need special shielding and construction according to the type of therapy provided. More information is given in Section 6.

TABLE 3.5. RADIONUCLIDE WEIGHTING FACTORS

Radionuclide	Factor
Se-75, Sr-89, I-125, I-131, P-32, Y-90, Mo-99, Sm-153	100
C-11, N-13, O-15, F-18, Cr-51, Ga-67, Tc-99m, In-111, In-113m, I-123, Tl-201	1.0
H-3, C-14, Kr-81m, Xe-127, Xe-133	0.01

TABLE 3.6. OPERATION WEIGHTING FACTORS

Type of operation or area	Factor
Storage	0.01
Waste handling — imaging or counting (radionuclide administration elsewhere) — patient waiting area — patient bed area (diagnostic)	0.1
Dispensing — radionuclide administration — imaging or counting (radionuclide administration in same room) — simple radiopharmaceutical preparation — patient bed area (therapy)	1.0
Complex radiopharmaceutical preparation	10.0

TABLE 3.7. HAZARD CATEGORIES

Weighted activity (MBq)	Category
<50	Low
50–50 000	Medium
>50 000	High

TABLE 3.8. RADIATION PROTECTION REQUIREMENTS FOR EACH HAZARD CATEGORY

	Hazard category		
	Low	Medium	High
Structural shielding	Nil	Possibly	Probably
Floor	Cleanable	Continuous and cleanable, welded to walls	Continuous and cleanable, welded to walls
Surfaces	Cleanable	Cleanable	Cleanable (where appropriate)
Fume hood	No	Yes (where appropriate)	Yes
Ventilation	Normal	Good	May need special forced ventilation
Plumbing	Normal	Normal	May need special plumbing
First aid	Wash basin	Wash basin and decontamination facilities	Wash basin and decontamination facilities

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Chapter 4

INSTRUMENTATION

4.1. INTRODUCTION

The quality and reliability of imaging instrumentation is critical to the practice of nuclear medicine. The design of equipment and the associated applications software have evolved rapidly and, to some extent, continue to be developed. This makes it difficult to take appropriate decisions as to what to purchase. Selection criteria should include flexibility in use, reliability and backup, with features determined by the desired function. It is important to ensure that equipment is specified to meet full requirements and, where possible, contractual conditions are in place to ensure the performance of the delivered system, as confirmed during acceptance testing. Nuclear medicine instruments are particularly sensitive to environmental conditions and consequently require strict control of temperature and humidity, as well as a continuous and stable power supply. Regular assessment is required to confirm stable operation using the quality control testing that is achievable in practice. It should be emphasized that National Electrical Manufacturers Association (NEMA) tests are primarily designed to permit meaningful comparison of specifications and are not intended for routine quality control. All three aspects (specifications, acceptance testing and routine quality control) are important to ensure effective clinical operation.

The instrumentation in nuclear medicine falls logically into three main sections: single photon imaging instruments (including SPECT), dual photon imaging instruments (combining the various approaches to PET) and various other non-imaging instruments. There are well established criteria for specification and testing of single photon instrumentation; however, the dual photon imaging field has only developed recently with the introduction of relatively inexpensive coincidence circuits for dual head gamma cameras. Guidelines for both specification and testing of PET equipment have recently been revised. The miscellaneous other equipment tends to utilize well established technology, even in the case of relatively new innovations (e.g. surgical probes). It is beyond the scope of this publication to provide a comprehensive coverage of instrumentation. The manual offers introductory information that may provide the reader with an improved understanding of performance specification and testing, referring the reader to more specific texts that can be used for a more detailed study.

4.2. PURCHASE OF IMAGING EQUIPMENT

4.2.1. General considerations

The following factors should be considered when purchasing nuclear medicine imaging equipment. It is recommended that a single head gamma camera with computer and SPECT capability be considered the minimum level of equipment for a new nuclear medicine department, although a dual head camera may be considered cost effective.

4.2.1.1. Required function

Consider the reason for buying a new imaging system carefully. An appropriate configuration should be selected to best match the desired end application, bearing in mind that the system may need to be used for other functions at some future date. The availability of specific features, software or accessories that meet the defined function is likely to be one of the main deciding factors in selecting a suitable system.

4.2.1.2. Service availability

It is critically important that there be demonstrated service capability in the country and a guaranteed support for the system. In considering the overall cost of a system, maintenance contract costs should be included and considered essential.

4.2.1.3. Performance characteristics

Direct comparison of performance characteristics is possible due to the standard use of NEMA specifications. International Electrical Council (IEC) standards that are applicable in the European Union (EU) are an extension of the tests suggested by the European Economic Community (EEC) but are not commonly applied. For details see the bibliography to this chapter. Competition between companies usually results in very similar specifications, so much so that other factors generally determine the system of choice.

4.2.1.4. Demonstrated capability

Care should be taken in selecting completely new designs, as it is common with new systems for problems to manifest themselves that will be resolved in later models. At the same time, it is inadvisable to select a system that has been

superseded. Users should be consulted on the performance of previously installed systems of the same design.

4.2.1.5. Ease of upgrade

It is important that systems can be easily upgraded and that software can be updated for several years after purchase.

4.2.1.6. Compatibility

In some circumstances, the system purchased should be compatible with existing systems in the department. Advantages include the familiarity of staff with operation, sharing of accessories and proven availability of support. Provision for transferral of data between systems and general networking has increasing importance.

4.2.1.7. Flexibility

The flexibility of the system selected is crucial. For example, any SPECT system should be able to be used for a range of studies, both planar and SPECT, rather than being restricted to specific studies. Some ability to adapt software to match local requirements is desirable.

4.2.1.8. Ease of use

Ideally, the system should be easy to use, with manual override available for any automatic features (e.g. collimator exchangers). The computer's user interface should also be easy to use.

4.2.1.9. Selection of accessories

A wide range of accessories is normally available, but should be chosen to meet anticipated needs. Special attention must be given to the selection of collimators. Accordingly, it would be advisable to include a high resolution collimator, particularly for SPECT studies.

4.2.1.10. Cost

Obviously cost is a critical factor. However, there are instances where increased cost may be justified in terms of more effective use of the equipment.

One example is the choice of a dual detector system rather than a single detector system.

4.2.2. Contractual considerations

When purchasing an imaging system it is imperative that a document be prepared that not only defines the requirements of the system to be purchased but also clearly outlines the obligations placed on both the supplier and the receiving institution.

A list of the required specifications of the imaging system is very important. In addition to the specification sheets made available by the vendors, the user should also consider the main studies to be performed on the camera and the specifications necessary to obtain optimal clinical results.

Complete operation and service manuals should be supplied with the gamma camera and should remain the property of the user. Appropriate radiation sources and phantoms needed for quality control tests should be purchased at the time of instrument acquisition. Software should be available to quantify quality control results.

Bids should clearly define the instrument's performance specifications, referring to the results of NEMA tests. Results of acceptance tests, performed immediately after installation, will be compared with these data. Most acceptance tests should be performed by the supplier, under the supervision of, and in cooperation with, a suitably experienced nuclear medicine physicist. All phantoms and test equipment required for acceptance testing should be made available free of charge by the supplier. A percentage of the purchase price (e.g. 20%) should be held back until the user is satisfied with the acceptance test results. A clause built into the purchase agreement should specify the procedures to be used during acceptance testing, minimum acceptable results and actions to be taken if acceptance test results do not meet pre-purchase agreements.

Training of personnel should be included in the company bid. Training on the operation and programming of the system, including acquisition and processing of patient studies, must be supplied. Furthermore, training on the operating system and computer maintenance (e.g. the steps required to reload software) must also be included.

It should be emphasized that the full installation, including acceptance testing and on-site training, is the responsibility of the supplier. A competent service person from the company, with training on the specified equipment, should be available.

The contract should state clearly which spare parts are available locally and the supplier's response time to supply those spare parts that are not available locally.

4.2.3. Site preparation and installation

Before installation takes place, steps should be taken to ensure that the environment is suitable for the installation. These will include the following:

(a) The room should be of an appropriate size and in an acceptable condition before installation takes place. Particular care should be taken to ensure that the floor is sufficiently robust to support the equipment. Floor loading details are provided in the supplier's specification, and local engineering staff should be consulted.
(b) The electric power supply should be stable; if not, a constant voltage transformer should be installed. An uninterrupted power supply system is essential for optimal utilization of the gamma camera system. The grounding of the equipment should be checked since this can be a source of electrical noise as well as being a potential hazard. Poor quality electric supply is recognized as a major reason for instrument malfunction and failure.
(c) The design of the department should ensure that background radiation levels in the vicinity of the equipment are not markedly influenced by the location of the radiopharmacy, the storage and movement of radioactive materials and the movement of patients incorporating these materials. Similarly, care should be taken that other radiation sources in the vicinity (X ray machines, linear accelerators or ^{60}Co devices) do not contribute to the background.
(d) The presence of a strong magnetic field in the vicinity may also influence the functioning of the gamma camera. If there is an MRI system in the same institution, the magnetic field levels should be monitored.
(e) Air-conditioning units with properly deflected airstreams and dehumidifiers should be provided and used permanently to provide continuous protection against the adverse effects of temperature changes and humidity.
(f) Instruments should not be installed where they could be exposed to dust, smoke or chemical fumes.

4.2.4. Acceptance tests

The first crucial step after installation of the imaging equipment is the initial evaluation or acceptance testing. This includes not only confirmation that the instrument performs according to specifications, but also evaluation of performance under conditions that will be encountered in clinical practice.

These tests should be carried out immediately after installation. The user should not accept an instrument that fails to conform to specifications. No instrument should be put into routine use unless it has been shown through acceptance testing to be performing optimally. Provided the equipment is operating according to specification and has been demonstrated to be safe, a limited number of patient studies should be performed as part of the acceptance procedure.

Acceptance tests require special test devices, phantoms and evaluation software. Quantification of tests is essential in order to compare results with specifications and to provide baseline values for future comparison. Therefore it is recommended that the specialized instruments and software are provided by the company for the purpose of acceptance testing, and that the tests are carried out on-site by the company engineer, under supervision of the user. The user may chose to perform additional tests to confirm the operation of the equipment and may chose to use these results as a reference for future quality control. If necessary, the user should invite a competent expert to participate in the acceptance tests and the evaluation of the results.

4.2.5. Warranty period

The warranty period (usually one year) should be clearly defined in the purchasing documents. The warranty period is very useful in exposing possible failures of electronic components at an early stage. The manufacturer has an obligation to repair the problem at no cost to the user. It is recommended that the warranty period should start only when the equipment has passed all acceptance tests. Equipment should be put into clinical use as soon as possible in order to optimize the warranty period.

There must be a clear understanding between the supplier and the end user as to how the warranty period will be influenced if a major part of the system needs to be replaced during the warranty period. The company should perform regular services and preventative maintenance procedures during this period.

4.2.6. Service contracts

A service contract should be negotiated, to include labour and either no spare parts, spare parts excluding the crystal, or all spare parts. The availability of spare parts and the terms of payment should be stated. The price of the service contract usually varies between 2 and 10% of the purchase price of the imaging system.

The supplier should make available a qualified person to perform preventative maintenance and servicing on the camera (proof of adequate training should be provided). In the event of system failure, the maximum response time of the service engineer should be specified (two hours is a typical figure). The maximum acceptable downtime per year should also be specified (10% of available working days is suggested). A penalty clause should be added to the contract if the supplier does not meet all the requirements.

The supplier should supply a checklist of what will be performed during the services for preventative maintenance. This checklist should be compiled to the satisfaction of the user. The service engineer should leave on-site a record of all tests and checks performed.

It is recommended that quality control tests such as those for uniformity and spatial resolution be performed before each service and repeated after completion, to evaluate the effectiveness of the service.

4.3. SINGLE PHOTON IMAGING

4.3.1. General considerations

The main imaging device in nuclear medicine is the gamma camera based on a sodium iodide detector, developed originally in 1958 by H. Anger. Although there are rectilinear scanners still in use, these will not be discussed. Similarly, while there continue to be various experimental detectors (e.g. solid state detectors) that may have particular appeal for specialized applications (e.g. breast imaging), their current limited use does not warrant their inclusion. The conventional gamma camera forms the basis of systems used for solely planar imaging and with appropriate mounting on a rotating gantry is used for SPECT. Multidetector systems are normally constructed using multiple gamma cameras that improve the efficiency of detection. Designs using multiple small detectors rather than conventional gamma cameras are also not in widespread use. The addition of coincidence circuitry to the conventional dual head gamma camera allows it to be used for ‘positron imaging’ as discussed in a later section of this manual. To a large extent the versatility of the conventional gamma camera is a

particular strength. The design of gamma cameras has improved dramatically over a long period, with current devices being very much digital systems rather than simply being interfaced to an acquisition computer. Over the years the performance of cameras has also improved; not only is their resolution, uniformity and count rate capability better but also, more importantly, their stability is improved. Sensitivity remains a constraint of the collimated detection geometry.

Although there have been various attempts to design specialized gamma camera systems for specific applications, in general the more successful designs are those that provide flexibility. In many centres, the camera is required for different applications and, at the time of purchase, it is often difficult to predict what the ultimate application may be. A system that permits efficient SPECT acquisition without compromising the utility for planar imaging is particularly attractive. Provided this flexibility is maintained, a dual head system has the advantage of improved throughput, and the low likelihood of both heads having problems means that a single head can be available for continued operation, even when the second head is non-functioning. A dual head system also offers the possibility for dual photon imaging, as discussed elsewhere. It is this flexibility that has resulted in the dual head camera currently being the most popular system. Although more expensive than a single head system, the dual head system is cost effective in terms of both throughput and flexibility.

The computer is now an integral part of any imaging system, and consideration of not only speed but also the range of available software, connectivity and ease of upgrade become important considerations. There has been a trend in recent years towards standard computer platforms that can keep abreast of developments more easily than the older manufacturer-specific systems. Even though these systems tend to lag behind the general release of systems software, they generally offer a wide range of available peripherals and general software (including free software). Although there is a wide selection of advanced clinical applications software, the ability to develop user defined applications, without the need for advanced programming skills, remains a requirement that is not always available. Confirmation of results arising from application software is the responsibility of the site concerned. Particular care needs to be taken to ensure that interpretation is correct for the population concerned (e.g. normal databases derived from specific populations may be inappropriate). Software phantoms, i.e. reference data sets that can be used to check the accuracy of analysis, are available via the Internet.

There are many accessories for gamma cameras, including some that reduce overall reliability. One example is automated collimator exchangers that do not permit manual override and therefore result in the system being inoperable in the event of malfunction. Collimators continue to be vital

components of single photon imaging. Although basic collimators have changed very little (except for construction), there is a range of specialized collimators now available including fanbeam and cone-beam collimators that provide improved efficiency as well as marginally improved resolution compared with that of parallel hole collimators. Some manufacturers strive for 'super-resolution' at the expense of counting efficiency; consequently specifications should be carefully examined as collimator names can be misleading. For cardiac SPECT applications, there are several accessories that are desirable, such as some form of transmission measurement to permit attenuation correction in the thorax and an electrocardiogram (ECG) with a suitable trigger pulse used for gated acquisition. In the case of transmission sources, there is a range of available options with no single system acknowledged as clearly superior, and effectiveness of correction is dependent to some extent on the software supplied. For example, it is now common for manufacturers to offer iterative reconstruction software as an alternative to filtered back-projection. The system choice is normally based on the underlying camera unless there is very high priority for a specific acquisition (e.g. cardiac SPECT).

4.3.2. Specifications

The tender specification list must include the items listed below. A more detailed questionnaire, aimed at manufacturers, is available. The use of such an approach enables comparison of bids, resulting in a possible scoring system that will assist in the decision making process.

(a) Gamma camera and accessories:

— Detector performance;
— Detector head and gantry design;
— Detector head motion;
— Collimators;
— Pulse height analysis;
— Imaging table(s) and attachments.

(b) Data acquisition:

— General acquisition features;
— Static acquisition;
— Dynamic acquisition;
— List mode acquisition;
— Gated cardiac acquisition;

— Whole body imaging;
— Automatic patient–detector distance sensing;
— Tomography.

(c) Data processing system:

— Data display;
— Image manipulation and arithmetic;
— Region of interest (ROI) generation and display;
— Curve generation, display and arithmetic;
— Processing of SPECT data;
— File and disk utilities;
— Data transfer;
— Archiving and backup;
— Quality control software and test data;
— Patient database management software;
— Software development tools;
— Network capability;
— Multi-tasking capability (ability to acquire and process simultaneously);
— Clinical software;
— Data security.

(d) Accessories:

— Hard copy;
— Physiological triggering;
— Anatomical landmarking;
— Phantoms: ^{57}Co, bar and SPECT.

(e) General:

— Warranty, maintenance and reliability;
— Room layout;
— Pre-installation work and requirements;
— Purchase, installation and training;
— Electrical and mechanical safety;
— Quality management systems;
— Documentation.

(f) Multidetector gamma camera systems:

— Performance requirements;
— Detector head motion;
— Detector performance assessment by comparison of the NEMA performance parameters from the various manufacturers.

4.3.3. Acceptance tests

4.3.3.1. List of acceptance tests for planar or SPECT gamma cameras

(a) Intrinsic NEMA procedures:

— Energy resolution;
— Flood field uniformity;
— Spatial resolution;
— Spatial linearity;
— Count rate performance and maximum count rate;
— Multiple window spatial registration.

(b) Extrinsic (system) NEMA procedures:

— Flood field uniformity;
— Spatial resolution with and without scatter;
— Sensitivity for each collimator;
— Detector head shielding leakage.

(c) Acceptance tests for SPECT (non-NEMA):

— SPECT centre of rotation;
— Angular linearity errors;
— Uniformity;
— Tomographic slice uniformity;
— Rotational uniformity;
— System volume sensitivity (NEMA);
— Tomographic resolution:
  - tomographic resolution in air (NEMA),
  - tomographic resolution in a scatter medium (NEMA),
  - test of slice thickness (IAEA),
  - total performance check (data spectrum phantom) (American Association of Physicists in Medicine (AAPM)),

- tomographic uniformity,
- tomographic resolution (spheres and rods),
- contrast.

The above tests should be done in addition to the following planar gamma camera tests.

(d) Specific tests for multiple detector systems:

— Multiple detector registration;
— Matched sensitivity;
— Matched pixel calibration;
— Matched centre of rotation (COR).

4.3.3.2. Minimum quality control requirements for gamma cameras

Routine quality control is an essential requirement for any nuclear medicine practice in order to ensure that equipment operation remains optimal. Quality control is commonly, but wrongly, viewed as a difficult and time consuming chore and, for this reason, is frequently neglected. This section provides guidelines for minimum quality control based on the Australian and New Zealand Society of Nuclear Medicine recommendations and is compatible with other recommendations. The guidelines are intended to provide a very basic practical approach to gamma camera quality control, requiring very little specialized equipment or expertise. It is therefore recommended that these guidelines be adopted by all nuclear medicine practices. The minimum quality control tests are intended to detect problems before they have an impact on clinical patient studies. They are not intended to provide a full evaluation of equipment performance. Further tests may be required to trace the cause of a problem and to ensure that the equipment is performing properly after service or adjustment. Exact quality control procedures vary between manufacturers and models, making it impractical to provide detailed quality control procedures covering all equipment.

In order to make quality control procedures as simple as possible, the following is a suggested list of the minimum test equipment required:

(a) Cobalt-57 sheet source

This source is recommended for high count extrinsic uniformity checks and the collection of uniformity correction floods. It is preferable to water filled flood tanks, which may introduce non-uniformities due to poor mixing, bulging

and air bubbles, although care needs to be taken with new sources. On some systems, a water filled flood tank may also be required to calibrate the system for non-^{99m}Tc radionuclides such as ^{67}Ga.

(b) Resolution phantom

A four quadrant resolution phantom is recommended. The finest bars should be small enough to test the intrinsic resolution of the system (i.e. 2–2.2 mm bar width) and the largest bar should allow some extrinsic tests to be carried out (i.e. 4.5–4.8 mm bar width).

(c) Centre of rotation jig

On some cameras, special jigs are required for calibrating COR offset and other calibration procedures.

It is imperative that quality control procedures be carried out in a consistent manner (i.e. the same collimator, orientation, activity and energy window) and that qality control results and settings be recorded. Proper record keeping greatly facilitates detection of gradual deterioration of performance over an extended period of time. A baseline set of quality results should be recorded after installation and acceptance testing to serve as a reference.

Quality control procedures for SPECT cameras in general are more stringent than those for planar cameras. Since corrections can be applied to SPECT data such as uniformity and COR offsets, it is more important that the quality control parameters are stable over time, rather than having an exact absolute value.

For each quality control test listed below, the aims and rationale are described first, followed by a general procedure for performing the test. Although recommendations are made on the frequency of the quality control tests, it must be pointed out that in some tests this depends on the equipment. It is recommended that, on the basis of these guidelines, an experienced nuclear medicine physicist draw up detailed quality control protocols for use with specific equipment.

In practice, the routine quality control tests should give data that permit the physician to decide whether:

— To image patients normally;
— To image patients but request that the equipment be serviced;
— To cease patient studies until the system is repaired.

4.3.3.3. Rationale and description of quality control tests: planar

(a) Visual inspection

A visual inspection of the collimators should be performed daily and whenever collimators are changed. Signs of new dents, scratches or stains should be followed up with a background and/or contamination check and an extrinsic uniformity check before a suspect collimator is used for patient imaging. It should be borne in mind, however, that not all collimator damage may be externally visible.

A general visual inspection for any other defects that may compromise patient or staff safety (e.g. frayed or damaged electrical cables as well as mechanical faults in the camera or scanning table) should also be carried out on a daily basis. If any such defects are detected, the equipment should not be used until it is established that it is safe to do so.

(b) Background/contamination

A background radiation check should be carried out with the collimator off, by means of the energy window which is most frequently used for imaging. The total number of counts acquired in a fixed time period and inspection of the energy spectrum will indicate the presence of any unusually high levels of background radiation. The measurement should be repeated with the camera head pointing in the full range of directions used for clinical scanning (including head positions at 0, 90, 180 and 270° for SPECT). A high reading in any particular direction may indicate background radiation from contamination (e.g. on the floor) or an unshielded source. A high reading that persists irrespective of the camera head orientation is indicative of contamination on the crystal face or the gamma camera head itself. Both of these conditions should be investigated and remedial action taken (e.g. decontamination or removal/shielding of the offending radiation source) before proceeding with any further checks or imaging.

The above background radiation checks should be repeated with the collimator switched on. This will serve as a check for possible contamination on the collimator itself. If such contamination is indicated, an extrinsic uniformity check should be carried out to assess the location of contamination and its effect on uniformity. Decontamination of the collimator may be necessary before imaging can proceed.

(c) Photopeak and window setting

Incorrect photopeak energy window setting(s) can degrade uniformity, reduce sensitivity or increase the scatter contribution to the image. The photopeak can change as a result of slight variations in the high voltage, photomultiplier drift, changes in temperature and other factors.

Photopeak settings should be checked and adjusted in a consistent manner, and the settings recorded to detect long term drift. Sudden changes in peak settings indicate a possible fault in the camera and should be fully investigated and rectified, if necessary, before the camera is used for clinical studies.

It is important to check the energy window settings for all radionuclides used on a particular gamma camera, since the fact that there are proper peak settings for one radionuclide (such as ^{99m}Tc) does not necessarily mean that the window settings for other radionuclides (such as ^{201}Tl and ^{67}Ga) are correct. If a change in the peak setting for one radionuclide is detected, it is likely that the settings for other radionuclides also need to be adjusted.

Peaking should preferably be performed intrinsically, to reduce scatter and ensure that an average peak for the whole field of view (FOV) is obtained. If peaking is performed extrinsically, a sheet source must be used to ensure that an average peak for the whole detector is obtained. Peaking should usually be performed at the same time as the uniformity check, as the same set-up and source are used.

(A) Intrinsic tests

(1) Suspend the source at a distance of more than 4 × FOV of the gamma camera away from the detector. The count rate should be between 10^4 and 3×10^4 counts/s.
(2) Check for proper centring of the window on the photopeak and, if necessary, adjust the peak.
(3) Record the peak setting and check for any large or gradual change from previous settings.
(4) Check the peak for each radionuclide used on the camera for the day. This is particularly important if adjustment was required in step (2).

(B) Extrinsic tests

(1) Place the sheet source on the collimator. If a flood tank filled with water is used, protect the collimator and/or the detector with a protective cover from possible contamination.

(2) Repeat steps (2)–(4) as for intrinsic tests.

(d) Uniformity

The uniformity quality control procedure checks that the response of the detector to a uniform irradiation is uniform within defined limits. Interpretation of clinical images taken with the gamma camera relies on the assumption that differences seen are due to differences in tracer distribution in the patient only and not differences introduced by the gamma camera itself.

Many problems that are possible in a gamma camera can degrade uniformity. Checking that the camera performs properly is thus a good general quality control test for these devices. Uniformity defects can be quite marked and focal, such as during a failure of a photomultiplier tube, or there can be general degradation of uniformity across the FOV due to inappropriate spatial linearity or energy corrections. Further quality control tests may thus be required to detect the cause of the observed non-uniformities.

Uniformity can be checked either without a collimator (intrinsic) or with a collimator (extrinsic). An intrinsic uniformity test is simpler to perform but it does not check for non-uniformities introduced by the collimators. Furthermore, on some multidetector systems, it may not be possible to perform an intrinsic uniformity check.

To detect a gradual deterioration in uniformity, it is important that uniformity measurements be carried out in a consistent manner (i.e. same orientation, same number of counts and same collimator) and that records be kept to allow comparisons over periods of weeks or even months. Regular analysis of uniformity by a computer can facilitate detection of a gradual deterioration prior to any visible change.

Uniformity can be different for different radionuclides and window settings. Thus, it is important to ensure that uniformity be consistent for all radionuclides used on the gamma camera. Furthermore, if non-standard or different window settings are introduced (e.g. a narrow window or an asymmetric window), their effect on uniformity should be assessed before clinical studies are performed.

(A) Intrinsic tests

(1) Suspend a point source (typically ^{99m}Tc) at a distance of more than 4 × FOV of the gamma camera away from the detector. The count rate should be between 10^4 and 3×10^4 counts/s. Ensure that the source is located centrally in the detector head.
(2) Adjust the energy window setting to peak.

(3) Make sure that a consistent set-up is used (e.g. same distance of source, orientation, peaking, radionuclide and formatter settings) and that there is no significant background radiation from other sources.
(4) Collect a uniformity image for at least 4×10^6 counts. Check for pronounced non-uniformity in the image. Windowing may be used to highlight non-uniform areas if the study is stored on a computer. A comparison should also be made with previous images for any gradual degradation in uniformity.
(5) File the image (if acquired on film) or archive the image (if collected on computer) for future comparison. Where available, use NEMA software to calculate integral and differential uniformity and record these figures for a specified FOV.

(B) Extrinsic tests

(1) Place the sheet source on non-attenuating supports to position the source approximately 10 cm in front of the collimator. If a flood tank filled with water is used, protect the collimator and/or detector from possible contamination with a protective cover.
(2) Repeat steps (2)–(5) as for intrinsic tests.

(e) Spatial resolution

The purpose of checking resolution is to detect gradual, long term deterioration of resolution, rather than to detect abrupt changes. Inappropriate adjustments carried out during service may affect the resolution without necessarily being apparent in the uniformity or other checks.

An intrinsic resolution test with a four quadrant bar phantom can be carried out as follows:

(1) Place a four quadrant bar phantom on the detector.
(2) Suspend a point source at a distance of more than 4 × FOV away from the detector. The count rate should be between 10^4 and 3×10^4 counts/s.
(3) Make sure that a consistent set-up is used (i.e. same distance of source, orientation, peaking, radionuclide and formatter settings).
(4) Collect an image of at least 10^6 counts on film. If a computer is connected to the camera, also collect an image on the computer using at least a 256 × 256 matrix. Check for any degradation in resolution between previous images and the current image.

(5) File the image (if acquired on film) or archive the image (if collected on computer) for future comparison.

(f) Multiple window spatial registration

A multiple window spatial registration checks that images acquired using different photopeak energies (e.g. 93 and 184 keV peaks of ^{67}Ga) are spatially co-registered (typically within 2 mm). Misregistration of the images will cause a deterioration in resolution, particularly towards the edge of the FOV. Inappropriate adjustment during servicing can cause excessive multiple window spatial misregistration without being apparent in the uniformity check.

The procedure described below should be followed:

(1) Place the four quadrant bar phantom on the detector.
(2) Suspend a point source of ^{67}Ga at a distance of 4 × FOV of the gamma camera away from the detector. The count rate should be between 10^4 and 3×10^4 counts/s.
(3) Peak the camera for the ^{67}Ga setting with windows over the three peaks of ^{67}Ga.
(4) Repeat steps (3)–(5) in (e) as for intrinsic tests.

Note that this test is simple but may not be as sensitive as the normal NEMA test.

(g) Whole body scan resolution

To avoid loss of resolution in the scanning direction during whole body scans, the relative physical position between bed and detector has to be accurately synchronized, with the electronic offset applied to the image data to form the whole body image. Both mechanical problems and drift, inappropriate adjustment of image offset, and size can cause a loss of resolution for whole body scans.

The procedure described below should be followed:

(1) Place the sheet source and the four quadrant bar phantom on the scanning bed such that the resolution phantom is between the sheet source and the collimator. Resolution loss normally only occurs in the direction of scanning. Position the phantom so that the bars are oriented at 45° to the direction of movement, to ensure that all bars measure to some extent in the direction of the motion.

(2) Bring the collimator as close as possible to the resolution phantom while still allowing a whole body scan to be carried out.
(3) Make sure a consistent set-up is used (i.e. use the same collimator and proper peaking of the camera).
(4) Collect a whole body scan at a speed to give 10^6 total counts over the resolution phantom.
(5) Collect a static image over the resolution phantom for the same number of counts.
(6) Compare the two images. There should be no appreciable loss in the resolution of the whole body image compared with the static image.

(h) Less frequent planar quality control tests: pixel size

On new equipment, accurate calibration of the pixel size is performed as part of the service and calibration of the camera. However, on older systems, particularly those connected to a separate computer, pixel size can change after servicing of the gamma camera or adjustment of the computer interface and should be checked regularly if studies which rely on proper calibration of pixel size for accurate results are performed on the system.

Pixel size can be calibrated with sources placed at known distances apart or with slit or grid phantoms.

(i) Planar spatial resolution (FWHM)

The bar phantom used for the resolution tests provides a quick method of checking resolution. Interpretation is, however, subjective, since this method may not detect minor changes in resolution and only gives semi-quantitative results.

Resolution is usually defined in terms of the full width at half maximum (FWHM) of a line spread function. A profile is generated on the computer across the image of a line source and the full width of the profile at half the maximum level is found, either by fitting a Gaussian function to the curve or by measuring the width directly from the curve using linear interpolation between the curve points. To convert the FWHM measurement from units of pixels to units of millimetres, the pixel size has to be accurately known. Use of multiple line sources permits estimation of pixel size in the same acquisition.

(A) Intrinsic tests

(1) To measure intrinsic FWHM resolution, a slit phantom is placed on the uncollimated detector. The slit phantom consists of 1 mm

wide slits, 30 mm apart in a lead mask. A point source is placed at a distance of more than 4 × FOV from the detector (the count rate should be between 10^4 and 3×10^4 counts/s) and an image is acquired.

(2) NEMA specifications for measuring FWHM require a pixel size of less than 0.1 times the expected FWHM, i.e. for a camera with an intrinsic resolution of 3.5 mm, a pixel size of less than 0.35 mm is required. Thus, a large matrix size and/or large zoom are required to achieve the small pixel size. If this pixel size is difficult to achieve, meaningful results can still be obtained by using a pixel size of around 1 mm. In this case it is important that for each test the same pixel size is used.

(3) Profiles are generated across the image and FWHM is measured at several points across the FOV. Programs are available on a number of gamma camera/computer systems which generate profiles and calculate mean, maximum and standard deviation of the FWHM automatically across the FOV.

(B) Extrinsic tests

For extrinsic FWHM measurements, one or more line sources (line source diameter of 1 mm) are placed at the required distance from the collimator (e.g. at 10 cm). Images are then taken of the line source(s), profiles are generated and the FWHM is calculated as above. The same comments regarding pixel size apply.

4.3.3.4. Rationale and description of QC tests: SPECT

(a) High count flood

Non-uniformities, particularly near the central axis of rotation, are substantially magnified by filtered back-projection reconstruction, resulting in ring artefacts. This places more stringent requirements on the uniformity of the camera. To achieve the required uniformity, flood correction is either applied during acquisition or post-acquisition. To allow accurate measurement and correction of non-uniformities, the variation per pixel as a result of counting statistics has to be small. For a pixel coefficient of variation (COV) of less than 1% due to counting statistics, the count per pixel needs to be more than 10^4. This requires 3×10^7 counts for a 64 × 64 matrix or 1.2×10^8 counts for a 128 × 128 matrix. The COV or the number of counts required can be reduced somewhat by smoothing the data.

This high count flood can typically be used to assess uniformity as well as to act as the flood correction for SPECT data. Drifts in differential uniformity of more than 1% should be investigated and usually require new uniformity corrections. Uniformity corrections should not be used as a substitute for proper camera tuning and adjustment.

As collimators can also introduce non-uniformities, high count floods should be performed extrinsically with each collimator used for SPECT. It is important that the flood source used for extrinsic high count floods is uniform across the FOV and that it does not introduce non-uniformities. A ^{57}Co sheet source with guaranteed uniformity is thus preferred to a fillable flood tank.

The procedure described below should be followed:

(1) At least 3×10^7 counts should be acquired using a 64×64 matrix or 1.2×10^8 counts using a 128×128 matrix.
(2) High count uniformity floods should be collected for each radionuclide–collimator combination in accordance with clinical needs. The acquisition frequency is dependent on system stability.
(3) Integral and differential uniformity should be calculated from the high count flood and recorded. The figures should be compared with previous results.

(b) Centre of rotation

The COR that is assumed by the reconstruction program has to accurately coincide with the mechanical axis of rotation in order to avoid loss of resolution and distortion in the reconstructed slices. COR offsets are easily corrected during the reconstruction process. Consequently, it is important that the COR offset remain stable, within a 2 mm variation, for a period of at least one week.

The COR offset can vary with the collimator, detector rotation, radius of rotation and zoom factor. It is important to establish which factors affect COR offset on each particular camera and make the appropriate allowances.

The procedure described below should be followed:

(1) The COR data should be collected as specified by the manufacturer and then recorded.
(2) Significant changes over previous values (>3 mm) and important changes in the COR with rotation angles of more than 1 mm should be investigated and, if necessary, corrected.

In addition, the detector head must be parallel to the axis of rotation. The variation in point source location along the patient axis as the camera rotates

can be assessed by viewing the COR data. In some cases, a *y* axis analysis of the COR data is provided. This should be checked periodically.

(c) Correction tables

Modern gamma cameras include on-line corrections for variations in energy response and linearity across the crystal. Some cameras also include on-line uniformity corrections. These corrections are designed to provide the uniform energy response and good linearity across the FOV, which are also prerequisites for good uniformity.

As the camera slowly drifts over time, the correction tables have to be updated in order to apply proper correction factors during collection of the image. The exact frequency of reacquiring the correction tables depends on the stability of the camera. In general, energy and on-line uniformity corrections require more frequent updating than linearity correction tables. Energy and uniformity tables are usually obtained by operators, whereas linearity correction tables are typically found by service engineers.

It should be stressed that energy and on-line uniformity corrections are designed for minor variations in response across the FOV. They are not a replacement for proper tuning of the gamma camera. While energy and on-line uniformity corrections can in some instances take care of relatively large non-uniformities, these should normally be corrected by a retuning of the camera since they can affect the linearity, resolution and overall sensitivity of the camera.

The procedure described below should be followed:

(i) Collect correction tables (e.g. energy) as specified in the instruction manual for the camera and at the frequency recommended by the manufacturer.
(ii) If the frequency of collecting correction tables increases in order to maintain acceptable performance, a service of the equipment may be required as it may be faulty.

(d) Less frequent SPECT quality control tests

The following tests require additional equipment resources (e.g. specialized phantoms) and expertise and are most likely to be undertaken by a nuclear medicine physicist. These quality control tests need only be performed less frequently.

SPECT spatial resolution

For a properly operating SPECT system, the reconstructed resolution should be within 10% of the planar resolution at a distance from the collimator equal to the radius of rotation of the SPECT acquisition. Incorrect COR correction, mechanical misalignment and instability, and problems with the reconstruction software can degrade the reconstructed SPECT resolution. Thus a SPECT resolution measurement provides a good check of the quality of a SPECT study.

To perform the SPECT resolution measurement, a point or preferably line source is placed near the centre of, and parallel to, the axis of rotation. A SPECT study is then made with sufficient counts to allow reconstruction with an unmodified ramp filter. The study is reconstructed and the FWHM resolution is calculated for the slices spanning the source.

With the same source and collimator, at a distance equal to the radius of rotation of the SPECT study, a planar image is collected and the FWHM of the source in the planar images is calculated and compared with the resolution results from the SPECT study. The measured SPECT resolution should be within 10% of the planar image if all the components are operating correctly.

SPECT total performance

Phantoms, such as the Jaszczak SPECT phantom, are designed to provide an evaluation of the overall performance of a SPECT system. They contain a uniform section for detecting ring artefacts, cold spheres of varying sizes for assessing contrast, and cold and/or hot rods.

The phantoms are typically filled with 400–600 MBq of ^{99m}Tc, and images are collected over 30 min or more to obtain a very high count SPECT acquisition. This does not reflect clinical conditions but is designed to demonstrate the limit of performance of the SPECT system.

These phantoms are particularly useful for detecting slow overall degradation of the SPECT performance provided that a reference study with the phantom was performed when the SPECT system was known to be working optimally, for example during acceptance testing. Subsequent phantom studies are then performed under the same conditions and compared with the reference study to detect changes in performance. These studies are also useful to check the proper functioning of SPECT acquisition and reconstruction software after major software upgrades.

4.3.3.5. Recommended frequency of quality control tests

The recommended frequency of quality control tests depends on the particular equipment available and its stability. Thus it is important to tailor quality control tests to specific equipment. Significant changes consistently detected between consecutive quality control tests may require the frequency of the tests to be increased. Conversely, the frequency may be reduced if only minor fluctuations are detected over a series of quality control tests. Manufacturers' literature may also provide some guidance on the required frequency of tests. An experienced nuclear medicine physicist may in addition provide advice on the frequency for specific tests and equipment.

Tests such as those on uniformity are specifically designed to detect malfunction of the equipment and sudden deterioration of performance before they affect a large number of patient studies. Thus the frequency of this type of test should not be reduced even if results remain consistent over a prolonged period of time.

The following schedule is thus recommended:

Daily:

— Visual inspection;
— Background and/or contamination;
— Photopeak and window setting;
— Uniformity.

Weekly:

— High count flood;

System dependent frequency:

— Centre of rotation;
— Energy and uniformity correction;
— Uniformity correction floods for all collimators used for SPECT.

After a major service:

— Spatial resolution;
— Uniformity with high count flood;
— Multiple window spatial registration;
— Whole body resolution.

Less frequent tests:

— Pixel size;
— Planar spatial resolution (FWHM);
— SPECT resolution;
— SPECT total performance.

4.4. DUAL PHOTON IMAGING

4.4.1. What is dual photon emission tomography?

In contrast to SPECT, which relies on the detection of single photon emissions, PET involves the detection of the dual photons that arise from each positron. When a positron, a positively charged electron, is emitted from a nucleus, it travels a short distance, losing energy until it reaches a resting state. It then interacts with one of the many electrons, whereupon the two annihilate (disappear), giving rise to two 511 keV gamma rays that travel in opposite directions. Unlike SPECT, where a single photon is emitted on each disintegration, in PET a pair of photons is emitted (hence the term dual photon imaging). Detection involves a pair of opposing detectors, which must record events at the same instant of time (i.e. in coincidence).

This unique process has two very important properties. Firstly, since the two photons travel in opposite directions, the point of annihilation will lie on a straight line joining the points of detection. This means that directional information is determined electronically, without the need for conventional collimation. Hence, unlike SPECT, detection is not limited to those photons travelling at right angles to the detector and consequently the sensitivity of PET is many times greater than that of SPECT. Collimation may be retained for separate data from different planes; however, within any detection plane no conventional collimation exists.

The second important property of dual photon imaging is that for it attenuation is dependent only on the total attenuating path through the patient, but is independent of the exact location of the annihilation event in the tissue. This is quite different from the case of SPECT where attenuation poses a much more difficult problem.

4.4.2. Dedicated PET instrumentation

Dedicated PET systems consist of multiple rings of detectors that surround the patient. Each ring normally consists of a set of small BGO

detectors, the high density of BGO giving it a very effective stopping power for 511 keV photons. Sodium iodide (NaI), the detector of choice for gamma cameras and SPECT systems, is also used; however, it has several properties which make it less attractive than BGO. It does have a higher light output and consequently better energy resolution than BGO (~10% compared with ~20%) but, besides its significantly lower stopping power, it is hygroscopic.

Dedicated PET systems may use lead collimation in the form of 1 mm x 65 mm lead septa, which significantly reduces scattering and random events. Recent developments have involved removal of these septa, so that there is no collimation within the imaging volume of the scanner, necessitating a 3-D reconstruction of the data. This can potentially improve sensitivity by a factor of six.

The differences between PET and SPECT in performance and applications are decreasing. Dedicated multidetector SPECT instruments with purpose designed collimators, for example fanbeam collimators, now permit SPECT studies to be performed with improved sensitivity compared with that of single head SPECT and comparable resolution to that of PET, although sensitivity is still lower than that of PET.

4.4.3. Hybrid PET–SPECT instrumentation

Although gamma cameras were used in the early days of PET, manufacturers have recently introduced commercial gamma cameras based on coincidence detection systems. Dual head gamma cameras with opposing heads, originally designed for multidetector SPECT, can be used with additional coincidence circuitry to detect positron events in exactly the same way as PET detectors. The clear advantage of these systems is their relatively low cost and the fact that the instrument can be used for either SPECT or coincidence detection (CD).

The term CD is often used to differentiate these systems from dedicated PET systems, although a more appropriate term is ‘hybrid PET–SPECT systems’. The only real difference is that the dual detectors must be rotated around the patient, as in normal SPECT acquisition, whereas dedicated PET systems are designed with a complete (or partial) ring of detectors that surround the patient.

The main differences in the design of a hybrid PET–SPECT system, compared with a dual head SPECT system, are as follows.

Coincidence circuitry must be added so that the two opposing detectors can detect the two annihilation photons in coincidence, i.e. within a very short time of each other. It is this coincidence that defines the path along which the photons must have travelled, eliminating the need for a conventional collimator.

The absence of a collimator means that the sensitivity for detection is much higher than normal. This introduces problems relating to count rate performance since, for each detected coincidence, there are many more single events (single gamma photons detected without a corresponding coincident event). Several approaches have been implemented to improve the count rate performance of gamma cameras, with some coincidence systems now capable of achieving count rates of several million counts per second.

Since for 511 keV photons the stopping power of sodium iodide is relatively poor, manufacturers offer thicker crystals than normal (up to 25 mm thick), with only slightly poorer resolution, due to uncertainty in the location of detected events. The ability to maintain performance is largely attributable to the improved design of recent gamma cameras. Despite the thicker crystals, the detection efficiency for 511 keV coincident photons is still relatively low compared with that for PET systems that use BGO detectors.

It should be recalled that the absence of a collimator means that the resolution is essentially defined by the intrinsic resolution of the gamma camera at 511 keV (typically 4.5–5.5 mm).

Dual head systems rotate to different angles around the patient, recording coincidences at each angle. The detected events are rebinned or sorted in the same way as in a dedicated PET system to form sinograms for each slice. These are reconstructed using filtered back-projection or iterative reconstruction in exactly the same way as in dedicated PET or SPECT. Although the earlier systems did not include attenuation correction, recent systems now have this as an option.

Despite the attraction of low cost and the versatility of the hybrid PET–SPECT systems, their place in clinical practice has still to be defined. The performance of CD is definitely limited compared with that of dedicated PET (Table 4.1) and this has imposed limitations in the technique for the detection of small lesions. Nevertheless, their introduction has resulted in the widespread use of positron emitting tracers in clinical practice.

4.4.4. PET versus SPECT

It is useful to make a direct comparison of performance between PET and gamma camera based SPECT, although this largely depends on the choice of collimator and number of heads. PET performance will also depend on several parameters, for example detector diameter and type of septa. The figures provided in Table 4.1 are therefore indicative only. Current PET systems provide a reconstructed resolution on-axis of around 6 mm. SPECT can achieve a resolution only slightly worse than PET using ultrahigh resolution fanbeam collimators (around 8–10 mm), but this can only be achieved with significantly

TABLE 4.1. COMPARISON OF PET VERSUS SPECT SYSTEMS

Characteristic	SPECT	Hybrid PET–SPECT	PET (2-D)	PET (3-D)
Sensitivity relative to SPECT	×1 (single high resolution) ×6 (triple head fanbeam)	≈×7	×15	×75
Reconstructed resolution (mm)	8–10 (head)	5–7	4–6	4–6
Scatter fraction (%)	≈30	≈30	≈15	≈40
Attenuation factor (thorax)	×7 (Tc-99m)	×30	×30	×30

less sensitivity: at best a factor of less than 2.5 (with fanbeam and triple head compared with 2-D PET).

4.4.5. Use of ultrahigh energy collimators

A very simple approach to imaging 511 keV photons is to use an ultrahigh energy collimator. In this case conventional SPECT or planar imaging is preferable to coincidence imaging, which is based on the detection of the single 511 keV photons. Although coincidence imaging was used initially in oncology studies, it has since become evident that only fairly large tumours can be detected. However, the need for high resolution is less critical in cardiac studies using FDG since normal or enhanced uptake is evidence of myocardial viability.

4.4.6. Purchase of dual photon imaging systems

All nuclear medicine physicians, assisted by a nuclear medicine physicist, acquire some experience during their careers in purchasing gamma cameras and other accessories for a nuclear medicine service. The decision making process, leading to the purchase of a system performing dual photon imaging, calls for knowledge of the basic physics of coincidence detection and of the differences between 2-D and 3-D acquisition in terms of sensitivity, the ratios between the true and the random events, and scatter fraction, as well as the different methods to overcome these problems.

There are a number of different ways to increase the sensitivity of the system and physicians should work closely with a physicist who has extensive knowledge of these areas. It is recommended that they should visit or contact a site that is already functioning. They should be allowed to review the

performance of the system from the work scheduling book in terms of its uptime and downtime. They should also have an opportunity to observe on the workstation the studies performed. The nuclear medicine physicist should be able to review the results of the various quality control tests performed.

There are many aspects of purchasing dual photon imaging systems that are common to the purchasing of single photon imaging systems; these have been covered in an earlier section of this chapter. In addition to specific advice on contractual arrangements, warranty and service, the reader should bear the following points in mind when purchasing dual photon imaging equipment.

In most cases the primary purpose of purchasing the equipment is to perform oncology studies, although specific centres may have research requirements in other areas. The main dilemma facing a purchaser is what type of system to purchase. At the time of writing there are basically two types of system: dedicated PET systems (including full ring systems and lower cost partial ring systems) and hybrid SPECT–PET systems based on standard gamma camera technology, and there is a significant difference in the cost of these systems. The main considerations in choosing between the systems can be summarized as follows.

(a) Sensitivity

In a hybrid PET–SPECT system, sensitivity is a primary concern and a thicker crystal than that normally used for SPECT is required. The effect of increasing crystal thickness on routine single photon nuclear medicine studies should be considered. Although a slight decrease in resolution is demonstrated in bar phantom studies, it has little effect on routine clinical studies. An advantage is the additional increase in the sensitivity for such radionuclides as ^{67}Ga, ^{111}In and ^{131}I. The trade-off between resolution and sensitivity in PET versus SPECT applications for these thick crystal systems is still under evaluation. Sensitivity is improved by using 3-D rather than 2-D acquisition as outlined in the sections earlier in this chapter. The exact trade-off in useful counts (with scatter correction) for whole body applications continues to be evaluated.

(b) Count rate

The use of a large single detector results in specific count rate limitations. There are several approaches to improve count rate capability with specific circuitry designed to enhance the performance of gamma camera based systems. Nevertheless, there is a limit to the activity that can be administered.

(c) Time for acquisition and processing

Both the relatively low sensitivity of hybrid systems and the count rate limitations result in the need for relatively long acquisition times, particularly for whole body imaging studies. A further constraint is the period required to measure attenuation in these studies. This makes the total time required for whole body acquisition a critical factor in determining the utility of a system. In addition, since iterative reconstruction is commonly used instead of filtered back-projection, processing can be relatively slow. The total time of examination including processing should be taken into consideration.

(d) Flexibility

Despite some constraints on performance, hybrid systems are considerably more flexible than dedicated systems. This can be a major consideration in situations where patient numbers or radionuclide supply may be limited. Hybrid systems have considerable appeal for certain centres. New developments in detector technology are likely to result in a wider range of hybrid systems.

It should be noted that the technology used in dual photon imaging is changing rapidly. As it develops, additional factors might need to be taken into consideration.

4.4.7. Specification

A new NEMA publication (Performance Measurements of Positron Emission Tomographs (see the bibliography to this section)) has been finalized on the specification of dual photon imaging systems and is applicable to both conventional PET systems and gamma camera based coincidence detection systems. The emphasis of this document is on instruments designed for whole body applications, although additional tests are included that provide comparative information related to other types of application. The major advance in this document is that no distinction is made between conventional and gamma camera based systems. A more direct comparison between the specifications should therefore be possible in the future.

The parameters specifically defined in the new document include those listed below:

(a) Spatial resolution

The report includes the radial and tangential FWHMs at the centre and 10 cm off-axis as well as the axial resolutions at the same positions.

(b) Scatter fraction, count losses and random events

This includes count rate plots for true, random, scatter and total events as well as noise equivalent count rate. Peak counts are specified for each type of event as well as scatter fraction.

(c) Sensitivity

System sensitivity is specified, with extrapolation to a zero attenuation situation. The axial sensitivity profile is also presented.

(d) Accuracy — corrections for count losses and random events

The deviance of the corrected count rate from the expected true count rate is specified, as are image quality and accuracy after attenuation and scatter corrections. The contrast is calculated for spheres in a whole body phantom.

The additional tests suggested for applications other than whole body studies are:

Scatter fraction

Count loss and random event measurements (dead time and true event rates) should be made.

4.4.8. Acceptance testing

As in the case of single photon imaging, it is important that all aspects of system performance are tested immediately after installation, and the ability of the system to meet the functionality standards specified in the purchasing document must be confirmed. These include:

— Sensitivities in 2-D mode (with interplane septa) and 3-D mode (with no interplane septa);
— Energy resolution;
— Width of the coincidence time window;
— Spatial resolutions in the transaxial and axial directions;

— Variation in the detection sensitivity in the detection volume,
— True random ratio;
— Scatter fraction;
— Accuracy of scatter and attenuation correction.

4.4.9. Quality control

As in the case of SPECT there is a need for quality control procedures on a daily basis, at regular intervals and on an as needed basis.

The daily quality control procedures include:

— Checking detector performance with a standard source;
— Updating detector normalization;
— Monitoring and recording any shift in parameters and environment.

The regular quality control procedures include:

— Setting up and recalibrating the detector;
— Checking the working parameter setting of the device;
— Making a phantom study of transmission and emission.

The less frequent quality control tests include:

— After power shutdown: checking detector set-up and normalization;
— After servicing: checking detector set-up, performance and normalization;
— After change of source: checking normalization and making a phantom study;
— When necessary: changing the transmission sources.

4.5. OTHER INSTRUMENTATION

4.5.1. Radiation protection and measurement equipment

Any nuclear medicine facility involves the use of radiation in many different ways, including:

— Handling, storage and disposal of small to large activities of radioactive material, potentially in gaseous, liquid and solid forms;
— Storage and handling of sealed radiation sources;

— Monitoring of radiation levels in the work environment;
— Dealing with accidents involving any source used;
— Therapy with radioactive materials.

Even small facilities involve most, if not all, of the above. As a result, different types of radiation measuring equipment are required as follows:

— Passive personnel dosimeters;
— Active (direct reading) personnel dosimeters;
— Contamination monitoring instruments (photons and beta radiation at least);
— Radiation field monitoring instruments (photons).

4.5.1.1. Types of radiation detectors

The various types of radiation detector are described briefly below, in particular their advantages, disadvantages and uses, all of which must be understood by the user.

(a) Gaseous detectors — Geiger counters

Common, rugged, relatively cheap and reasonably accurate, the Geiger counter is probably the most versatile detector available for nuclear medicine. It operates by measuring individual radiation events, which can also be smoothed out into a continuous signal of radiation exposure rate. Geiger counters can be calibrated to read in units of absorbed dose or equivalent dose, with, however, limited accuracy.

The detector itself is usually in the form of a cylinder of varying size, from 2 cm long by less than 1 cm diameter, to around 10 cm long by 3 cm diameter. The detector may have a thin entry window for more efficient detection of low energy photons and particles. Geiger counters are characterized as side window, end window or pancake types. The first two usually have a shield to filter out particles so that only photons are measured. Removing the shield also allows particles to be detected, for example in contamination measurements.

End window type detectors can be made very thin to allow beta and even alpha particles with energies greater than about 50 keV to be detected, whereas side window types (with a larger surface area) are thicker and will only allow photons and more energetic beta particles to pass. Pancake type detectors also have a thin window but a larger area, and are designed for contamination measurements.

Geiger counters have some disadvantages; in particular their detection efficiency decreases rapidly below around 70 keV, although some models can be used at lower energies. In nuclear medicine, most of the photon and beta energies used are above 60 keV, so the energy limitation is not a major problem. The detector should also be 'quenched' to allow use at higher dose rates.

The most versatile and useful Geiger counter for nuclear medicine use should have the following features:

— Have a thin window (but with protection against accidental damage) for particle detection;
— Have a window shield for discrimination against particles;
— Produce an audible signal of radiation events (for contamination detection);
— Be calibrated in dose rate units allowing a wide measurement range, say 10 Sv/h to 10 mSv/h;
— Be energy compensated to give the lowest possible detectable energy;
— Be powered by easily available batteries, or have an inbuilt battery charger.

A detachable probe may be of use for contamination measurements, but inbuilt probes will suffice in most cases. Some Geiger counters are miniaturized, for use as personal dosimeters. They may have a digital readout of total dose and dose rate. Some models have an audible indication of dose rate and/or an alarm which sounds at predetermined steps of total dose. These devices are very useful where staff may be exposed to high levels of radiation, for example, when using ^{131}I for therapy.

(b) Gaseous detectors — ionization chambers

Ionization chambers are only used to measure radiation exposure rates, but are very sensitive and accurate and can be used at low photon energies. They are, however, more bulky and expensive than Geiger counters and may not be as rugged. They are usually used where more accurate measurements are needed. While they (like Geiger counters) measure exposure, they can be calibrated to measure absorbed dose or equivalent dose.

The detector chamber may be less than 1 cm^3 in size or as large as 1500 cm^3. The chamber volume determines the sensitivity. They may operate at ambient pressure or be pressurized for higher sensitivity and stability.

If an ionization chamber survey meter is required for radiation safety use in nuclear medicine, it should:

— Have a chamber volume of around 100 cm^3;
— Be calibrated in the SI unit of equivalent dose (deep dose, H*10);
— Have a usable range of around 10 μSv/h to 10 mSv/h.

The use of ionization chambers in larger nuclear medicine departments is justifiable, and they may also be useful in smaller facilities.

(c) Gaseous detectors — proportional counters

The proportional counter, a special type of ionization chamber, produces a pulse proportional to the energy of the detected photon or particle, and does not produce an average current. Proportional counters are used for sensitive radiation detection where energy discrimination is important. Their main radiation safety use is in contamination detectors, which can be set for a particular radionuclide.

(d) Scintillation detectors

The sodium iodide scintillation detectors used in radiation safety have crystals of the order of 2–5 cm diameter, and are between a few millimetres and a few centimetres long.

Scintillation detectors are used for in vitro sample counting, for probes designed for organ counting or surgical exploration and for general counting. Owing to their energy discrimination capability, they are used in some larger nuclear medicine facilities for spectroscopic investigations to identify radionuclides. These instruments have a very high sensitivity and provide a reading in counts per minute or counts per second.

(e) Semiconductor detectors

This class of detector can be thought of as solid state ionization chambers. Until recently, the most common type was the germanium detector — an expensive and complicated device used for high resolution photon spectroscopy, and rarely used in nuclear medicine.

There are now many miniaturized solid state detectors available as personal dosimeters, with the ability to provide integrated dose, dose rate and dose or dose rate alarms. These devices are affordable and are recommended in situations where staff may be involved in higher radiation level work.

Personal semiconductor dosimeters should:

— Provide an easily readable digital display of integrated absorbed or equivalent dose that can be reset;
— Provide an audible alarm at user selectable integrated dose levels, and/ or provide an audible indication of instantaneous dose rate;
— Be powered by an easily obtainable battery.

(f) Pocket electrometer dosimeters

Also known as quartz fibre electrometers (QFEs), these devices have been available for many years. They allow direct reading of integrated exposure (or calibrated as absorbed dose), and are simple and cheap. Recharging is performed with a small battery powered charger. They are available in full scale ranges from 1 mSv to 100 Sv.

QFEs are still a simple and inexpensive means of instantaneous dose assessment, but are not suitable for routine personal dosimetry in nuclear medicine since a low dose rate is usually encountered.

(g) Film badges

The most common type of personal dosimeter for many years, the film badge, still remains in widespread use. The film badge uses a special type of photographic film in a special holder fitted with filters of various types to allow discrimination between beta and photon (and in some cases neutron) radiation, at various energy levels. As a result, the wearer's radiation exposure can be estimated as an effective dose.

The sensitivity of a film badge varies according to the supplier, but the lower limit of readable dose is of the order of 200 μSv. Cheap, and widely available, film badges still remain an effective means of assessing doses to staff.

(h) Thermoluminescent dosimeters

Thermoluminescent detectors (TLDs) operate on the principle that certain materials, such as lithium fluoride, 'store' energy from incident radiation more or less indefinitely and that the stored energy is released under heating, in the form of a weak emission of light. The amount of light released is in proportion to the stored radiation energy. In many personnel monitoring services, TLDs have replaced film badges.

TLDs are more sensitive than film badges (around 10 μSv lower limit), capable of being reused many times, more rugged and versatile, but more

expensive. It is also possible to obtain TLD monitors for the fingers or hands, which can be valuable for monitoring hot laboratory staff who handle large quantities of radioactive material.

4.5.1.2. Radiation monitors

A nuclear medicine department needs to have, or have immediate access to, at least one radiation monitor. Instruments may be designed to measure dose rate (in μSv/h), integrated dose (μSv) or contamination level (Bq/cm^2). Dose rate measurement is necessary to ensure that levels of radiation in working environments are within the limits required by legislation and also to confirm dose rates from packages that may be despatched from the radiopharmacy. Suitable monitors may be based on ionization chambers, Geiger–Müller counters, scintillation detectors or proportional counters. The choice of instrument is governed by the nature and level of radiation anticipated in the environment. The suitabilities of the various types of dose rate monitor are given in Table 4.2.

Contamination monitors are necessary for routine use to detect any spillage of radioactivity that may have occurred. In view of the fact that gamma emitting radionuclides are most commonly used, a monitor based on a scintillation detector will be suitable, although in situations where beta emitters are used, a Geiger–Müller counter is also valuable.

Quality assurance

Any device used for radiation detection must be regularly calibrated, with the calibration traceable to a recognized primary or secondary standard. Any of the types of radiation instruments mentioned above can drift over time to become inaccurate. Even film badges and TLD badges are calibrated. It is not

TABLE 4.2. COMPARISON OF DIFFERENT TYPES OF DETECTOR

Equipment	Energy response	Sensitivity	Useful for
Ionization chamber, betas 100–500 keV	Flat over a wide range	Poor	Photons, betas 100–500 keV
Geiger–Müller counters	Flat over a limited range	Good	Gammas >40 keV
Proportional counters	Flat over a limited range	Good	Gammas >30 keV
Scintillation detectors	Energy dependent	Very good	Gammas, betas >500 keV

always easy to obtain a calibration, however; sometimes an acceptable alternative is to purchase a calibrated radiation source, and in turn to use this to check the instrument. As far as contamination monitors are concerned, the calibration source must be spread over a known area, and different radionuclides should be used.

It is important to distinguish between calibration and consistency testing. Calibration is performed to ensure that the instrument readings are as accurate as possible for the type of instrument concerned. Consistency testing can be performed on a calibrated instrument to check for drift. All that is required is a radiation source that has a reliable output (^{137}Cs for example) and a reproducible testing geometry.

4.5.1.3. Radionuclide calibrators (dose calibrators)

(a) Choice of instrument

Dose calibrators are a special type of ionization chamber for measurement of radionuclide activity in hot laboratories or radiopharmacies. Such a piece of equipment is essential in order to measure the activities of radiopharmaceuticals received, of generator eluates and kits prepared from them and also of syringes containing individual patient injections.

A range of designs is available, but measurement is based on ionization of a gas by the radioactive sample, which produces a proportional electric current. These chambers are usually pressurized and have a large detector volume. They are calibrated for a number of individual radionuclides so that the activity can be measured directly. Alternative models designed for the measurement of beta emitting radionuclides are also available but are less likely to be required.

The instrument chosen will be influenced by the range, geometry and activities of the nuclides handled. Recent publications that deal with design, calibration and use of calibrators should be consulted for further information.

(b) Quality assurance

The aspects of the calibrator that need to be assessed for acceptance are considerably more extensive than those which should be assessed on a daily basis once the calibrator has been brought into use. The important features are listed in Table 4.3. Radionuclide calibrators should be tested daily. Consistency testing is useful, particularly when calibration is not easily performed, or can be done only occasionally. The department will need a long lived comparison source such as ^{137}Cs (half-life 30 years). Alternatively ^{57}Co can be used since its

TABLE 4.3. RADIONUCLIDE CALIBRATOR PERFORMANCE TESTING

Test	Acceptance	Daily	Monthly	Annually
High voltage	X[a]	X	X	X
Background	X	X	X	X
Zero adjustment	X	X	X	X
Accuracy	X			X
Precision	X		X	X
Geometry	X	X	X	X
Constancy	X (all settings)	X (nuclides in use)	X (all settings)	X (all settings)
Linearity	X			X
Electrical safety	X			X

[a] X indicates 'Yes' and a blank 'Not required'.

gamma energy is similar to that of ^{99m}Tc. However, the half-life of ^{57}Co of 271 days means the source will need to be replaced every few years.

High voltage checks are necessary to ensure the supply to the ionization chamber is adequate. Background measurements and adjustment to zero ensure that any unnoticed radioactive contamination of the calibrator can be detected so that artefacts can be eliminated from measurements.

The accuracy of the instrument should be tested with a reference source of activity whose activity has been certified by an appropriate authority. This same source can be used to test the precision of the instrument by performing at least ten repeated measurements of its activity.

The constancy of response can be determined using the comparison source to ensure that the calibration factors used are appropriate and do not vary. The value of a reading on the individual settings should decline according to the half-life of the radionuclide in the comparison source.

The linearity of the instrument should be checked by measuring a source of ^{99m}Tc whose initial activity is as high as possible, over a period of several half-lives, in order to check that the response of the instrument is linear over the range of giga- to kilobecquerels.

4.5.1.4. Minimum recommended monitoring equipment

From all the monitoring devices described, the minimum requirements for a nuclear medicine department are given below. It should be recalled that in

most hospitals the nuclear medicine department is often called upon to deal with radiation accidents or incidents involving radionuclides (or even radiation sources) that may occur outside the department. Monitoring equipment is therefore a resource for the whole institution.

(a) Small departments

Survey meter/contamination monitor

A Geiger counter is required with a removable beta shield, audible count rate signal, dose rate calibration and a range of around 10 μSv/h to 10 mSv/h.

Radionuclide calibrator

A simple device is required with a digital readout and preset settings for common radionuclides.

Scintillation counter

A simple counter with a single sample well for in vitro tests is required.

Personnel monitors

Film or TLD badges (usually from a national radiation regulatory body) are required. Finger badges are recommended for staff working in a hot laboratory.

(b) Medium sized departments (additional items)

Medium sized departments should have the same equipment as small departments plus the following items:

Survey meter, contamination monitor

A portable ionization chamber meter with a flat energy response from about 30 keV and a dose rate range as for a small department meter is required. An integrating mode option is suggested.

Radionuclide calibrator

A simple device with a digital readout and preset settings for common radionuclides, as well as a variable user defined setting, is required — a dedicated label printer is desirable.

Personnel monitors

At least one direct reading dosimeter (QFE or electronic), with a measurement range of 0–1 mSv, is required.

(c) Large departments performing therapy procedures (additional items)

Large departments should have the same equipment as for a medium department plus the following items:

Survey meter, contamination monitor

A dedicated contamination monitor, ideally calibrated for common radionuclides, is required.

Scintillation counter

A gamma spectroscopy system with a well or cylindrical scintillation detector is required.

Personnel monitors

Direct reading dosimeters, multiple QFEs and/or at least two electronic personnel meters, with range 0–1 mSv, are required.

Zone (area) monitor

An ionization chamber, Geiger counter or scintillation counter at a fixed position is required, with either a visual or an audible alarm (or both) at variable preset values.

4.5.2. Probe systems

4.5.2.1. Probes for external organs

In vivo counting probe systems are used for measurement of thyroid uptake and kidney function, as well as for other more specialized counting. Whatever the application, however, counting systems have a common specification:

(a) A large volume NaI scintillation detector, typically 5 cm diameter by 5 cm thick, to allow both good sensitivity and high detection efficiency for a range of radionuclide photon energies.
(b) A collimator to limit the FOV of the detector, with some form of distance indication to ensure a repeatable geometry.
(c) A strong stand to hold the probe and collimator, and to allow freedom of movement to set the probe up to count at a variable height and orientation.
(d) Counting electronics including a high voltage supply, pre-amplifier, pulse amplifier, single channel spectrum analyser (with variable counting window and threshold); if available, a multichannel analyser is ideal.
(e) A pulse counter and timer, preferably with a printer, are required.

The collimator is made of lead or another high density material; it is designed to allow a reasonably sized sensitive area, whilst minimizing interference from other sources of radiation in the body. For thyroid examinations, a special thyroid collimator can be used.

For measurement of kidney function, the ideal counting system has three probes: one for each kidney plus one to measure the blood and tissue background. Each probe has its own set of electronics.

When purchasing a probe system, one must ensure that a set of low activity spectrum calibration sources is provided. These are crucial, as they are used to set the single channel spectrum analyser to the required counting energy. The sources should have a relatively long half-life, a distinct photon energy (or energies), and cover the required energy range, usually 60–511 keV. The following sources are suitable: ^{241}Am, ^{109}Cd, ^{57}Co, ^{22}Na, ^{137}Cs and ^{133}Ba. The system should be checked before each use.

4.5.2.2. Surgical gamma probes

The use of surgical probes for localization of activity which can be traced and surgically excised has been of interest since 1949. Recently, surgical probes

have grown in importance for localization of sentinel nodes in early stage malignant melanoma and breast cancer surgery, using ^{99m}Tc labelled radiopharmaceuticals. Attempts to use surgical probes in other applications, including monoclonal antibody studies and even ^{18}F FDG studies, are under investigation. In this section emphasis is placed on the use of probes employed for detection of ^{99m}Tc radiopharmaceuticals.

The technique of sentinel node localization needs multidisciplinary cooperation between nuclear medicine physicians, surgeons and pathologists. Success in detecting the sentinel node depends on many factors related to the sensitivity of the detector, the spatial and energy resolution and geometric efficiency of the detector, the radiopharmaceutical injected, the rate of clearance from the site of injection and the uptake in the sentinel node. Detectors are made by various manufacturers, and the preference between different types requires knowledge of the basic physics principles, which are summarized below.

It is important to know the reason why the surgical probe is purchased. Is it for the purpose of sentinel node localization only for early breast cancers and malignant melanomas or for the intraoperative detection of residual or recurrent tumours such as colorectal cancer, thyroid cancer or parathyroid adenoma? The energy of the radionuclides used and the expected depth of the target from the skin or the surface of the probe are important because of the attenuation of the photons, the FOV of the detector, the angular scatter and the geometric efficiency. The user must familiarize him/herself with different probes and have experience in operating them.

A surgical gamma probe is based on either a scintillation detector or a semiconductor detector. Scintillation detectors consist of either NaI or CsI, either 14 or 19 mm in diameter, with a photomultiplier tube and amplifier. The signal intensity of scintillation detectors is higher than that of semiconductor detectors, but their energy discrimination is inferior. Scintillation detectors are also bulkier.

Semiconductor detectors consist of either cadmium telluride or, more recently, cadmium zinc telluride. These have high stopping power materials and accordingly are more sensitive. They are significantly more compact than scintillation detectors and therefore more suitable for intraoperative use. Their spatial resolution is also better. However, the useful energy range for these detectors is limited to 200–300 keV.

When purchasing a probe system for use in surgery, the following factors should be taken into consideration:

(a) Shielding (collimation) from scattering is important for improved localization and improved spatial resolution. However, shielding will reduce

the sensitivity of the collimator. Shielding may be either integral in the design of the probe or in the form of removable collars of a heavy attenuating material. It should only be used when necessary. It adds to the weight of the detectors and reduces their manoeuvrability. It is advisable to use collimators when there is adjacent activity next to the sentinel node. The thickness of the collimator depends on the energy of the radionuclide used.

(b) Count rate should be recorded by both a digital display at time intervals in the range of 1–10 s and by an audible sound, the intensity of which is proportional to the count rate.

(c) The probe should have a long cable between the detector at the surgical field and the associated electronics in order to allow sufficient space between the surgeon and her/his associates.

(d) The response of the detector should be constant over its useful life and can be checked using a calibration source (dependent on the radionuclide used). For ^{99m}Tc applications ^{57}Co can be used.

(e) The probe should be battery operated for several hours and should have a rapid charging system.

(f) Sterilization is easily achieved by covering the detector with a sterile, disposable plastic sheath.

4.5.3. Well counters

Well counters are used for low activity, high efficiency counting of in vitro samples, and are available either as manually operated single sample (or limited number of samples) devices or as fully automatic, multiple sample counters.

All well counters use large volume NaI detectors in the form of a well, where the sample is virtually surrounded by the detector. Ideally, they should have the following capabilities:

— Automatic photon spectrum calibration, with continuous correction for drift;
— Ability to select and count multiple radionuclides;
— Automatic radioactive decay correction for the selected radionuclide(s);
— Variable counting time;
— Sample identification;
— A printed report for each sample including sample identification, counting time, energy selected and counts;
— An indication of errors in the electronics or mechanical sample changer.

Manual systems are satisfactory for small numbers of samples, such as for glomerular filtration rate (GFR) measurement, but automatic sample changing systems will be needed where in vitro testing is widely used.

4.6. COMPUTERS AND NETWORKING

4.6.1. Purchasing a nuclear medicine computer

Computers have been central to the practice of nuclear medicine for many years, particularly as the extraction of functional information commonly necessitates image analysis. Computers form an integral part of imaging equipment, providing on-line acquisition and data correction to improve instrument performance, essential functions such as tomographic reconstruction and flexible display of images. As computer speed increases exponentially, and with memory and disk capacity showing similar growth, the capacity of the computer to tackle more complex and challenging tasks in a clinically acceptable time increases. Patient throughput and efficiency of operation are greatly aided by the computer tools available.

In recent years most vendors of nuclear medicine imaging equipment have moved away from proprietary computer designs towards general purpose computer platforms such as the IBM PC, the Macintosh and various desktop workstations running the UNIX operating system. By adopting these relatively highly developed and widely used computer systems, for which numerous hardware and software options are available, the vendors are now able to offer support for industry standards in several important areas, including networking.

When purchasing nuclear medicine equipment it is usual to include a computer supplied from the same manufacturer, although there are instances where computers may be purchased separately. Choice of equipment should be based on the criteria outlined in earlier sections, with choice of computer being secondary to general considerations such as the amount of support available. Since computers are increasing in performance so rapidly, the main problem with them is that they have a much shorter life than that of the associated imaging equipment. The limitation therefore is in the ability to upgrade systems so that software and new features are available. Continuation of a software support contract is advisable as this will normally include any improvements, fixes of any bugs and new releases for a reasonable time. However, at some stage, hardware will also need to be upgraded at the customer's cost to permit operation of the current software. The adoption of industry standards by manufacturers has reduced the problem of hardware

support, although most suppliers will still utilize customized hardware for special functions (e.g. data acquisition).

When specifying requirements it is important to define the expected functionality rather than the technical specifications. Acceptance testing can then be based on the capability to provide results for a specified clinical analysis in an acceptably short time. Comparison of the time for processing various clinical studies is a useful indicator of system performance, independent of underlying technical specifications. Many manufacturers now offer package software that is common across multiple manufacturers (e.g. cardiac SPECT analysis). The software supplied sometimes requires an exact acquisition protocol, otherwise its application may be invalid. Every effort should be made to ensure that the software purchased is validated in-house. Software phantoms and test data sets available via the Internet may assist in the validation of some programs.

When purchasing computers the following factors should be carefully considered:

(a) Advice should be sought regarding the version of the operating system used. System software tends to be fairly stable but major changes occasionally occur that may limit the availability of future releases. Care should be taken to avoid manufacturers whose system software lags well behind the current release (as available directly from the system software supplier, e.g. Sun or Microsoft).
(b) Although most systems are supplied with all the clinical functionality that can be envisaged, it is important that there is some ability to customize protocols to match individual requirements. In particular, the ability to easily add simple programs is important to permit flexibility in use. In most cases some level of programmability is available suited to a non-expert user.
(c) Training in the use of the computer is as important as training in the use of the equipment itself. Ensure that adequate provision for training is included with the equipment purchase.
(d) The connectivity of the system to other equipment in the department or institution is an important consideration which may require additional hardware and software to be purchased. This is further addressed in the next section.
(e) Choice of system may be based on personal preference for a particular user interface and speed of response rather than choice of options.

4.6.2. An introduction to networking

An important development in computing has been the ability to connect computers via a network so that there can be communication and data transfer between systems. A network that extends over a limited geographical area (e.g. a single hospital department) is called a local area network (LAN). However, networking is not limited to a department but permits communication between computers in different institutions, even if these are located in different countries, via the Internet and the World Wide Web. A brief overview of the components of a typical network is provided below in order to ensure familiarity with some of the jargon used. The most important applications of networks in nuclear medicine are:

(a) To permit interconnection of imaging equipment in a department or hospital;
(b) To permit transfer of images for reporting or provision of an opinion remote from the site of data acquisition;
(c) To permit access to educational information, technical advice or software.

Networking is made possible by the adoption of a set of standards that define how information can be sent via electrical signals in a cable and deciphered by a computer interfaced to the cable. The underlying standard model for networking as defined by the International Standards Organisation (ISO) is the Open Systems Interconnect Model (OSI). Networking therefore uses specialized hardware involved in interconnecting computers and the software used to interpret or translate the information transferred.

By far the most common means of connecting computers in LANs is the Ethernet, a standard that defines both the protocol for data transfer and the cable used. It uses a method called carrier-sense multiple access with collision detect (CSMA/CD) to share a common cable among several computers. CSMA/CD operates as follows. When it is desired to transmit data from one computer to another computer on the network, the computer first 'listens' to determine whether the cable is in use. If the cable is in use, the computer waits for a random period of time before listening again. If the cable is not in use, the computer transmits, and at the same time listens to ensure that another computer did not commence transmitting at the same time. When two or more computers attempt to transmit at the same time, a 'collision' is said to occur. All the computers involved detect the collision and each waits for a random period of time before trying again. Until recently Ethernet networks operated at a transmission rate of 10 megabits per second (Mb/s). However, the fast Ethernet protocol, which runs at 100 Mb/s on standard, high quality (CAT5) Ethernet

cables, is gaining rapid acceptance, and hardware also exists for gigabit transfer rates, usually via optical fibres. Alternative approaches to the Ethernet exist for transfer via optical fibre (fibre distributed data interface (FDDI)) and for transfer (usually fast) via other media (e.g. asynchronous transfer mode (ATM) for use with microwaves). However, these are beyond the scope of this overview.

Hardware linking computers involves a network interface card (NIC) on each connected device and various 'boxes' that interconnect cables or control the flow of traffic, limiting connection to specific Internet addresses. Lack of this traffic control would result in all computers worldwide receiving communication from all others, clearly an impossible situation. Examples of network devices are hubs that simply connect cables without any attempt to alter traffic flow, switches that permit interconnection between cables with transfer rate maintained and routers that simply direct or filter traffic. The simplest network in nuclear medicine involves direct connection between two machines, with appropriate software handling the network communication (usually with one machine acting as a server that effectively takes control of the network). Where more devices and interconnection to larger networks are involved, additional devices are necessary.

Another important networking standard which, like the Ethernet, has been implemented on all commonly used computer platforms is Transmission Control Protocol/Internet Protocol (TCP/IP). Although other protocols exist (e.g. Internetwork Packet Exchange (IPX) and Netbios Extended User Interface (NetBEUI)), TCP/IP is by far the most widely used and forms the basis for worldwide communication via the Internet. The term Internet dates back to the earliest days of TCP/IP in the early 1980s, when it was used to describe any network built using IP. Since that time, the power and flexibility of TCP/IP have resulted in the creation and explosive growth of the Internet, a worldwide network of networks linked by TCP/IP.

TCP/IP is a set of protocols designed to facilitate the interconnection of dissimilar computer systems, and it makes no assumptions about the nature of the connection between the computers. TCP/IP provides computer users with a number of useful services, and new services are being added regularly. Until the early 1990s, most Internet usage involved three TCP/IP protocols — Simple Mail Transfer Protocol (SMTP), Terminal Emulation Program for TCP/IP Networks (TELNET) and File Transfer Protocol (FTP). SMTP handles electronic mail delivery. With TELNET, an interactive session can be established with another computer connected to the Internet. FTP facilitates file transfer between computers. The network file system (NFS) is another useful TCP/IP protocol, which allows computers connected to the same LAN to share files.

The advent of the TCP/IP protocol and, later in the early 1990s, the HyperText Transfer Protocol (HTTP), brought about the creation of the World Wide Web. The World Wide Web is made up of millions of documents containing many sources of information (including text, graphics, sound and video) that are stored on computers called web servers. A web browser program such as Netscape is needed to fetch documents from a web server and display them with links appearing as highlighted text (hypertext). Clicking on a link fetches and displays the hypertext document addressed by the link. Web browsers provide users with a much friendlier, more intuitive interface to Internet resources than do FTP or TELNET.

4.6.3. Connecting imaging equipment via computer networks

Section 4.6.2 refers to general networking details that are common to all networks and that are not specific to nuclear medicine. In medical imaging, the transfer of image data between computers is common, either within nuclear medicine or between different imaging modalities. The networking of computers in this environment, with the possibility of circulating images for reporting purposes and review and central archiving, is commonly referred to as a Picture Archiving and Communication System (PACS). If fully implemented, this will link closely with both a Radiology Information System (RIS) and a Hospital Information System (HIS), both of which handle the administrative aspects of diagnostic imaging. Interconnection of remotely sited imaging modalities via the Internet (rather than a LAN) is usually referred to as teleradiology or telenuclear medicine. In this case the objective is usually to transfer images for remote reporting.

It should be recognized that medical images usually occupy fairly large files and therefore the speed of transfer remains a major concern, even with modern technology, particularly if there are many file transfers occurring. In a PACS system, direct high speed cabling can be arranged between critical components, usually dedicated to this purpose. In contrast, teleradiology is dependent on the slowest link between centres, which may be a modem connected to a telephone. Table 4.4 illustrates some typical figures for files encountered in diagnostic imaging and the time required for transfer via different media; clearly, nuclear medicine poses no problem compared with some other modalities.

In order to optimize speed for medical image transfer, images can be compressed by methods that may lose some information (lossy or irreversible methods) or preferably by lossless or reversible methods. Reversible methods are capable of retrieving exactly the same image data as originally compressed and are essential for most diagnostic reporting, whereas irreversible methods

TABLE 4.4. SOME TYPICAL FIGURES OF DIFFERENT DIAGNOSTIC IMAGING FILES AND RATES OF TRANSFER VIA DIFFERENT MEDIA

Study	Matrix size	Number of images	File size (Mb)	Time via 100 Mb/s	Time via 32 kb/s
Mammography	4096 × 5120 × 12	4	125	10 s	31250 s = 8.7 h
Functional MRI	256 × 256 × 12	300	30	2.4 s	7500 s = 2.1 h
CT	512 × 512 × 12	30	12	1 s	3000 s = 50 min
Nuclear medicine	128 × 128 × 8	24	0.4	0.03 s	25 s

may still be acceptable in some non-diagnostic situations. The most popular currently used approach was developed by the Joint Photographic Experts Group (JPEG), with lossless or lossy compression achieved depending on the degree of compression used. A popular alternative involves wavelet compression. For lossless compression, reduction in file length, savings in storage space or improvement in transmission speed by a factor of 2–3 is possible; for lossy compression the factor can be much higher (e.g. 10–20), depending on the modality.

Even with established networks that permit image transfer between systems, there remains a further obstacle to establishing a workable system. Most suppliers of medical imaging equipment have developed their own database structure for images, with a proprietary format for the structure of image files. After transferring a file to a second system, translation between file formats is necessary and is not a trivial step. Certain file formats have become useful as intermediaries between individual manufacturer's equipment. Examples are Interfile, which was developed specifically for nuclear medicine and includes a readable header that is easily edited, or the file structure developed initially by the American College of Radiologists and NEMA (ACR–NEMA). Fortunately, more sophisticated standards have been established that define not only the file format but also the method to be used to establish communication between systems. The result is DICOM (Digital Imaging Communication in Medicine) which extends to ACR–NEMA but adheres to the more general networking standards summarized earlier in this section. Even with this standard available to link computers there are frequently incompatibilities in different manufacturers' implementations of DICOM conversion, resulting in missing administrative data or, in the worst case, inability to transfer data. Most suppliers are actively improving their DICOM software as they now recognize the importance of connectivity. Useful tools for checking DICOM conformance are readily available.

For useful information on issues related to medical image transfer, the reader is referred to the web site maintained by D. Clunie (http://www.dclunie.com/medical-image-faq/html).

4.7. GLOSSARY OF TECHNICAL TERMS

analog-to-digital converter (ADC). These devices convert continuous electrical voltages to discrete integer numbers in a defined range. When a digital image is acquired from an analog gamma camera an ADC converts the electrical signal that represents the x and y positions for a detected photon to a matrix location in the ranges of, for example, 1–64 or 1–128.

annihilation photons. When a positron is emitted it travels a short distance in tissue, losing energy. It eventually combines with an electron and the two annihilate (disappear), with the mass being converted into energy in the form of two gamma rays (511 keV) that travel in opposite directions.

asymmetric energy window. Normally, the energy window is centred on the main peak(s) of the radionuclide being imaged. To reduce scatter, an off-centre energy window, shifted up on the peak, is sometimes used. This is referred to as an asymmetric window.

back-projection. This is the process used in reconstruction, which allocates counts in the reconstructed image at each voxel proportional to the number of recorded counts on the projection, defined by the geometry of detection. In the simplest case assuming a parallel hole collimator, each voxel will be allocated counts from a projection pixel, defined by a line drawn at right angles to the projection that passes through the voxel.

bar phantom. These are phantoms consisting of lead bars of varying widths and separations set in Perspex or another plastic material. The bars can be arranged either parallel to each other across the entire phantom, or more usually into four quadrants of parallel bars. Bar separation and width is then different for each quadrant. Bar phantoms are primarily used for measuring resolution.

bismuth germanate oxide. This is a detector material commonly used in PET cameras. It has a higher density than NaI and is therefore well suited to detection of the high energy (511 keV) annihilation photons.

centre of rotation. This defines the point that should correspond to the exact centre around which the detectors rotate; it should correspond exactly to the centre of the projections recorded at all angles. Any error in this point will lead to loss of resolution.

coincidence detection. In order to detect the two gamma rays emitted from a positron annihilation event, two detectors are used and a valid event is recorded when both detectors record an interaction at the same time (or within a very short time of each other). The detectors operate in electronic coincidence. This term is used with detectors in dedicated PET systems as well as in gamma camera based PET systems.

collimator angulation. A parallel collimator should normally be constructed so that the holes and septa are exactly at right angles to the detector. Any difference in this angle is referred to as a collimator angulation error. Any such error will lead to some artefacts due to acquired data not being correctly treated during reconstruction.

converging collimator. These collimators are similar to parallel hole collimators, except that the holes are angled to converge to a focal point at some distance in front of the collimator. These collimators can be used to obtain magnified images of small organs.

convolution. Convolution is the filtering procedure undertaken in the spatial domain. It involves replacing each pixel value by a weighted sum of the neighbouring values and the value itself. The result will depend on the weighting values, usually resulting in a smoother image (e.g. nine point smooth).

cut-off/critical frequency. The shape of a filter is defined by some mathematical function, with the value 1 at zero frequency and lower values at progressively higher frequencies. The cut-off or critical frequency is a parameter that defines the shape of the function, a lower cut-off frequency defining a curve that drops to zero faster, resulting in a smoother result. In the case of the Butterworth filter the cut-off frequency defines the point when the amplitude reaches half the maximum value.

DICOM. This is a relatively new standard that defines both the image format and the communication protocol to permit transfer of image data

between medical imaging modalities. On the basis of earlier approaches developed by ACR–NEMA, the method is now widely available.

diverging collimator. These are similar to parallel hole collimators, except that the holes diverge outwards. They can be used to fit a large organ onto a small FOV gamma camera, i.e. they can be used to reduce image size.

electronic collimation. Since annihilation photons travel in opposite directions, the origin of the annihilation can be defined by the straight line joining the points of detection of the two photons without the need for conventional collimation.

energy spectrum. A plot of the number of gamma photons detected as a function of the energy of the gamma rays. Such spectra are useful for setting energy windows with the pulse height analyser and for observing the amount of scatter present.

energy window. Setting a lower and upper energy threshold, the energy window determines which gamma ray energies are accepted and displayed.

Ethernet. This is a standard that defines the type of cable and interface, as well as the protocol, for data transfer between computers.

extrinsic. This usually refers to measurements performed with a collimator attached to the detector.

fanbeam collimator. Most collimators are parallel as the holes are straight and parallel to each other. However, the fanbeam collimator holes are focused in one direction only and are parallel in the other direction (fan shaped). As a result the collimator focuses on a line at the focal distance. This collimator results in magnification in one direction (normally in the plane corresponding to projections) with improved sensitivity and improved resolution compared with a parallel hole collimator.

filter kernel. The filter kernel defines the relative weights to be applied to neighbouring pixels during a convolution process; the result is normally divided by the sum of the weights. As an example in a nine point smooth fit the central kernel value is 4, the nearest neighbours 2 and the corners or next nearest neighbours 1; the result is divided by 16.

filter order. This is a further parameter used to define the shape of a filter function. It is specific to the Butterworth filter and defines the slope of the filter function (i.e. the rate at which the curve drops to zero).

FOV. This abbreviation stands for field of view.

full width at half maximum (FWHM). This term refers to resolution measurements (e.g. spatial and energy resolutions). FWHM is usually measured from a profile through an image of a line or point source, or, in the case of energy, from the energy spectrum of a single gamma emitting radionuclide. The spread is due to resolution effects and is measured by the full width of the profile at a point which is half the maximum height of the profile.

gated SPECT. An ECG can be used to synchronize SPECT data acquisition on each projection with the patient's heartbeat in the same way as in conventional planar gated blood pool studies. Each time interval can be reconstructed to produce a set of 3-D images throughout the cardiac cycle.

Interfile. This has become a widely used standard file format for exchange of image data in nuclear medicine. The method was developed by the ANZ Society of Nuclear Medicine's Technical Standards Committee on the basis of an early AAPM recommendation before being adopted by the EEC's COST B2 project.

Internet. Historically this referred to any network that utilized the Internet Protocol (i.e. TCP/IP), a protocol that is now widely used. It tends now to be used to refer to the general worldwide network that interconnects the many thousands of networks that exist.

intrinsic. Refers to measurements performed without a collimator.

iterative reconstruction. This general term applies to a number of reconstruction algorithms that involve a repetitive process of comparison to find the best estimate of the activity distribution that matches the measured projections.

JPEG (Joint Photographic Experts Group). A commonly used method for image compression was developed by JPEG, and so the term JPEG now refers to an image compressed by this method. Compression can be lossy

or lossless depending on the degree of compression applied. It is one of the most commonly used compression methods, particularly for transmission via the Internet.

line source. A thin line (such as a capillary tube) filled with activity, which is used for measuring resolution. The diameter of the line source should typically be 1 mm.

local area network (LAN). A set of computers located in a department or institution that are connected, usually via Ethernet cabling, so that they can communicate with each other.

lutetium oxyorthosilicate (LSO). This is a new detector material currently being considered for PET systems.

multidetector SPECT. It is common for SPECT systems to have two or three separate detectors rather than a single gamma camera. These multidetector systems simply acquire more than one projection at a time; each detector being a standard gamma camera. A few special instruments have been designed that use many individual detectors which are more similar to the most common PET systems.

multiple coincidence. When more than two photons are detected at the same time it is not possible to identify which two events are related to a single positron emission. This event is called a multiple coincidence.

NEMA (National Electrical Manufacturers Association). NEMA develops standard specifications for imaging equipment including gamma cameras (SPECT) and PET. These form the basis for specification and acceptance testing of equipment, and some tests, with modification, can also be used for routine quality control.

Nyquist frequency. The Nyquist frequency F_N defines the maximum frequency that can be recorded for a given pixel size, $F_N = 1/(2 \times \text{pixel size})$. It is common to refer to frequencies relative to the Nyquist frequency as a form of unit, for example the cut-off frequency for a Butterworth filter can be defined as $0.5F_N$. Care should be taken as the Nyquist frequency will be different for images with different pixel sizes.

positron emission tomography (PET). Tomography based on detection of the dual annihilation photons that originate from positron emission. The

technique involves detection of the dual photons in coincidence (at the same time).

primary photons. These are gamma rays that have not undergone any scattering.

projections (count profiles). This term refers to the counts recorded during tomographic acquisition. The counts in a single row of the images recorded in SPECT at a given angle represent a projection of the emitted counts. These can also be referred to as count profiles. The set of projections, recorded at different angles, form the data that are used for tomographic reconstruction.

pulse height analyser (PHA). These instruments analyse the size of the energy signal and produce an output only if the size of the energy signal is within the range specified by the predefined energy windows.

random coincidence. When two gammas originating from quite independent sources (e.g. two separate positron emissions) are detected at the same time, the path defined by the points of detection does not correspond to a positron emission. This incorrectly located coincidence event is referred to as a random event.

reconstruction. This is the process of obtaining a cross-sectional image from a set of projections.

resolution. This refers to the ability of imaging systems to distinguish between two closely spaced small sources. Usually expressed in terms of FWHM, which describes the spread of the image obtained from a line or point source.

resolution recovery. This is the opposite of smoothing, and is achieved by filtering or deconvolution. By use of an appropriate filter the loss of resolution due to some measurable effect (e.g. due to a detector's finite resolution) can be partially recovered. However, any noise in the original image will normally be amplified.

ring artefacts. These are a common error in reconstructed images which are caused by a localized non-uniformity in the detector.

scatter coincidence. When one or both photons originating from a positron event are detected in coincidence, the path defined by the points of detection does not necessarily correspond to the point of positron emission. This event is referred to as a scatter coincidence.

scattered photon. A gamma ray which has changed direction at least once due to Compton interaction and loss of energy in the material through which it is travelling.

sensitivity. Fraction of the emitted gamma rays which pass through the collimator (collimator sensitivity) or are detected by the gamma camera (system sensitivity).

single event. In a PET system, when a photon is detected without a corresponding coincident photon, this is referred to as a single event. Owing to the probability of detection, there are many more single events detected than coincidences.

single photon emission computed tomography (SPECT). Cross-sectional slices of the radionuclide distribution in the patient are generated by taking images of the patient from various angles and then using these to construct the slices with a computer. SPECT refers to tomography based on the use of a single photon emitter.

sinogram. The image formed by placing projection values in sequential rows (i.e. arranging pixels corresponding to projection position versus projection angle) is called a sinogram. It is so called since the projections from a single point describe a perfect sine wave when plotted in this form.

slit phantom. A phantom consisting of a lead mask with thin slits cut into it. Typically the slits are 1 mm wide and 30 mm apart. They are used for measuring intrinsic FWHM resolution and also linearity.

smoothing. Smoothing is an operation that involves spreading values across neighbouring pixels; the averaging effect reduces statistical noise but degrades resolution. Smoothing is a filtering operation often achieved by convolution.

spatial frequency. Frequency normally refers to cyclic variations as a function of time (units: s^{-1}). However, if a curve represents variations in values over

distance (units: 1/distance), the number of oscillations per unit distance is referred to as a spatial (rather than temporal) frequency.

tomography. Literally this means 'drawing a body slice'. Tomography involves measurement from different angles around an object with the intention to 'reconstruct' an image of the internal distribution of some parameter (e.g. activity in SPECT or PET).

true coincidence. When two annihilation photons originating from a single positron annihilation are detected in coincidence (without being scattered), this is referred to as a true coincidence.

uniformity. A measure of how uniform the observed counts across the FOV are when the detector is irradiated by a uniform source. Integral uniformity is a measure of the maximum count deviation ((max – min)/(max + min)) over a given FOV. Differential uniformity is a measure of the maximum rate of change over a specified distance. Both shall be measured for the UFOV and the CFOV.

voxel. If one considers a digitized 3-D volume rather than a digitized 2-D image, each digital value within the volume can be considered to occupy a small volume element (e.g. a small cube) or voxel. One therefore refers to planar projections as having pixels, but to each reconstructed slice as having voxels, which also have a thickness corresponding to the spacing between adjacent slices.

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Chapter 5

GUIDELINES FOR GENERAL IMAGING

5.1. INTRODUCTION

5.1.1. Purpose

The purpose of this chapter is to provide nuclear medicine practitioners with general guidelines on imaging using a single photon scintillation camera. Recommendations specific to individual procedures are included in their respective procedure guidelines.

5.1.2. Planar image acquisition using a gamma camera

5.1.3. Peaking

The scintillation camera must be peaked correctly for the energy (energies) of the emitted photon for the radionuclide used. This should be checked at least once daily and when different radionuclides are used. A 15 or 20% energy window is typically used. The window is placed symmetrically about the photopeak, or asymmetrically if an appropriate energy correction is available, in order to minimize scattered radiation.

A physicist can help in determining the limits of asymmetry that are desirable for a range of energies. Caution must be taken not to affect uniformity.

5.1.3.1. Multiple energy windows

The use of multiple energy windows for radionuclides that have more than one energy peak is advantageous. It is necessary to check the spatial registration for the combination of windows.

A physicist can help determine if the co-registration is adequately adjusted for all of the windows in order to maintain the best spatial resolution and contrast. A collimator offering adequate resolution for the most energetic photons must be used. Intrinsic uniformity should be checked for imaging multiple energy windows for such radionuclides. Again, a physicist can help determine the need for special uniformity corrections.

5.1.3.2. Dual radionuclide studies

When using two radionuclides in a sequential study, images from the lower energy radionuclide should be obtained first.

In principle, it is possible to use multiple energy windows to image two radionuclides simultaneously. Such a technique involves many pitfalls however, and the results will depend on the equipment used and special quality control tests. The different energies will render different spatial resolutions. The procedure must account for the detection of scatter from the higher energy photons into the energy window used for lower energy photons (normally referred to as downscatter). This procedure should be designed carefully by an individual with the necessary expertise. If, on the other hand, the two radionuclides are imaged separately, it will be necessary to consider the effects of motion, especially if subsequent processing of the two images assumes co-registration.

5.1.3.3. Matrix size for planar imaging

The matrix size is primarily dependent on resolution and is independent of counting statistics. It is a requirement of sampling theory that the pixel size be less than the FWHM divided by 2 for planar imaging, otherwise resolution is lost and aliasing artefacts may occur. The matrix size is determined by the field size divided by the pixel size. Increasing the matrix size beyond this limit does not improve resolution. Magnification (zoom) can be applied for imaging of small areas. If desired, a larger matrix size can be used for display using interpolation.

5.1.3.4. Static imaging

The specific imaging parameters for a given static acquisition will vary in accordance with the above sampling considerations, depending on the desired clinical information. For computer acquired images, matrix size will depend on the specific requirements of each type of study. For example, whole body scans require large matrices to ensure that sampling is maintained over the large area scanned. Typical matrix sizes for spot images are 128×128 and 256×256. When large matrices are used for smaller areas, statistical fluctuation (noise) may be excessive unless reduced by smoothing: this will result in decreased spatial resolution. The digital appearance of smaller matrix sizes can be improved by interpolation to large matrices for display, although this will not improve resolution.

5.1.3.5. Whole body imaging

Scan time varies depending on the count rate and count density required. Matrix size must match the expected resolution for a given radionuclide. Because a whole body image covers about 200 cm, the matrix dimension along the length of the patient should be at least 512 pixels. Acquisition times greater than about 30 min are not practical for routine use in unsedated patients.

5.1.3.6. Dynamic imaging

The time per frame selected depends on the temporal resolution needed for the processing of the study and the organ function under investigation. Shorter times are preferred for quantitative functional studies, provided adequate statistics are obtained, in order to measure physiological changes. For purposes of qualitative imaging alone, somewhat longer times are generally used or multiple frames summed together in order to provide sufficient imaging statistics for each frame.

For computer acquired images, the matrix size chosen for dynamic studies may be smaller than that required for static imaging provided that the resultant loss of resolution is acceptable for image interpretation. It is worth noting that sometimes a choice has to be made between word and byte mode acquisitions. If there is any doubt, word mode should be used to avoid pixel saturation that may occur in byte mode. For high count rate studies, dead time effects may be important. Count rate loss should be ascertained by dead time measurements, about which a physicist can provide advice.

5.1.3.7. Gated imaging

ECG gating is used in functional cardiac studies to synchronize image acquisition with the patient's heart rate.

The number of frames per RR interval (the time duration between two consecutive R waves of an ECG) should be no less than 16 for planar cardiac blood pool ejection fraction measurements, 32 for time based measurements (filling rates, etc.) and 8 for gated myocardial SPECT studies (although 16 frames should preferably be used if accurate volumetric parameters are required).

For planar gated cardiac blood pool imaging, an electronic zoom should normally be used to magnify the FOV to approximately 25 cm during acquisition. A matrix size of 64 × 64 is sufficient provided that magnification is applied.

Typically, a total of at least 5 million counts in the entire study will provide sufficient statistics for quantitative and functional image processing.

5.1.3.8. Pinhole imaging

Pinhole imaging provides the spatial resolution that most closely approaches the intrinsic limit of the camera at the expense of sensitivity. The distance between the collimator and the patient determines both the degree of magnification and the sensitivity (or count rate). Smaller pinhole apertures (2–3 mm) provide better resolution but lower sensitivity. The largest pinhole in routine clinical use should be 5 mm. For bone scintigraphy, 4 mm is commonly used. For typical collimators with a 25 cm diameter FOV, a matrix size of 256 × 256 or 128 × 128 is generally sufficient.

5.1.4. SPECT image acquisition

In SPECT, a similar approach to that used for planar imaging is taken to determine appropriate matrix size, except that pixel size must be less than reconstructed FWHM divided by 2.5 (or 3). The acquisition matrix size will normally be 64 × 64 or 128 × 128 depending on the reconstructed resolution and field size. The manufacturer's processing protocols should be consulted for compatibility with specific data acquisitions.

The number of projections is likewise determined from similar sampling considerations. Consider a region, centred on the centre of rotation that includes the organ of interest. Then the arc at the edge of this region, defined by the detector position in two adjacent projections, should be approximately equal to the defined pixel size. In general, at least 60 (64) views are used for 360º acquisition or 30 (32) views for 180º acquisition. However, 120 (128) views should be used for high resolution studies such as those of the brain, irrespective of the matrix size used.

Statistics play an important role in the reconstruction process and typically can prolong imaging times. Total imaging time should not exceed 30 min, to minimize patient motion.

SPECT data can be acquired using step-and-shoot, continuous motion or a hybrid technique, depending on the camera design and the type of study to be performed. Continuous rotation will provide the most efficient image gathering capability, especially if 120 (128) views are acquired.

5.2. NUCLEAR CARDIOLOGY

5.2.1. Introduction

Nuclear cardiology is a superspecialty, in which nuclear physicians with training in cardiology, or cardiologists with nuclear medicine training, use nuclear imaging technology to investigate a variety of physiological and pathological aspects of the cardiovascular system. The major techniques used in nuclear cardiology can be categorized as: first pass angiocardiography, multigated blood pool imaging, myocardial perfusion imaging, and receptor and metabolic imaging. The data derived from these studies can be used for diagnosis, prognosis, treatment monitoring and assessment of viability in heart diseases, particularly in coronary artery disease.

5.2.2. First pass angiocardiography

5.2.2.1. Principle

First pass radionuclide angiocardiography (FPRNA) is a rapid, reliable and reproducible method of assessing cardiac function based on a few monitored heartbeats. It involves the imaging of an intravenously injected radionuclide bolus during its initial transit through the central circulation. A time–activity curve is generated, and the temporal separation of the right and left ventricular phases allows evaluation of individual ventricular function. This is based on the assumption that thorough mixing of the tracer has occurred in the blood pool and that the detected count rate reflects the changes in ventricular volume during contraction and relaxation.

Left and right ventricular function assessed at rest, or during stress with first pass imaging, gives a comprehensive evaluation of short duration changes that may affect the ventricles. This includes evaluation of global and regional wall motion, estimation of ejection fraction and other systolic and diastolic parameters. Such information has proved significant in the diagnosis, prognosis, decision making and management of certain clinical problems such as coronary artery disease and chronic obstructive lung disease, as well as congenital and valvular heart disease.

5.2.2.2. Clinical indications

(a) Coronary artery disease

Rest and exercise FPRNA has been used extensively in diagnosing and managing patients with known or suspected coronary artery disease, as well as with complications of acute myocardial infarction.

Wall motion abnormalities, changes in end systolic volume and changes in diastolic filling rate are suggestive of ischaemia and the presence of coronary artery disease. Evaluation of the left ventricular regional wall motion is, however, limited since the FPRNA is done one projection at a time. The anterolateral, apical and inferoseptal walls are visible in the anterior view while the right anterior oblique (RAO) view provides images of the anterior, apical and inferior walls. Regional wall motion in the RAO first pass study correlates well with contrast angiocardiography. Correlation is likewise good between the left ventricular ejection fraction (LVEF) calculated using FPRNA and the values obtained by contrast and equilibrium radionuclide ventriculography. For the measurement of right ventricular ejection fraction (RVEF), FPRNA is considered the method of choice. The technique allows images to be acquired in the RAO view, giving a maximal separation of the right atrium from the right ventricle. The tracer bolus might not, however, mix completely with the right atrial blood prior to entering the right ventricle and may exit without mixing completely with apical blood, giving rise to potential sources of errors.

(b) Congenital heart disease

Detection and quantification of left-to-right shunts are possible using FPRNA. The presence of a shunt is confirmed by simultaneous tracer appearance in the right and left ventricles. In a normal study, the left ventricle is free of any right ventricular activity. Quantitation of a left-to-right shunt is dependent on the quality of the bolus injected. A delayed or fragmented bolus may affect the shape of the pulmonary curve generated, which should be monoexponential, even in the absence of a shunt. Shunting separates the pulmonary activity curve into two components, which are proportional to the systemic and shunt flows, respectively, giving an index of the severity of the shunt.

(c) Valvular heart disease

Assessment of valvular insufficiency is possible with resting FPRNA. Studies showing prolonged tracer transit through the left side of the heart may

reflect mitral or aortic insufficiency. From the pulmonary and left ventricular time–activity curves, the degree of regurgitation may be calculated and quantified. Data from FPRNA have been shown to correlate with measurements obtained by catheterization. Resting studies performed serially can be helpful in monitoring the severity of the valvular insufficiency and in deciding when valve replacement is necessary.

5.2.2.3. Radiopharmaceuticals

The ideal radionuclide as a first pass imaging agent must remain intravascular as it moves through the central circulation. It should also be safe for application in large doses in order to generate the necessary high count rates.

(a) Technetium-99m agents

The high specific activity of ^{99m}Tc makes it suitable as a first pass agent. For multiple or sequential studies, ^{99m}Tc diethylene triamine pentaacetic acid (DTPA) is preferred to ^{99m}Tc-pertechnetate. DTPA has rapid renal excretion, making possible a repeat injection 20 min later. Technetium-99m pertechnetate can be used when a single assessment of ventricular function is needed. The usual dose for ^{99m}Tc agents is 925 MBq and a maximum of three injections, 370 MBq (10 mCi) each, can be given (Table 5.1).

Other technetium based compounds such as sestamibi and tetrofosmin are also suitable. First pass imaging can be performed upon injection of the tracer during peak exercise, thus combining information on regional and global ventricular function as well as myocardial perfusion in one setting.

(b) Short lived radioisotopes

The need for first pass studies to be performed repeatedly in a short period of time presents some restrictions with ^{99m}Tc agents. The half-life of 6 h and varying biological clearance times limit the number of acquisitions that can be done in a given period. In order to reduce the patient's radiation exposure and allow for a greater number of studies to be performed, radionuclides with half-lives in terms of seconds or minutes would be ideal.

Tantalum-178 produces suboptimal results when used with standard gamma cameras because of its low energy; more satisfactory results have been reported with a multiwire proportional gamma camera. The short half-life of ^{191m}Ir makes it suitable for paediatric patients. Gold-195m is ideal for adult patients, and the calculated ejection fraction correlates well with that obtained using ^{99m}Tc agents. Evaluation of myocardial perfusion could possibly be done

TABLE 5.1. FIRST PASS STANDARD Tc-99m DOSES

Type of study	Rest	Exercise
LV/RV[a] function – multicrystal camera	370–925 MBq (10–25 mCi) (0.3–0.5 mL)	925 MBq (25 mCi) (0.3–0.5 mL)
LV function – single crystal camera	925 MBq (25 mCi) (0.3–0.5 mL)	925 MBq (25 mCi) (0.3–0.5 mL)
RV function – single crystal camera	740–925 MBq (20–25 mCi) (0.3–0.5 mL)	740–925 MBq (20–25 mCi) (0.3–0.5 mL)
Shunt study	370–555 MBq (10–15 mCi) (0.3–0.5 mL)	

[a] LV, left ventricular; RV, right ventricular.

with ^{195m}Au in combination with ventricular function assessment. Table 5.2 summarizes the physical characteristics of the above mentioned short lived radioisotopes.

5.2.2.4. Equipment

Monitoring of the radionuclide bolus during its transit through the central circulation requires a gamma camera with a high count rate capability, a high sensitivity collimator, a computer software capable of processing high temporal resolution data and a small matrix acquisition, as well as an ECG gating device. A bicycle ergometer is an additional requirement for first pass studies during exercise.

More than 200 000 counts/s are needed to achieve an adequate image quality. The use of cameras with low count rate capabilities leads to an inaccurate measurement of ejection fraction and assessment of wall motion. Originally, only multicrystal gamma cameras could record such high counts, although with some loss of spatial resolution. Newer generations of multicrystal cameras can now acquire the same range of counts with enhanced energy and spatial resolutions. Modern single crystal cameras are also capable of achieving rates of up to 200 000 counts/s, as opposed to older cameras with rates of only up to 60 000 counts/s.

The choice of collimator depends on the objective of the study and the dose to be injected. Computer software should allow acquisitions to be performed with 64×64 or smaller matrices.

An ECG signal is unnecessary for high count rate studies, since there are enough counts to distinguish between end-diastolic and end-systolic frames. It

TABLE 5.2. CHARACTERISTICS OF SHORT LIVED RADIOISOTOPES

Radioisotope	Half-life	Photon energy (keV)
Ta-178	9.3 min	55–65
Ir-191m	4.9 s	129
Au-195m	30.5 s	262

may be more helpful with single crystal camera acquisitions to facilitate data processing of low count rate studies.

5.2.2.5. Procedure

(a) Tracer injection

First pass studies require the injection of a small volume of radionuclide bolus. The quality of the study largely depends on the integrity of the bolus. Large proximal veins must be used as injection sites, since smaller, peripheral veins may cause bolus fragmentation. The injection parameters appropriate to the various kinds of study are listed in Table 5.3.

For left ventricular evaluation or shunt studies, it is important that the bolus arrive in the heart as a single front. Rapid injection of the radionuclide and a 10–20 mL saline flush (within 2–3 s) is necessary. In right ventricular studies, since the bolus reaches the right ventricle without significant dispersion, an antecubital vein is preferred since the use of the external jugular vein may result in too rapid transit of the bolus through the chamber. A slower bolus is preferred to increase the number of beats available for analysis; the saline flush may be then infused without interruption for 3–4 min.

(b) Radionuclide dose

The appropriate dose depends upon the sensitivity of the gamma camera, the number of times the study will be performed and the specific radionuclide used. Recommended doses for ^{99m}Tc agents are listed in Table 5.1. Short lived radionuclides have been given at doses as high as 50 mCi. The acquisition parameters for FPRNA are given in Table 5.4.

TABLE 5.3. INJECTION PARAMETERS

	LV[a] function studies	RV[b] function studies	Shunt studies
Site	Medial antecubital or external jugular vein	Antecubital vein	Antecubital or external jugular vein
Cannula	18–20 gauge	18–20 gauge	18–20 gauge
Rate	Rapid (FWHM < 1 s)	Slow (FWHM 2–3 s)	Rapid (FWHM < 1 s)

[a] LV, left ventricular.
[b] RV, right ventricular.

(c) Imaging angles

Proper positioning of the patient must be verified prior to the start of the study. A radioactive source or dose syringe may be used to check areas of interest in the FOV and allow identification of the lungs.

Temporal separation of the cardiac chambers allows the study to be acquired in any view, although the RAO and anterior projections are most commonly used. The shallow RAO view — best for direct comparison with contrast angiography — separates the atria from the ventricles and the left ventricle from the descending aorta. The 30° RAO view enhances the separation of the right chambers, making it the ideal view for right ventricular assessment.

The upright straight anterior view is best for exercise studies since the chest is stabilized against the detector. The pulmonary background is also reduced, enhancing study quality. The descending aorta and the basal portion of the inferoseptal wall may, however, overlap with the left atrium and basal portion of the left ventricle. The left anterior oblique view is useful when the circumflex artery territory is in question, but may result in underestimation of the LVEF.

(d) Frame rates

A standard frame time of 25 ms/frame can be applied for RV and LV function studies regardless of the type of camera used, but theoretically the frame time should be adjusted according to the heart rate. Fifty ms/frame is adequate at heart rates lower than 80 beats per minute decreasing to 10–20 ms/frame for faster heart rates, especially if diastolic function is of interest. Two thousand frames are sufficient to encompass the entire left ventricular phase.

TABLE 5.4. ACQUISITION PARAMETERS FOR FPRNA

Parameter	LV[a] function	RV[b] function	Shunt study
Position	Upright	Upright	Upright
Angle	Anterior	20–30° RAO	Anterior
Frame time (ms)	25	25	50
Total frames	1500–2000	1500–2000	2000

[a] LV, left ventricular.
[b] RV, right ventricular.

Frame rates are not as essential in a shunt study since data analysis uses curves of lower temporal resolution.

(e) Stress protocol

Bicycle ergometry is the method of choice for exercise studies. Although supine bicycle exercise results have been shown to correlate with catheterization, upright bicycles are more often used since they minimize chest motion and are better tolerated by patients. Any graded exercise protocol is acceptable and no time is required to stabilize the heart rate. Exercise should be continued until the bolus clears from the left ventricle.

(f) Data processing

Processing of a first pass radionuclide study can be divided into four major steps:

(1) Generation of an initial time–activity curve over the cardiac region;
(2) Beat selection;
(3) Background subtraction;
(4) Creation of the final representative cardiac cycle.

The initial time–activity curve is important for quality control. It permits inspection of the separation of the right and left ventricular phases, allows the estimation of the peak count achieved, and detects the presence of irregular beats. An ROI is drawn around the ventricle, grouping frames from the raw data. This is used to generate a time–activity curve from which an initial representative cycle is created and used to draw separated end-diastolic and end-systolic ROIs.

Once the left ventricular ROI has been identified, cardiac cycles to be included in the final analysis may be selected from the ventricular time–activity curve. The cycles before and after the beat with the maximum number of counts are selected. Premature ventricular beats and post-extrasystolic beats should be excluded. Beats whose end-diastolic counts are below 50% of the maximum end-diastolic count should also be omitted if they do not preclude a statistically adequate representative cycle. Only beats around the peak of the time–activity curve (80% or more of maximum activity) are to be used. This leaves one or two beats during the right ventricular phase and four to five beats during the left ventricular phase available for analysis. Averaging of several individual beats can also be done to form a summed representative cycle.

Background subtraction can be performed using several methods. The most accurate appears to be the lung frame method. Image counts in the ROI just prior to the appearance of tracer activity in the left ventricle are chosen as background counts and used to correct the left ventricular phase. Background correction is crucial for the LVEF, and any variations in background counts can lead to changes in the calculated ejection fraction, volumes and wall motion.

Once the background has been corrected, initial ROIs are adjusted and the final ROIs are used to regenerate the time–activity curve from which the final representative cycle is created from the previously selected beats. This cycle may be displayed in a cine-loop for analysis of regional wall motion. All quantitative data are also derived from this cycle.

The LVEF and RVEF are calculated from the end-diastolic (ED) and end-systolic (ES) counts as follows: (ED counts – ES counts)/ED counts. The systolic emptying rates and diastolic filling rates are calculated with appropriate software using a Fourier filter applied to the representative cycle and taking the first derivative of the filtered curve. Left ventricular end-diastolic volume may be measured using the geometric or count proportional method. The geometric method measures the area of the left ventricle and the length of the major axis in pixels. Converted to centimetres, the pixels are used to calculate the volume. In the count proportional method, volume is derived from the total counts and the counts in the hottest pixel in the left ventricle. This method requires validation for each laboratory.

5.2.2.6. Interpretation

The radionuclide bolus appears sequentially in the superior vena cava, right atrium, right ventricle, pulmonary circulation, left side of the heart and aorta. Any changes in this pattern would suggest the presence of a congenital abnormality. Delayed tracer transit through the right side of the heart suggests

pulmonary hypertension, tricuspid or pulmonary valve insufficiency or a left-to-right shunt. Delayed tracer transit on the left side of the heart would suggest mitral or aortic insufficiency.

Regional wall motion is analysed by superimposing the end-diastolic outline against the end-systolic image or by viewing the representative cycle in cine-mode. However, it has to be noted that since the study was acquired in only one projection, regional wall motion abnormalities may be difficult to identify in overlapping segments. Ischaemic responses applicable to the diagnosis of coronary artery disease are typically a new onset of a regional wall motion abnormality or a worsening of a previous one, an increase in the end-systolic volume and alterations in diastolic filling parameters.

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5.2.3. Equilibrium radionuclide angiography

5.2.3.1. Principle

Equilibrium radionuclide angiography (ERNA) is a non-invasive means of quantitatively assessing cardiac function. It has been demonstrated to be a

highly accurate and reproducible technique, capable of assessing left and right ventricular function even if infarction, hypertrophy or dilation has distorted the shape of the ventricle. Assessment of right ventricular function, however, may not be as accurate as with the first pass radionuclide angiography method.

This imaging modality makes use of an intravenously injected radionuclide that remains in the cardiac chambers in a concentration directly proportional to the blood volume. Data are collected from several hundred cardiac cycles to create an image of the beating heart, presented as a single cardiac cycle. It can be used to assess global and regional wall motion, chamber size and morphology, and ventricular function including ejection fraction. Acquisitions are made at rest or during exercise, or under pharmacological, isometric mechanical, cold-pressor or mental stress. The procedure is also known as gated cardiac blood pool imaging, multigated acquisition (MUGA) or radionuclide ventriculography (RVG). ERNA studies are superior in counting statistics to FPRNA studies, but are affected by arrhythmia.

5.2.3.2. Clinical indications

(a) Coronary artery disease

ERNA is an excellent test for the assessment of regional and global ventricular dysfunction by detecting changes in regional wall motion, end-systolic volume, ejection fraction and diastolic filling, making it useful in the diagnosis of coronary artery disease and myocardial infarction. It is also used to monitor therapeutic response and for long term follow-up.

(b) Congestive heart failure

Patients may undergo ERNA to distinguish ischaemic from non-ischaemic, as well as systolic from diastolic, causes of congestive heart failure.

(c) Valvular heart disease

Changes in ventricular stroke volumes can be used to detect and quantify valvular regurgitation. Periodic monitoring of cardiac function helps in the determination of the optimal timing for valvular surgery.

(d) Doxorubicin cardiotoxicity

Serial studies evaluate the cardiac function of patients receiving chemotherapy to determine if therapy should be discontinued or possibly reinstituted.

(e) Other indications

ERNA may be helpful in cardiomyopathy, asymmetrical septal hypertrophy and chronic obstructive pulmonary disease (COPD).

(f) Contraindications

The following conditions are contraindications for ERNA:

— Severe arrhythmia;
— Uncontrolled unstable angina;
— Decompensated congestive heart failure;
— Uncontrolled hypertension (blood pressure more than 200/120 mm Hg);
— Acute myocardial infarction of less than two days evolution.

Stress testing should be avoided in cases of particular contraindications for exercise, pharmacological procedures or other forms of cardiac challenge.

5.2.3.3. Radiopharmaceuticals

Technetium-99m is the only radionuclide that has been used for ERNA studies. Historically, ^{99m}Tc human serum albumin was the agent of choice for ERNA, but image quality was poor due to albumin trapping in the pulmonary arterial tree. This was then replaced by ^{99m}Tc labelled red blood cells (RBCs), which have a more favourable target-to-background ratio than albumin. Activities are given in Table 5.5.

Labelling of RBCs requires reduction of technetium by stannous ions. The reduced form binds to the globin chain of the haemoglobin molecule. The optimal dose of stannous ions will maximize the amount of technetium bound inside the cell and limit the proportion of circulating free pertechnetate that would be taken up by the thyroid, kidneys and gastric mucosa. Three labelling procedures can be used (Table 5.6): in vivo, in vitro and modified in vivo

TABLE 5.5. RECOMMENDED ACTIVITIES FOR Tc-99m AGENTS

	Adults	Paediatrics
Tc-99m labelled RBCs	555–1100 MBq (15–30 mCi)	8–16 MBq (0.2–0.4 mCi)/kg
Tc-99m albumin	370–740 MBq (10–20 mCi)	4–12 MBq (0.1–0.3 mCi)/kg

TABLE 5.6. COMPARISON OF RBC LABELLING PROCEDURES

Procedure	Labelling efficiency (%)	Advantages	Disadvantages
In vivo	75–85	Easy to perform Low exposure of personnel	Low labelling efficiency
In vitro	>95	Highest labelling efficiency	More complex Time consuming
In vivtro	90–93	Better labelling than in vivo	Lower labelling efficiency than in vitro

('in vivtro'). For in vivo labelling, the stannous ions, usually provided as a pyrophosphate bone kit, are injected first, followed 20 min later by the ^{99m}Tc pertechnetate dose.

Interference with the RBC labelling may be seen in patients receiving heparin, which oxidizes stannous ions and reduces the labelling efficiency. With such patients, human serum albumin may be used instead of RBCs. Dextrose, mannitol and sorbitol, or the presence of antibodies to the RBCs, as seen in certain autoimmune diseases or after receiving methyldopa or quinidine, may also reduce tagging efficiency.

5.2.3.4. Equipment

(a) Cameras

Both large and small FOV cameras may be used for the procedure. The more frequently used large FOV camera provides diagnostic quality images. Small FOV cameras provide higher resolution images and are easily manipulated into the required position. With either type of camera, the detector must be positioned as close as possible to the patient's chest during acquisition. Multicrystal cameras are not recommended due to their lower spatial resolution. An ECG gating device should be interfaced with the camera.

(b) Collimator

A standard parallel hole, low energy, all-purpose collimator is sufficient for most ERNA studies. High resolution collimators improve image quality but require longer imaging times.

(c) Computer systems

Current nuclear imaging computers are capable of acquiring ERNA data. The software should be capable of handling 64×64 and 128×128 acquisitions at rates of 8–32 frames per cycle in frame and list mode, contain temporal, spatial and Fourier filters, and allow for manual, automatic and semi-automatic approaches.

5.2.3.5. Patient preparation

Resting ERNA studies require no special preparation. For exercise studies, 3–4 h fasting prior to the procedure is recommended, and the patient should be haemodynamically and clinically stable. Pharmacological stress is recommended for patients unable to exercise. Cardiac medication, particularly that affecting heart rate, should be withheld unless contraindicated by the patient's medical condition or if there is interest in testing the efficacy of the drug.

5.2.3.6. Procedure

(a) Positioning

The patient should lie down comfortably to prevent movement during the procedure. Three standard projections — left anterior oblique (LAO), anterior and left lateral views — are acquired for 10–15 min each. The best septal view is taken with the detector in a 40–50° LAO position. Some argue that this is the only necessary view for ERNA studies since most referring physicians request a study primarily to obtain an accurate LVEF value. The other views (details of which are given in Table 5.7) should be obtained depending on the cardiac structures being studied.

(b) Acquisition parameters

An adequate study contains 250 000–500 000 counts per frame, acquired in approximately 5 min from 300–400 heartbeats. The data collected over the

TABLE 5.7. IMAGING PROJECTIONS FOR ERNA (MUGA) STUDIES

View	Structures seen
Left anterior oblique	Right and left ventricles, clearly separated Left circumflex artery territory Left atrium
Anterior	Right atrium Right ventricle (inferior and apical aspects) Left ventricle (anterolateral wall and apex) Pulmonary artery Ascending aorta
Left lateral	Long axis of the left ventricle Posterobasal segment of the left ventricle Right ventricular outflow tract Pulmonary artery Left atrium Descending aorta
Right anterior oblique	Right ventricle Superior vena cava

multiple cycles are synchronized or 'gated' with the patient's R wave on an ECG. This circumvents the problem of images becoming blurred by cardiac motion. The standard method for gating is the forward gating technique, where the ECG signal is used to identify the beginning of the acquisition. Another method is reverse gating, where the last frame ends on the R wave instead of the first frame being assigned to the R wave. Early systolic data are more accurate with forward gating, while end-diastolic data are preserved with reverse gating.

All commercially available systems exclude premature beats from the data by setting an RR interval acceptance window around the average, typically 10–15%, depending on the patient's rhythm. A narrow window means more homogeneous beats, making the study more accurate but with a prolonged acquisition time if some arrhythmia is present. Increasing the window will reduce the acquisition time at the expense of the diastolic portion of the time–activity curve.

Frame mode is the typical acquisition method but list mode is the more memory demanding one. List mode is particularly appropriate for studies of diastolic function and is more flexible in adjusting the beat length window,

which can be manipulated until the correct combination of width and acceptable number of beats is reached.

The number of frames depends on the clinical problem, software capabilities and acquisition time available. A higher number of frames improves the temporal resolution, making the image more representative of the variations in chamber volume. Sixteen frames per cycle are enough to assess the systolic phase, while 32–48 frames per cycle are ideal in studying the diastolic phase but longer acquisition times are required to achieve good frame statistics.

(c) Stress protocols

Treadmill exercise is inappropriate for ERNA because of chest motion. Bicycle exercise is preferred and can be performed in both the upright and supine positions: both place similar overall stress on the heart at any given workload. Exercise in the supine position, however, places more strain on the legs and may cause patients, particularly the older or those out of condition, to stop exercising before an adequate cardiovascular stress is reached. Exercise in the upright position is usually better tolerated.

A resting ERNA is usually performed first and in the same position that will be used in the exercise study so that any changes reflect true cardiac conditions rather than positional changes. Sufficient time should be allowed at each workload for the heart rate to stabilize and for enough image statistics to be acquired for reliable quantification. The period of peak exercise should be of sufficient length for superior image quality. However, prolonging the exercise by reducing the workload may lead to an immediate improvement of the ventricular function and to an underestimation of an eventual ischaemic reponse. An optional post-exercise image may be valuable in predicting functional recovery after revascularization in segments with severe wall motion abnormalities at rest.

Alternatives for patients unable to exercise include atrial pacing, cold pressor testing, catecholamine infusion and coronary vasodilators such as dipyridamole or adenosine.

(d) Data processing

Processing begins with a review on a cinematic display to evaluate the adequacy of the counting statistics, ECG gating, RBC labelling and positioning of the heart. Visual assessment of left ventricular systolic function is done before calculation of LVEF. The whole activity from the left ventricle must be encompassed by the ROI, drawn either manually or automatically. Discrepancies between the calculated LVEF and the visual assessment of left

ventricular systolic function may reflect an error in data processing or edge positioning.

It is recommended that the entire cycle be reviewed to obtain optimal information. Fourier transform analysis of the data and the first and third harmonics are used to filter the images and curve, to obtain functional parametric images such as those of phase or amplitude, or fit ventricular volume curves in order to determine systolic and diastolic function.

The peak left ventricular filling rate is often a useful parameter to detect early dysfunction. The various processing parameters are listed in Table 5.8.

5.2.3.7. Interpretation

Step-by-step interpretation starts with the assessment of image quality, particularly the target-to-background ratio, ECG gating and views obtained. Next, the morphology, orientation and sizes of the cardiac chambers and great vessels are evaluated and reported. Global left ventricular function is assessed qualitatively, followed by a segmental analysis of regional function using a cinematic display. Contraction abnormalities are reported as hypokinesia, akinesia and dyskinesia. Resting and stress images are displayed side by side to assess changes in chamber size, wall motion and ejection fraction. Quantitative measurements of ventricular systolic and diastolic functions are made. Findings of ERNA studies have been shown to be reliable and reproducible, with an important influence on patient management.

For patients with coronary artery disease, wall motion abnormalities can develop on exercise, with a fall in ejection fraction. With myocardial infarction, there are regional wall motion abnormalities or ventricular dilatation and a reduced LVEF. Distortion of the left ventricular contour and paradoxical wall motion, usually in the anterior or anteroapical myocardium, are characteristic findings of ventricular aneurysm. In patients under chemotherapy, a decrease in LVEF by 10 units or more indicates cardiotoxicity. In cardiomyopathies, there are diffuse wall motion abnormalities and a dilated left ventricle with decreased LVEF. When left ventricular hypertrophy is present, a photopenic area surrounding the left ventricle may be seen on the LAO view. If asymmetrical septal hypertrophy exists, there is usually a small left ventricle with a thickened septum and increased LVEF.

TABLE 5.8. PROCESSING PARAMETERS

Parameter	Method
LV[a] volume curve generation	Obtained from a single ROI drawn at the ED[b] or using multiple ROIs at each time point.
Background	ROI 5–10 mm away from the ED border, with atrial, splenic, descending aortic and LV counts excluded, time–activity curve from background ROI should be flat.
LVEF	Calculated from a Fourier filtered curve, or apply an ED ROI to the ED image, an ES[c] ROI to the ES image, or computed from LV counts from a single ED ROI.
Wall motion	Visual assessment of cinematic display or analysis of phase and amplitude images.
LV filling	Measurement of the peak slope of the diastolic portion of the LV curve.
LV emptying	Measurement of the slope of a line connecting the ED and ES time points.
LV volumes	Calculated using count based or geometrical methods.

[a] LV, left ventricle.
[b] ED, end diastole.
[c] ES, end systole.

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5.2.4. Myocardial perfusion single photon emission tomography

5.2.4.1. Principle

Myocardial perfusion scintigraphy uses perfusion radiotracers that are distributed in the myocardium (primarily the left ventricle) in proportion to coronary blood flow. Areas of normal flow exhibit a relatively high level of tracer uptake, while ischaemic regions present a relatively low uptake. Regional coronary blood flow may be compared in conditions of rest, stress or pharmacologically induced vasodilation. Thus, the coronary flow reserve can be assessed, which is usually affected by significant coronary artery disease (CAD).

Myocardial perfusion tracers are not taken up by an infarcted myocardium. In addition to evaluating relative regional blood flow these tracers are, therefore, also markers of myocardial viability. Myocardial perfusion scintigraphy may be performed using either single photon or positron emitting radionuclides. Among the commonly used single photon emitting perfusion tracers are ^{201}Tl and the various ^{99m}Tc labelled perfusion tracers (e.g. sestamibi and tetrofosmin). While having different physical and pharmacokinetic properties, these tracers have considerably overlapping clinical uses and will therefore be considered in parallel in this section.

5.2.4.2. Clinical indications

The clinical indications for myocardial perfusion tomography are summarized in Table 5.9.

(a) Detection of myocardial ischaemia and myocardium at risk

Myocardial perfusion imaging is a sensitive means to determine the presence, location and extent of myocardial ischaemia. The presence of extensive ischaemia or myocardium at risk indicates the need for more invasive work-up, such as coronary angiography. Conversely, the absence of significant ischaemia or myocardium at risk generally rules out the need for intervention.

Myocardial perfusion imaging can be performed in various settings: in patients with suspected coronary artery disease, after myocardial infarction or for the assessment of therapy.

TABLE 5.9. CLINICAL INDICATIONS FOR MYOCARDIAL PERFUSION IMAGING

Information	Clinical settings
Detection of myocardial ischaemia and myocardium at risk	Intermediate pre-test probability of coronary artery disease, post-myocardial infarction, assessment of medical or operative therapy
Risk stratification and prognostication	Suspected coronary artery disease, post-myocardial infarction, pre-operative cardiac risk assessment
Detection of stunned myocardium	Post-myocardial infarction, post-revascularization, post-stress testing
Significance of borderline angiographic studies	Consideration for revascularization procedures (PTCA[a], CABG[b])
Detection of hibernating myocardium	Left ventricular dysfunction, dilated cardiomyopathy of possible ischaemic aetiology
Assessment of ventricular function (ECG-gated SPECT study)	Ischaemic heart disease, cardiomyopathy

[a] PTCA, percutaneous transluminal coronary angioplasty.
[b] CABG, coronary artery bypass graft.

Myocardial perfusion imaging can also be used to evaluate the pathological significance of coronary lesions already detected by angiography. Angiographic coronary artery disease with a normal stress myocardial perfusion scan has little prognostic significance according to accumulated data.

(b) Risk stratification and prognostication

Myocardial perfusion imaging has the ability to distinguish patients at high risk of cardiac events from those at low risk. This helps clinicians to determine which patients to manage aggressively with invasive procedures and which ones to manage conservatively.

As with detecting myocardium at risk, stratification using mycardial perfusion imaging can be done in various settings: in patients with suspected coronary artery disease, after myocardial infarction as well as before non-cardiac surgery (to determine the risk of perioperative cardiac events).

Some studies have shown decreased accuracy in predicting future cardiac events in patients who have undergone thrombolytic therapy for acute myocardial infarction.

(c) Assessment of myocardial viability

One of the more important factors in deciding whether to refer a patient with left ventricular dysfunction due to coronary artery disease for revascularization (whether coronary bypass or angioplasty) is the presence or absence of viable myocardium. The term 'viable myocardium', in its broadest sense, denotes any myocardium that is not infarcted. This includes normal, stunned or hibernating myocardium. For the cardiologist, however, the search for myocardial viability is primarily a quest for myocardial hibernation. Myocardial hibernation is classically defined as chronic hypoperfusion and dysfunction that reverses after revascularization. It can be distinguished from myocardial stunning, which denotes acute but transient hypoperfusion and dysfunction, typically after a myocardial infarction in adjacent tissue that does not require intervention because it recovers spontaneously. It is now accepted, however, that the line separating hibernation from stunning is not as clear as was once thought.

Various modifications to basic myocardial perfusion imaging protocols have been devised in order to distinguish hibernating, viable myocardium from non-viable, infarcted myocardium. These include late redistribution, re-injection imaging (both protocols using ^{201}Tl) and nitrate augmented rest imaging (using either ^{201}Tl or ^{99m}Tc labelled agents).

The current best non-invasive method of detecting myocardial viability is the comparison of perfusion and metabolism using PET tracers, although this still underestimates the presence of viable myocardium in roughly 10% of patients.

(d) Assessment of ventricular function

Combining myocardial perfusion imaging with the ECG gating technique (synchronization of acquisition to the ECG signal) allows the investigation of ventricular wall motion and thickening throughout a typical cardiac cycle. This may then be evaluated qualitatively by viewing the images in an endless loop cine-display, or quantitatively using commercially available software. By drawing ROIs around the endocardial boundaries of the ventricle, either manually or through automatic edge detection algorithms, a volume curve can also be generated for the entire cardiac cycle, from which quantitative parameters such as end-systolic and end-diastolic volumes as well as ejection fraction can be derived.

Ventricular function may be also assessed by performing a first pass acquisition of the injection of the perfusion tracer, either at rest or during stress. This is feasible only using ^{99m}Tc labelled perfusion tracers.

Finally, ventricular function may be indirectly evaluated from non-gated images. The presence of global dilatation, thinned out walls, ventricular aneurysms and increased lung uptake are all suggestive of left ventricular failure.

5.2.4.3. Radiopharmaceuticals

A number of single photon emitting radiopharmaceuticals may be used for imaging myocardial perfusion. The three most commonly used at present are ^{201}Tl and the ^{99m}Tc labelled tracers sestamibi and tetrofosmin. Some of their most important properties are summarized in Table 5.10.

(a) Thallium-201

This radionuclide is used in the chemical form of thallous chloride. The photons of interest are mostly mercury X rays of 68–80 keV. Thallium-201 also has gamma rays of 135 and 167 keV, which contribute little to the total image counts.

Thallium-201 has a very high single pass extraction fraction in the myocardium, for which the major mechanism is active transport through the Na-K ATPase pump. Uptake of this tracer therefore denotes intact sarcolemmal membranes. The extraction fraction is linearly proportional to blood flow over a wide range of physiological flow levels, plateauing only at very high flow rates and logarithmically decreasing towards the very low flow range. Relative accumulation in the myocardium thus reflects relative regional perfusion.

This radiotracer is characterized by redistribution in the myocardium, settling in equilibrium between the myocardial and blood pool concentrations. This makes ^{201}Tl a marker of myocardial viability, which is perhaps its greatest advantage.

(b) Technetium-99m labelled perfusion agents

There are two ^{99m}Tc labelled myocardial perfusion agents in common use today. A third agent, ^{99m}Tc-teboroxime, characterized by extremely avid myocardial uptake but rapid myocardial washout, is no longer commercially available, while some others such as ^{99m}Tc-NOET are under investigation.

TABLE 5.10. PROPERTIES OF SINGLE PHOTON MYOCARDIAL PERFUSION TRACERS

Property	Tl-201	Tc-99m-sestamibi	Tc-99m-tetrofosmin
Chemical type	Electrolyte	Isonitrile	Diphosphine
Myocite localization	Cytosol	Mitochondria	Mitochondria
Physical half-life (h)	73	6	6
Mode of decay	Electron capture	Isomeric transition	Isomeric transition
Photon energy (keV)	68–80	140	140
Usual activity at single dose (MBq/mCi)	74–111/2–3	<1110/30	<1110/30
Effective dose equivalent (mSv/MBq)	26/74	18/1110	8/1110
Critical organ	Kidneys (1.2 cGy/mCi)	Upper colon (0.18 cGy/mCi)	Gall bladder wall (48.6 μGy/MBq)
Adverse effects at recommended dose ranges	No serious reported	No serious reported	No serious reported
Advantages	Redistribution property ideally suited for assessment of myocardial viability.	Short half-life allows larger doses for increased photon flux. Lack of significant redistribution allows early injection with delayed acquisition (e.g. in an emergency room).	More easily prepared compared with sestamibi. More rapid hepatobiliary clearance compared with sestamibi. Same advantages as Tc-99m-sestamibi over Tl-201.
Disadvantages	Relatively long half-life limits allowable dose. High incidence of attenuation artefacts.	High hepatobiliary activity needs delay in acquisition.	—

(1) Technetium-99m-sestamibi

Sestamibi (sometimes called hexamibi or simply MIBI) is an isonitrile lipophilic complex. Uptake of this tracer requires sarcolemmal and mitrochondrial integrity. The major route of excretion is through the hepatobiliary

system, and intense liver and intestinal activity occasionally causes significant interference with image quality. Protocols employing ^{99m}Tc-sestamibi involve post-injection waiting times of 45–90 min, to allow for adequate clearance of subdiaphragmatic activity.

Technetium-99m-sestamibi is characterized by a minimal yet discernible amount of redistribution, which may sometimes be used as a marker of recoverable myocardium.

(2) Tc-99m-tetrofosmin

Tetrofosmin is a diphosphine compound whose pharmacokinetics largely parallel that of Tc-99m-sestamibi. Its main advantages are ease of preparation and faster hepatic clearance, allowing shorter post-injection waiting times of 20–30 min.

5.2.4.4. Equipment

(a) Cameras

A single-crystal gamma camera is the basic piece of equipment required for myocardial perfusion imaging using both ^{201}Tl and ^{99m}Tc agents. Most commercial models are equipped with a rotating gantry for SPECT imaging. Planar imaging is no longer recommended for myocardial perfusion imaging. Planar imaging is not considered optimal for myocardial perfusion due to its lower sensitivity.

Acquiring images in a single, symmetric energy window is adequate, although an asymmetric window as well as multiple window capability allow minimization and correction of scattered radiation. A single-detector camera is adequate for heavy patient workloads; however, a dual-detector camera, with the heads oriented at 90° to each other, would reduce the acquisition time for a typical 180° SPECT orbit. A small FOV camera, which only admits counts from the heart and some adjacent part of the lungs, allows closer positioning of the detector to the patient compared with a large FOV camera and is ideal for departments with high patient throughputs.

Many current gamma cameras provide an option for non-uniform attenuation correction using an attenuation map acquired with a transmission source. By reducing or eliminating the incidence of attenuation artefacts on SPECT, the specificity of the study is potentially enhanced. It is probable that transmission attenuation correction will become the standard technique in the future. With caution and experience, however, most attenuation artefacts can be identified even without special techniques or manoeuvres.

(b) Collimators

Two low energy collimators should be sufficient for imaging with ^{201}Tl and ^{99m}Tc agents: a general purpose collimator to obtain adequate count statistics in ^{201}Tl imaging and a high resolution collimator to take advantage of better tolerance of patient-to-detector distance with ^{99m}Tc myocardial agents.

General purpose collimators may also be used for ^{99m}Tc imaging; however, high resolution collimators may yield inadequate count statistics using ^{201}Tl.

(c) Gating devices

An ECG synchronizer or gating device is required for gated cardiac SPECT if one is not already included with the camera. The ideal is gated acquisition software that automatically adjusts RR interval windows to changing heart rates (for a more equal distribution of counts across the cardiac cycle) coupled with premature beat rejection.

(d) Processing computers

An automated reconstruction and reorientation program is not a requirement. For accuracy, there is still no substitute for a trained and experienced human operator. Most manufacturers, however, provide for automated endocardial border ROI placement for quantitation of ventricular function, a feature that may provide greater consistency. Nonetheless, automated drawings always require human verification, especially in cases of ventricles with extensive and severe perfusion defects.

An effective quality control program should be strictly observed for myocardial perfusion imaging. Any error in the acquired image resulting from a failure of quality control will be magnified many times upon tomographic reconstruction.

(e) Exercise stress

A programmable motorized treadmill and ECG machine is preferred for exercise testing, although a bicycle ergometer is an acceptable alternative. Pharmacological stress modalities should be selected for patients who are unable to perform upright leg exercise. A common protocol for treadmill exercise is the Bruce protocol with symptom limited stress. The exercise is terminated if there are significant ECG changes, drop in blood pressure, severe chest pain, severe arrhythmia or exhaustion of the patient.

(f) Pharmacological stress

No special equipment is required for dipyridamole infusion, other than a syringe and an intravenous line. An infusion pump is essential with dobutamine, because of the tight control required of the infusion rate. Adenosine may be manually infused, but its extremely short duration of action requires special attention to be paid to the timing in order to maintain an adequate vasodilatory effect during tracer uptake, so an infusion pump is preferable.

(g) Intravenous administration of dipyridamole

The standard protocol consists of a slow intravenous injection of the drug ($0.142\ mg \cdot kg^{-1} \cdot min^{-1}$) for 4 min, followed 3 min later by intravenous administration of the tracer. This is necessary because the peak effect of dipyridamole on coronary blood flow occurs 2–2.5 min after injection and then declines exponentially with a half-life of 33 min. ECG, heart rate and blood pressure should be monitored every minute for ten minutes and longer if necessary. There is usually a mild reduction in systemic blood pressure, an increase in heart rate and cardiac output and a significant increase in coronary blood flow up to five times the resting values.

The common side effects of dipyridamole are angina, nausea, ST depression, headache, dizziness, facial flush, vomiting and ventricular arrhythmia.

5.2.4.5. Patient preparation

In order to obtain optimal image quality and maximal diagnostic benefit from myocardial perfusion imaging, certain steps must be strictly observed for patient preparation.

(a) Clinical examination

Ascertaining the reason for the request for myocardial perfusion imaging, i.e. the precise information desired from the study, is the first step in preparation for the test. This may be indicated on the attending physician's request. If not, a discussion with the referring physician is advisable.

It is essential to obtain an adequate history and conduct a physical examination in order to interpret the images properly. What is the patient's risk profile for ischaemic heart disease? Are there any predisposing conditions for small vessel disease, such as diabetes mellitus? Any complaints of chest pain

must be characterized, as well as any symptoms of congestive heart failure. A history of eventual hospitalization for chest pain, shortness of breath or syncope should be taken, as well as therapeutic measures adopted. Awareness of previous operations, especially those involving implants such as breast implants, pacemakers or valve prostheses, is important. A description of any previous revascularization procedure should be available. It is also important to know the results of recent cardiac diagnostic studies, especially catheterization as well as imaging studies, provided this will not bias the interpretation of the myocardial perfusion images. Obtaining a current and recent drug history is always necessary.

A physical examination should include a description of the patient's body habitus, as well as breast size; note should be taken of any thoracic deformities that could affect the orientation of the heart within the thorax and/or the positioning of the patient under the camera.

(b) Diet and medication

A fasting state is required for both ^{201}Tl and ^{99m}Tc imaging at rest, except for diabetic patients on insulin or oral hypoglycemic agents.

Patients undergoing exercise or vasodilator testing should discontinue medications and foods that could interfere with the test. For exercise testing, these include drugs that limit the tachycardic response to exercise, such as beta blockers and calcium antagonists and drugs that increase flow to segments subtended by coronary stenoses, such as nitrates. For dipyridamole or adenosine testing, drugs and foods that antagonize the effects of adenosine should be withheld, especially caffeine and other methylxanthine containing drugs and beverages. For purely resting (viability) studies, no medication needs to be withheld; the intake of nitrates may in fact optimize the sensitivity for detecting viable myocardium.

Radiopaque objects should be removed from the area of the thorax. Note should be taken of any implanted radiopaque devices (e.g. pacemakers and silicone breast implants).

5.2.4.6. Procedure

(a) Viability studies

When the clinical situation simply requires an assessment of the distribution and extent of viable myocardium, no cardiac stress is required. A rest redistribution ^{201}Tl protocol will detect resting ischaemia as well as myocardial

viability with satisfactory accuracy. This is done by simply injecting the patient with 2–3 mCi of ^{201}Tl and commencing imaging 15 min later.

Alternatively, a single injection of a ^{99m}Tc agent at rest after administering a short acting nitrate will yield an image from which myocardial viability may be inferred by uptake alone; however, it sometimes may not differentiate hibernating myocardium from a mixture of normal myocardium and scar tissue. With ^{99m}Tc sestamibi, by delayed imaging 2–4 hours later, it may be possible to detect a slight redistribution and thus infer the presence of myocardial hibernation; however, this is currently not widely recommended for evaluation of viability.

Where there is a question of both inducible ischaemia and viability, exercise or pharmacological stress imaging should be performed either on a separate day or as a second procedure in a same day protocol (using ^{99m}Tc) or with late redistribution or re-injection imaging (with ^{201}Tl). Late redistribution imaging should be done no more than 18–24 hours after injection; later times often result in images too degraded for interpretation.

(b) Ischaemia studies

In order to detect inducible myocardial ischaemia when the resting perfusion is normal, a stress study is performed, whether physical or pharmacological.

From an imaging standpoint, a two day protocol is preferable over a one day protocol for ^{99m}Tc labelled tracers because a full allowable dose of ^{99m}Tc may be given for both stress and rest studies. The main advantage of a one day protocol is convenience for the patient, although a two day protocol could also spare the patient a return trip for the rest study if the stress study is normal. For a one day protocol, a rest stress sequence is preferable, not only because of improved detection of defect reversibility but also because it may avoid the effect of post-exercise stunning in the rest images.

(c) Gated myocardial perfusion imaging

This may be done in conjunction with either an ischaemia or a viability study. When a two day protocol is used, both studies should be gated, although for practical purposes only the rest study can be gated and used to evaluate function in the true basal state.

5.2.4.7. Interpretation

Information useful for the interpretation of patient studies is summarized in Tables 5.11–5.13.

Anatomical and physiological variations should be kept in mind, such as low uptake at the apex and the membranous part of the interventricular septum as well as low activity at the anterior wall (due to breast attenuation) and the inferior wall (due to diaphragmatic attenuation). Artefacts such as metallic implants, motion artefacts and bowel tracer should be excluded.

(a) Ischaemia

For ^{201}Tl, early redistribution in stress defects indicates stress induced ischaemia. Late redistribution, redistribution after a resting injection or improvement after re-injection implies hibernation or critical stenosis of the

TABLE 5.11. TYPICAL[a] PERFUSION ABNORMALITIES IN VARIOUS CLINICAL CONDITIONS

Condition	Typical regional perfusion findings (assuming no artefacts)		
	Stress	Rest	Late imaging – re-injection (Tl-201)
Normal	Normal	Normal	—
Stunned	Variable	Variable	No further change
Inducible ischaemia	Decreased	Normal	—
Mixture of ischaemia and infarcted tissue	Decreased	Partially improves	No further change
Subendocardial infarction	Normal	Worsens	Partially improves or no further change
Critical stenosis/ hibernating myocardium	Decreased	No change to partial improvement	Partial improvement to normal
Infarction (transmural)	Decreased (moderate to severe)	No change	No change
Dilated idiopathic cardiomyopathy	Patchy, decreased	No change	No change

[a] Considerable variation may occur, depending on the clinical circumstances.

TABLE 5.12. MARKERS OF HIGH RISK FOR FUTURE CARDIAC EVENTS WITH POOR PROGNOSIS IF UNTREATED[a]

Without prior infarction	Defects in multiple vascular territories Redistribution in perfusion defects (partial or complete) Transient ventricular cavity dilatation Persistent global ventricular dilatation Elevated pulmonary tracer activity under stress Left ventricular ejection fraction less than 40%
With prior infarction	Any of the above, plus: Redistribution in infarct zone Extent of infarct more than 40% of entire ventricular wall

[a] For pre-operative assessment, this means a 10–20% risk of perioperative cardiac events.

supplying artery. Defects that persist after these latter procedures, especially after enhancement by nitrates, very likely represent an infarct.

For ^{99m}Tc-sestamibi or -tetrofosmin, higher relative uptake with rest imaging compared with stress implies induced ischaemia. Rest defects that improve after nitrate enhanced rest imaging indicate hibernation with a critical stenosis of the supplying artery. Defects that persist after such manoeuvres are probably due to infarction.

(b) Prognosis

The finding of normal stress–rest myocardial perfusion indicates a very low (<1%) annualized risk for myocardial infarction and cardiac death, regardless of positive findings on a stress ECG or the findings of a coronary

TABLE 5.13. MARKERS OF POTENTIALLY RECOVERABLE MYOCARDIAL SEGMENTAL DYSFUNCTION

Perfusion	Stress or pharmacologically induced (reversible) perfusion defects
Nitrate enhanced	Uptake improvement relative to rest
Redistribution	Late Tl-201 redistribution or redistribution after resting injection
Re-injection	Increased uptake on Tl-201 re-injection
Relative uptake	>50% of maximum myocardial uptake
Gated images	Significant wall thickening

angiogram. Some studies indicate caution when making a prognosis from normal scans in patients taking beta blocking agents, which may be associated with a more advanced state of disease.

The finding of totally fixed defects with an otherwise normal cardiac global function also indicates a benign prognosis.

(c) Viability

General rules for interpreting viability studies include:

— Totally or partially reversible defects in a dyskinetic region have a high predictive value for functional recovery after revascularization.
— Persistent mild to moderate defects (i.e. relative regional uptake ≥50%) frequently, but not always, indicate hibernating myocardium.
— The presence of significant systolic thickening (systolic counts ≥120% of diastolic counts) in a defect favours viability in that segment.
— Absolute (i.e. activity no higher than background) or near absolute defects without redistribution have a low probability for functional improvement after revascularization.

Diagnosis of myocardial viability with SPECT has an accuracy of about 80%, to some extent varying according to the protocol employed and the population under study. The gold standard for assessing viability has been segmental functional recovery after revascularization. However, in the opinion of an increasing number of authors, a significant improvement in life quality and/or in life expectancy after interventions should be instead considered.

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5.3. CENTRAL NERVOUS SYSTEM

5.3.1. Cerebral angiography and blood–brain barrier studies (brain scans)

5.3.1.1. Principle

The human brain is protected by several mechanisms. The skull provides bony coverage against outside impact and the blood–brain barrier (BBB) offers protection from toxic influence from inside. The integrity of the BBB is broken as a consequence of a variety of brain lesions. Brain scan is an old term referring to scintigraphic detection of the integrity of the BBB. Dynamic imaging of the head immediately after tracer injection, referred to as radionuclide cerebral angiography, depicts the cerebral vasculature.

Since the introduction of other imaging technologies with finer anatomical resolutions, such as CT, MRI and DSA, brain scanning is no longer the first choice to reveal an abnormality of the brain. However, in some developing countries, or areas where other modalities are not readily available, the brain scan is still a useful investigation to neurologists, neurosurgeons and oncologists.

5.3.1.2. Clinical indications

Diagnostic investigations are carried out for any of the following purposes:

(a) To detect the existence, location, extent and distribution of brain lesions (tumour, infarct, inflammation, haemorrhage or trauma) or intracranial lesions;
(b) To assess the integrity of the BBB in systemic disorders such as infection, intoxication or connective tissue diseases;
(c) To detect the patency and morphology of major intracranial vessels;
(d) To diagnose brain death.

5.3.1.3. Radiopharmaceuticals

Several ^{99m}Tc labelled simple compounds, such as ^{99m}Tc-DTPA, ^{99m}Tc-pertechnetate and ^{99m}Tc-glucoheptonate, are widely used for brain scans and first pass radionuclide cerebroangiography. The adult dose is 370–740 MBq (10–20 mCi). To avoid confusing uptake by the choroid plexus in the case of pertechnetate, 300–400 mg of potassium perchlorate is given orally prior to administration of the radiopharmaceutical.

5.3.1.4. Protocols

The protocols listed below should be followed:

— The patient should rest quietly for a few minutes before the study. Sedation is indicated for those unable to cooperate.
— Potassium perchlorate should be given orally, if indicated, 30 min before injection.
— The patient is positioned for cerebral angiography, usually in the anterior view.
— The selected radiopharmaceutical is then given as a bolus into a peripheral vein.
— Data acquisition is started immediately (angiography) or 30–90 min (static scan) after injection.

5.3.1.5. Acquisition

The procedures listed below should be followed:

— Brain scans and angiography are usually undertaken as planar imaging.
— Brain scans are usually performed in the anterior, posterior, vertex and both lateral projections, but occasionally additional views may help delineate a lesion better.
— Cerebral angiography is acquired in dynamic mode at 1–2 s/frame for 20–40 s, using a 64 × 64 matrix, with or without zoom.
— Brain scans are usually started in the anterior view, using a 256 × 256 matrix, for 600 000 counts. The acquisition time is used to determine the time for the other views, for comparison purposes.
— Tomography (SPECT) might be used, in which case acquisition is carried out 40–60 min after injection, using a 360° orbit and preferably in a 128 × 128 matrix.

5.3.1.6. Data processing and interpretation

The viewing and interpretation of brain scans and angiography are usually straightforward. Since most radiopharmaceuticals cannot pass through an intact BBB, there is low radioactivity in normal brain and cerebellum. More activity is noticed in the skull and scalp, making the normal image look like a hot outer rim around a hollow centre. Activity is also visible in venous sinuses and facial bones. The mouth and nose are usually 'hot'. Owing to limitations in resolution, only the carotids, middle cerebral arteries and anterior cerebral arteries together are shown on the arterial phase of cerebral angiography. The arterial phase starts 6–8 s after injection, lasting for 3–5 s; the capillary or parenchymal phase lasts for 6–8 s, then the radioactivity appears inside the venous sinus, producing a venous phase.

Any concentration of radiotracer outside the normal cranial distribution asymmetries or change in blood flow pattern indicates a brain or intracranial lesion. The findings are not specific except in the case of subdural haemorrhage.

Occasionally, bilateral choroid plexus uptake in the middle of the hollow area of the brain might be mistaken for a lesion. Carotid obstruction may introduce a 'hot nose' sign on an angiogram due to collateral flow.

5.3.2. Cerebral perfusion tomography (brain SPECT)

5.3.2.1. Principle

The human brain relies on continuous blood flow to supply all needed nutritional elements. Owing to the high extraction of oxygen from the blood, and the rapid adjustment of the blood flow to meet function demands, the brain has a special mechanism to regulate its blood flow. This regulation is relatively independent of the systemic circulation and is determined by regional cerebral function and metabolism. This is sometimes referred to as the 'trinity' of metabolism–function–blood-flow of the brain. The perfusion, i.e. the distribution of blood supply at the tissue level of the brain, is a key aspect to revealing brain function.

The objective of this section is to assist nuclear physicians to perform, interpret and report the results of SPECT studies of brain perfusion using ^{99m}Tc labelled radiopharmaceuticals. This technique has been used to reflect the regional cerebral blood flow (RCBF) distribution in different areas of the brain.

5.3.2.2. Clinical indications

The purposes for which an investigation is needed include:

(a) Detection and evaluation of cerebrovascular disease:
  — Differentiation of cerebral infarction,
  — Prediction of the outcome of patients with cerebrovascular accident (CVA),
  — Work-up of patients with transient ischaemic attack (TIA);
(b) Evaluation of patients with suspected dementia;
(c) Presurgical localization of epileptic foci;
(d) Evaluation of symptomatic traumatic brain injury, especially in the absence of CT and/or MRI findings;
(e) Diagnosis of encephalitis;
(f) Monitoring and assessment of subarachnoid haemorrhage;
(g) Verification of brain death;
(h) Assistance in planning and monitoring treatment for intracranial or brain disorders.

Other indications include neuropsychiatric disorders such as depression, obsessive–compulsive disorder, chronic fatigue syndrome, neural degenerative diseases, for example Huntington's chorea, and functional disorders, for example schizophrenia, where the findings of SPECT brain perfusion imaging have not been fully characterized. In HIV-positive encephalopathy, CO_2 intoxication and connective tissue diseases, SPECT brain perfusion can detect organic changes in the brain, and point to appropriate treatment and monitoring.

5.3.2.3. Radiopharmaceuticals

There are several kinds of radiopharmaceutical suitable for cerebral perfusion imaging, whose characteristics are listed in Table 5.14. Different agents may provide different distributions in the CNS, especially in pathological cases, so they should be carefully selected.

All are highly lipophylic agents freely able to cross the intact BBB. The radiopharmaceuticals have different mechanisms for accumulating in the brain. For example, hexamethyl-propylene-amine oxime (HMPAO) is thought to convert to a hydrophylic form unable to recross the BBB. ECD is retained because of hydrolysis of its ester group, and IMP is probably combined to some receptor site in the brain. Whatever the mechanism, retention of the tracer in proportion to cerebral blood flow is the primary requirement for imaging. IMP,

TABLE 5.14. CHARACTERISTICS OF RADIOPHARMACEUTICALS USED IN CEREBRAL PERFUSION

Radiotracer	Adult dose (MBq)	Image time post-injection
Tc-99m-d,l,HMPAO[a]	555–1110	0.5–1.5 h
Tc-99m-l,l,ECD[b]	555–1110	5–60 min
I-123-IMP[c]	150–185	20–60 min

[a] HMPAO, hexamethylpropyleneamine oxime.
[b] ECD, ethyl cysteinate dimer.
[c] IMP, N-isopropyl-p-iodoamphetamine.

no longer commercially available, exhibits some redistribution over time, while ^{99m}Tc based agents do not.

In the preparation of ^{99m}Tc radiopharmaceuticals, fresh (<2 h old) elution must be used. After reconstitution, the radiopharmaceutical should be allowed to stand for 10 min before injection. Owing to its instability, ^{99m}Tc-HMPAO must be injected within 30 min of its preparation. Technetium-99m-HMPAO in stabilized form can be injected for up to 4 h after reconstitution. For seizure disorders, it is important to use stable agents since the exact time of injection cannot be anticipated.

5.3.2.4. Protocols

The following protocols apply:

(a) Patients should be instructed to stop taking caffeine, alcohol or other drugs known to affect cerebral blood flow (CBF) at least 1–3 days before the study. Patients should be instructed to avoid smoking for at least the day of the test. Other medication should be discussed with the referring physician.
(b) Patients must be clearly informed about the test procedures and necessary precautions. The most important aspect of patient preparation is to evaluate and ensure the ability of the patient to cooperate. A written consent form should be obtained.
(c) Patients should void prior to the study for maximum comfort.
(d) Patients are placed in a quiet dimly lit room with no direct light source facing their eyes. Whether patients are instructed to keep their eyes open or closed depends on each department's protocol, which should be followed in all studies.

(e) Intravenous access should be established and maintained for at least 10 min prior to injection.
(f) The radiotracer should be injected through the intravenous route at the recommended time. After a specified interval, patients are comfortably positioned to tolerate the long imaging time.
(g) Each department should have a specific form, to be completed by the nuclear medicine physician, that includes the relevant patient data suggested for optimal interpretation of scans, covering the patient's history (including any past drug use or trauma), neurological and psychiatric findings, mental status (e.g. Folstein mini-mental exam or other neuropsychological test), recent morphological imaging studies (e.g. CT and MRI), current medication and when this was last taken. It is also important to know if the patient has had previous studies and their results. All these data should be reported on the patient information sheet.
(h) If patients are unable to cooperate (e.g. mental deficiency or young children), sedation is needed, but it should not precede the injection of the radiopharmaceutical. Preferably, to minimize the duration of sedation, it should start just prior to the acquisition of the study.
(i) If an intervention study is needed, vasodilatory challenge can be induced by slow intravenous injection of acetazolamide (Diamox) or an equivalent, at a dosage of 1000 mg, or 14 mg/kg for children, 15–20 min before injection of the tracer. Inhalation of CO_2 or mental stress tests can also be used as an alternative.

5.3.2.5. Image acquisition

The following points should be noted:

(a) Multiple detectors or other dedicated SPECT cameras generally produce superior results than single-detector general purpose units. However, with meticulous attention to procedure, high quality images can be obtained on single-detector instruments with appropriately longer scan times (5 million total counts or more are desirable).
(b) Patients should be positioned for maximum comfort. There should be minor obliquity of the head, although the orientation can be corrected in most systems during processing.
(c) The patient's head should be positioned in the midline with the orbit line at a 90° angle to the horizontal line. The patient's head should be slightly restrained to facilitate patient cooperation in minimizing motion during acquisition.

(d) The smallest possible radius of rotation is used with appropriate patient safeguards. The radius of rotation should not exceed 14 cm. Non-circular orbits are preferred, allowing a shorter distance to the patient at all angles.
(e) The use of high resolution or ultrahigh resolution collimators is recommended. All-purpose collimation is not suitable. As a general rule of thumb, the highest resolution collimator available should be used.
(f) Fanbeam or other focused collimators are generally preferable to parallel hole ones as they provide improved resolution and higher count rates. However, these collimators should be used with caution because of the possibility of missing areas of the brain. Slant hole collimators may also be used.
(g) A 128 x 128 or greater acquisition matrix is preferred over a 64 x 64 matrix unless magnification is used. Most protocols recommend 120–128 angular steps for 360° rotation.
(h) It may be necessary to use a hardware zoom to achieve an appropriate pixel size. Different zoom factors may be used in the x and y directions of a fanbeam collimator.
(i) Continuous acquisition may provide a shorter total scan duration and reduced mechanical wear to the system when compared with the step and shoot technique.
(j) The segmentation of data acquisition into multiple sequential acquisitions will permit the exclusion of bad data, for example, removing segments of projection data with patient motion. This data acquisition type is recommended when available. Each department should develop a protocol in data acquisition that would allow technical staff to optimize utilization of resources and reproducibility of results.

5.3.2.6. Data processing

The following points should be noted:

(a) Image processing filters are applied in 3-D (x, y and z directions). This is achieved either with 2-D pre-filtering of the projection data or by applying a 3-D post-filter to the reconstructed images.
(b) Low-pass (e.g. Butterworth) filters should generally be used. Resolution recovery or spatially varying filters should be used with caution, as they may produce artefacts. Iterative reconstruction methods give better results and are now available in modern systems.
(c) When processing, it is important to include the entire brain from the cerebellum to the vertex.

(d) Data should be reconstructed at the highest pixel resolution, i.e. one pixel thick. Summation of pixels for display should be performed after complete reconstruction and oblique reorientation.
(e) Attenuation correction should be performed in all cases unless a specific application or circumstance dictates otherwise. Shape contouring can be used if available. The contour should include scalp and not just grey matter. Whenever possible, the surface contour should be defined individually for each transaxial slice.
(f) Transaxial data are reformatted into at least three orthogonal planes. Transverse sections should be generated relative to a repeatable anatomical orientation, while coronal and sagittal sections should be orthogonal to the transverse. Additional sections along a plane parallel to the long axis of the temporal lobes are often useful.

5.3.2.7. Image interpretation

All studies should be interpreted first without the benefit of clinical information and the findings of other morphological imaging modalities. Each department should have an individual assigned for brain image processing and display in order to standardize the reproducibility.

Each department should also have examples of normal brain perfusion studies to be used as a reference in interpretation. There is substantial variability among normal individuals and among scans of a single subject obtained at different times. Each laboratory should develop criteria for defining the normal and abnormal findings according to its method of processing and displaying the studies. Individual centres in the area should cooperate towards the development of a normal database to be used for this purpose.

Unprocessed projection images should be reviewed in a cinematic display prior to evaluation of tomographic sections. Projection data should be assessed for the presence and degree of patient motion, target-to-background ratio and other potential artefacts. Inspection of projection data in sinogram form may also be useful. These data should be reviewed before the patient leaves, in case a repeat study is needed.

Images should be viewed on a computer screen rather than on a film or paper copy to permit interactive adjustment of contrast, background subtraction and colour table. It is recommended that studies be first displayed without background subtraction. It is also recommended that the studies be displayed at a thickness of one or two pixels. The brain should be positioned using the sagittal sections and the midline centred with the thalamus; the frontal lobes should be tilted upwards by 10–15°. The coronal and transaxial

sections should be aligned to avoid any tilting of the brain. Slight tilting of the brain produces artefacts in the interpretation. The colour scale is easy to use because it may be set with increments of 10% of changes in colour. The range should be normalized to maximum cerebellar or occipital counts. A two pixel display will present all sections of the brain — the transaxial, sagittal and coronal — on one screen.

Three dimensional volume renderings may be useful in appreciating overall patterns of disease. Care must be taken in the choice of threshold, as artefactual defects are easily generated.

A rule of thumb for the interpretation is that asymmetry between both sides of more than 15% and any decreased perfusion in the cerebral cortex of less than 70% of maximum uptake is pathological. Overinterpretation of the study is to be avoided.

Functional images must be evaluated with knowledge of the structural information. In cases of CVA, the extent of the perfusion abnormality compared with CT is important for defining the penumbra versus infarct, and for prognostic reasons.

In cases of epilepsy, images must be correlated with the relevant electroencephalography (EEG) data and clinical observations in seizure patients. The exact timing of tracer injection relative to observed seizure activity must be known. Ictal studies are more reliable for localization of seizure foci.

It is very important for the interpreter to be aware of the non-specificity of the findings in certain diseases such as neuropsychiatric and mild or moderate traumatic brain injury.

5.3.2.8. Precautions

Patients with neurological deficits or dementia may require special care or close monitoring at all times.

If sedation is required, it should be given at least 5 min after injection of the radiopharmaceutical.

In patients with known sulphonamide allergy, migraine history, and within three days of an acute stroke, acetazolamide or other vasodilatory challenge is contraindicated.

Some patients might experience mild vertigo, tinnitus, paresthesias and nausea after use of acetazolamide (Diamox). Postural hypotension might occur, and patients should be appropriately warned before the study.

Radiochemical purity determinations should be performed on each vial of radiopharmaceutical prior to injection using the method outlined in the package insert. A shortened one step technique may also be used for ^{99m}Tc-HMPAO.

5.3.2.9. Reporting

The report should describe the location, extent and severity of defects, their correlation with morphological and clinical abnormalities and, when relevant, a differential diagnosis and/or statement of the significance of the abnormalities. The report should include the radiopharmaceutical used, the dose injected, the delay period post-injection, the type of equipment used for acquisition of the data, as well as any interventional medication used and any side effects noted. The report should also state whether the eyes were open or closed at injection, and whether the patient is right or left handed.

There should be an assessment of the technical quality of the scan (good, adequate, poor, including presence of patient motion and deviations from the usual protocols, if relevant). There should also be a description of abnormalities (including the criteria for definition of abnormal, i.e. visual inspection criteria, ROIs and comparison with the database).

5.3.2.10. Interpretation and conclusions

The referring physician must be given an answer to the reason for the referral of the patient when feasible. The physician should relate the findings of the SPECT studies to the patient's condition.

The scan is to be interpreted in the context of known clinical history, associated co-morbid conditions, medication and other diagnostic studies (CT, MRI and EEG). There is a limitation regarding differential diagnosis if relevant clinical data are not available, and additional tests may be recommended.

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5.3.3. Brain tumour imaging

5.3.3.1. Principle

Current literature on the clinical use of ^{201}Tl chloride or ^{99m}Tc-sestamibi supports their usefulness for tumour localization in general and for intracranial lesions in particular.

The purpose of this guideline is to assist nuclear medicine practitioners in recommending, performing, interpreting and reporting the results of brain ^{201}Tl chloride or ^{99m}Tc-sestamibi studies for the purpose of CNS tumour evaluation.

5.3.3.2. Clinical indications

Imaging is required for the following purposes:

(a) Differentiation of tumour recurrence from oedema, fibrosis or necrosis post-treatment;
(b) Evaluation of tumour response to various kinds of treatment;
(c) Differentiation of benign opportunistic infection lesions from malignant intracranial lesions in both immunosuppressed and non-immunosuppressed patients.

5.3.3.3. Radiopharmaceuticals

The following doses of radiopharmaceuticals should be used:

(a) Thallium-201 chloride

The dose for an adult is 111–148 MBq (3–4 mCi), or 1.5–5.0 MBq (0.04–0.05 mCi)/kg body weight, intravenously injected, with a minimum dose of 37 MBq (1 mCi).

(b) Technetium-99m-sestamibi

The dose for an adult is 555–740 MBq (15–20 mCi), or 7.5–12 MBq (0.2–0.3 mCi)/kg body weight, intravenously injected. Use of ^{99m}Tc-sestamibi is encouraged in paediatric patients because of higher injected dose, less radiation burden and better physical characteristics. In paraventricular lesions, ^{201}Tl chloride is preferred because of physiological choroid plexus and pituitary uptake with ^{99m}Tc-sestamibi.

5.3.3.4. Protocols

The following protocols should be made:

(a) Patient preparation

(i) No special recommendation such as fasting or stopping medications is needed. Studies can be performed after a few days of chemotherapy or radiotherapy but are not advisable in the immediate post-operative period. Waiting for a few weeks is advisable in the post-operative period. In AIDS patients with suspicion of intracranial lymphoma versus toxoplasmosis, the study should be performed before the patient has started medical treatment for toxoplasmosis. Patients having antibiotic treatment for toxoplasmosis might give a false positive.

(ii) Pre-injection preparation includes:
- Explaining to the patient the procedure, the time needed for it and the need for their cooperation;
- Obtaining an informed written consent;
- If sedation is needed, giving this just before starting acquisition of the study and minimizing the sedation period to the shortest time possible.

(b) Information pertinent to performing the procedure

A full clinical history, a clinical examination of the patient and the findings of other morphological imaging modalities are essential for the proper interpretation of a study. Previous radiation therapy, chemotherapy or surgery, and the time period from the current study should be specifically mentioned. Any previous studies should be made available for comparison.

5.3.3.5. Image acquisition

Attention should be paid to the following points:

(a) Waiting time after injection

Imaging can start at any time after a 15 min waiting period from intravenous injection of either ^{201}Tl or ^{99m}Tc-sestamibi.

In the case of differentiation of post-operative changes, oedema or inflammation, from residual tumour or recurrence, delayed images after a minimum of two hours from the time of injection of the radiopharmaceuticals are essential.

(b) Acquisition parameters

The following points should be noted:

— Multiple head gamma camera systems are preferable for the purpose of shortening the time of study and acquiring higher counts for better resolution. However, single head systems can be used with high levels of confidence.
— The patient should be positioned for maximum comfort for the whole period of the study. Preferably the patient should void before positioning on the table.
— The patient's head should be lightly restrained so as to minimize motion during acquisition. It is not possible to rigidly bind the head in place. If the patient's cooperation is unsatisfactory, sedation may be used according to the previous guidelines.
— The smallest radius of rotation possible is used with appropriate patient safeguards. The maximum radius allowed is about 14 cm.
— General purpose collimators can be used for both radiopharmaceuticals; however, for ^{99m}Tc-sestamibi it is possible to use high resolution collimators.

— A matrix size of 64 × 64 is adequate.
— Angular sampling: 3–6º is satisfactory (60–120 projections for a 360º rotation).
— Time per projection: 40 s for Tl and a minimum of 20 s for sestamibi.
— Zooming is necessary in order to obtain the whole brain within approximately a 25 cm FOV. The zoom factor will vary according to different gamma cameras.
— Step and shoot or continuous acquisition mode can be used, either with elliptical or circular obits.

5.3.3.6. Data processing

Filter all studies in three dimensions (x, y and z). This can be achieved either by two dimensionally prefiltering the projection data or by applying a 3-D post-filter to the reconstructed data.

Low pass (e.g. Butterworth) filters should generally be used. Resolution recovery or spatially varying filters should be used with caution, as they may produce artefacts. It is highly recommended to reconstruct the whole brain. Iterative reconstruction methods, available in modern systems, are preferred.

Reconstruct data at one pixel resolution. If slices are to be summed, this should be done after reconstruction and oblique orientation (if performed).

Attenuation correction is encouraged especially in paraventricular lesions and in the presence of low-grade lesion uptake.

Each institution should develop its own technique for calculating the lesion-to-background ratio.

5.3.3.7. Image interpretation

Interpretation should be performed in conjunction with X ray CT, MRI, full history and clinical data.

There is a higher confidence in the interpretation of CT and MRI images of solid lesions than in those of necrotic lesions. The same is true for large lesions.

Paraventricular small lesions (especially with ^{99m}Tc-sestamibi) must be interpreted with caution, as well as lesions close to the calvarium, the petrous bone, the temporal bone, the cribriform plate in the base of the anterior cranial fossa and near the orbits.

Benign lesions such as tuberculosis, histiocytosis, sarcoidosis and brain abscesses may produce high intensity uptakes. It is not the purpose of such

studies to avoid biopsy of intracranial lesions. Low grade gliomas can show no uptake of either radiopharmaceutical and be the cause of false negatives.

In post-operative studies, interpretation can be improved by taking into consideration the site, extent, intensity and location of the abnormal uptake, and also by comparing the early (if available) and delayed images.

A negative study following recent treatment does not indicate that a disease has been cured. It only indicates a good response and does not exclude microscopic residual disease.

If the studies are performed for the purpose of differentiation of intracranial toxoplasmosis from lymphoma in the immunosuppressed patient, recent treatment for toxoplasmosis might produce a false positive uptake.

5.3.3.8. Reporting

The report should include the radiopharmaceutical used, dose, route of injection, waiting period, clinical history and the reason for referring the patient for the study. If sedation has been given it should be mentioned, as well as any adverse reactions.

The report should include and mention the findings of other morphological imaging modalities and their correlation with the nuclear medicine procedure.

The intensity of uptake should be graded as low, medium or high, together with the size of the lesion as accurately as possible, and its location. A tumour-to-background ratio calculation might be useful.

Comparison with previous studies, if they have been done, is very important.

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5.3.4. Cerebral metabolic imaging

5.3.4.1. Principle

The trinity of metabolism–function–blood-flow of the brain are closely interrelated. An abnormality in one aspect will be reflected in the other two. Brain function can be studied with PET and SPECT through imaging radio-labelled substrates or RCBF markers. With PET, regional cerebral perfusion can be imaged repeatedly at short intervals using ^{15}O-water. The RCBF images reflect neuronal activities, which are used for the localization of activated areas of brain after specific stimulation or performance of tasks. The subtraction of rest images from activated images enables a clearer identification of activated regions of the brain. Activation paradigms include visual, audio and finger motion stimulations as well as speech and thinking. Activation studies may elucidate higher brain functions in healthy volunteers and neuropsychiatry patients.

5.3.4.2. Clinical indications

The indications are the same as those for the cerebral perfusion studies (Section 5.3.2).

5.3.4.3. Radiopharmaceuticals

Positron emitting radiopharmaceuticals are used for metabolic imaging. Three aspects of cerebral metabolism are of interest clinically, namely glucose and oxygen utilization and protein synthesis. Oxygen-15-O_2, ^{18}F-FDG and ^{11}C-methionine, used to study these activities, require there to be a cyclotron in close proximity for their production. Oxygen-15-O_2 can be continuously inhaled, with little dissolved in plasma and most bound to haemoglobin. It is the bound portion of ^{15}O that is transported to, and utilized by, the brain. Fluorine-18-FDG is transported to the brain and taken up by cells as glucose but cannot be metabolized any further. It reflects the regional glucose utilization of tissue. Carbon-11-methionine shows protein synthesis and is used mainly for brain tumour imaging.

5.3.4.4. Protocols

Patient preparation and pre-test precautions are similar to those described for perfusion. Data acquisition is performed with either dedicated PET or other forms of positron imaging devices. The start of the acquisition

depends on the radiopharmaceutical selected, for example by 10–20 min when using ^{11}C compounds, 40–90 min using ^{18}F compounds and immediately using ^{15}O compounds. Because of the very short half-life of radionuclides, brain metabolic imaging may be repeated at short intervals to facilitate assessment of different brain states. It is important to maintain very strict protocols and stable test conditions.

5.3.4.5. Acquisition setting

The procedure is best performed with PET dedicated systems. Acquisition is usually accompanied by a transmission session for attenuation correction. If a kinetics analysis is required, dynamic or fast repeated acquisitions are needed.

5.3.4.6. Data processing and interpretation

Cerebral metabolic images are similar to those of cerebral perfusion. Usually the metabolic and perfusion images are similar in pattern under normal circumstances. Metabolic images should be interpreted with the structural data available, and co-registration techniques are of great value.

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5.3.5. Brain receptor imaging

5.3.5.1. Principle

Neuroreceptors can be altered in a variety of clinical settings. Characterization of neuroreceptor and neurotransporter activities has a more specific impact on the diagnosis of many neurodegenerative and neuropsychiatric

disorders than do CT or MTI studies. Most brain functions are linked to certain neurotransmitters or receptors.

5.3.5.2. Clinical indications

Most receptor–transporter studies have been performed to evaluate movement disorders, epilepsy and psychiatric illnesses, but clinical indications are still investigational.

5.3.5.3. Radiopharmaceuticals

The radiotracers used in the functional imaging of the brain are listed in Table 5.15.

TABLE 5.15. RADIOPHARMACEUTICALS FOR NEURORECEPTOR IMAGING

PET	SPECT
Dopamine receptors	
C-11, 3-N-methylspiperone (NMSP)	I-123, Iodobenzamide (IBZM)
F-18, Fluoroethylspiperone	I-123, Iodobenzofuran (IBF)
F-18, Fluoropropylspiperone	I-123, Epidepride
C-11, Raclopride	I-123, Lisuride
C-11, YM-09151-2	I-123, 2-Iodospiperone
C-11, FLB457	I-123, SCH23982
Benzodiazepine receptors	
C-11, Flumazenil	I-123, Iomazenil (IMZ)
C-11, Suriclone	
C-11, Diazepam	
Opiate receptors	
C-11, Carfentanil	
C-11, Diprenorphine	
F-18, Acetylcyclofoxy	
Muscarinic acetylcholine receptors	
C-11, Nicotine	I-123, Iododexetimide (IDEX)
C-11, Dexetimide	I-123, (R)-3-quinuclidinyl-4-iodobenzilate (QNB)

TABLE 5.15. RADIOPHARMACEUTICALS FOR NEURORECEPTOR IMAGING (cont.)

PET	SPECT
Serotonin receptors	
F-18, Setoperone	
Dopa metabolism	
F-18, 6-fluorodopa	
Monoamine oxidase enzyme activity	
C-11, Clorgyline	
C-11, Deprenyl	
C-11, Dimethylphenylethylamine	
Acetylcholine enzyme activity	
C-11, MP4A	
Neurotransporter	
C-11, Cocaine	Tc-99m, TRODAT
F-18, CFT (WIN 35,428)	I-123, Iodobenzovesamicol (IBVM)
F-18, N-3-fluoropropyl-2-β-carbomethoxy-3-β-(4-iodophenyl) nortropan	I-123, N-α-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) tropan (FP-CIT)
	I-123, β-Carbomethoxy-3β-(4-iodophenyl) tropan (β-CIT)

5.3.5.4. Protocol

Preparation, basic requirements and operational procedures are almost identical to those used in perfusion and metabolic studies. Intervention, for example audiovisual stimulation, task performance tests and complicated conditioning, are more widely used in neuroreceptor studies.

5.3.5.5. Acquisition setting

The same setting is used as for metabolic imaging.

5.3.5.6. Data processing and interpretation

This is similar to the metabolic study. Since the receptor study requires detailed spatial and timing information, the use of specific analysis and image fusion with an anatomically informative modality (e.g. MRI) is recommended.

5.3.5.7. Special notes for receptor imaging

(a) Neuroreceptors

Neuroreceptors are membrane bound proteins that bind to exogenously administered agents in addition to endogenously released neurotransmitters. There are two types of receptor:

(1) Those that are a part of the structure of the so-called ligand-gated channels that directly affect membrane potential and ionic permeability;
(2) Those that act by affecting intracellular second messengers via G proteins.

The benzodiazepine (γ-aminobutyric acid (GABA)-benzodiazepine complex) receptor is an example of a ligand-gated channel receptor and is part of a pore in the cell membrane that operates as an ion channel for the transport of chloride ions. Dopamine receptors are examples of the second type of receptor, which modulate the levels of intracellular second messengers such as cyclic adenosine monophosphate (CAMP).

(b) Ligands

Ligands for imaging are generally selected from agonists for, or against, neuroreceptors. They have no pharmacological effects because of the very small amounts administered. An ideal ligand for neuroreceptor imaging should demonstrate:

(1) High extraction across the BBB with rapid clearance from the blood;
(2) A high affinity constant;
(3) High specificity for the site of interest;
(4) No or small metabolites;
(5) Appropriate kinetics of binding.

Slow clearance from the sites of interest compared with non-specific sites of interaction is required for quantitation.

(c) Quantitation of neuroreceptors

In vivo PET imaging enables the quantitative measurement of the density of a receptor and the affinity of a ligand by means of compartment analysis. Quantitative analysis of receptor imaging is important for the interpretation of images and for a better understanding of the mechanism of neuronal disorders.

The in vivo distribution of a radiolabelled ligand is time dependent. The early distribution reflects the delivery of the ligand by the circulating blood. The specific binding of the labelled ligand with the target receptor gradually increases, to reach the maximum after a certain time lapse. Simultaneously, the ligands are dissociated or the label is released from the receptors. The freed substrates are then cleared from the brain by the blood. The dynamic equilibrium varies according to the characteristics of the labelled ligands and receptors. When the specific activity of the radiolabelled ligand is low, the receptor is easily occupied by the labelled ligand. The labelled ligands that bind to non-specific sites and remain in the blood may obscure the tracer activity specifically bound to receptors. On the other hand, if the specific activity is very high and the mass of injected ligands is small, most of the labelled ligands bind to receptors occupying only a fraction of the receptors. As a result, the free ligand pool remains nearly empty. Under such conditions, PET images continue to reflect delivery of the ligand by circulating blood or its transport across the BBB. Kinetics models permit the calculation of density (B_{max}, maximal binding capacity of receptor sites (pmol $\cdot$ g^{-1} $\cdot$ mL^{-1})), affinity ($1/K_d$, where K_d is the dissociation constant at equilibrium (nM)) and binding potential (B_{max}/K_d).

Many of the kinetic principles and models developed for PET have been applied to the analysis of receptor imaging by SPECT.

(d) Imaging receptors and their clinical application

For details of the various receptors, Table 5.15 should be consulted.

(1) Dopamine receptors

Dopamine was found to be involved in Parkinson's disease. This finding led eventually to the treatment of the disease by administration of the dopamine precursor, L-dopa. Many neurons secreting dopamine as a neurotransmitter are located in the substantia nigra, the limbic cortex, hippocampus, anteromedial frontal cortex and medial and lateral habenula. The direct action of dopamine on neurons is inhibitory. Dopamine receptors are classified into two groups, namely the D1 group (D1 and D5)

and the D2 group (D2, D3 and D4). Imaging of D2 receptors, which are located at the basal ganglia, has been investigated mainly in association with schizophrenia, Huntington's disease and pituitary adenomas. Imaging of D1 receptors with ^{123}I-SCH23982, which distributes in the corpus striatum, has also been studied.

(2) Benzodiazepine receptors

The use of benzodiazepines in clinical practice as anxiolytics, antiepileptics and sedatives was successful, since the benzodiazepines potentiate and prolong the synaptic actions of γ-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the mammalian brain. Imaging of a benzodiazepine receptor with ^{11}C-flumazenil and ^{123}I-IMZ, which are disseminated in the cerebral cortex, basal ganglia and thalami, has been studied in relation to neurological disorders such as epilepsy, anxiety, ethanol dependency, Huntington's disease, Parkinson's disease and hepatic encephalopathy, as well as strokes.

(3) Opiate receptors

Opiates have been recognized both for their effective relief of pain and for their mood altering properties. Three major opiate receptor subtypes exist: μ-receptors, δ-receptors and κ-receptors. The μ-receptors have a high affinity to morphine and related compounds, while δ-receptors have their highest affinity to encephaline. The κ-receptors are distinguished by their high affinity to dymorphins and certain benzomorphan synthetic opioids. Although multiple neurotransmitters and their receptors have been implicated in human epilepsy, it has also been observed that interactions of endogenous opioids with the μ-opiate receptor can produce both proconvulsant and anticonvulsant effects. Investigation of μ-opiate receptor imaging with ^{11}C-carfentanil, ^{11}C-diprenorphine and ^{18}F-acetylcyclofoxy, which are distributed in the basal ganglia, thalami, frontal cortex and temporoparietal cerebral cortex, can throw light on epilepsy and addiction.

(4) Muscarinic acetylcholine receptors

The muscarinic acetylcholine receptors are present in the CNS, myocardium, pancreas, salivary glands and intestinal smooth muscles. Autopsy samples have shown that the concentration of muscarinic acetylcholine receptor is altered in a number of neurological disorders such as Huntington's disease, Parkinson's disease, Alzheimer's disease and sleep disorders, although

there are conflicting reports concerning the receptor concentration changes. Imaging of the muscarinic acetylcholine receptor has been studied in patients with neurological disorders.

(5) Serotonin receptors

Serotonergic innervations and serotonergic receptors are widespread in the central and peripheral nervous systems, although little is known about the role of serotonin in human health and disease. Animal studies and clinical pharmacological investigations suggest that the serotonin system has an important role in mental disorders. Serotonin brain receptors can be divided into two subtypes on the basis of their affinities to serotonergic agonists and antagonists:

— The 5-hydroxytryptamine-1 (5-HT1: 1A, 1B, 1C, 1D, 1P) receptor class for those receptors displaying high affinity (dissociation constants in the nanomolar range for serotonin).
— The 5-HT2 class displaying low affinity (micromolar range) for serotonin but high affinity for selective serotonergic antagonists. Imaging of 5-HT2 receptors by [F-18] setoperone (which distributes in the striatum and cerebral cortex) has been investigated in patients with neurological and mental disorders.

(6) Dopa metabolism

Doperminergic neurons that project from the substantia nigra to the striatum are known to be involved in the control of movement. Parkinson's disease is characterized by nigral cell loss that gives rise to a profound decline in striatal dopamine levels. In the processes of dopamine metabolism, tyrosine hydroxylase is the initial enzyme in the biosynthetic pathways. This enzyme catalyzes the hydroxylation of tyrosine to form 3,4-dihydroxy-L-phenylalanine (L-dopa) and is located in dopamine synthesizing neurons. L-dopa is decarboxylated to dopamine by aromatic L-amino acid decarboxylase. The highest concentration of this enzyme is present in the striatal dopaminergic nerve endings. The conversion of tyrosine to L-dopa, and L-dopa to dopamine, is followed by dopamine uptake within storage vesicles in the nerve terminals. Imaging dopa metabolism with the L-dopa analogue 6-[F-18] fluoro-L-dopa has been studied in Parkinson's disease.

(7) Monoamine oxidase enzyme activity

Upon nerve activation, dopamine stored in vesicles in the terminal is released into the synaptic cleft. Monoamine oxidase located on the mitochondria is responsible for the catabolism of dopamine. Monoamine oxidase activity is related to psychiatric illness, and a number of intriguing studies link low platelet monoamine oxidase activity to vulnerability to psychiatric illness. Monoamine oxidase A and B, which are identified by their substrate selectivity and their sensitivity to different inhibitors, are subtypes of the enzyme. Monoamine oxidase A oxidizes 5-hydroxytryptaine and is selectively inhibited by clorgyline. Monoamine oxidase B oxidizes benzylamine and is selectively inhibited by L-deprenyl. Monoamine oxidase enzyme activity imaging has been used in patients with Parkinson's disease and Alzheimer's disease.

(8) Acetylcholine enzyme activity

In the cholinergic nervous system, acetylcholine released into the synaptic cleft is catabolized by acetylcholine esterase, inhibiting the effect of the neurotransmitters. Imaging of acetylcholine enzyme activity has been utilized for the early diagnosis of Alzheimer's disease.

(9) Neurotransporters

When an action potential reaches the nerve terminal, neurotransmitters stored in vesicles in the terminal are released into the synaptic cleft. Transporters in the presynaptic neuron are also responsible for the control (reuptake) of the neurotransmitters. Imaging of the dopaminergic transporter and acetylcholinergic transporter has been shown to be useful for the evaluation of Parkinson's and Alzheimer's disease, respectively.

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5.3.6. Radionuclide cisternography

5.3.6.1. Principle

After intrathecal injection, sterilized, pyrogen-free radiotracers circulate within the cerebrospinal fluid (CSF) from the lumbar level upwards to the cranial portion of the subarachnoid space. The tracer then flows along the convexity of the brain to the superior longitudinal sinus where it is absorbed with the CSF via the arachnoid villi. The process can be followed by external imaging devices.

The purpose of this section is to assist nuclear medicine practitioners in recommending, performing, interpreting and reporting the results of radionuclide cisternography.

5.3.6.2. Clinical indications

Investigations of patients should be made for the following purposes:

(a) To detect and differentiate obstructive and non-obstructive hydrocephalus;
(b) To detect other abnormalities in the subarachnoid space;
(c) To assess the patency of a shunt or pathway in surgical treatment of hydrocephalus;
(d) To detect the site and severity of a CSF fistula or leakage.

Studies are contraindicated in patients with severe intracranial hypertension, where there is a risk of brain herniation.

5.3.6.3. Radiopharmaceuticals

Both ^{99m}Tc and ^{111}In can be used to label DTPA. The labelled product must pass a series of quality control processes on sterility and apyrogenicity to ensure its safe intrathecal use. The common adult doses are 185–370 MBq (5–10 mCi) for ^{99m}Tc-DTPA and 18.5–37 MBq (0.5–1 mCi) for ^{111}In-DTPA.

5.3.6.4. Protocols

The appropriate protocols are the following:

(a) The patient should be well informed about the procedure.
(b) Preparations should be made for a standard lumbar puncture (regarding patient positioning, sterilization and anaesthesia).
(c) In the case of CSF leakage, a swab should be carefully inserted into each nostril.
(d) A lumbar puncture using the finest needle possible (22-gauge or finer) should be conducted.
(e) After measuring pressure and withdrawing samples of CSF, the tracer should be injected slowly and steadily.
(f) The patient should lie in the supine position with no pillow under the head, resting for four to six hours.

(g) Images should be taken, swabs removed, counts from the well counter recorded and results interpreted.

5.3.6.5. Acquisition setting

The following procedure is adopted:

(a) Radionuclide cisternography is usually undertaken in planar mode, although SPECT can be performed.
(b) The images are taken at 1, 2, 3, 6, 24 and 48 (^{111}In only) hours after injection.
(c) The displacement of radioactivity from lumbar to intracranial subarachnoid space is imaged.
(d) Multiple views (anterior, posterior, vertex and both lateral) of the head are needed to better visualize cisterna.

5.3.6.6. Data processing and image interpretation

The flow of activity in the spinal subarachnoid space is fast and smooth. A low activity segment is noted in the thoracic region due to a thickening of the spinal cord. The activity reaches the basal cisterna after one to three hours, and then enters the sylvian and interhemispheric fissures. The lateral views display the cisterna magna, quadrigemina, interpeduncularis, suprasellar and pontis. The distribution of activity on both sides is symmetrical in the anterior and posterior views.

After 24 hours, activity is distributed around the convexity of the brain, especially along the superior sagittal sinus. Most of the activity in the cisterna is cleared or diminished in intensity.

If any sign of lateral ventricles appears on the image, then communicating hydrocephalus, with normal pressure or obstructive in nature, is suspected. Interruption or loss of symmetry may indicate blockage of the CSF pathway.

Leakage or fistula is diagnosed if any activity is dispersed outside the outline of the subarachnoid space. Counting each swab from the nose cavity might help to locate the leak. To facilitate detection, the patient should lie sitting with the head in hyperflexion.

5.3.6.7. Precautions

The radiopharmaceutical must meet all quality requirements for intrathecal use. Two to three millilitres of 10% dextrose solution may be added to help injection.

5.4. NEPHROLOGY AND UROLOGY

5.4.1. Introduction

Renal radionuclide studies are commonly used procedures, particularly in paediatrics. The goal is to obtain reliable functional and structural information in a non-invasive way and to provide the clinician with both diagnostic and prognostic information.

5.4.1.1. Paediatric considerations

Nuclear medicine techniques are essential in the initial diagnosis and follow-up of many genito-urinary diseases in children, such as urinary tract infection (UTI), neonatal hydronephrosis and vesicoureteral reflux.

Renal function parameters such as GFR and renal plasma flow (RPF) are low in newborns; there is an increase in GFR from approximately 30 $mL \cdot min^{-1}$ per 1.73 m^2 body surface area at birth to a nearly adult value by two years of age. The tubules are even less mature than the glomeruli at birth, but maturation of the tubules is more rapid. Renal immaturity in neonates reduces to some extent the utility of radionuclide studies during the first months of life.

In infants, the relatively large extravascular space gives a low plasma concentration of any freely diffusible injected substance. This, together with renal immaturity, explains why ^{99m}Tc-DTPA scintigraphy in neonates is characterized by poor visualization of the kidneys, high background activity with low signal-to-noise ratio yet a rapid kidney transit time.

Using ^{99m}Tc-mercaptoacetyltriglycine (MAG3), the high protein binding (90%) ensures a high plasma concentration and a low extravascular distribution space. This, coupled with the greater extraction efficiency of ^{99m}Tc-MAG3 compared with that of ^{99m}Tc-DTPA, results in better renal delineation and a higher signal-to-noise ratio. Technetium-99m MAG3 is therefore the radiopharmaceutical of choice for dynamic renal studies in children, particularly below the age of two. High quality dynamic renal scans can be obtained with ^{99m}Tc-MAG3 from 2 to 4 weeks of age.

In paediatric studies, the dose of radiopharmaceuticals has to be scaled down. Table 5.16 suggests the fraction of the adult dose to be used in children. For convenience, only the body weight (BW) of the child needs to be known. The dose fraction is, however, based on body surface area.

TABLE 5.16. FRACTION OF ADULT DOSE USED FOR CHILDREN

BW (kg)[a]	Fraction of adult dose	BW (kg)	Fraction of adult dose	BW (kg)	Fraction of adult dose
3	0.10	22	0.50	42	0.78
4	0.14	24	0.53	44	0.80
6	0.19	26	0.56	46	0.82
8	0.23	28	0.58	48	0.85
10	0.27	30	0.62	50	0.88
12	0.32	32	0.65	52–54	0.90
14	0.36	34	0.68	56–58	0.92
16	0.40	36	0.71	60–62	0.96
18	0.44	38	0.73	64–66	0.98
20	0.46	40	0.76	68–70	0.99

[a] BW, body weight.

5.4.2. Renal scintigraphy with ^{99m}Tc DMSA

5.4.2.1. Principle

The functional mass of the kidneys can be demonstrated by means of Tc-99m dimercaptosuccinic acid (DMSA), a radiopharmaceutical that is taken up by the proximal tubular cells of cortical and juxtamedullary nephrons after extraction from the peritubular space and, to some extent, after filtration and reabsorption. Technetium-99m DMSA is deposited in the proximal tubule and is not released thereafter.

5.4.2.2. Clinical indications

Investigations with ^{99m}Tc-DMSA may be made for the following purposes:

(a) Detection of renal scars in the follow-up of UTI in children;
(b) Detection of parenchymal involvement during acute febrile pyelonephritis;
(c) Assessment of relative renal function when one kidney has poor function or a space occupying lesion;

(d) Detection of associated abnormalities: abnormal duplex kidneys, small kidneys, dysplastic kidneys and horseshoe kidneys;
(e) Distinguishing pseudotumours from tumours;
(f) Ectopic kidneys;
(g) Allergy to iodine contrast agents precluding radiological investigations.

5.4.2.3. Radiopharmaceuticals

Technetium-99m DMSA should be reconstituted with ^{99m}Tc-pertechnetate and used within 30 min. Oxidation of the product reduces tubular reabsorption and increases urinary excretion, so care should be taken to prevent oxidation. As renal function becomes more impaired, increasing liver uptake of DMSA occurs. The agent should be prepared according to the manufacturer's instructions for kidney studies.

5.4.2.4. Equipment

A single- or double-headed gamma camera is required with a low energy, high resolution (LEHR) parallel hole collimator, or a pinhole collimator for infants. SPECT is unnecessary for routine work but can be done as a complement. Data should be recorded in a 256 × 256 matrix into a computer for subsequent analysis. Alternatively the electronic zoom (factor 2) can be used for recording in a 128 × 128 matrix.

5.4.2.5. Patient preparation

An explanation to the patient and/or the child's parents should be given before the start of the study. For adults, no preparation is necessary. Infants should be fed before imaging. Anaesthetic skin preparation before an injection is advised for children. An intravenous injection of 80–100 MBq (2–2.5 mCi) of ^{99m}Tc-DMSA is given for adults. For children and infants, the dose is scaled down according to the body surface area; however, a minimum of 15 MBq (0.4 mCi) should be administered.

Imaging is performed between 2 and 4 hours after injection. A child should be cushioned comfortably against the camera face, and in an inclined or supine position. An infant should be cushioned and supported in place with Velcro strapping, lying supine on the face of the camera, which has been covered by a protective sheet.

In adults, the acquisition of 500 000 counts each of the posterior, anterior, left and right posterior oblique views is recommended. Additional oblique images are recommended for scan evaluation only. In children, a posterior

300 000 count acquisition is considered sufficient and the anterior view is needed only if one or both kidneys are displaced. A pelvic view is obtained if one or both kidneys are not seen.

5.4.2.6. Measurement

Relative function is determined from the background subtracted renal activities in the posterior view according to right or left/(right + left). In the case of displaced kidneys, the geometric mean of the background subtracted renal activities in the posterior and anterior views is used. The relative function may be inaccurate if there is pelvic retention of activity. Absolute uptake may be used as an indication of renal growth.

5.4.2.7. Interpretation

Normally the kidney contours are smooth and rounded with a contrast between the outer cortical part and the less active medial portion of the kidneys. A smearing of the uptake and 'soft' kidney contours may indicate patient motion during acquisition.

(a) The normal image variants are:
- A contour can be flattened without suggesting a lesion; this is particularly the case for the upper lateral aspect of the left kidney (splenic impression).
- In young children, the kidney may have a triangular shape, with flattened external sides.
- A 'slender' kidney, characterized by a short transverse axis in the posterior view, is usually normal and corresponds to a rotated kidney.
- The transverse axis is sometimes shorter at one pole (upper or lower), giving the kidney a pear shaped appearance.
- The poles, and particularly the upper pole, may appear diffusely hypoactive simply because of the contrast with the hyperactive columns of Bertin below the pole or because of respiratory movement of the kidneys.
- The number and size of the columns of Bertin differ from patient to patient.

(b) Abnormal DMSA scintigraphy:
- The relative function is outside the normal range of 45–55%.
- Areas of reduced uptake, without cortical defects, are relatively common. These kinds of defect are seen in acute pyelonephritis and may either heal or develop into permanent lesions. Dilated calyces

may cause parenchymal defects, as do cysts and space occupying lesions.
— The diagnosis of a 'scar' requires both a cortical defect and a subjacent parenchymal uptake defect for interpretation. The number and sites of defects should be recorded.
— The timing of imaging is important. Imaging at the time of urinary tract infection may show defects due to renal infection. Imaging three, or preferably six, months later will show whether these defects have healed or left scars. Scarring may be found without demonstrable vesicoureteric reflux, and reflux may be present without demonstrable scarring.
— Poor renal function leads to hepatic uptake.
— A suspected space occupying lesion on intravenous urography that shows normal DMSA uptake at that site is called a pseudotumour.

5.4.2.8. Pitfalls

In the case of a unilateral duplex kidney with normal parenchyma, the duplicated kidney may correspond to more than 55% of the total function. The single moiety kidney may then be erroneously classified as abnormal. Bilateral small kidneys may be overlooked.

Pelvic retention, in the case of hydronephrosis, may cause an artefactually high differential function. Imaging after 24 hours will give the true relative function.

5.4.3. Dynamic renal radionuclide studies

This procedure is also called renography for convenience, although modern gamma camera studies include much more than simply recording time–activity curves from the various regions of the kidneys.

5.4.3.1. Principle

The uptake by the kidneys of a tracer substance, its transit through the nephrons and its excretion into the pelvis and then through the urethers into the bladder are evaluated. The tracer should be non-reabsorbable and can be filtered by the glomeruli, excreted by the renal tubules or a combination of both. The amount filtered depends on the degree of protein binding of the agent in the plasma. The amount secreted depends on the affinity of the transport sites in the proximal tubules for the agent.

Changes of kidney activity with time are recorded and time–activity curves from the kidney regions are created (renograms). On the basis of renographic curves, descriptive indices or measurements related to renal physiology may be obtained (e.g. uptake function, transit time and outflow efficiency).

5.4.3.2. Clinical indications

Renography can be used for any of the following purposes:

(a) Measurement of the contribution of each kidney to global renal function.
(b) Evaluation of obstructive nephropathy and obstructing uropathy (for definitions see Section 5.4.5).
(c) Determination of the presence of renovascular disorder as a cause of hypertension.
(d) Evaluation of renal transplantation.
(e) Use prior to indirect radionuclide cystography (IRC).
(f) Investigation of lumbar pain.
(g) Investigation of acute or chronic renal failure.
(h) Investigation of renal disorder in patients who are allergic to contrast media.
(i) Renal trauma.

5.4.3.3. Radiopharmaceuticals

(a) Technetium-99m DTPA

This agent may be used as a marker of the GFR after injection into the plasma as it shows almost no protein binding and blood clearance takes place only by filtration. Technetium-99m DTPA is no longer the agent of choice for renal radionuclide studies except when GFR measurements are required, although it is low cost and readily available. It should be prepared according to the manufacturer's instructions.

(b) Technetium-99m MAG3

This is currently the radiopharmaceutical of choice for radionuclide renography. It is strongly protein bound and less than 2% of it is filtered by the glomeruli. It is tubularly excreted with about 65% of the efficiency of ^{123}I ortho-iodohippurate (^{123}I-OIH (Hippuran)). It should be prepared according to the manufacturer's recommendations. Some preparations have lipophilic

contaminants that are taken up and excreted by the liver, which increase with temperature and exposure to light after preparation. Thus, liver uptake is not dependent on renal function but on the impurities contained. These can be minimized by dividing the preparation after the boiling and cooling step into between two to five syringes, which are capped and kept in the refrigerator at 4°C for use as required.

(c) Iodine-123 OIH

This is an effective renal pharmaceutical which is rarely used now because of expense and reduced availability. It is actively excreted by the tubules and weakly protein bound with approximately 6% glomerular filtration.

(d) Iodine-131 OIH

This is no longer recommended for renal radionuclide studies, since ^{131}I is both a beta emitter and a gamma emitter with an energy unsuitable for gamma camera imaging. It requires a medium energy collimator and gives a high radiation dose to the kidneys in the case of obstruction.

(e) Technetium-99m EC

Technetium-99m ethylene di-cysteine (EC) is tubularly secreted to a greater extent than ^{99m}Tc-MAG3 and does not require a boiling step. It is less strongly protein bound than ^{99m}Tc-MAG3. It is also recommended as an agent of choice but is less easily available commercially.

5.4.3.4. Equipment

A large FOV gamma camera is required so that the left ventricle of the heart, the kidneys, the pelvis and the proximal ureters are in the FOV. A low energy, parallel hole collimator with high resolution is preferred for the most widely used ^{99m}Tc agents. The data are transferred to a computer on-line in a 128 × 128 or 64 × 64 matrix. Electronic zoom should be used for small children.

5.4.3.5. Procedure

The procedure should be explained to the patient or parents before entering the gamma camera room. An anaesthetic cream can be applied to relieve discomfort of the venipuncture. The patient should be hydrated before the study. Normally, 500 mL of water is given to the patient half an hour before

the procedure so that the patient is hydrated or slightly overhydrated. Parenteral hydration can also be used. The bladder should be emptied before entering the camera room and the time should be noted. In infants unable to void on demand, bladder emptying will be spontaneous so catheterization is not usually needed.

The patient should void again at the end of the test, and the volume and time noted to give a measure of the urine flow. Diuresis should ideally be in the range of 1–3 mL min^{-1}.

The patient should lie in the supine position on the couch with a camera positioned below or preferably reclining against the camera face, which is set 15° off the vertical so that the kidneys drop back. This is the most comfortable position and allows free gravitational drainage of the pelvis and easy observation of any tendency for the kidneys to descend. In children, the study is performed more easily if the patient is lying in the supine position on the couch. Velcro straps, sandbags or an inflatable cushion can be used to reduce movements. The bladder should be in the FOV whenever possible, particularly in the case of children. An image of the pelvis and bladder before and after micturition and/or after five minutes in the upright position to ensure gravitational drainage is recommended in the event of pelvic retention at the end of the study.

The injection consists of 200 MBq (5 mCi) ^{99m}Tc-DTPA, 100 MBq (2.5 mCi) ^{99m}Tc-MAG3, 100 MBq (2.5 mCi) ^{99m}Tc-EC or 80 MBq (2 mCi) ^{123}I-Hippuran. It should be given as a bolus. For children, the adult dose is scaled down on a surface area basis, with a minimum of 20 MBq (0.5 mCi) for ^{99m}Tc-DTPA and 15 MBq (0.4 mCi) for ^{99m}Tc-MAG3.

The arm should be abducted and a deep antecubical vein chosen for puncture. The injection should be less than 1 mL in volume and either given rapidly or pushed by a bolus of saline through a three way stopcock. The injection should be given in one single continuous movement of the syringe plunger.

Acquisition should last for a minimum of 30 min.

A flow study is not generally useful unless vascular problems are suspected. If it is performed, one frame per 2 s for the first 60 s is suggested. The rest of the study should be recorded at one frame per 10 s.

The use of frame times greater than 15 s reduces the temporal resolution of the study so that the sharpness of the peak of the renogram and the quality of the analysis can be impaired.

At completion, the study should be reviewed in cine-mode and background corrected time–activity curves generated from kidney ROIs.

5.4.3.6. Interpretation

A holistic approach to interpretation should be made combining images, renograms, numerical results and interventions (see below).

A report should contain the demographic data, the name of the test, type and activity of the injected radiopharmaceutical, any interventions and any patient reactions (e.g. fainting). It should also include a description of the images and curves, the numerical data, a separate conclusion and a separate recommendation or clinical advice when appropriate.

A description of the images should consider relative renal size, cortical or parenchymal defects and retention of activity in the parenchyma or pelvis. Unusual anatomy features such as an ectopic, duplex or horseshoe kidney should be recorded.

Normal renogram curves are symmetric in shape and height, and three phases can be identified: an uptake phase with rapid upslope, a parenchymal transit phase with less pronounced upslope ending in a peak of maximum activity, and an excretion phase.

The background subtracted renograms should be described in terms of:

— The characteristics of the uptake and parenchymal phases;
— The presence and sharpness of the peaks;
— Whether the peaks occur at the same time (time to maximum activity);
— The shape of the third phases, or the continuing rise of the curve with no excretion phase.

The relative function considering the normal range of 43–57% for each kidney should be noted. If there is a duplex kidney, the relative function of the upper and lower portions should also be given. The report should also note the peak time and the difference in peak time.

There are various measurements that can be made from the time–activity curve to characterize its shape, typically ratios of one point on the second phase or peak activity time and one point on the third phase. These may be helpful in straightforward cases but give disappointing results when renal function is poor or in more complex cases.

Besides relative function, there are other physiological measurements that can be done. Firstly, there are the times for the tracer to reach the nephrons, cortex and pelvis. These are measured as transit time indices, for example whole kidney transit time, mean parenchymal transit time (MPTT), pelvic transit time, parenchymal transit time index (PTTI) and minimum transit time. A whole kidney transit time index (WKTTI), which is the combination of PTTI and pelvic transit time, may also be stated. Secondly, there is outflow

efficiency, which compares the cumulative output and input of the kidneys. The value is given as a percentage for a specified time, usually 30 min, and has the merit of being independent of the level of renal function.

Excretory indices (time to half-peak of the third phase and the ratio of peak time to time of injection (*T*) plus 20 min) take no account of the level of renal function, and NORA (the ratio of the 2 min value to the 20 min value) should be used with caution. The following are normal values:

Peak time:	2.5–5 min depending on hydration
Whole kidney transit time:	2.5–5 min
MPTT:	<240 s
PTTI:	<156 s
Pelvic transit time:	20–75 s
WKTTI:	<170 s.

Outflow efficiency is calculated as the percentage of the activity entering the kidney that is discharged in 30 min. The normal value is above 78%. It is independent of the level of renal function but is affected when MPTT is very prolonged.

5.4.4. Interventions — renovascular disorder

5.4.4.1. Principle

Renovascular hypertension (RVH) is the name given to hypertension caused by renovascular disorders. Correction of this disorder in one kidney leads to a normalization of blood pressure, provided the other kidney is functioning normally. This can be determined by a renal radionuclide study.

Renovascular disorders may be symmetrical when caused by systemic pathology such as glomerulonephritis, diabetes, autoimmune diseases and accelerated hypertension. It may be asymmetrical when caused by small vessel disease such as in pyelonephritis, tuberculosis, endarteritis, amyloid or renal vein thrombosis and large vessel disease, for example unilateral or bilateral renal artery stenosis or fibromuscular hyperplasia, or in association with a resistance to outflow. The features of renovascular disorder are a reduced relative function, an impaired second phase of the renogram, a delayed peak of over 60 s compared with the contralateral kidney and a prolonged mean parenchymal transit time of over 240 s. These abnormalities may be revealed or enhanced by the use of Captopril.

5.4.4.2. Captopril intervention

Captopril is an angiotensin II converting enzyme (ACE) inhibitor that can be used as a stressor to the kidneys, exaggerating or producing renal dysfunction in the case of renovascular disease but not in essential hypertension.

Essential hypertension is associated with increased vasoconstriction of the afferent arterioles of cortical nephrons, which is partly under angiotensin II control. Captopril inhibits this vasoconstriction and increases the blood flow in essential hypertension, thereby improving the uptake function, reducing peak time, restoring a normal MPTT and improving the shape of the renogram.

Renovascular disorders are made worse with Captopril. There is no action on the afferent arterioles, which are maximally dilated through autoregulation in response to the renovascular disorder. It has a major effect on circulating Angiotensin II, inhibiting its vasoconstrictor action on the efferent arterioles of primarily juxtamedullary nephrons, which are thickly muscled, whereas those of the cortical nephrons have almost no muscular layer in humans. Captopril of 25 mg strength given orally is appropriate. Blood pressure is monitored before and at 5 min intervals after the oral administration of Captopril. If the diastolic pressure falls by 10 mmHg or more during the subsequent hour, this is an indication that Captopril has been absorbed and the test may be started. If this is not the case, the test is started after one hour. It is sometimes recommended that the patient fasts for at least four hours before the Captopril test, during which time a normal amount of fluid is given to assure hydration. Infusion of saline during the study is not necessary unless it is known or suspected that the patient is salt depleted, in which case a severe hypotensive response may be observed. Intravenous Enalapril (2.5 mg) can also be used.

5.4.4.3. Patient preparation

The patient should be taken off ACE inhibitors for at least seven days, and angiotensin II inhibitors and diuretics for at least two days, in order to reduce the likelihood of salt depletion. Other hypotensive therapy needs no interruption.

5.4.4.4. Interpretation

The images may show parenchymal retention of activity at the side of the renovascular disorder, persisting longer after use of Captopril compared with a baseline study because the absence of filtration fluid precludes washout of the tubulary secreted agents. With DTPA, however, since it is filtrated by the

glomerulus, disappearance of the affected kidney or a significant decrease in uptake may be noted. Numerical indices such as the corticopelvic transfer time (measuring the time of first appearance of activity in the kidneys and the first appearance of activity in the pelvis) may be recorded and compared between baseline and Captopril values.

The time–activity curve should deteriorate in shape in comparison with the baseline; in particular there should be impairment of the second phase, further prolongation of the peak time and deterioration or absence of the third phase. The relative function should usually fall by 5%, although some authors require a 10% fall for a positive diagnosis of RVH.

MPTT should increase to over 240 s, or 60 s more than the baseline value. If unilateral renovascular disorder is suspected, the contralateral kidney should show a normal renogram and indices.

5.4.4.5. Clinical indications

Renovascular disorder may be suspected in the following situations:

(a) Hypertension that becomes more difficult to control or resistant to medical therapy;
(b) Hypertension occurring before the age of 35;
(c) Deterioration of renal function during therapy with ACE inhibitors;
(d) Accelerated hypertension;
(e) Deteriorating renal function of no obvious cause;
(f) Evidence of vascular disease in other organs;
(g) Renal artery narrowing shown incidentally by angiography done for another reason;
(h) Abdominal bruit.

It should be recognized that renal artery stenosis, common in the elderly as a result of atheroma, might co-exist with essential hypertension, which is also very common in this population. This does not mean that renal artery narrowing, as seen on renal artery angiography, is the cause of renovascular disorder or hypertension. Only renal radionuclide studies can distinguish whether narrowing of a renal artery is functionally significant.

A baseline study may be conducted in patients on regular ACE inhibitor medication, but if it is abnormal a true baseline study should be performed once the patient has been taken off ACE inhibitors for one week. An improvement in the result would indicate that ACE inhibitors are having a detrimental effect on kidney function, pointing to the presence of renovascular

disorder. A Captopril renal radionuclide study should then be performed to confirm that the deterioration can be ascribed to an ACE inhibitor.

Diabetic nephropathy is a common cause of renovascular disorder. This may be due to small vessel disease although additional large vessel disease can be present. When the response to Captopril is symmetrical, small vessel disease is most likely.

An asymmetrical response to Captopril, with the poorer kidney having initially a prolonged MPTT of more than 240 s, which becomes further prolonged over 300 s, suggests the presence of functionally significant renal artery stenosis. Improvement of the study over the baseline is an indication that ACE inhibitors will not cause deterioration of renal function in diabetes when used to treat proteinuria, which will help to delay the progression of diabetic nephropathy.

5.4.5. Diuretic renography

5.4.5.1. Principle

Furosemide is a potent diuretic which inhibits the reabsorption of salt and secondary water in the ascending limb of the loop of Henle. Its diuretic action is dependent on the level of renal function, particularly the number of nephrons in the kidney, the absence of both sodium and chloride depletion, and the absence of hypotension.

5.4.5.2. Definitions

Dilatation of the collecting system does not necessarily mean obstruction. It may, for example, be due to reflux or previous damage to the pelvis. Absence of dilatation does not exclude obstruction, particularly in an oliguric patient. Obstruction can be defined as an increased resistance to urine outflow.

The following definitions may be helpful:

(a) Obstructing uropathy is a change in the outflow tract due to an obstructing process.
(b) Obstructive nephropathy is the effect of an obstructing process on the kidney function.
(c) An obstructing process is an increase above normal of the resistance to outflow, which may be chronic. There is still fluid flowing down the ureter in the presence of a chronically increased resistance to outflow. The resistance to outflow has two main effects. Firstly, the intratubular luminal pressure is marginally greater (fractions of a millimetre of water)

than the peritubular capillary pressure so that the passive component of salt and water reabsorption is enhanced. This slows the flow and prolongs the transit time, in particular the PTTI of non-reabsorbable solutes such as ^{99m}Tc-MAG3 and ^{99m}Tc-DTPA. Secondly, the resistance also causes a reduction in the amount of excreted activity compared with the amount that has been initially taken up. This is demonstrated by a reduction of outflow efficiency to below 78%. The consequence of these processes is that the third phase of the time–activity curve fails to fall as rapidly as expected or may even continue to rise.

5.4.5.3. Clinical indications

Clinical indications for diuretic renography are:

(a) Suspected obstructive nephropathy or obstructing uropathy associated with hydronephrosis or renal stones, malignancy or retroperitoneal fibrosis, etc.
(b) In children, suspected vesicoureteral or pelvo-ureteral stenosis is a common indication.

5.4.5.4. Procedure

The procedure should be explained to the patient and, in the case of a child, to the parents or carers. The patient should be well hydrated and not salt depleted. Some authors recommend infusion of saline and bladder catheterization in children. This is an area of controversy, however, and there are several drawbacks. It makes the study invasive and unpleasant for the child and there is a significant risk of ascending infection in children with gross reflux. This subgroup requires intravenous antibiotics after catheterization: the usual single dose per oral prophylactic regimen is insufficient. Ideally, the child should be allowed to void naturally.

The usual dose of Furosemide for an adult is 40 mg administered intravenously, while for a child the dose is 0.5 mg/kg. In children under one year of age, it is better to give 1 mg/kg due to immaturity of the tubular cells.

Furosemide is usually given intravenously 18–20 min after the start of the study through a three way tap. This is the so-called F+20 protocol. In children and occasionally in adults it may be necessary to wait 25–30 min until the pelvis appears maximally dilated before injecting the diuretic. Diuresis usually starts within 2 min of the administration. Some authors recommend administering Furosemide simultaneously with the injection of the radiopharmaceutical,

particularly if an earlier study has been equivocal (F0). Others recommend that it should be given 15 min before the study (F–15).

Administration 18–20 min after injection of the radiopharmaceutical (Section 5.4.3) permits recording a baseline study followed by the diuretic response, facilitating interpretation. However, F0 or F–15 protocols may enhance sensitivity.

5.4.5.5. Interpretation

Since the amount of activity leaving a kidney cannot be greater than the amount of activity entering the kidney, it is useful to compare the second phase with the third phase. If the third phase is appropriate to the second phase, then there is unlikely to be a resistance to outflow, whereas if the third phase is inappropriately reduced in comparison with the second phase, resistance to outflow is likely. Excretory indices, which only consider the third phase and not its relation to the second phase, may incorrectly suggest outflow disorder when renal function is poor and the absence of outflow disorder when renal function is good. It is, therefore, better to report a Furosemide response as appropriate, not appropriate or indeterminate rather than as good or poor. Excretion indices are not recommended. NORA may be measured if renal function is good.

Outflow efficiency compares renal input with renal output and gives a numerical result that aids this interpretation. PTTI is prolonged in obstructive nephropathy due to the increased salt and water reabsorption in the proximal tubules and reduced intraluminal flow. MPTT is shortened, reflecting the loss of concentrating ability of the medulla. These are typical features of an increased resistance to outflow.

Using the F+20 protocol, O'Reilly's classification of diuretic renograms can be applied, according to the shape of the curve: Type I (normal baseline renogram), Type II (obstructive), Type IIIa (dilated, non-obstructive), Type IIIb (equivocal or partially obstructed).

5.4.6. Radionuclide cystography

5.4.6.1. Principle

In the presence of reflux, activity in the bladder moves through the incompetent vesicoureteric valve towards or into the renal pelvis. This typically occurs during micturition but can be observed during passive repletion. The valve may be incompetent due to a congenital abnormality or become

incompetent due to a urinary tract infection, neurogenic bladder or other cause.

5.4.6.2. Clinical indications

The indication of a reflux study is usually limited to patients with recurrent urinary tract infection, usually children. The presence of reflux is likely to increase the risk of recurrent renal infection and renal scarring, and may eventually lead to renal failure.

Vesicoureteric reflux may be graded by X ray cystography (VCUG):

Grade 1: reflux into the distal ureters;
Grade 2: reflux into the renal collecting system without calyceal dilatation or blunting;
Grade 3: Grade 2 but with pelvic dilatation;
Grade 4: Grade 3 but with greater dilatation of pelvis and calyces with some clubbing;
Grade 5: Grade 4 associated with severe tortuosity of the ureters.

The anatomical definition is made by VCUG but this involves catheterization with a radiation dose to the bladder and genital organs of about 100 times higher than that with a radionuclide technique. It is required also to decide whether surgery for reflux is indicated and for follow-up.

A radionuclide cystography may be done either directly or indirectly. During direct cystography (DRC) the activity is instilled into the bladder, typically ^{99m}Tc sulphur colloid or ^{99m}Tc-pertechnetate, by means of a transurethral or a suprapubic catheter. This is the radionuclide equivalent to VCUG. IRC is performed during micturition with the activity accumulated in the bladder after a conventional intravenous renographic study.

5.4.6.3. Radiopharmaceuticals

For DRC, a catheter is inserted under fully aseptic conditions. Technetium-99m sulphur colloid is prepared according to the manufacturer's instructions and is instilled into the bladder either by slow infusion or after an instillation of 20 mL physiological saline followed by the colloid (20 MBq/kg) and then a further volume of 20 mL saline.

Technetium-99m MAG3 is recommended for indirect cystography. The minimum intravenous dose is 20 MBq (0.5 mCi).

5.4.6.4. Procedure and equipment

A full explanation of the procedure is given to the patient or the parents and child if feasible. Older children are studied sitting on the commode with the camera behind the back covering the kidneys and the bladder areas.

Infants are better studied lying supine, for reasons of safety, on the face of the camera, with an impermeable sheet over the collimator.

During direct radionuclide cystography, the bladder is slowly filled to capacity. The filling rate should be adjusted so that the process takes not less than 10 min. Micturition may occur spontaneously or in association with pressure over the lower abdomen. The filling volume should be adjusted to the child's size. Overfilling should be avoided. Direct cystography can be performed on a child of any age.

In performing indirect radionuclide cystography, the renal radiopharmaceutical is allowed to accumulate in the bladder. When the child is willing to void, he or she is placed on the commode in front of the camera. Indirect cystography should only be performed on toilet trained children.

Data acquisition is done in a 64×64 matrix with a frame rate of 2 s (range 1–5). Zoom may be used for small children. In DRC, acquisition should be performed both during the passive (vesical filling by instillation) phase and the active (voiding) phase.

5.4.6.5. Interpretation

The acquisition may be reviewed in slow cine-mode. In DRC, any increase in activity in the renal pelvis area may indicate reflux. ROIs are set up over the area of the kidneys and the bladder, and activity–time curves are generated at 10 s per frame. An upward deflection of the renal curve is indicative of reflux. Very low background activity in the renal area makes it easy to identify any refluxing activity during DRC. When activity from the renographic phase is still retained in the pelvis, it may be difficult to detect refluxing activity in IRC. During the renographic study, reflux may occasionally be observed, particularly in adults, as a sudden transient increase of renal activity during the third phase and in the images. There is no generally agreed grading system for radionuclide cystographies, although a system similar to X ray cystography has been attempted.

5.4.7. Renal transplantation

5.4.7.1. Principle

Renal transplantation can be performed from either a live donor or a cadaver. In the case of a cadaver, the kidney demonstrates acute tubular necrosis on transplantation and the recovery of blood flow can be monitored with radionuclide renography by serial measurements using the perfusion index. Both types of transplant may suffer rejection, which usually starts at about seven days and is associated with a progressive reduction in blood flow. Similar findings are seen with Cyclosporin toxicity but tend to occur in the first few days if the initial doses are too high. Toxicity that occurs at a later stage cannot be distinguished from rejection. The perfusion index should continue to improve as the transplanted organ improves function. A failure of the perfusion index to improve or a deterioration indicates a reduction in renal function, although the cause cannot be elucidated by this means. Septicaemia can lead to a reduction in renal function and an increase in the perfusion index.

Other early complications seen by renal radionuclide studies after transplantation include renal artery or venous thrombosis, which gives an unperfused kidney or 'black hole' at the background activity. A haematoma causes a halo around the transplant, and lymphocele produces a defect in activity. Leakage of urine may be seen when there is a failed anastomosis or ureteric rejection, which can cause an increased resistance to outflow without pelvic dilatation. Another later complication is obstructing uropathy; again there may be abnormal resistance to outflow without pelvic dilatation so that ultrasound may miss this diagnosis. Renal artery stenosis at the anastomosis usually develops later with worsening or new onset of hypertension. The Captopril study is positive if the transplant causes hypertension, but this is not fully reliable.

5.4.7.2. Clinical indications

Radiography studies may be made after a renal transplantation for the following purposes:

(a) Evaluation of the progress of the transplant shortly after the operation;
(b) Evaluation of the transplant for chronic rejection, drug toxicity or renovascular hypertension.

5.4.7.3. Radiopharmaceuticals

The following radiopharmaceuticals are used in renal studies:

(a) Technetium-99m MAG3 is preferred (see dynamic renal radionuclide study).
(b) Technetium-99m DTPA is less satisfactory in a poorly functioning kidney, and may give an initial spike (vascular activity artefact) immediately after injection.
(c) Technetium-99m DMSA may be used to show cortical defects caused by peripheral infarction or scarring, but is not the radiopharmaceutical for routine evaluation of a renal transplant.

5.4.7.4. Procedure and equipment

It is important to place the gamma camera over the correct side of the transplant. The gamma camera is set up as for a renal dynamic radionuclide study.

Patients with renal disease often have poor veins. Since a good perfusion index requires a bolus of activity, permission may need to be sought to use the arteriovenous fistula, which is optimal for a good bolus. If there appears to be no activity in the transplant, it is important to monitor the injection site with the gamma camera to check for extravasation of the dose.

Imaging should be performed within the first 24–48 hours of the transplant, to verify perfusion and to serve as a baseline study. It should be performed every second or third day to monitor the improvement of the perfusion index. At each imaging session it is important to determine activity in the bladder and the catheter bag. The study should be performed before, and not after, dialysis.

The data are recorded at one frame per second for 60 s, followed by one frame per 10 s up to 30 min. The data are collected in a 64 × 64 or 128 × 128 matrix after an injection of up to 300 MBq (8 mCi) of ^{99m}Tc-MAG3. ROIs are drawn around the kidney and over the iliac artery distal to the kidney and a background region, which may be on the contralateral side or peripheral to the kidney. If the transplant overlaps the iliac artery, ROIs should be drawn excluding the artery. The ROI over the iliac artery should be within its boundaries and not stray outside the vessel. Activity–time curves should be displayed to compare the shape of the arterial curve and the transplant curve. In an ideal situation, the initial upslopes of the two curves should be similar. The worse the perfusion of the transplant, the lower the slope of the renal curve in comparison with the arterial activity curve.

The perfusion index may be calculated from the peak of the iliac artery time–activity curve. A vertical line is drawn through the curve of the transplant and the slopes of the two curves are compared. The iliac vessel slope divided by the renal slope multiplied by 100 gives the perfusion index. It is in fact a resistance index, which increases as renal function deteriorates and decreases as renal perfusion improves.

5.4.7.5. Interpretation

The images are viewed with particular attention to the early phase (0–30 s). A transplant uptake that appears more intense than that of the iliac artery is considered to be associated with good early perfusion. If the transplant and the iliac artery have the same activity, this demonstrates moderate early perfusion, while a transplant with lower uptake than the iliac vessel demonstrates poor perfusion. Good and moderate perfusion are usually seen in acute tubular necrosis, whereas moderate to poor early perfusion is seen in established rejection. Images are scrutinized for defects that can be caused by a branch artery infarct. The time of appearance of urine in the ureter or bladder is noted. Lack of urinary activity in the first 30 min is typical of acute tubular necrosis, and as the kidney recovers the time to urinary activity shortens.

Native kidneys can occasionally be seen according to pre-transplant functional status. Urinary activity can thus be detected even if the transplant has no excretion.

(a) Acute tubular necrosis

Shortly after transplantation, acute tubular necrosis shows moderate to good early perfusion with a parenchymal image that persists throughout the study. Serial perfusion index determinations show a gradual reduction of the number towards 100%. Any superimposed adverse event such as rejection, septicaemia or Cyclosporin toxicity will have a negative effect on the improvement of the perfusion index.

(b) Rejection

Hyperacute rejection is rarely seen but is associated with poor early perfusion and a high perfusion index. Rejection usually has its onset at about 7 days and is characterized by a reduction of uptake on the early image, moving from moderate to poor early perfusion. The time of urinary activity appearance, however, may not change. The image may take on a mottled

appearance as rejection becomes more marked. The response to treatment is a fall in the perfusion index towards 100%.

(c) Cyclosporin toxicity

This is associated with moderate early perfusion, a rising perfusion index and impairment of renal function. It may occur even if serum Cyclosporin levels are satisfactory. It is difficult to distinguish from rejection.

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5.4.8. Clinical measurement of the glomerular filtration rate

5.4.8.1. Principle

GFR tracer substances, for example ^{51}Cr-EDTA or ^{99m}Tc-DTPA, are given intravenously. The distribution of these tracers is in the extracellular space. They are almost exclusively excreted via the kidneys by glomerular filtration. In adults with normal renal function, final distribution is attained after two hours and the excretion can then be described by a monoexponential function.

5.4.8.2. Clinical indications

The clinical indications for measuring the glomerular filtration rate are:

(a) Investigation and evaluation of chronic nephro-urological diseases;
(b) In conjunction with renography before surgery on the kidneys and/or the urinary tract;
(c) Evaluation in association with transplantation;
(d) Monitoring of renal function during treatment with potentially nephrotoxic pharmaceuticals;
(e) For dose calculation of potentially toxic pharmaceuticals that are mainly excreted by the kidneys.

5.4.8.3. Radiopharmaceuticals

Chromium-51 EDTA is the agent of first choice and probably allows the most precise GFR measurements among available tracers.

Technetium-99m DTPA also allows very accurate values and is more available than ^{51}Cr-EDTA. Its use, however, requires standardization, since the amount of protein binding varies among different manufactures. Significant protein binding makes a tracer unsuitable for GFR studies.

Iodine-125 iothalamate can be used but it is a high-osmolar ionic contrast agent whose intravenous administration some countries no longer endorse.

Adults are given 4 MBq ^{51}Cr-EDTA, 10 MBq ^{99m}Tc-DTPA or 4 MBq ^{125}I-iothalamate. Children receive 0.074 MBq/kg body weight (maximum 4 MBq) ^{51}Cr-EDTA, 5–7 MBq ^{99m}Tc-DTPA or 2–3 MBq ^{125}I-iothalamate. The minimum volume of injection is 1.0 mL.

5.4.8.4. Equipment

The equipment required for glomerular filtration rate studies is:

— A Venflon hypodermic needle;
— A three way stopcock;
— A 100 mL volumetric flask;
— A 50 mL volumetric flask;
— Glass counting vials;
— A 1 mL tuberculin (TB) syringe;
— A 1.000 mL precision pipette;
— Water;
— A centrifuge;
— A dose calibrator;
— Counting vials;
— A gamma ray vial counter (well type).

5.4.8.5. Patient preparation

Patients should be in a well hydrated condition. It is desirable for children to come to the laboratory with an intravenous route already established.

5.4.8.6. Procedure

(a) Strategy for drawing blood sample(s)

The number of blood samples and the timing for drawing of the samples should be based on an estimation of the expected clearance (GFR) value.

A preliminary estimation of GFR can be made by entering the weight, age, sex and serum creatinine into a Kampmann nomogram (Appendix 2). For reasons of feasibility, the blood sample needed for the determination of serum creatinine can be taken together with the blood sample that is taken for determination of background activity immediately before injection of the radiopharmaceutical.

Patients with an expected GFR of more than 30 mL/(min · 1.73 m^2) should be examined through a single sample method. The plasma sample should be drawn 4–5 hours after injection in adults and after 90–120 min in children.

Patients with an expected GFR of less than 30 mL/(min · 1.73 m^2) should, as the first choice, be studied with a procedure that includes urine collection. Urine sampling should always be included in the procedure for patients with oedema and ascites.

Alternatively, for patients with renal failure, their GFR can be calculated from a single plasma sample taken after 24 hours (^{51}Cr-EDTA only), or with a multisample method.

When the estimated GFR is 15–20 mL/(min · 1.73 m^2), blood samples should be taken between 3 and 5 hours post-injection. When the estimated GFR is less than 15 mL/(min · 1.73 m^2), blood samples should be obtained 5 and 24 hours post-injection.

Formulas for calculation according to the single sample and multisample methods are presented in Appendix 1 to this section.

(b) Urine collection

When a urine collection technique is utilized, urine should be collected at 2–3 and 3–4 hours with blood samples at 150 and 210 min. Correction for the time delay due to the transport of urine from the kidneys to the bladder in the well hydrated patient can be made as follows:

$$\text{Time delay (min)} = 3.6 + 2.6 \times [\text{amount over time (mL/min) of diuresis}]^{-1}$$

When a urine collection is made, the amount of residual urine in the bladder should be estimated.

(c) Correction for body size

Plasma concentrations should be corrected to the concentration expected in an individual with a body surface area of 1.73 m^2. This can be done by the equations from Ham (see Bibliography to this section).

Procedural issues concerning preparation of standards, injection of radiopharmaceutical, drawing of blood samples and centrifugation are described in Appendix 2 to this section.

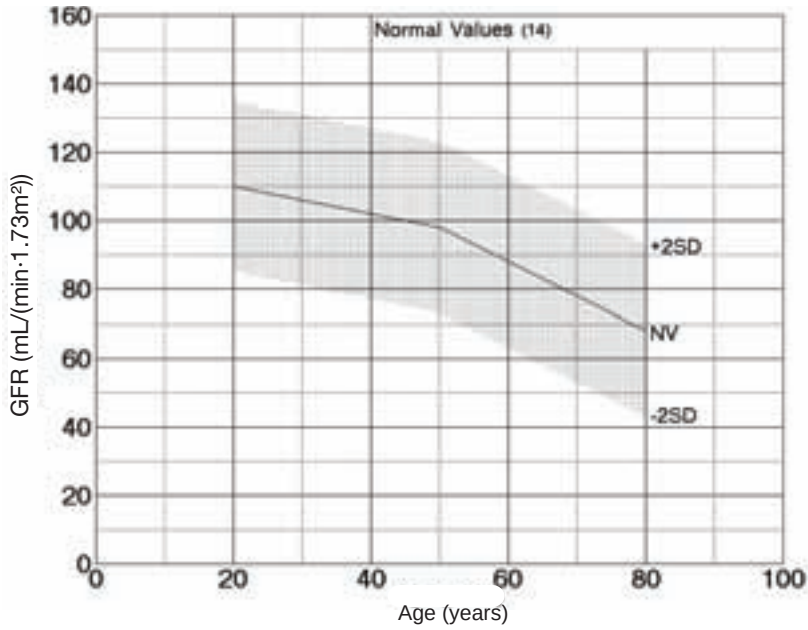

FIG. 5.1. Normal values of GFR (SD stands for standard deviation and NV for normal value).

5.4.8.7. Interpretation

Interpretation should be made with reference to a set of normal values (Fig. 5.1). Children aged 2 years and above should have a kidney function corresponding to that of a 20 year old, provided the result is normalized to a body surface area of 1.73 m^2. For children younger than 2 years, special reference values should be used.

The 95% confidence interval for the day-to-day variation of a single GFR measurement in adults is ±14%.

Appendix 1 to Section 5.4.8

FORMULAS FOR CALCULATION OF GFR

(a) Multisample method in adults and children

The plasma time–activity curve usually fits a two exponential model. It is, however, only necessary to determine the slow exponential component, since the contribution of the fast component to the total area under the curve is small and can be corrected for.

The first step of the multisample method is to calculate a preliminary clearance, Cl_p, as

$$Cl_p = Q_0 / (A/b)$$

where Q_0 is the injected amount of radioactivity, b is the slope of the slow component, as determined from one blood sample drawn at the beginning and another drawn at the end of the recommended time interval for drawing of the blood samples, and A is the intercept of the extrapolated slow component with the y axis.

In adults the final clearance, Cl_f, can then be obtained by insertion of Cl_p into:

$$Cl_f = 0.99Cl_p - 0.0012Cl_p^2$$

In children, the formula to be recommended is

$$Cl_{ch} = 0.87Cl_p$$

(b) Single sample methods in adults

(1) Clearance of ^{99m}Tc-DTPA in adults is calculated using the following formula:

$$Cl'_n = \frac{-\ln\left(C(t)\frac{V}{Q_0}\right)V}{t\,g(t)_{corr}(n-1)}$$

where

V = $8116.6A - 28.2$
$g(t)_{corr}(n-1)$ = $(1.70 \times 10^{-6}t - 1.20 \times 10^{-3})Cl_{n-1} - 7.75 \times 10^{-4}t + 1.31$
$C(t)$ = background subtracted plasma activity in the sample taken t minutes after the injection
Q_0 = injected activity
V = ^{99m}Tc-DTPA distribution volume (mL)
A = body surface area (m^2)

Start by calculating $g(t) = 9.55\exp(-0.0336t) + 1.18\exp(-0.000461t)$ to yield an initial value Cl′. Use this initial value to calculate an initial $g(t)_{corr}$ value. Insert this value in the formula above and iterate until $Cl'_n - Cl'_{n-1} < 0.1$.
This formula is applicable for any $t > 180$ and < 300 min.

(2) Clearance of ^{51}Cr-EDTA in adults is calculated using the following formula:

$$Cl'_n = \frac{-\ln\left(C(t)\frac{V}{Q_0}\right)V}{tg(t)_{corr}(n-1)}$$

where

V = $10800A - 5578.6$
$g(t)_{corr}(n-1)$ = $(-4.18 \times 10^{-6}t + 6.43 \times 10^{-4})Cl_{n-1} + 1.60 \times 10^{-6} \times t^2 - 0.00103t + 1.25$
$C(t)$ = background subtracted plasma activity in the sample taken t minutes after the injection
Q_0 = injected activity
V = ^{51}Cr-EDTA distribution volume (mL)
A = body surface area (m^2)

Start by calculating $g(t) = 0.324\exp(-0.0121t) + 1.13\exp(-0.000289t)$ to yield an initial Cl′ value. Use this to calculate an initial $g(t)_{corr}$ value. Insert this value in the formula above and iterate until $Cl'_n - Cl'_{n-1} < 0.1$ mL/min. This formula is applicable for any $t > 180$ and < 300 min.

(3) Clearances of ^{51}Cr-EDTA of less than 21 mL/min in adults are calculated using the following formula:

$$C' = -9.2\ln\left(C(t)\frac{A\times 10^8}{Q_0}\right) + 87.2$$

where

$C(t)$ = background subtracted plasma activity in the sample taken 1440 min after the injection
A = body surface area (m^2)
Q_0 = injected activity

(c) Single sample methods in children

(1) Clearance of ^{51}Cr-EDTA in children is calculated using the following formula:

$$\mathrm{Cl} = \frac{-\ln\left(C(t)\frac{V}{Q_0}\right)V\times 1.73}{tg(t)A}$$

where

$V = 5867A^{1.1792}$
$g(t) = 1.01\exp(-0.000110t) + 0.538\exp(-0.0178t)$
$C(t)$ = background subtracted plasma activity in the sample taken t minutes after the injection
Q_0 = injected activity
V = ^{51}Cr-EDTA distribution volume (mL)
A = body surface area (m^2)

This formula is applicable for any $t > 90$ and < 120 min for children between 1 and 15 years of age.

(2) Clearance of ^{51}Cr-EDTA in infants is calculated using the following formula:

$$C' = \frac{-\ln\left(C(t)\frac{V}{Q_0}\right)V}{t \times g(t) \times A}$$

where

V = $6980A^{1.23}$
$g(t)$ = $1.01\exp(-0.00008t) + 0.42\exp(-0.017t)$
$C(t)$ = plasma activity in the sample taken t minutes after the injection
Q_0 = injected activity
V = ^{51}Cr-EDTA distribution volume (mL)
A = body surface area (m^2)

The formula above is applicable for any $t > 90$ and < 120 min for infants less than 1 year of age.

Appendix 2 to Section 5.4.8

PROCEDURE FOR PREPARATION OF SOLUTIONS AND HANDLING OF BLOOD SAMPLES

(a) Preparation of stock solution:

(1) Fill the 50 mL volumetric flask half full with water. Mark it properly, identifying it as the stock solution. Add date.
(2) Withdraw an aliquot from the radiopharmaceutical solution using the 1.0 mL TB syringe. Do not exceed 55 MBq (1.5 μCi) or well counter dead time count losses may occur.
(3) Add water to bring the syringe volume to 1.0 mL.
(4) Record the activity in the syringe by means of a dose calibrator.
(5) Transfer the syringe contents into the volumetric flask; flush at least once. Then fill the volumetric flask with water to the 50 mL level. Shake gently.
(6) Record the emptied syringe in the dose calibrator to determine residual activity.
(7) Calculate the activity per millilitre of the stock solution. Note date and time in a protocol.

(b) Preparation of standards:

(1) Fill the 100 mL volumetric flask half full with water. Mark it carefully as the standard solution.
(2) Carefully pipette 1.0 mL of the stock solution into the 100 mL volumetric flask.
(3) Fill the flask to 100 mL with water. Shake the flask gently.
(4) Carefully pipette 1.0 mL of the thus produced standard solution into a glass counting vial. Use a clean pipette. Label as 'Standard 1'. Add date.
(5) Using separate pipettes prepare by a similar procedure two additional standards (Standards 2 and 3).
(6) Set the standards aside for later counting with plasma samples.
(7) The three standards should be recounted for each clearance measurement performed on that same day. A substantial variation in counts between standards indicates a pipetting error and new standards should be prepared.

(c) Procedure for dose injection:

(1) Start an intravenous injection and attach a three way stopcock to the intravenous line.
(2) Attach a 10 mL saline syringe to one of the ports containing approximately 10 cm^3 of 0.9% saline for injection.
(3) Attach the dose to the last port of the three way stopcock.
(4) Inject the dose through the three way stopcock.
(5) Flush the dose injection syringe twice by drawing saline into the dose syringe and inject into the patient. Inject the remainder of the saline through the three way stopcock.
(6) Remove the assay syringe, stopcock and intravenous tubing for calculation of residual activity.

(d) Procedure for blood sampling:

(1) Insert an intravenous line for drawing blood samples.
(2) Attach a three way stopcock to the intravenous line.
(3) Attach a 10 mL syringe containing heparinized saline to the second port.
(4) Attach a syringe for the blood sample.

(5) Approximately 30 s before the time the blood sample is needed, start to withdraw blood back into the heparinized saline syringe to clear the line of heparin.
(6) After approximately 15 s, turn the stopcock so that about 5 mL of blood can be withdrawn into the blood sample collection syringe.
(7) Withdraw blood and note the midpoint of the blood collection period as the time when the sample was collected.
(8) Turn the three way stopcock back so that the heparinized saline can be injected back into the patient. Inject approximately 5 mL of heparinized saline to clear out the stopcock and the tubing.
(9) Inject the withdrawn blood into a tube containing acid citrate dextrose (ACD) or a minute volume of heparin to prevent clotting.
(10) Clean out the three way stopcock with sterile cotton swabs to remove any residual blood and radioactivity.
(11) Replace as necessary the heparinized syringe with a new syringe containing heparinized saline.
(12) Repeat the procedure for all subsequent blood samples.
(13) Inject the anticoagulated blood into a centrifuge tube and spin it to separate the red cells from the plasma.
(14) Carefully pipette 1.0 mL of plasma into the counting vial. Do not disturb the interface between the plasma and the red cells.

(e) Procedure for centrifugation:

(1) Ensure that the centrifuge is balanced.
(2) Centrifuge for 15 min at a speed to exceed 2000*g*.
(3) When the centrifuge stops, remove the clear, colourless ultrafiltrate and pipette immediately. Pipette 1.0 mL from each assembly into test tubes. Cap each tube and count immediately.
(4) Record the counts and the time each sample was counted.

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5.5. RESPIRATORY SYSTEM

5.5.1. Introduction

Radionuclide methods are available for the study of lung ventilation and perfusion. The main indication for lung scintigraphy is suspected pulmonary embolism. Other indications are for assessment of residual lung function if surgery is planned for lung tumours, ventilation scans to assess alveolar capillary permeability in smoke inhalation injuries and studies of mucociliary clearance (tracheobronchial clearance).

5.5.2. Pulmonary scintigraphy

5.5.2.1. Principle

(a) Airways

The airway runs from: trachea → main stem bronchi → segmental bronchi → bronchioles → terminal bronchioles → alveolar ducts → alveolar sacs → alveoli.

Ventilation or inhalation studies are performed using radioactive gases (^{133}Xe, ^{127}Xe and ^{81m}Kr) or submicronic (particle size less than 2 μm) radioaerosols (^{99m}Tc-DTPA or colloid). Technegas, a vaporized ^{99m}Tc-carbide from a special device, has a particle size of less than 0.02 μm, which avoids bronchial trapping and is suitable but still expensive. The images obtained show the distribution of gases or radioaerosols in the lungs. Using a positive pressure, larger aerosol particulates such as ^{99m}Tc human serum albumin (HSA) can be used and tracheobronchial epithelial function can be studied.

(b) Vascular system

The lungs are divided into lobes and discrete bronchopulmonary segments, each with its own artery, vein and bronchus. In adults, there are approximately 280 billion pulmonary arterioles small enough to trap the 20–40 μm particles used for perfusion scanning.

Perfusion lung imaging permits an evaluation of the pulmonary arterial blood flow. Usually ^{99m}Tc macroaggregated albumin (MAA) is employed, with a particle size of 10–40 μm; given intravenously, the labelled particles lodge in the pulmonary arterioles in proportion to the regional blood flow, giving a map of the pulmonary functional circulation.

5.5.2.2. Clinical indications

The most common indication for lung scintigraphy is to confirm or exclude pulmonary embolism. Thrombi, usually from the deep venous system of the lower extremities, and globules of fat and particulate amniotic fluid can embolize the pulmonary arteries and produce acute pulmonary hypertension. A ventilation study, performed in conjunction with the lung perfusion images, improves the sensitivity of the lung perfusion image up to 90%. As a general rule, normal ventilation is found in regions of pulmonary embolization.

Clinical suspicion of pulmonary embolism should lead to immediate heparinization (unless there is a contraindication), with a lung study conducted at the same time or on the following day in order to confirm or exclude pulmonary embolism. Alternatively, spiral X ray CT may be considered.

Less common indications include the evaluation of lung function pre-operatively, alveolar capillary permeability after smoke inhalation injury, mucociliary function and lung transplant evaluation. Lung perfusion imaging in conjunction with ventilation imaging has added a non-invasive component to the proper evaluation of patients with bronchitis or obstructive forms of chronic pulmonary disease.

Bronchogenic carcinoma, the most common form of lung carcinoma, causes a decrease or absence of pulmonary blood flow to the affected bronchial segment. Lung perfusion images can provide a direct quantitative estimate of the amount of perfusion remaining in the total lung field, to enable a prediction as to whether or not the patient will become respiratorily disabled if the portion of the lung involved in the malignant process is surgically removed.

5.5.2.3. Radiopharmaceuticals

(a) Perfusion agents: Technetium-99m labelled MAA

Most of the MAA particles are 10–40 μm in size, with a biological half-life of 2–9 hours. Albumin microspheres, although less available, give a more homogeneous particle size. The minimum number of particles necessary to obtain an even distribution of radioactivity in the vascular bed is 60 000; hence it is reasonable to use about 100 000 particles, which will transiently occlude one in 1500 arterioles of the lung. The usual adult administered activity is 40–150 MBq (1–4 mCi). The usual paediatric administered activity is 0.5–2.0 MBq/kg (20–80 μCi/kg), with a minimum of 7–8 MBq ($\approx$200 μCi).

Vials should be agitated prior to withdrawing a dose since labelled MAA particles will settle in the vial with time; the syringe should be inverted prior to injection.

(b) Ventilation agents: Aerosols

Technetium-99m DTPA is the preferred radiopharmaceutical.

The usual administered activity of ^{99m}Tc-DTPA is 900–1300 MBq (25–35 mCi) in the nebulizer, from which approximately 20–40 MBq (0.5–1.0 mCi) reach the patient's lungs.

Aerosol imaging is usually performed before perfusion imaging because it is more difficult to deliver a larger dose of the ^{99m}Tc aerosol than to deliver a larger dose of ^{99m}Tc-MAA. Since both agents are labelled with ^{99m}Tc, it is extremely important for the count rate of the second study to be at least four times that of the first study.

The radioactive gases ^{133}Xe or ^{81m}Kr are unavailable in many countries so that radioaerosols are preferred.

5.5.2.4. Equipment

The following equipment is useful for pulmonary scintigraphy studies:

(a) Large FOV gamma cameras with low energy, general purpose (LEGP) collimators or LEHR collimators.
(b) Large FOV gamma cameras with a rotation capability, as well as a computer with appropriate software, for SPECT imaging. An LEGP collimator is recommended for SPECT in order to minimize acquisition time.

5.5.2.5. Preparation and procedure

(a) Patient preparation

A chest radiograph in both the anterior–posterior position and with lateral projections should be obtained before lung scintigraphy for pulmonary embolism. A portable anterior–posterior chest radiograph is acceptable only if the patient cannot tolerate a routine upright examination. In patients who have no changes in signs or symptoms, a chest radiograph within one day of scintigraphy is adequate. A more recent radiograph (preferably within 1 hour) is necessary in patients with evolving clinical status.

Before intravenous administration of the pulmonary perfusion radiopharmaceutical, the patient should be instructed to cough and to take several deep breaths. The patient should be in a supine position during injection or, in the case of a patient with orthopnea, as close to the supine position as possible, since particle distribution is affected by gravity.

In women of childbearing age, pregnancy and lactation status should be noted and the procedure performed in such a manner as to minimize radiation exposure. For example, half the usual activity may be used for the perfusion study and the ventilation study is omitted if possible.

The pertinent clinical history should include details on:

— Right-to-left shunt(s);
— Severe pulmonary hypertension;
— Chest pain;
— Dyspnea;
— Haemoptysis;
— Syncope;
— Symptoms of deep venous thrombosis;
— Oral contraceptive use;
— Recent surgery;
— Prior pulmonary embolism(s);
— Cancer;
— Congestive heart failure;
— Underlying or previous diseases;
— Smoking;
— Intravenous drug abuse;
— Long air flights.

Other factors may also be relevant; a physical examination includes vital signs, chest cardiac examination and leg findings, among other aspects.

(b) Review of prior lung scintigraphy

Pertinent chest radiographic findings include, but are not limited to, consolidation, atelectasis, effusions, masses, cardiomegaly and decreased pulmonary vasculature. The chest radiograph may be normal in patients with pulmonary embolism. Treatment with anticoagulants or thrombolytic therapy should be noted, as should the results of tests for deep venous thrombosis, for example compression ultrasonography.

The referring physician's estimate of the prior probability of pulmonary embolism may be helpful, or may be assessed from a properly completed request form.

(c) Precautions

Reduced numbers of MAA particles should be considered for patients with pulmonary hypertension or right-to-left shunting, and for infants and children. In adults, the number may be reduced to between 100 000 and 200 000 particles without significantly altering the quality of the images for detection of perfusion defects. Inhomogeneous distribution of activity may result from a reduction in the number of particles to below 100 000 in adults.

(d) Procedure

A chest radiograph should be obtained and reviewed before lung scintigraphy. When ^{99m}Tc labelled aerosol imaging is performed before ^{99m}Tc-MAA perfusion imaging, smaller amounts (40 MBq (1.0 mCi)) of activity should be administered to the lungs.

In aerosol ventilation imaging, the aerosol is administered through a mouthpiece with the nose occluded and the patient performing tidal breathing. An advantage of aerosols is that images can be obtained in multiple projections to match those obtained for perfusion.

It is preferable to have the patient inhale the aerosol in the upright position, although the supine position can be used if necessary. Aerosol ventilation imaging can be performed at the bedside.

In perfusion imaging the patient is asked to cough and take several deep breaths. Technetium-99m MAA is then injected slowly during 3–5 respiratory cycles, with the patient in the supine position.

A well flushed indwelling line can be used if venous access is difficult. The physician should not administer the radiotracer in the distal port of a Swan–Ganz catheter or any indwelling line or port that contains a filter, for example a chemotherapy line.

Imaging is preferably performed in the upright position to increase chest cavity size and minimize diaphragmatic motion. If necessary, images can be obtained in the supine or decubitus position.

Planar images should be obtained in multiple projections including anterior, posterior, both posterior oblique, both anterior oblique and both lateral projections. The anterior oblique or the lateral projections may be omitted. In some patients only a limited number of views are possible. A minimum of six views, each of ventilation and perfusion, are required for reliable interpretation.

5.5.2.6. Interpretation

(a) Criteria for prospective investigation of pulmonary embolism diagnosis (PIOPED) and modified PIOPED

These criteria are derived for interpretation but lead to a high indeterminate rate because they are based on limited ventilation scan views. Interpretation is improved with six perfusion and ventilation images:

(1) High probability (≥80%, in the absence of conditions known to mimic pulmonary embolism):

- At least two large mismatched segmental perfusion defects or the arithmetic equivalent in moderate or large and moderate defects;
- Two large mismatched segmental perfusion defects, or the arithmetic equivalent.

(2) Moderate probability (70–80%):

- One large to two moderate mismatched perfusion defects or the arithmetic equivalent in moderate or large and moderate defects.

(3) Low probability (<20%):

- Single matched ventilation–perfusion defects with clear chest radiograph or very extensive matched defects;
- Non-segmental perfusion defects (e.g. cardiomegaly, enlarged aorta, enlarged hila or elevated diaphragm);
- Any perfusion defects with a substantially larger ventilation defect or chest radiographic abnormality;
- Perfusion defects matched by ventilation abnormality provided that there are:
  - a clear chest radiograph,
  - some areas of normal perfusion in the lungs;
- Any number of small perfusion defects with a normal chest radiograph.

(4) Indeterminate probability:

- Those that cannot be categorized as low, moderate or high probability.

(5) Normal perfusion:

— No perfusion defects or perfusion exactly outlining the shape of the lungs seen on the chest radiograph (note that hilar and aortic impressions may be seen and the chest radiograph–ventilation study may be abnormal).

(b) Further interpretive considerations

Ventilation–perfusion mismatch can result from any cause of pulmonary arterial blood flow obstruction. Although a very long list of differential diagnoses exists for ventilation–perfusion mismatch findings, the most common causes include only a few:

— Acute pulmonary embolism;
— Old pulmonary embolism (without reperfusion);
— Obstruction of a pulmonary vessel by a tumour;
— Previous radiation therapy to the thorax.

On perfusion scintigraphy, extrapulmonary activity (which may be seen at the edges of lung images in the thyroid or kidneys) may be due to right-to-left shunt, free ^{99m}Tc-pertechnetate or reduced technetium compounds, or a recent nuclear medicine procedure. An image of the head can be used to differentiate free pertechnetate or reduced technetium from a shunt.

The stripe sign (activity at the periphery of a perfusion defect) lowers the chance of pulmonary embolism in the zone of the perfusion defect that shows the stripe.

(c) Sources of error

Perfusion images can show hot spots in the lung if clotting of blood occurs in the syringe during injection, or if the injection is made through an indwelling catheter that is not well flushed.

Ventilation scintigraphy is obtained at a different point in time than perfusion scintigraphy. In the intervening time, there can be changes in ventilation and perfusion. Similarly, ventilation scintigraphy may be obtained in an upright position and perfusion scintigraphy injected in the supine position. These changes in position may also affect the comparability of the two scintigrams.

Injection of ^{99m}Tc-MAA through a central line can result in inadequate mixing of activity in the pulmonary artery. This inadequate distribution of

activity is especially common if the activity is injected through a pulmonary artery line.

(d) Communication

Any urgent lung scan results should be discussed with the referring physician or her/his representative.

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5.6. LIVER AND GASTROINTESTINAL SYSTEM

5.6.1. Liver and spleen imaging

These procedures are used less often nowadays, having largely been replaced by ultrasound, CT and MRI. However, some techniques remain effective for specific clinical indications.

5.6.1.1. Principle

Liver–spleen imaging is performed following the injection of a ^{99m}Tc labelled colloid, which is rapidly phagocytized by the reticuloendothelial cells of the liver, spleen and bone marrow.

Liver blood pool imaging is performed following the injection of ^{99m}Tc labelled RBCs for the detection of cavernous hemangiomas of the liver.

Hepatic perfusion studies are performed following the injection of ^{99m}Tc-MAA through a hepatic artery catheter to determine whether intra-arterially administered chemotherapeutic agents are being optimally delivered.

Splenic imaging is performed following the injection of ^{99m}Tc labelled heat damaged RBCs. Damaged RBCs are selectively taken up by functioning splenic tissue.

5.6.1.2. Clinical indications

(a) Liver–spleen imaging

These studies can be used for determining the size and shape of the liver and spleen as well as for detecting functional abnormalities of the reticulo-endothelial cells of these organs. Specifically, these studies are occasionally performed for:

(1) Suspected focal nodular hyperplasia of the liver. These lesions often have normal or increased uptake on sulphur colloid imaging.
(2) Assessment of reticuloendothelial system (RES) function in patients with suspected liver disease. The decision to perform a liver biopsy or to continue treatment with a hepatotoxic agent may be influenced by the severity of the liver disease that is seen on liver–spleen imaging as a complement to blood tests.

(b) Liver blood pool imaging

This procedure is highly specific for the diagnosis of cavernous hemangiomas of the liver. The sensitivity for detecting large lesions (more than 2–3 cm) is very high, but hemangiomas as small as 0.5 cm may be detected with SPECT.

(c) Hepatic perfusion imaging

These studies are useful for demonstrating that hepatic artery catheters used to infuse chemotherapeutic agents are optimally positioned to perfuse liver tumours and to avoid drug delivery to normal extrahepatic tissues (e.g. the stomach).

(d) Splenic imaging

These studies are used for detecting functional splenic tissue. They are often performed:

— In children, to rule out congenital asplenia or polysplenia;

— In adults with recurrent thrombocytopenia previously treated by splenectomy;
— For characterizing an incidentally noted mass as functional splenic tissue;
— For the evaluation of abdominal trauma with suspected splenic rupture;
— For viability assessment of splenic grafts.

5.6.1.3. Radiopharmaceuticals and doses

(a) Liver–spleen imaging

Technetium-99m sulphur colloid (SC) is preferred for liver–spleen imaging because the biodistribution and biokinetics of this agent are more reproducible than those of ^{99m}Tc-albumin colloid (AC). Adult doses should be in the range of 110–220 MBq (3–6 mCi) and those in children should be properly adjusted.

(b) Liver blood pool imaging

Technetium-99m labelled RBCs can be labelled using in vitro, in vitro and in vivo, or in vivo methods. Methods with higher labelling efficiency (in vitro and in vivo, or in vitro) may improve the results of imaging. Recommended adult doses are 370–1110 MBq (10–30 mCi) according to body weight. Large doses allow better delayed images.

(c) Hepatic artery perfusion imaging

Technetium-99m MAA is used, with doses in the range of 110–220 MBq (3–6 mCi).

(d) Splenic imaging

Technetium-99m heat damaged RBCs are used.

5.6.1.4. Equipment

A large FOV gamma camera equipped with a low energy, all purpose or high resolution collimator is usually used. For a small FOV gamma camera, a diverging collimator may be needed.

5.6.1.5. Preparation

(a) Patient preparation

No special patient preparation is required.

(b) Information pertinent to performing the procedure

The following information should be collected:

— Relevant history and results of physical examination;
— Results of other relevant imaging studies;
— Results of liver function tests;
— For splenic imaging, the results of a complete blood count with platelet count;
— For hepatic perfusion studies, the position of the hepatic artery catheter.

(c) Precautions

When RBCs are labelled, it is essential to adhere to a strict procedure designed to maintain sterility and prevent the administration of one patient's labelled RBCs to other patients. Appropriate procedures and quality assurance for the correct identification of patients and the handling of blood products are imperative.

5.6.1.6. Procedures

(a) Image acquisition

(1) Liver–spleen imaging

Imaging is begun 10–15 min or longer after the intravenous administration of ^{99m}Tc-colloid. Anterior, posterior, right lateral, right anterior oblique and right posterior oblique images of the liver are commonly obtained. Left posterior oblique and left lateral views are added to evaluate the spleen.

For small FOV gamma cameras and standard amounts of administered activity, images are usually collected for 300 000–500 000 counts. For large FOV gamma cameras, an anterior image is usually acquired for 500 000–1 000 000 counts. Subsequent images are then obtained for the same length of time as for the anterior image. SPECT imaging may be helpful, particularly if focal disease is suspected or if organ volume determinations are needed.

Views taken with the patient holding their breath may sometimes help clarify ambiguous findings by eliminating image degradation due to respiratory motion. A size marker and a costal margin marker are needed for measuring liver and spleen size and for identifying anatomical landmarks.

(2) Hepatic blood pool imaging

A rapid sequence of images (1 frame per 2–3 s for 60 s) immediately following injection may reveal useful information about regional variations in blood flow. Such dynamic studies should be performed in the view that is most likely to show the lesion. This view should be selected on the basis of the location of the lesion of interest, which has usually been documented in a previous imaging study (i.e. CT, ultrasound or MRI). Immediate blood pool images should be obtained in the view most likely to show the lesion, as well as in anterior, posterior and right lateral views. These views are generally acquired for 1 000 000–2 000 000 counts each.

Delayed (45–180 min post-injection) blood pool images are obtained in the anterior, posterior and right lateral views for 1 000 000–2 000 000 counts each. When the lesion is small (less than 2–3 cm), or if there are multiple lesions, SPECT imaging is preferred. If a high quality delayed SPECT study is obtained, planar images are then optional. SPECT facilitates comparison with CT and MRI, and permits calculation of liver–spleen absolute volumes.

A hepatic perfusion index, comparing the hepatic artery and portal counts to total blood flow, may also be obtained from the dynamic flow study and the corresponding hepatic time–activity curve. This index can aid in the characterization of focal lesions.

(3) Hepatic perfusion imaging

The radiopharmaceutical ^{99m}Tc-MAA should be infused very slowly at a measured rate through the hepatic catheter to demonstrate the tissue perfused by the catheter. Imaging is performed immediately after the infusion of the agent. Anterior, posterior and right lateral images of the liver containing 500 000–1 000 000 counts are typically acquired. Images of the lung are required to identify intrahepatic arteriovenous fistulas.

(4) Splenic imaging

Imaging may commence 30–120 min after the injection of the heat damaged labelled RBCs. Anterior, posterior and posterior oblique images of the expected location of the spleen should be acquired for 300 000–750 000

counts. If ectopic splenic tissue is a concern, the entire abdomen should be imaged. If the patient has had prior trauma that may have resulted in a diaphragmatic rupture, the chest should also be imaged. SPECT imaging facilitates comparison with CT and MRI.

(b) Interpretation

(1) Liver–spleen imaging

Most focal lesions in the liver will have less activity than the normal tissue. Focal nodular hyperplasia may have activity equal to, or greater than, the surrounding liver in about 50% of patients. Normal activity or increased activity found in a lesion is very specific to focal nodular hyperplasia. Visualization of the caudate lobe only (with splenic enlargement) is typical of the Budd–Chiari syndrome due to hepatic vein thrombosis.

A relative radiocolloid 'shift' (increased radionuclide deposition in the spleen and bone marrow relative to the liver) may occur in liver cirrhosis but also in any diffuse form of hepatic dysfunction, portal hypertension, hypersplenism and marrow-active anaemia as a response to chemotherapy, as well as in some patients with malignant melanoma. In patients with diffuse parenchymal disease, serial studies can document the progression and severity of the disease.

(2) Hepatic blood pool imaging

The finding of markedly increased blood pool activity within a liver lesion is pathognomonic of a cavernous hemangioma. Hemangiomas typically have reduced or normal initial blood flow with increased activity on delayed images. Other tumours of the liver (e.g. angiosarcomas) have rarely been reported to have an increased blood pool on delayed images; however, they can usually be distinguished from cavernous hemangiomas by an increased blood flow in dynamic studies.

Cavernous hemangiomas that are 3 cm or greater in size almost always demonstrate a markedly increased blood pool even on planar images. The sensitivity for detection of lesions smaller than 3 cm is improved by the use of SPECT imaging. SPECT imaging is also helpful when there are multiple lesions in the liver, and facilitates comparison with CT and MRI. A hepatoma usually shows increased early perfusion followed by a defect, whereas abscesses and cystic lesions are hypoactive in all phases of the study.

(3) Hepatic perfusion imaging

The images should be assessed for the presence of extrahepatic accumulation of activity (e.g. in the stomach and pancreas). Some activity in the lungs may be seen with a properly positioned catheter due to arteriovenous fistulas in the liver resulting in trapping of the radiolabelled MAA in the pulmonary circulation. The presence of extrahepatic subdiaphragmatic activity indicates that the catheter is not optimally positioned.

(4) Splenic imaging

Functional splenic tissue is preferentially visualized when heat damaged blood cells are used. Hepatic activity is present but usually with less intensity than splenic tissue.

(c) Reporting

(1) Liver–spleen imaging

The size and shape of the liver and spleen, and the relative amount of activity in the liver, spleen and bone marrow, should be noted. Focal lesions should be described. It is not always necessary to include absolute measurements of liver and spleen size, although volume measurements are feasible with SPECT and appropriate computer software.

(2) Hepatic blood pool imaging

Lesion location, approximate size and uptake characteristics should be described. The report should include the results of other imaging studies where available. When multiple lesions have been noted in other imaging studies, the presence or absence of an increased blood pool should be reported on a lesion-by-lesion basis when possible.

(3) Hepatic perfusion imaging

The approximate rate (mL/min) of the injection of the radiopharmaceutical should be included in the report. The presence of any extrahepatic activity should be noted.

(4) Splenic imaging

The time between injection and imaging should be reported as well as the number, approximate size and location of any focal areas of uptake.

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5.6.2. Hepatobiliary scintigraphy

5.6.2.1. Principle

Hepatobiliary scintigraphy is a diagnostic imaging study that evaluates hepatocellular function and patency of the biliary system by tracing the production and flow of bile from the liver through the biliary system into the small intestine. Sequential images of the liver, biliary tree and intestinal tract are obtained. Computer acquisition and analysis as well as pharmacological interventions are frequently employed.

5.6.2.2. Clinical indications

(a) Functional assessment of the hepatobiliary system.
(b) Evaluation of integrity of the hepatobiliary tree.

These two categories include investigation of:

— Suspected acute cholecystitis;
— Suspected chronic biliary tract disorders;
— Common bile duct obstruction;
— Bile extravasation;
— Atresia of the biliary tree (differential diagnosis in neonatal jaundice);

— Patency of bilio-enteric surgical anastomosis;
— Enterogastric reflux.

5.6.2.3. Radiopharmaceuticals

Technetium-99m labelled disofenin (2,6-diisopropylacetanilido iminodiacetic acid (DISIDA)) or mebrofenin (bromo-2,4,6-trimethylacetanilido iminodiacetic acid (BRIDA)) is administered intravenously in activities of 50–200 MBq (1.5–5 mCi) for adults; higher doses may be required in hyperbilirubinaemia with activities of 100–370 MBq (3–10 mCi). Mebrofenin may be selected instead of disofenin in moderate to severe hyperbilirubinaemia due to its higher hepatic extraction. For infants and children, the administered activity is 2–7 MBq/kg (0.05–0.2 mCi/kg), with a minimum of 15–20 MBq (0.3–0.5 mCi).

5.6.2.4. Equipment

A large FOV gamma camera equipped with a low energy, all purpose or high resolution collimator is generally used. For a smaller FOV gamma camera, a diverging collimator may be needed. Whenever possible, continuous computer acquisition should be performed (1 frame/min for 30–60 min).

5.6.2.5. Patient preparation

To permit gall bladder visualization, the patient must have fasted for a minimum of two and preferably four hours prior to administration of the radiopharmaceutical. If the patient has fasted for longer than 24 hours or is on total parenteral nutrition, a false positive study for cholecystitis may occur. In those cases, especially with parenteral nutrition, the patient may be pre-treated with sincalide (Section 5.5.2.7).

(a) Information pertinent to performing the procedure

The physician should review all the pertinent clinical, laboratory and radiographic information available prior to the study for the patient. Additional information specifically related to hepatobiliary scintigraphy includes:

— History of previous surgeries, especially biliary and gastrointestinal surgery;
— Time of most recent meal;

— Current medications, including the time of their most recent administration (with particular attention to opioid compounds);
— Results for bilirubin and liver enzyme levels;
— Results of ultrasound investigations.

(b) Precautions

The test should be performed under the optimal state of fasting to avoid a false positive result. Interference by opioids can be minimized by delaying the study for four hours after the last dose.

(c) Procedure

The protocol should be adjusted to the clinical situation, but normally imaging should commence at injection and continue serially for 60 min or until activity is seen in both the gall bladder (which confirms the patency of the cystic duct) and the small bowel (which confirms the patency of the common bile duct). Additional views (e.g. right lateral, left or right anterior oblique) may be obtained as needed to clarify the anatomy. The digital data can be reformatted to 5–15 min images for display and hard copying. Cinematic display of the data may reveal additional information not readily apparent on the film.

When acute cholecystitis is suspected and the gall bladder is not seen within 40–60 min, 3–4 hour delayed images should be obtained, or morphine augmentation may be employed in lieu of delayed imaging. Delayed imaging at 18–24 hours may be necessary in some cases (e.g. patients who are seriously ill and those with suspected common bile duct obstruction or suspected biliary atresia). If the patient is being studied for a biliary leak, imaging delayed by 3–4 hours and patient positioning manoeuvers (e.g. decubitus views) may be helpful.

5.6.2.6. Interventions

A variety of pharmacological or physiological interventions may enhance the diagnostic value of the examination. Appropriate precautions should be taken to promptly detect and treat any adverse reactions caused by these manoeuvres.

(a) Sincalide pretreatment

Sincalide, a synthetic C-terminal octapeptide of cholecystokinin (CCK), in doses of 0.01–0.02 μg/kg, may be given intravenously 30–60 min prior to the

hepatobiliary tracer injection to minimize the potential for a false positive study in acute cholecystitis. This may occur in patients who have fasted longer than 24 hours, are on parenteral hyperalimentation or have a severe intercurrent illness. Sincalide should be administered slowly (over 3–5 min) to prevent biliary spasm and abdominal cramps. An even slower infusion may also be used.

(b) Morphine sulphate

When acute cholecystitis is suspected and the gall bladder is not seen by 40–60 min, morphine sulphate, 0.04–0.1 mg/kg, may be administered intravenously over 2–3 min. If the cystic duct is patent, the flow of bile into the gall bladder will be facilitated by morphine induced temporary spasm of the sphincter of Oddi. The intrahepatic biliary tree and common bile duct (CBD) must contain radioactive bile, and tracer activity should be present in the small bowel at the time of morphine injection. A second injection of radiopharmaceutical (a booster dose of approximately 1 mCi) may be necessary prior to morphine injection if the remaining liver and/or biliary tree activity appears insufficient to permit gall bladder visualization. Imaging is usually continued for another 30 min following morphine administration but may be extended if desired. Contraindications to the use of morphine include respiratory depression in non-ventilated patients (absolute), morphine allergy (absolute) and acute pancreatitis (relative).

(c) Sincalide stimulation

Gall bladder contractility may be evaluated by determining the gall bladder ejection fraction (GBEF) response to sincalide. The study involves a 3 min intravenous injection or a 15–45 min infusion of 0.01–0.04 μg/kg sincalide after the gall bladder has been maximally filled with radiopharmaceutical (usually 60 min after the injection) and there is minimal remnant activity in the liver. Computer acquisition (1–2 frames/min) then continues for 20 to 30 min. Numerous protocols can be employed, but when performing and interpreting this procedure, the physician must adhere to a specific technique (i.e. dosage and duration of infusion) and normal values must be validated, preferably at the local institution.

(d) Fatty meal stimulation

Measurement of gall bladder ejection fraction using a fatty meal challenge instead of sincalide has also been described. If visual assessment of gall bladder emptying is adequate, a fatty snack may be used.

(e) Phenobarbital

In jaundiced infants in whom biliary atresia is suspected, pretreatment with phenobarbital, 5 mg/(kg · day), may be given orally in two divided doses daily for a minimum of 3–5 days prior to the hepatobiliary imaging study, to enhance the biliary excretion of the radiotracer and increase the specificity of the test. Mebrofenin may be preferred to Disofenin in suspected biliary atresia.

5.6.2.7. Processing

(a) Gall bladder ejection fraction

Using the immediate pre-sincalide and the post-sincalide data, ROIs are drawn around the gall bladder (taking into account patient motion) and adjacent liver (background) using any standard nuclear medicine software package. The liver background ROI is selected taking care to exclude ductal activity. GBEF is calculated from the gall bladder time–activity curve as:

$$\text{GBEF}\,(\%) = \frac{(\text{net GB counts}_{\text{max}}) - (\text{net GB counts}_{\text{min}}) \times 100}{(\text{net GB counts})_{\text{max}}}$$

where GB stands for gall bladder.

(b) Hepatic extraction fraction (HEF)

This index of hepatocellular function can be obtained by deconvolution analysis of curves derived from ROIs over the liver and heart.

5.6.2.8. Interpretation

(a) Normal

A normal hepatobiliary scan is characterized by immediate demonstration of hepatic parenchyma, followed sequentially by activity in the intra-extrahepatic biliary ductal system, gall bladder and upper small bowel. All these structures should be evident within one hour. Gall bladder visualization implies a patent cystic duct and excludes acute cholecystitis with a high degree of accuracy. Some renal excretion of the tracer may be seen, and bladder activity should not be regarded as pathological.

(b) Acute cholecystitis

The hallmark of acute cholecystitis (acalculous as well as calculous) is persistent gall bladder non-visualization 30 min post-morphine or on the 3–4 hour delayed image. A pericholecystic hepatic band of increased activity (the rim sign) is often associated with severe phlegmonous and/or gangrenous acute cholecystitis, and constitutes a surgical emergency.

(c) Chronic cholecystitis

Chronic cholecystitis and clinical settings associated with physiological failure of the gall bladder to fill with radiotracer (e.g. after prolonged fasting for more than 24–48 hours, or in severely ill or post-operative hospitalized patients) may result in gall bladder non-visualization within the first hour, but may be distinguished from acute cholecystitis using low dose intravenous morphine (see above) or delayed imaging. In chronic cholecystitis, the gall bladder will usually be seen within 30 min of morphine administration or on 3–4 hour delayed images, while true cystic duct obstruction (acute cholecystitis) will result in persistent gall bladder non-visualization. Visualization of the gall bladder after activity in the bowel has been observed has a significant correlation with chronic cholecystitis. Further evaluation with ejection fraction determination may be useful. Severely ill patients and those on total parenteral nutrition will have a high incidence of gall bladder non-visualization even after morphine despite a patent cystic duct, and a larger dose of morphine (0.1 mg/kg) may be necessary to reduce the false positive rate of the study.

(d) Reduced gall bladder ejection fraction

Reduced gall bladder ejection fraction in response to sincalide may be indicative of chronic cholecystitis, gall bladder dyskinesia or the cystic duct syndrome.

(e) Common bile duct obstruction

Delayed biliary-to-bowel transit beyond 60 min raises the suspicion of partial CBD obstruction, although it may constitute a normal variant found in up to 20% of individuals. Conversely, activity in the small bowel observed within 60 min does not exclude partial CBD obstruction. High grade CBD obstruction should be suspected when neither the gall bladder nor the small bowel are found within 18–24 hours after tracer injection. Severe hepatocellular dysfunction may appear similar but is associated with persistently high

background activity, dominant renal excretion of the radiopharmaceutical and low liver uptake.

(f) Biliary leaks

A biliary leak is present when tracer is found in a location other than the liver, gall bladder, bile ducts, bowel or urine. This may be seen more easily using a cinematic display and when the patient is imaged in the decubitus position.

(g) Biliary atresia

Biliary atresia can be excluded by demonstrating the transit of radiotracer into the bowel. Failure of the tracer to reach the intestinal tract can be caused by hepatocellular disease or immature intrahepatic transport mechanisms and is not necessarily related to biliary atresia or CBD obstruction. However, no evidence of hepatobiliary excretion in a jaundiced neonate having received phenobarbital is probably due to biliary atresia. Urinary excretion of the tracer (especially into a diaper) may be confused with bowel activity and is a potential source of erroneous interpretation.

(h) Duodenogastric bile reflux

During a hepatobiliary scan, activity may reflux from the duodenum into the stomach. This abnormal bile reflux is highly correlated with bile gastritis, a cause of epigastric discomfort.

(i) Post-cholecystectomy sphincter of Oddi dysfunction:

Following pretreatment with sincalide, a combination of visual and quantitative indices (the ‘scintigraphic score’) may be used when this condition is suspected.

5.6.2.9. Reporting

In addition to patient demographics, the report should include the following information:

(a) The indication for the study (e.g. suspected acute cholecystitis, suspected common bile duct obstruction or suspected bile leak).

(b) Procedure:
- Radiopharmaceutical and dose administered;
- Other medications given and their dosage (e.g. pretreatment with cholecystokinin or morphine, or post-treatment with cholecystokinin);
- Duration of imaging, special or delayed views obtained.

(c) Findings: The appearance of the liver, the presence and time of visualization of the gall bladder, small bowel, any unusual activity (e.g. a bile leak or enterogastric reflux) and any quantitative data generated (e.g. GBEF).

(d) Study limitations and patient reactions to drugs administered.

(e) Comparative and/or correlative imaging data.

(f) Impression: This should be concise, as precise as possible, address the clinical question, provide a differential diagnosis and make recommendations if appropriate.

(g) Any urgent or unexpected findings should be directly communicated to the referring physician and documented.

5.6.2.10. Sources of error

(a) The causes of a false positive study (gall bladder non-visualization in the absence of acute cholecystitis) include:

- Insufficient fasting (<2–4 hours);
- Prolonged fasting (>24–48 hours), especially total parenteral nutrition (despite sincalide pretreatment and morphine augmentation);
- Severe hepatocellular disease;
- High grade CBD obstruction;
- Severe intercurrent illness (despite sincalide and morphine);
- Pancreatitis (rare);
- Rapid biliary-to-bowel transit (insufficient tracer activity remaining in the liver for delayed imaging);
- Severe chronic cholecystitis;
- Previous cholecystectomy.

(b) False negative studies (gall bladder visualization in the presence of acute cholecystitis) are rare; their causes include:

- A bowel loop simulating the gall bladder (drinking water may help clarify the anatomy).
- Acute acalculous cholecystitis.
- The presence of the ‘dilated cystic duct’ sign simulating the gall bladder. If this sign is present, morphine should not be given.

— A leak of bile due to gall bladder perforation.
— Congenital anomalies simulating the gall bladder.
— Activity in the kidneys simulating the gall bladder or small bowel (this may be clarified by a lateral image).

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5.6.3. Gastrointestinal bleeding

5.6.3.1. Principle

Gastrointestinal bleeding scintigraphy is performed using ^{99m}Tc labelled RBCs in patients suspected of having active gastrointestinal bleeding. Sites of active bleeding are identified by the accumulation and movement of labelled RBCs within the bowel lumen. Since activity within the lumen of the bowel can move antegrade and retrograde, frequent images will increase the accuracy of localization of the bleeding site. Technetium-99m SC can be used but it has a short residence time within the intravascular space, which limits sensitivity. The agent is cleared from the blood by the reticuloendothelial system with a half-time as short as 2–3 min, while radiolabelled RBCs remain for hours.

5.6.3.2. Clinical indications

Gastrointestinal bleeding can be either upper, originating above the ligament of Treitz, or lower, distal to the ligament of Treitz. Frequent causes of upper gastrointestinal bleeding include esophageal varices, gastric and duodenal ulcers, gastritis, esophagitis, Mallory–Weiss tears or neoplasms. Causes of lower gastrointestinal haemorrhage include angiodysplasia, diverticula, neoplasms and inflammation, and, in children and young adults, Meckel's diverticulum. Endoscopy and angiography provide accurate localization of bleeding sites and potential therapeutic control. Scintigraphy with

labelled RBCs is complementary to endoscopy and angiography because it permits continuous monitoring over a period of hours. This is a major advantage since most gastrointestinal bleeds are intermittent and therefore are frequently missed by other methods.

The clinical picture for active gastrointestinal haemorrhage is often unreliable and misleading. There is frequently a marked temporal lag between the onset of bleeding and clinical presentation. While it may be clinically apparent that the patient has bled from the presence of melena or a haemorrhage, the blood may pool in the colon for hours before being evacuated. A drop in the haematocrit and elevated serum blood urea nitrogen (BUN) also lack the temporal resolution needed to indicate active bleeding. Orthostatic hypotension and tachycardia occur more acutely but are insensitive and non-specific signs.

In cases where there is occult bleeding detected only by positive stool tests, gastrointestinal bleeding scintigraphy is unlikely to be useful, although the method can detect bleeding rates as low as 0.1–0.35 mL/min. The guaiac test detects bleeds at rates well below the necessary threshold to be seen by scintigraphy.

The goals of gastrointestinal bleeding scintigraphy are to locate the bleeding site and to determine which patients require aggressive treatment as opposed to those who can be medically managed. In some patients, the bleeding site is identified with sufficient confidence for specific surgical intervention (e.g. right hemicolectomy in the case of the ascending colon). If bleeding is detected, the site is usually localized well enough to direct the next diagnostic test (e.g. endoscopy or arteriography).

5.6.3.3. Radiopharmaceuticals

The in vitro method for labelling RBCs is preferred due to its higher labelling efficiency. The in vivo/in vitro method can be used, while the in vivo method is not recommended because of potential high free pertechnetate activity giving confusing results. Adult doses should be in the range of 370–1110 MBq (10–30 mCi) according to the patient's weight.

5.6.3.4. Equipment

A large FOV gamma camera is required and a low energy, all purpose, parallel hole collimator is preferred. When the study has to be performed at the bedside with a small detector, a diverging collimator is useful in order to include the maximum abdominal area.

5.6.3.5. Patient preparation

Patients suspected of acute gastrointestinal bleeding should have blood pressure and heart rate measured upon their arrival in the nuclear medicine department to confirm that they are haemodynamically stable. Vital signs should be monitored periodically while the patient is being imaged. The patient should have an intravenous catheter in place so that hypotension can be rapidly treated with replacement of fluids or blood.

The removal of blood for radiolabelling and re-injection poses the risk of misadministration to the wrong patient. The handling and administration of blood products must be subject to special safeguards and procedures, in order to prevent errors or contamination accidents.

5.6.3.6. Procedure

The procedure for gastrointestinal bleeding scintigraphy is as follows:

(a) A dynamic acquisition is important in order to accurately localize the bleeding site:
  — Photopeak, typically a 20% window at 140 keV;
  — Computer, 128 × 128 matrix.
(b) Patient position: supine.
(c) Imaging field: abdomen and pelvis.
(d) Acquisition protocol:
  (1) Abdominal flow study: anterior flow images (1–5 s/frame for 60 s).
  (2) Dynamic abdominal imaging:
    — Dynamic images are acquired at a frame rate of 10–60 s/frame over a 60–90 min period. Acquiring these images in multiple sets of 10–15 min each may facilitate review by the physician as the study is in progress.
    — In case a computer is not available, sequential analog images of 1 000 000 counts each should be obtained every 5 min for 60–90 min; localization might be aided by acquiring images at shorter intervals.
    — Delayed imaging: for ^{99m}Tc-RBCs, if no bleeding site is identified on the initial 60–90 min dynamic images, delayed images may be acquired at 2–6 hours and/or at 18–24 hours after the injection of the radiopharmaceutical. Delayed images are useful in showing subsequent bleeding and categorizing severity, but may result in incorrect localization of the bleeding site.

(3) Additional views: owing to overlying bladder activity, activity in the rectum can be obscured. Lateral views may be needed to detect rectal bleeding. Anterior oblique and posterior views are frequently helpful in deciding if activity is located anteriorly or posteriorly.
(4) ROI counts over extravasated blood in the bowel may be used to estimate blood loss when normalized to counts obtained from a blood sample drawn simultaneously from the patient and corrected for attenuation. The precision and accuracy of estimates should be determined for each institution.
(5) The following procedures may be useful to provide more exact localization of a bleeding site:
— Review of prior barium studies;
— Oral ^{99m}Tc-SC to outline the upper gastrointestinal and small bowel anatomy;
— Technetium-99m SC enema to outline the colon.

5.6.3.7. Interventions

Pharmacological intervention is controversial and is not widely used. Glucagon studies have been suggested as an adjunct to gastrointestinal bleeding studies. Glucagon decreases intestinal peristalsis and increases vasodilatation, although it is not widely used.

Heparin also has been suggested as an adjunct to gastrointestinal bleeding studies in selected patients with recurrent significant bleeding and negative standard diagnostic tests. Six thousand units of heparin are administered intravenously as a loading dose, followed by 1000 units every hour. The patient's baseline coagulation status should be evaluated before giving heparin. Surgical coverage should be immediately available as a precautionary measure and close monitoring of the patient is necessary with protamine sulphate on hand to reverse the effects of heparin.

5.6.3.8. Processing

Subtraction and/or contrast enhancement should be used, with no other routine processing parameters to be observed.

5.6.3.9. Subtraction cinematography

The first frame or normalized summed set of data can be subtracted from the latter images to improve contrast. When using this technique, the patient

must remain still during the examination or appropriate motion correction software should be used.

5.6.3.10. Interpretation

In addition to patient demographics, the report should include the following information:

(a) Reasons why the study was indicated.
(b) Procedure:
    (1) Radiopharmaceuticals:
        — Dose;
        — Radiolabelling method for RBCs (e.g. in vivo);
        — Method of administration (intravenous).
    (2) Acquisition:
        — Duration of acquisition (e.g. 1 hour);
        — Frame rate (e.g. 10 s/frame);
        — Projections acquired (e.g. anterior or laterals).
    (3) Display (e.g. static versus cine).
    (4) Findings:
        — Onset;
        — Location;
        — Characteristics:
            - Size and shape (e.g. focal or diffuse);
            - Pattern of movement (e.g. moving or stationary, serpentine small bowel pattern or colonic pattern, antegrade or retrograde);
            - Severity (e.g. waxing or waning intensity, qualitative intensity compared with the liver, and qualitative volume (large or small)).
(c) Study limitations or confounding factors.
(d) Interpretation (e.g. positive, negative or indeterminate) and location of bleeding site.
(e) Sources of error:
    (1) A delay in implementing the procedure should be avoided because bleeding may have stopped.
    (2) Subtle areas of bleeding may go undetected or the location of the bleeding may be inaccurately identified if images are not displayed in cine-mode. Use of windowing levels and different colour tables on a computer display further facilitate the detection of subtle abnormalities.

(3) It is important to continue to acquire images after abnormal activity has been detected. Accurate localization of the bleeding site is dependent upon identification of the focus of initial blood collection, and upon the movement of the blood away from the bleeding site.
(4) The entire abdomen must be examined before concluding that no bleeding has been detected. A lateral, posterior and/or subpubic view is best to help in identifying activity in the rectum that would otherwise not be detected due to overlying bladder activity or soft tissue attenuation.
(5) Inexperienced readers may mistake mesenteric varices or penile blood pools for areas of bleeding. A full urinary bladder may obscure sigmoidal or rectal bleeding. Radioactive urine in the renal pelvis of a transplanted kidney, in either the right or left lower quadrant of the abdomen, may mimic colonic activity.
(6) Gastric mucosal and renal activity are seen when free ^{99m}Tc-pertechnetate is present. This potential source of error can be avoided by using the in vitro RBC labelling method and performing a quality control for free pertechnetate, and by recognizing that intraluminal blood moves in a distinct pattern. Images of the thyroid and salivary glands can confirm the presence of free ^{99m}Tc-pertechnetate as the source of an artefact.

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5.6.4. Meckel's diverticulum scintigraphy

5.6.4.1. Principle

A Meckel diverticulum is a vestigial remnant of the omphalomesenteric duct located in the ileum, about 50–80 cm from the ileocecal valve. About half of Meckel diverticuli contain gastric mucosa. Bleeding may result from ileal mucosal ulceration from acid secretion. Technetium-99m pertechnetate avidly accumulates in gastric mucosa and is the study of choice for identifying ectopic gastric mucosa in a Meckel diverticulum.

5.6.4.2. Clinical indications

The indication for the study is to localize ectopic gastric mucosa in a Meckel diverticulum as the source of unexplained gastrointestinal bleeding. Bleeding Meckel diverticula usually occur in young children. A Meckel scintigram should be used when the patient is not actively bleeding; even in young children, active bleeding is best studied by radiolabelled RBC scintigraphy.

5.6.4.3. Radiopharmaceuticals

Inject 370–450 MBq (10–15 mCi) of ^{99m}Tc-pertechnetate intravenously, adjusting the dose according to age, but not less than 100 MBq (3 mCi).

5.6.4.4. Equipment

A large FOV gamma camera is required and a low energy, all-purpose, parallel hole collimator is preferred.

5.6.4.5. Patient preparation

Pretreatment with histamine H_2 blockers is reported to enhance the sensitivity and specificity of the Meckel scan. Histamine H_2 blockers (cimetidine and ranitidine) block secretion from the cells and increase gastric mucosa uptake, preventing release and accumulation of the tracer in the intestinal lumen, which constitutes a common cause of false positive studies. An oral dose of 300 mg cimetidine should be administered four times a day for two days in adults, and doses of 20 mg/(kg · day) for two days in children or 10–20 mg/(kg · day) in neonates, prior to starting the procedure. Intravenous cimetidine should be administered at a rate of 300 mg in 100 mL of saline dextrose over 20 min, with imaging starting 1 hour later. Ranitidine dosage is 1 mg/kg for infants, children and adults up to a maximum of 50 mg, infused intravenously over 20 min with imaging starting 1 hour later, immediately after injection of ^{99m}Tc-pertechnetate.

It should be determined whether the patient has received recent in vivo RBC labelling, where all circulating RBCs were exposed to stannous ions via intravenous administration of a 'cold' pyrophosphate kit. If so, the Meckel scan may be compromised, since ^{99m}Tc-pertechnetate will label RBCs rather than concentrate in the ectopic gastric mucosa. This may occur for days after the administration of a stannous pyrophosphate but is usually not a problem with in vitro labelling. Patients may also be placed in the left lateral decubitus

position to decrease small bowel activity arising from the stomach. Nasogastric tube suction has also been used for this purpose.

5.6.4.6. Procedure

The procedure for Meckel's diverticulum scintigraphy is as follows:

(a) Photopeak: typically a 20% window at 140 keV;
Computer: 128 × 128 matrix.
(b) Patient position: supine (the left lateral decubitus position is optional).
(c) Imaging field: abdomen and pelvis.
(d) Acquisition protocol:
— Optional acquisition of anterior abdominal flow images (1–5 s/frame for 60 s).
— Anterior abdominal images at one image every 30–60 s for at least 30 min (60 min is sometimes recommended).

Additional static images in the anterior oblique, lateral and posterior views are recommended at the end of the dynamic acquisition. Stopping the acquisition to obtain these images when abnormal activity is first seen can be helpful in distinguishing activity in a Meckel diverticulum from that in the kidney, ureter or bladder. Post-void images can also be helpful in detecting activity in a Meckel diverticulum obscured by the urinary bladder. A urinary catheter can be helpful if the Meckel diverticulum is adjacent to the bladder. Alternatively, the decubitus or upright views may at times cause the diverticulum to separate from the bladder.

5.6.4.7. Interpretation

Activity in the ectopic gastric mucosa should appear simultaneously with normal gastric mucosa. A Meckel diverticulum may appear anywhere within the abdomen, although it is more often located in the right lower quadrant. Frequently confused with a Meckel's diverticulum is activity in the kidneys, ureter or bladder. Activity in the urinary tract usually appears after that in the normal gastric mucosa. A small Meckel diverticulum may appear at a later time than that for the stomach. Pertechnetate that is secreted by the gastric mucosa will gradually accumulate in the small bowel. This activity can be distinguished from that in a Meckel diverticulum by its delay and by its appearance as an area of mildly ill-defined increased activity. It is also helpful to view the dynamic study in cine-mode with an upper threshold adjustment for enhancement of low activity areas.

5.6.4.8. Reporting

In addition to patient demographics, the report should include the following information:

(a) The indication for the study.
(b) Procedure:
    (1) Radiopharmaceutical:
        — Dose;
        — Method of administration (intravenous).
    (2) Acquisition:
        — Duration of acquisition (e.g. 1 hour);
        — Frame rate (e.g. 60 s/frame);
        — Projections acquired (e.g. anterior or lateral projections).
    (3) Display (e.g. static or cine).
    (4) Findings:
        — Onset (e.g. early or late, correspondence including gastric activity);
        — Location;
        — Characteristics;
        — Size and shape (e.g. focal or diffuse);
        — Movement (if any).
    (5) Study limitations and confounding factors.
    (6) Interpretation (e.g. positive, negative or indeterminate).

5.6.4.9. Sources of errors

The sources of error that arise in Meckel's diverticulum scintigraphy are as follows:

(a) Procedures that may cause interference:
    (1) False negative results can be due to:
        — Barium enema (attenuation);
        — Upper gastrointestinal contrast studies;
        — Perchlorate (uptake blockage);
        — Recent in vivo RBC labelling (stannous effect).
    (2) False positive results can be due to:
        — Laxatives or endoscopy causing bowel irritation (non-specific uptake).
(b) Anatomical/physiological causes of errors:
    (1) False negative results:

— Too small amount of gastric mucosa in a Meckel diverticulum;
— Ischaemia or necrosis of diverticulum;
— Obscured by urinary tract activity, e.g. in the bladder.

(2) False positive results:
— Urinary tract activity;
— Gastric secretion into the small bowel;
— Lesions with increased blood pool;
— Ulceration;
— Inflammation;
— Irritation;
— Tumour;
— Intussusception;
— Gastric activity secreted into small bowel.

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5.6.5. Gastric emptying and motility

5.6.5.1. Principle

Radionuclide studies of gastric emptying and motility are the most physiological procedures available for evaluating gastric motor function. These studies are non-invasive, use a labelled physiological meal (solid or liquid) and are quantitative. Serial testing can determine the effectiveness of therapy.

5.6.5.2. Clinical indications

Clinical indications relating to gastric emptying and motility are:

(a) Post-prandial:
— Nausea and vomiting;
— Upper abdominal discomfort and bloating;
— Chronic aspiration.

(b) Suspected gastroparesis.
(c) Poor diabetic control.
(d) Gastroesophageal reflux.
(e) Following response to therapy for previously documented motility disturbances.

5.6.5.3. Radiopharmaceuticals

The composition of radiolabelled meals varies widely. An important consideration is that normal emptying rates must be established for any specific meal, patient position, imaging protocol and environment. The radiolabel stability in gastric fluids for any solid meal should be established.

Meals are most often labelled with ^{99m}Tc-SC and may include:

(a) Solids. Prior to cooking the meal, the radiotracer is added to:
— Eggs (scrambled, whole, egg whites or hard boiled);
— Beef stew;
— Liver paté.
(b) Liquids. Almost any liquid can be used, but liquid emptying alone is not as sensitive as solids or semi-solids for the detection of delayed gastric emptying:
— Orange juice;
— Water;
— Milk.

Non-absorbable liquids or solids can be labelled with either ^{99m}Tc (7.4–14.8 MBq (0.2–0.4 mCi)) or ^{111}In (3.7–7.4 MBq (0.1–0.2 mCi)).

5.6.5.4. Patient preparation

Patient preparation for radionuclide studies of gastric emptying and motility require that:

(a) No food or drink should be taken for a minimum of 8 hours prior to imaging. It is preferable that the patient has been fasting since midnight; then administer the radiolabelled meal in the morning.
(b) The patient should be advised of the logistical demands of the procedure (e.g. the meal to be used, the time required for imaging and the position that he/she will be required to maintain throughout the study). Diabetics need to be instructed to bring insulin with them. The dose of insulin is to be adjusted at the time of the meal. If the patient cannot tolerate the

standard solid or liquid meal protocols, the procedure should not be carried out.

(c) Pre-menopausal women should be studied if possible on days 1–10 of their menstrual cycle to avoid the effects of hormonal variation on gastrointestinal motility.

5.6.5.5. Information pertinent to performing the procedure

The following information is relevant to this procedure:

(a) Related diseases and conditions:
— Hiatal hernia;
— Gastroesophageal reflux.

(b) Previous interventions:
— Indications (e.g. cisapride, metoclopramide, domperidone or erythromycin usage);
— Surgery.

5.6.5.6. Clinical contraindications

The following are clinical contraindications to this procedure:

(a) Allergy to the meal;
(b) Fasting in diabetic patients resulting in hypoglycaemia.

5.6.5.7. Procedure

Ingestion of the radiolabelled test meal should be completed as quickly as possible, optimally within 10 min. The technologist should record how long it took to ingest the meal, and if any portion of it was not eaten. The method should be standardized as to patient positioning and environmental conditions such as ambient noise and lighting or other factors affecting patient comfort. The normal values should be based on this standard methodology.

Images are obtained in a format of at least 64×64 pixels using a general purpose collimator. Recommended photopeak settings are 20% at 140 keV for ^{99m}Tc. For ^{111}In, 20% energy windows should be established around both the 172 and 246 keV photopeaks. If ^{111}In is used, a medium energy collimator must be employed for image acquisition. Dual isotope imaging can be performed, which allows for simultaneous evaluation of solid and liquid gastric emptying phases, provided both type of meals are labelled with different radionuclides.

Planar images with the distal oesophagus, stomach and proximal small bowel included in the FOV should be obtained immediately following ingestion of the meal.

Images are optimally obtained for at least 90 min, although a longer period (2–3 hours) is suggested for meals with larger volume or higher caloric content. Anterior and posterior views allow calculation of a geometric mean/square root of the product of the counts in the ROI and more consistently represent the amount of tracer in the stomach, independent of the effect of fundus and antrum motility. This can be performed sequentially with the patient on a rotatable stool using a single head camera or, preferably, simultaneously with a double head camera. Alternatively, the study can be acquired in the LAO view with a single head camera, in which case no mathematical compensation for attenuation is required.

Continuous data collection with a framing rate of 30–60 s is recommended. If data acquisition is interrupted at intervals, the emptying half-time is not as accurately determined and phase lag information may be unavailable. Intermittent data acquisition may be more suitable than continuous data for imaging patients in the upright position.

Images may be obtained standing, sitting or in the supine position, but the position should not change during the study. Normal values must be established for the position used (i.e. there should be separate normal values for upright or supine).

(a) Interventions

Metoclopramide or other prokinetic drugs can be used diagnostically in conjunction with gastric emptying studies to evaluate the effectiveness of a particular therapy.

(b) Processing

The steps involved in processing data are:

(1) An ROI is drawn around the area of tracer activity in the stomach in the anterior and posterior views (or in the LAO view). A cine-display may be helpful to confirm the stomach outline and to determine the extent of patient motion so that the ROI may be appropriately adjusted. Alternatively, if continuous imaging is used, the stomach contour may be identified with initial images combined with later images in the study, after the radiolabelled meal has distributed within the stomach. Using

initial or late images exclusively may under-represent the extent of the fundus and antrum.

(2) Data points must be corrected for radioactive decay.

(3) A time–activity curve obtained from the geometric mean or attenuation corrected counts of ROI activity should then be displayed.

(4) Measurements of gastric emptying may be derived and reported in several ways. Normal values should be available for the specific protocol being used. The half-emptying time reported should be accompanied by a brief description of what the value represents or how it was obtained. Values may be obtained by:
   — Direct determination of the time taken to evacuate half the peak counts;
   — A least squares fit of the emptying data to derive a half-emptying time at 50% of the peak counts;
   — Comparison with a graphic display of normal values plotted as a percentage against time.

(5) In addition, rate of emptying and per cent emptying at the end of the study may be reported together with other information that can be obtained from gastric motility studies, including:
   — Regional motility (e.g. antral contraction frequency and amplitude);
   — Response to medical interventions;
   — Effect of varying meal composition on emptying.

(c) Interpretation

Normal values for the specific meal and environment used should be established before results can be reported. The shape of the curve varies according to the labelled meal: solids exhibit an initial ‘lag phase’ with further almost lineal downslope, while liquids present an exponential downslope with no ‘lag phase’.

Display of images in a cine-format demonstrate better gastric anatomy and findings such as oesophageal reflux, overlap of small bowel with gastric ROI, and possible movement of gastric contents outside the ROI.

Previous surgical procedures and current medications should be considered during the interpretation of findings.

(d) Reporting

The report should include details about the following:

— The type of meal, imaging protocol and techniques for data analysis should be outlined.
— The gastric emptying data reported should be compared with normal values.
— The study should be compared with previous studies, if available. If the previous study protocol differed from the current procedure (e.g. type of meal and patient position), the differences should be reported.
— Any medications being taken that may alter gastric emptying should be documented.

(e) Quality control

The ingested meal must be controlled for caloric content (amount of carbohydrates, fat and protein), volume and temperature.

(f) Sources of error

Errors can arise for the following reasons:

— Food in the airways or the small bowel. (Before a liquid meal is administered to an infant or to a patient with severe neurological impairment through a feeding tube, an abdominal radiograph should be obtained to ensure that the meal is placed in the stomach and not in the airways or the small bowel.)
— Poor labelling of the food.
— Non-standard meal.
— Marked variations in the environment such as noise, lighting or temperature during imaging.
— Emotional fluctuations such as fear of the procedure, anxiety about results or anger after a long waiting time.
— Nausea caused by a meal that may be unfamiliar to the patient.
— Food eaten by the patient immediately prior to the study.
— Slow transit of the ingested meal from the mouth or oesophagus into the stomach.
— Gastroesophageal reflux.
— Overlap of small bowel activity with the stomach ROI.
— Excessive time for patient to ingest the meal.
— Lack of attenuation correction particularly in obese patients.
— Failure to recognize that patient has not eaten the entire meal.

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5.6.6. Oesophageal studies

5.6.6.1. Principle

By using radionuclide techniques, the function of the oesophagus and the gastro-oesophageal junction, and the presence and severity of gastro-oesophageal reflux, can be studied. The main advantages of the procedure are that it is non-invasive, is physiological and can be modified to reproduce the circumstances in which the patient's symptoms occur.

5.6.6.2. Clinical indications

(a) Adults

Oesophageal motility disorders usually present with dysphagia or chest pain (often mimicking ischaemic cardiac disease). The following symptoms and conditions often occur in combination:

— Reflux oesophagitis;
— Hiatus hernia;
— Oropharyngeal dysfunction;
— Primary and secondary achalasia;
— Diffuse oesophageal spasm;
— Oesophageal atresia and stricture;
— Connective tissue disorders;
— Other systemic, neurological and myopathic disorders.

(b) Children

Gastro-oesophageal reflux (GOR), especially in infants, is one of the commonest problems studied in the child presenting with recurrent pneumonia or lower respiratory tract symptoms. GOR can also present in more subtle ways such as:

— Abnormal vomiting;
— Failure to thrive;

— Episodes of apnoea;
— Haematemesis.

5.6.6.3. Radiopharmaceuticals

Many different radiolabelled compounds, foods and liquids have been used, but the most commonly employed is ^{99m}Tc-SC or DTPA mixed with water or milk. Krypton-81m (half-life of 13.3 s) in an aqueous solution allows for repeated studies to be done in a short period.

The volume of tracer swallowed ranges between 5 and 20 mL, and the administered activity of ^{99m}Tc ranges between 37 and 74 MBq (1–2 mCi).

For infants, 8–12 MBq (0.2–0.3 mCi) of ^{99m}Tc colloid are diluted to 2 mL in saline and 3 mL of milk, following which the preparation is added to an empty bottle for the next feed.

5.6.6.4. Equipment

A low energy medium resolution collimator and a standard FOV camera are adequate. In order to evaluate the dynamics of oesophageal transit, dynamic studies using an image matrix of 64×64 pixels are required.

5.6.6.5. Patient preparation

No special preparation is required. Patients are usually studied in a fasting state:

— Infants under 6 months are kept fasting for 3 hours.
— Infants between 6 and 12 months are kept fasting for 4–6 hours.
— Infants over 1 year are kept fasting for 6 hours.
— In older children, the intake of some liquids during the fasting period (but no solid food) can still be allowed.

For infants, an empty bottle (for administering the radioactivity) as well as a bottle containing the next feed should be brought to the nuclear medicine department.

5.6.6.6. Procedure

For the study of deglutition the patient is usually in the supine or an erect position. When a search is made for GOR, the supine position is required. Data acquisition is usually done in the anterior projection, with frame rates of

1–20 per second for the first 1–2 min, followed by a frame rate of 6 per minute for a further period of 20–30 min.

During the deglutition phase of the study, care should be taken to include both the mouth and the stomach in the FOV. In infants, the rest of the feed is administered after completion of the deglutition study. Breast feeding is allowed.

Since GOR, especially in infants, can be secondary to delayed gastric emptying, early and late images of the stomach at 2 hours and preferably also at 6 hours should be obtained.

Visual assessment of oesophageal transit is usually done before quantitative analysis is performed. A cine-display of the images is helpful to identify subtle retrograde motion or retention of the tracer. A useful additional method of display is to condense each dynamic image into a single column of pixels (y axis), with time expressed on the x axis. The resulting image of composite vertical lines is often useful to recognize subtle abnormalities.

Using ROIs over the lower oesophagus and stomach, the amount of reflux and the rate of gastric emptying can be quantitated.

5.6.6.7. Interpretation

The steps listed below should be taken:

— Note the activity, positioning and time frames used for the study.
— Evaluate oesophageal transit.
— Note any evidence of reflux.
— Calculate rate of gastric emptying.

5.7. NUCLEAR MEDICINE IMAGING STUDIES IN ENDOCRINOLOGY

5.7.1. Thyroid scintigraphy

5.7.1.1. Principle

Thyroid scintigraphy is based on iodide physiology involving the following: iodine ingestion, trapping and concentration in the thyroid, oxidation and organification to produce iodotyrosines, and a coupling process to form thyroid hormones. In thyroid imaging, the radioiodine is readily taken up by the thyroid gland, where it is trapped and concentrated from the plasma, and then undergoes the organification process. Similarly, the pertechnetate ion is also trapped and concentrated by the thyroid gland but it does not undergo

organification. The presence of high concentrations of these radiotracers in the thyroid gland provides excellent visualization of the gland by the gamma camera.

5.7.1.2. Clinical indications

Thyroid scintigraphy may be required for any of the following purposes:

(a) To determine the size of the thyroid gland;
(b) For localization of thyroid nodules;
(c) To determine the activity of thyroid nodules;
(d) To determine functional status of the thyroid gland;
(e) To evaluate presence of ectopic thyroid tissues, thyroglossal duct cysts and substernal masses.

5.7.1.3. Radiopharmaceuticals

Details of the radiopharmaceuticals used in thyroid scintigraphy are given in Tables 5.17 and 5.18.

Some centres have tried using other radiopharmaceuticals for evaluation of the thyroid gland. Thallium-201 is preferred by many specialists when

TABLE 5.17. CHARACTERISTICS OF THE RADIOPHARMACEUTICALS USED IN THYROID SCINTIGRAPHY

Property	Radioisotope		
	I–131	I–123	Tc-99m pertechnetate
Physical half-life	8 days	13 hours	6 hours
Mode of decay	β^-	Electron capture	Isomeric transition
Photon energy (keV)	364	159	140
Abundance (%)	81	85	89
Dose	1.8–2.2 MBq (45–55 μCi)	3–16 MBq (80–400 μCi)	80–200 MBq (2–5 mCi)
Method	Oral	Oral	Intravenous
Timing of imaging	24 hours after administration	3–4 hours after administration	15–30 min after administration
Thyroid dosimetry	78 rad (100 μCi)	7.7 rad (400 μCi)	0.13 rad/mCi

TABLE 5.18. ADVANTAGES AND DISADVANTAGES OF THE RADIOPHARMACEUTICALS USED IN THYROID SCINTIGRAPHY

Radioisotope	Advantages	Disadvantages
I–131	Useful in delayed imaging, particularly for thyroid metastases and mediastinal masses	High radiation dose to the thyroid and unfavourable dosimetry Longer time for imaging
I–123	Appropriate for visualization of substernal thyroid tissues	More expensive Longer time for imaging May contain long lived radionuclidic impurities that increase radioactive burden. Consequently, doses which are already 24 hours old cannot be used
Tc-99m pertechnetate	Less expensive and readily available More rapid examination Provides lowest radiation dose/ unit of administered activity	Oesophageal activity can be mistaken for ectopic thyroid tissue Organification function cannot be evaluated

thyroid replacement treatment cannot be discontinued and for looking for cancer metastases in patients with high serum thyroglobulin but with negative radioiodine scans. Other myocardial perfusion agents (^{99m}Tc-sestamibi and tetrofosmin) have also been utilized primarily to search for residual or recurrent thyroid cancer, but their clinical usefulness has not yet been fully assessed. Technetium-99m pertechnetate or low-dose radioiodine ^{131}I should be used for routine thyroid scanning.

5.7.1.4. Equipment

A gamma camera with a pinhole collimator is preferred, to allow multiple views of the thyroid and better resolution of thyroid nodules.

5.7.1.5. Patient preparation

Discontinuation or avoidance of medications or agents that interfere with the thyroid uptake of the radiotracers (radioiodine and ^{99m}Tc-pertechnetate) include:

— Thyroid hormones (T3 and T4 for at least two and four weeks, respectively);
— Anti-thyroid agents (for at least one week);
— Iodine-containing food, for example kelp (for at least one week);
— Iodine-containing medications (e.g. iodinated contrast agents for weeks, and lipid soluble media and amiodarone for months).

5.7.1.6. Clinical contraindications

Radiopharmaceuticals are contraindicated in pregnant women. Enquiries should be made about the menstrual history of female patients in the reproductive age group.

Discontinuation of breast feeding for nursing mothers (12 hours for ^{99m}Tc, permanently for current child with ^{131}I).

5.7.1.7. Procedure

The following procedure should be adopted:

(a) Patient position:
Supine with neck extended to elevate the thyroid.
(b) Timing of imaging:
— For ^{123}I: Imaging can be done 3–4 hours after oral administration. Delayed images at 24 hours have lower body background but with a lower count rate.
— For ^{131}I: Images are obtained 24 hours post-administration.
— For ^{99m}Tc-pertechnetate: Images are obtained 15–30 min after intravenous administration.
(c) Acquisition parameters:
— Obtain 100 000 counts or 5 min observation time, whichever occurs first, with ^{99m}Tc, 20 000 counts or 10 min with ^{131}I, 50 000 counts or 10 min with ^{123}I for images in the following projections:
  ● Anterior view;
  ● 45° right anterior oblique view;
  ● 45° left anterior oblique view.
— Note that the image of the thyroid should occupy at least two thirds of the FOV, necessitating adjustments in the distance between the pinhole aperture and the neck.
— Radioactive markers may be used to identify anatomical landmarks (e.g. sternal notches).
— Note the position of and mark the palpable nodules and surgical scars.

— In cases of oesophageal activity in ^{99m}Tc-pertechnetate or ^{123}I due to salivary excretion, ask the patient to rinse their mouth and drink a glass of water.

5.7.1.8. Interpretation

A clinical examination is mandatory for adequate interpretation.

Note the size, shape and location of the thyroid gland: the thyroid is normally a bilobed or a butterfly shaped organ with each lobe typically measuring 4–5 cm by 1.5–2.0 cm. The right side is slightly larger than the left. There is extreme variability in the appearance of the isthmus. The thyroid lies superior to the suprasternal notch, though this is dependent on the degree of neck extension present at the time of imaging.

Assess the tracer distribution in the thyroid gland: the tracer uptake in the gland should be homogeneous and uniform. Intensely increased uptake in the gland denotes a diffusely hyperplastic gland (e.g. Graves' disease) and in around 40% of cases may also show the pyramidal lobe. Uptake in only one portion or one lobe is commonly seen post-surgery or in hyperfunctioning autonomous adenomas. Diffusely decreased tracer uptake or non-visualization may be seen in cases with concomitant anti-thyroid medication, in patients with an increased iodine pool and in patients under thyroid suppression secondary to thyroid replacement therapy. In early subacute thyroiditis (de Quervain's syndrome), there is very poor tracer localization in the thyroid gland rendering visualization of the gland poor.

Make an evaluation for the presence of nodules and their functional status. Correlate with the clinical findings on palpation: evaluation of the nodules is one of the most frequent clinical indications of thyroid scanning. Identification of these nodules is based on areas of altered uptake in comparison with the rest of the gland and should always be interpreted in correlation with the palpation findings. The presence of increased uptake denotes a metabolically active nodule ('hot nodule'), most often a result of a benign process (autonomous adenoma) as may be seen in Plummer's disease. However, functioning nodules are not very common, occurring in less than 10% of all demonstrable palpable nodules. In comparison, the presence of nodules with decreased to absent tracer uptake connotes a non-functioning nodule ('cold nodule'). Although the majority of nodules represent a benign process (e.g. thyroid adenoma), the incidence of thyroid cancer in cold nodules ranges from 5 to 15%, and most thyroid cancers present scintigraphically as a solitary cold nodule. Solitary cold nodules are commonly due to an adenoma, colloid cyst or primary thyroid carcinoma. Malignant thyroid lesions are very rarely seen in association with hot nodules.

Make an assessment for the presence of ectopic thyroid tissues or the functional activity of the thyroglossal duct cyst and the substernal masses.

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5.7.2. Thyroid uptake measurements

5.7.2.1. Clinical indications

Thyroid uptake measurements can be made for the following reasons:

(a) To determine the functional status of the thyroid gland;
(b) To calculate specific doses for the treatment of hyperthyroidism and ablation therapy of thyroid cancer;
(c) To differentiate forms of thyrotoxicosis (thyroiditis, factitious hyperthyroidism and Graves' disease).

5.7.2.2. Radiopharmaceuticals

The following radiopharmaceuticals are used:

(a) I-131: 0.04–0.4 MBq (1–10 μCi) orally. Choose the dose that is closest in activity to the standard for that batch of in-house prepared doses.
(b) I-123: 3.2–4.8 MBq (80–120 μCi) orally.

Protocol for thyroid uptake tests

(a) Objectives

To measure the per cent uptake of a tracer dose of ^{131}I by the thyroid gland. This is the simplest and most widely used test to evaluate thyroid function. After oral administration of radioiodine, the 2, 24 and 48 hour uptake measurements are done to see the rate of uptake, total buildup and discharge of radioiodine by the thyroid gland.

(b) Equipment required

— Spectrometer;
— Flat field collimated scintillation crystal probe;
— Standard phantom;
— Standard lead shield 4 in × 4 in × 0.5 in (101.6 mm × 101.6 mm × 12.7 mm);
— Marker;
— Carrier-free sodium iodide (^{131}I) capsules (25 μCi).

(c) Procedure for calibration

— Switch on the main supply and the power switches on the spectrometer.
— After 1–2 min switch on the high voltage (HV).
— Increase the HV to the optimum value.
— Set the amplifier gain.
— Let the instrument stabilize for at least 30 min.
— Put the integral/differential switch on differential and the window on 1.0 V.
— Keep the standard capsule in the phantom 30 cm away from the probe and find out the photopeak for ^{131}I starting from a baseline of 300 V and increasing by intervals of 0.5 V (i.e. five divisions), each time counting for 50 s or until the maximum counts are obtained (calibration procedures may vary from instrument to instrument). Note the baseline reading.
— Set the window from 1.0 to 5 V and decrease the baseline setting by 20 divisions. At 1 V, the baseline is 360. Hence at 5 V, the baseline is 360 – 20 = 340.
— Count all the capsules and discard those that show a gross discrepancy in counts.
— Keep one capsule as the standard; the remaining capsules are used in patients to study their respective uptakes.

(d) Procedure for uptake measurement

— Count the standard capsule by keeping it in a phantom 30 cm away from the probe by means of a marker, take two readings of 2 min each and calculate the counts/min (S1).
— Place the standard shield near the capsule and count the standard again for 2 min and calculate the counts/min (S2).
— S1 – S2 = net counts of the standard capsule.
— Administer the radioiodine capsule to the patient after screening.
— Two hours later measure the radioactivity in the region of the patient's neck, keeping a distance of 30 cm from the probe, take two readings of 2 min each and calculate counts/min (P1).
— Ask the patient to hold the standard shield in front of their neck, count for 2 min and calculate counts/min (P2).
— P1 – P2 = net counts of the patient's thyroid.

$$\text{Percentage uptake in the thyroid} = \frac{\text{P1} - \text{P2}}{\text{S1} - \text{S2}} \times 100.$$

— Repeat the counting at 24 and 48 hours, and calculate the percentage uptakes.

(e) Limitations

— The test cannot be performed on pregnant women.
— There is a possible radiation hazard to children, which, unless it is essential, should be avoided.

(f) Disadvantages of the technique

— The prior administration of iodine containing drugs, thyroid hormones, anti-thyroid drugs and several other compounds may invalidate the test for a period of a number of weeks to several months.
— Serial readings are necessary for 3 days for a proper diagnosis.

(g) Advantages of the technique

— This is a simple test.
— The test provides dynamic functional information.

5.7.2.3. Interpretation

The normal range should be established locally. This is normally determined by the dietary iodine intake, types of equipment, standard applications and uptake phantoms.

Hyperthyroid individuals (with Graves' disease, toxic adenoma or toxic multinodular goitre) have elevated uptake values, while patients with subacute thyroiditis or factitious hyperthyroidism will have low to normal uptake values.

A low uptake value has a lower precision, brought about by decreased counting statistics. It is not a primary diagnostic criterion for hypothyroidism.

The interpretation of uptake should be made in conjunction with the patient's history and drug medication intake.

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5.7.3. Whole body imaging for differentiated thyroid cancer

5.7.3.1. Principle

Whole body scanning is primarily used for detection of thyroid metastases or thyroid tissue with residual function. Radioiodine is extracted by the residual thyroid tissue and by 75% of well differentiated thyroid cancers with similar iodide physiology. For functioning thyroid cancers to be visualized by

scanning, the thyroid remnant must be first destroyed or ablated. Metastatic deposits may then be visualized by therapeutic doses of ^{131}I.

5.7.3.2. Clinical indications

Whole body imaging can be used to:

(a) Determine the presence and extent of residual thyroid tissue after surgery;
(b) Localize metastases of thyroid carcinoma.

5.7.3.3. Radiopharmaceuticals

Iodine-131 is the recommended agent, with a dose of 80–200 MBq (2–5 mCi) taken orally. Most centres favour this dose range in order to avoid the possibility of thyroid stunning. Other investigators have proposed conducting diagnostic imaging coincident with the therapeutic dose of ^{131}I. In some cases, small metastatic deposits can only be visualized after therapeutic doses of ^{131}I. Alternatively, the use of 185 MBq (5 mCi) of ^{123}I has been proposed.

Thallium-201 or ^{99m}Tc-sestamibi have also been utilized in detecting residual thyroid tissues. However, the scintigraphic search should be confined to the neck and chest as there is high background radioactivity in the abdomen.

There are advantages to using ^{201}Tl as it does not require the discontinuation of thyroid hormone, as is the case with ^{131}I. In thyroid cancers that do not concentrate radioiodine (medullary cancer and Hürthle cell cancer), ^{201}Tl is very useful. It can also demonstrate metastases when the TSH level is normal or suppressed. In patients with an oxyphilic subtype of differentiated thyroid carcinoma, where there is negative immuno-histochemical staining for thyroglobulin, a ^{201}Tl whole body scan is strongly recommended. Some centres prefer ^{201}Tl in patients with high serum thyroglobulin but where the radioiodine scan is negative. On the other hand, other investigators proposed the use of FDG PET to detect lymph node metastases in conjunction with a CT scan on such patients. European researchers have had promising results with the use of ^{111}In-pentreotide somatostatin receptor scintigraphy to detect recurrent thyroid cancer (both undifferentiated and medullary) in patients without detectable iodine uptake.

Thallium-201, however, does not give information about the avidity of the tumour to radioiodine, especially if ablation with ^{131}I therapy is being contemplated.

A ^{99m}Tc-sestamibi or ^{99m}Tc-DMSA (V) scan can be complementary to the CT scan in patients with recurrent medullary thyroid cancer who have

extremely elevated levels of serum calcitonin. At low levels, however, scan usefulness appears to be limited.

5.7.3.4. Equipment

A gamma camera with a high energy collimator for ^{131}I is needed.

5.7.3.5. Patient preparation

Patient preparation should include:

(a) Discontinuation or avoidance of medications or agents that interfere with the thyroid uptake of radioiodine:
  (1) Thyroid hormones (T4 for 4–6 weeks, T3 for 2 weeks). Thyroid hormones must be stopped to attain a high TSH level. Some centres advocate replacement of T4 by T3 for 6–8 weeks to minimize the risk of cancer progression during the time thyroid hormone is withheld as T4 has a longer half-life of 1 week compared with 1.5 days for T3. This replacement is then discontinued 2 weeks prior to scanning. Ideally, TSH should be greater than 30 μIU/mL.
  (2) Iodine-containing food (e.g. kelp, for at least 1 week). It is preferable that the patient be on a low iodine diet for at least 1 week prior to the study, to increase the sensitivity of the procedure.
  (3) Iodine-containing medications (e.g. iodinated contrast, amiodarone and iodine-containing expectorants for at least 2 to several weeks)
(b) An ^{131}I tracer dose is given when TSH levels are high. This reflects the maximum stimulation of metastases to be seen in the scan.
(c) Contraindicated in pregnant patients. Find out about the menstrual history of female patients.
(d) Discontinuation of breast feeding by nursing mothers is essential for at least two months, preferably completely.

5.7.3.6. Procedure

The following procedure should be adopted:

— The patient should be in the supine position.
— Timing of imaging: images are obtained at 2 and 4 or more days after radioiodine administration. By this time, iodine initially extracted by the salivary glands and gastric mucosa has already been cleared and excreted via the urinary tract.

— In cases of ^{201}Tl- and ^{99m}Tc-sestamibi, images are obtained after administration and may be repeated at 3 hours.
— Images are then recorded in either a whole body or spot view format. In the latter case, imaging should be performed for 5–10 min over several areas: neck, chest spine, pelvis and proximal extremities in both the anterior and posterior projections. For the skull, a posterior image should be considered. For images with a whole body camera, the scan speed should be slow, usually less than 10 cm/min, and appropriate to the count rate.

5.7.3.7. Interpretation

Assess the size, shape and location of any areas of tracer uptake that correspond to normal or abnormal thyroid tissue, more particularly in the anterior neck area. Note also the tracer distribution in the residual thyroid tissue. The study should be compared with any prior scan and correlated with the recent thyroglobulin assay.

Activity in the gastro-intestinal tract, including the salivary and nasal glands, and the genito-urinary tract is considered normal, while tracer localization in the head and neck, liver, lungs and bones is considered to be due to metastatic deposits or functioning thyroid remnants. Uptake in the thymus in young patients should be recognized as normal.

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5.7.4. Parathyroid scintigraphy

5.7.4.1. Principle

Hyperfunctioning parathyroid tissues are primarily due to parathyroid adenomas (85–90% of cases) and hyperplasia of several or all the parathyroid glands (10–15%). Carcinoma of the parathyroid is extremely rare (2% of cases). Many centres have experience with ^{201}Tl- and ^{99m}Tc-pertechnetate subtraction scans for parathyroid scintigraphy. The rationale is based on the fact that ^{201}Tl, being a potassium analogue, is taken up by all tissues with high cellularity and vascularity, including parathyroid adenomas and hyperplastic parathyroid glands and the thyroid gland. The ^{99m}Tc-pertechnetate thyroid image would then be subtracted from the ^{201}Tl image. However, many other tissues can take up ^{201}Tl, mimicking parathyroid adenomas (e.g. thyroid adenomas and cervical lymph nodes). Since the average parathyroid gland is only 40 mg in size, it is not possible to see normal parathyroids by this technique.

Recent developments have shown that ^{99m}Tc-sestamibi and tetrofosmin have an advantage in detecting abnormal parathyroid glands. Sestamibi washes out of the normal thyroid more rapidly than out of abnormal parathyroid glands. A persistent increase in tracer localization in the delayed views would then be construed as hyperfunctioning parathyroid glands.

There are two problems with a direct subtraction technique. Firstly, it is not possible to normalize the two images in a rigorous enough way to know how much of one to subtract from the other, making subtraction subjective. Secondly, there is always some slight movement between the two images. These problems can be addressed by using a formal translation rotation programme (a) to superimpose the two images and (b) to compare the two images using a change detection analysis, the result of which is a colour coded probability map where significant differences are displayed in red ($P < 0.001$) or orange ($P < 0.01$). The use of different energy radionuclides also creates a problem. This can be avoided by using ^{99m}Tc-pertechnetate thyroid imaging instead of ^{99m}Tc-MIBI.

5.7.4.2. Clinical indications

Parathyroid scintigraphy can be used for:

(a) Localization of parathyroid adenomas;
(b) Localization of ectopic parathyroid adenomas.

5.7.4.3. Radiopharmaceuticals

The following radiopharmaceuticals can be used for parathyroid scintigraphy:

(a) Thallium-201 chloride:
  — Physical half-life of 73 hours;
  — Photopeak due to characteristic X rays of 68–80 keV (98%);
  — Emission gamma rays of 135 keV (2%) and 167 keV (8%);
  — Intravenous dose of 80–120 MBq (2–3 mCi).
(b) Tc-99m pertechnetate:
  Pertechnetate is taken up by the thyroid and is subtracted from the ^{201}Tl image. The dose is dependent on which radionuclide (^{201}Tl- or ^{99m}Tc-pertechnetate) is administered first – the dose is generally within 80–400 MBq (2–10 mCi). There are many reports on the sequence of administration of radionuclides (^{201}Tl- followed by ^{99m}Tc-pertechnetate or vice versa), each having advantages and disadvantages. Giving ^{201}Tl first is

preferred as it has lower energy as well as the ability to image the mediastinum. The patient must, however, be properly instructed to remain immobile throughout the procedure.

(c) Tc-99m sestamibi or tetrofosmin is the preferred radiopharmaceutical with an activity of 200–800 MBq (5–20 mCi).

5.7.4.4. Equipment

A gamma camera with a high resolution collimator is needed.

5.7.4.5. Patient preparation

No special preparation is needed. However, clear instructions should be given to the patient regarding movement, particularly in ^{201}Tl–^{99m}Tc-pertechnetate digital subtraction scans. Sedation is sometimes inevitable.

5.7.4.6. Procedure

The following procedure is used for parathyroid scintigraphy:

(a) Thallium-201–Tc-99m pertechnetate digital subtraction scans:
- Position the patient in the supine position with imaging done in the anterior projection and with the patient's head immobilized.
- Set up the gamma camera to acquire images in a dual isotope study. Digital data are acquired in a 256 × 256 matrix.
- Inject ^{201}Tl intravenously. Begin to acquire images of the mediastinum between the heart and the thyroid in the ^{201}Tl energy window recorded for 3–5 min not later than 2–3 min after injection. After the upper mediastinum image has been completed, image the entire neck for 15 min with the collimator placed closer to the patient. The thyroid is now centred.
- Inject Tc-99m pertechnetate intravenously. After 5 minutes, image the neck with the same total count as the 15 minute ^{201}Tl image. The patient's immobilization should be maintained.
- Process the images by serial subtraction of 10% increments of the pertechnetate image from the thallium image until the entire thyroid is removed.

(b) Dual-phase ^{99m}Tc-sestamibi
- Position the patient in the supine position with their neck extended to include the mediastinum in the FOV.

— Inject ^{99m}Tc-sestamibi and after 15 min start the imaging for 10 min. This is to be repeated every 15 min for the first hour, and at 2 and 3 hours post-injection.

5.7.4.7. Interpretation

Normal parathyroid glands are not seen scintigraphically. Abnormal parathyroid tissue usually presents as a focal area of increased tracer deposition, which would become increasingly intense on the delayed views. Hyperfunctioning parathyroid glands, more particularly parathyroid adenomas, have a slow washout in comparison with the thyroid tissues. Hyperplastic glands, on the other hand, have a more rapid washout than adenomas, and are not visualized in late images.

The most common cause of a solitary focus of radioactivity pertaining to the parathyroid is an adenoma. Parathyroid adenomas are usually seen unilaterally. The presence of two abnormal glands strongly suggests parathyroid hyperplasia since the prevalence of double adenoma is extremely rare (around 2–4% of patients with hyperparathyroidism). Change detection analysis is able to identify adenomas down to 100 mg and four gland hyperplasia in patients with renal failure with 87% accuracy.

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5.7.5. Adrenal medulla scintigraphy

5.7.5.1. Principle

Adrenal medulla scintigraphy is primarily indicated for the evaluation of functioning paragangliomas. These are catecholamine-secreting tumours (paragangliomas, in particular pheochromocytomas, neuroblastomas, ganglioneuroblastomas and ganglioneuromas). These paraganglionic cells belong to the amine precursor uptake and decarboxylation (APUD) system and have the ability to store catecholamines in intracellular cytoplasmic vesicles.

Meta-iodobenzyl-guanidine (MIBG) is an analogue of guanethidine and norepinephrine. Localization of MIBG in adrenergic tumours is thought to be via the energy dependent active amine transport mechanism and further storage in the cytoplasmic storage vesicles in pre-synaptic adrenergic nerves. However, MIBG does not bind to post-synaptic receptors and does not induce a pharmacological response. MIBG also localizes to other organs with sufficient sympathetic innervation (heart, salivary glands, spleen and occasionally the colon).

5.7.5.2. Clinical indications

Adrenal medulla scintigraphy is used for:

(a) Localization of adrenal pheochromocytomas and extra-adrenal paragangliomas;
(b) Localization of metastatic adrenal medullary cancer secondary to pheochromocytoma;
(c) Prior to ablation therapy of metastatic adrenal medullary cancer secondary to pheochromocytoma;
(d) Evaluation of patients with suspected neuroblastomas;
(e) Evaluation of patients with carcinoid and medullary thyroid cancer, to determine if there is a likelihood that ^{131}I-MIBG therapy would be of benefit.

5.7.5.3. Radiopharmaceuticals

MIGB is available labelled with either ^{123}I or ^{131}I. Because of unfavourable dosimetry properties, ^{131}I-MIBG places a high radiation burden on the adrenal medulla. It is recommended to block thyroid uptake with iodine administration (saturated solution of potassium iodide (SSKI) or Lugol's solution) and this is continued for five days. Its disadvantage lies in the requirement for delayed imaging.

Iodine-123 MIBG, on the other hand, shows some superiority in visualizing the adrenal glands but is usually not available. Iodine-131 MIBG is preferred in developing countries.

The dose of ^{131}I-MIBG is 20–40 MBq (0.5–1.0 mCi). Excretion is via the kidneys, with about 85% being excreted unchanged in the urine.

5.7.5.4. Equipment

A gamma camera with a high energy, parallel hole collimator for ^{131}I-MIBG is required.

5.7.5.5. Patient preparation

Patient preparation consists of the following:

(a) Check the patient's current intake of medication. All the drugs in the following list have been shown to interfere with MIBG uptake. All of these should be discontinued from three days before the study:
   — Tricyclic antidepressants (amitriptyline and imipramine);
   — Decongestants (phenylpropanolamine and pseudoephedrine – common in nose drops and cough mixtures);
   — Sympathomimetics and amphetamines;
   — Reserpine;
   — Antipsychotics (phenothiazines);
   — Calcium channel blockers;
   — Adrenergic blockers (long acting beta blockers);
   — Cocaine.
(b) Administer SSKI or Lugol's solution one day prior to the study and continue for five days, or 60 mg potassium iodide (KI) twice daily for five days.
(c) Do not administer to pregnant and nursing patients.

5.7.5.6. Procedure

Ensure that the patient has stopped all medications known to interfere with the uptake of ^{131}I-MIBG.

Check if the patient has taken SSKI, KI tablets or Lugol's solution prior to the study.

Slowly inject 20–40 MBq (0.5–1.0 mCi) ^{131}I-MIBG intravenously over 15 s. Higher doses have been used post-operatively to look for residual remnant tissues.

Acquire images for 20 min obtained at 24 and 48 h post-injection. Obtain anterior and posterior spot views of the body from the skull to the pelvis. For pheochromocytomas, anterior and posterior views of the mid-abdomen to include the region of the adrenal glands are most important. An image at 10 min will show the renal excretion, which may be misinterpreted as adrenal activity if there is pelvic retention of tracer.

5.7.5.7. Interpretation

The adrenal glands are usually either not identified or faintly identified with ^{131}I-MIBG. A normal adrenal gland is visualized with ^{123}I-MIBG and with therapeutic doses of ^{131}I-MIBG. Pheochromocytomas usually present as unilateral focal tracer depositions. In general, most pheochromocytomas demonstrate a more intense uptake than the liver (80%). The sensitivity is reported to be in the range of 85–90% with a specificity of 95–99%. Computed tomography scans or MRI are more accurate in detecting primary tumours of the adrenal glands but they are inferior to MIBG in the detection of extra-adrenal tumours, which account for about 10% of lesions. For neuroblastomas, sensitivity is about 90–95% with 95–100% specificity. In detecting bone metastasis, MIBG is superior to bone scans for neuroblastomas, while in pheochromocytomas a bone scan is also required. Liver metastasis is difficult to ascertain since MIBG is normally taken up by the liver, although ^{123}I-MIBG allows this distinction to be made.

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5.8. MUSCULOSKELETAL SYSTEM

5.8.1. Introduction

This section provides guidelines for nuclear imaging of the musculoskeletal system using ^{99m}Tc-methylene-diphosphonate (MDP) or hydroxyethylene diphosphonate (HEDP) in a broad spectrum of diseases of the bone, joint, muscle and musculotendinous unit. Bone infections can additionally be imaged using ^{111}In-leucocytes and ^{67}Ga. Bone scan modes include whole body planar scintigraphy, planar spot scintigraphy, SPECT, planar pinhole scintigraphy and pinhole SPECT.

5.8.1.1. Radiopharmaceuticals

Technetium-99m MDP is universally used for bone scintigraphy. It is a monoenergetic emitter of 140 keV gamma rays with a physical half-life of 6.02 hours and efficiently labels phosphonates or pyrophosphates, yielding ^{99m}Tc-MDP and ^{99m}Tc-HEDP among other compounds. These have a strong avidity for hydroxyapatite crystals in the mineral phase of the bone, especially at sites where new bone is actively formed as in the physes of growing bones and fractures. The diphosphonate molecule is adsorbed onto the calcium of hydroxyapatite in bone. Following intravenous injection, ^{99m}Tc-diphosphonates are rapidly distributed in the extracellular fluid space and approximately half of the injected dose is taken up by bone, with the unfixed portion excreted into the urine by glomerular filtration. The amount of radiopharmaceutical accumulated in bone at 1 hour after injection is 58% with MDP, 48% with HEDP and 47% with pyrophosphate. The blood levels of HEDP are 10% at 1 hour after injection and continue to fall to 6, 4 and 3% at 2, 3 and 4 hours, respectively.

The usual dose for adults is 740–1110 MBq (20–30 mCi) injected intravenously. For markedly obese adults, the administered dose may be increased to

11–13 MBq/kg (0.3–0.35 mCi/kg). For children, the administered dose is 9–11 MBq/kg (0.25–0.3 mCi/kg), with a minimum of 40–90 MBq (1.1–2.4 mCi). In children, the administered dose can be scaled on the basis of body surface area. Bone radiopharmaceuticals are subject to oxidation, hence care should be taken to avoid introducing air into the multidose vial. Quality control should be performed prior to administration of the radiopharmaceutical.

5.8.1.2. Equipment: Gamma camera system

A large FOV gamma camera is best suited for bone scintigraphy. A low energy, high resolution collimator and a pinhole collimator are the two most widely used collimators for bone scanning. For SPECT, a high resolution parallel hole should be used but some systems also accept pinhole collimators.

5.8.1.3. Modes of bone scintigraphy

Scintigraphic modes for the imaging of bone, joint and musculotendinous units include planar whole body scintigraphy, planar spot scintigraphy, three phase planar scintigraphy, planar pinhole scintigraphy, conventional SPECT and pinhole SPECT.

(a) Planar whole body bone scintigraphy

Planar whole body bone scintigraphy produces a pair of the anterior and posterior images of the entire skeleton, including cranially from the skull and caudally from the cervical spine, to the complete upper and lower extremities.

(b) Planar spot bone scintigraphy

Planar spot bone scintigraphy portrays a localized portion of the body, usually the ROI, with most of the rest of the skeleton in additional views.

(c) Three phase planar bone scintigraphy

Three phase planar bone scintigraphy consists of dynamic arterial blood flow images, static blood pool images and delayed static bone images. The blood flow phase is obtained in sequence as the tracer is injected in a bolus. Sixteen to 20 frames are taken, with the acquisition time per frame varying from 2 to 4 seconds according to the site imaged. The static images of the blood pool, one or two in number, are taken within 10 min of the injection. The delayed planar images are taken 2–3 hours after injection. Scanning can be

started as early as 1.5 hours after injection when HEDP is used. By commencing scanning earlier, more photons are yielded per injected amount of tracer, resulting in an improvement of the system sensitivity. Delayed static imaging can be reinforced with planar pinhole magnification, conventional SPECT and pinhole SPECT when necessary (see the following paragraphs).

(d) Planar pinhole bone scintigraphy

Planar pinhole bone scintigraphy is performed using either single head or dual head gamma camera systems simply replacing a multihole collimator(s) with a pinhole collimator(s). Pinhole scintigraphy significantly enhances the resolution through optical magnification. This mode generates either a single magnified view or a pair of magnified views of a selected portion of the skeleton in any desired projection. Dual head planar pinhole scintigraphy eliminates the 'blind zone' seen on single head pinhole images and reduces the scan time by half. Optimal pinhole scintigraphy of a portion of the skeleton takes 15–20 min using a pinhole with a 4 mm aperture.

(e) Conventional single photon computed tomography of bone

Bone SPECT produces sectional images of a portion of the skeleton. The standard projections are transverse, coronal and sagittal. SPECT can separate selected plane or small volumes of tissue from other tissues that overlie or underlie them, improving the image contrast up to sixfold. It is useful in assessing the distribution of radioactivity qualitatively, although it does not improve resolution. SPECT cameras can be single, dual or triple headed. SPECT is used in the diagnosis of complex structures such as the spine, pelvis, hip and temporomandibular joint. One of the most typical applications is the evaluation of lower back pain or facet joint syndrome. In general, SPECT is not suitable for the imaging of small, flat or thin bones. Sectional images can be reconstructed three dimensionally.

(f) Pinhole single photon computed tomography of bone

Pinhole SPECT, a hybrid of pinhole scanning and SPECT, generates magnified sectional images of a portion of the skeleton. Pinhole SPECT can be achieved using any single head SPECT gamma camera system provided with a filtered back-projection algorithm and a Butterworth filter. Pinhole SPECT simultaneously enhances both the resolution and the contrast by optical magnification and tomography, respectively. The value of the limiting spatial resolution of the planar pinhole scintigraphy is 2 line pair/cm, which is greater

than the 1 line pair/cm resolution of an ordinary planar scan and similar to those of CT scans, MRI and ultrasonography.

5.8.1.4. Patient preparation

The rationale for performing the imaging and the details of the procedure itself should be explained to patients in advance. Unless contraindicated, patients should be well hydrated by drinking at least two glasses (500 mL) of water or other beverages between the time of injection and the time of delayed imaging. Patients should be instructed to urinate immediately prior to delayed imaging and to drink plenty of fluids for at least 24 hours after radiopharmaceutical administration.

The following information should be obtained before commencing the procedure:

— Clinical questions to be resolved by bone scintigraphy;
— History of trauma, infection, oedema, arthritis, neoplasm, metabolic bone disease or limitation of function;
— Presenting symptoms and signs;
— History of recent scintigraphy, especially with ^{131}I, ^{67}Ga and ^{111}In;
— Results of prior bone scintigraphy;
— Results of radiography, CT and MRI studies;
— History of therapy that might affect the results of the current study (e.g. antibiotic, steroid, radiation, diphosphonate or iron therapies, as well as chemotherapy);
— History of orthopaedic procedures (e.g. nailing repairs and prosthetic implants) and non-orthopaedic surgery (e.g. ileal conduits), which might affect the results of bone scintigraphy;
— Breast surgery and implants;
— Relevant laboratory data (e.g. PSA level in patients with prostate cancer);
— History of anatomical–functional renal abnormalities.

5.8.1.5. Clinical contraindications

If possible, elective bone scintigraphy should be deferred in pregnant women. Similarly, breast feeding should be discontinued for 24 hours after the injection of the radiopharmaceutical.

5.8.1.6. Observation and interpretation

Attention should be paid to the following points:

— Observe altered tracer activity in bone, joint, muscle and tendon.
— Note whether increased (or sometimes decreased) activity in the individual lesion is intense, moderate or mild.
— Identify specifically the bone, joint, muscle and tendon affected.
— Observe if lesions are:
  ● Single or multiple;
  ● Focal, diffused or generalized;
  ● Unilateral, bilateral, axial or appendicular;
  ● Well or ill defined.
— Describe as accurately as possible the location of the lesion in terms of its topography (e.g. the epiphysis, metaphysis or diaphysis, cortex, medullary space, periosteum, joint, muscle or tendon).
— Describe the location in as precise and anatomical terms as possible.
— On follow-up scintigraphy, describe morphologically and semi-quantitatively the changes that have occurred since the previous study. The lesions may be improved, aggravated or unaltered. If any therapy was performed, evaluate the change in terms of the response. Use caution not to interpret 'flare-up' as aggravation.
— Abnormalities should be interpreted with other available information including clinical history, physical examination, other imaging studies and laboratory tests.
— Observe abnormal tracer activity in extramusculoskeletal organs such as the lungs, liver, spleen, kidneys and bladder.
— Check for artefacts (motion, attenuation objects, urine contamination, etc.).

5.8.1.7. Reporting

In reporting, be sure to:

(a) Describe the technique used:
  — Flow images;
  — Blood pool images;
  — Delayed images;
  — Injection site;
  — Magnification or SPECT images (if performed).
(b) Describe abnormal tracer accumulation:
  — Increased;
  — Decreased;
  — Number, site and pattern of abnormal accumulation;
  — Bone and joint findings;

— Soft tissue findings.

(c) Correlate with other studies.

(d) Compare with previous bone scans.

(e) Draw conclusions:
   — Use a narrow differential diagnosis as much as possible.
   — Recommend further definitive stud(ies) if the differential diagnosis is broad.

5.8.1.8. Sources of error

The following sources of error should be noted:

— Injection artefacts;
— Urine contamination or a urinary diversion reservoir;
— Prosthetic implants, radiographic contrast materials or other attenuating materials that obscure normal structures;
— Surgical deformation;
— Homogeneously increased bone activity (e.g. superscans);
— Motion of patient, table or camera head;
— Greater than necessary collimator-to-patient distance;
— Imaging too soon after injection before radiopharmaceutical has been optimally cleared from soft tissues;
— Restraint artefacts caused by soft tissue compression;
— Prior administration of a higher energy radionuclide (^{131}I, ^{67}Ga or ^{111}In), or of a ^{99m}Tc radiopharmaceutical that accumulates in an organ that can obscure or confound the skeletal activity;
— Radioactivity extraneous to patient;
— Significant findings outside the ROI may be missed if a limited study is performed;
— Radiopharmaceutical degradation or improper tagging;
— Changing bladder activity during SPECT of pelvic region;
— Lesions without alteration of tracer activity;
— Pubic lesions obscured by underlying bladder activity;
— Renal failure.

5.8.1.9. General methodological considerations

Bone scintigraphy usually starts by imaging the whole skeleton in both anterior and posterior projections. This is followed by planar spot or pinhole imaging to highlight the ROI. Standard views may be supplemented by an oblique or other special view as indicated. Among the special views are the

Waters' view of the maxillary bone, the seated view of the sacrum and coccyx, the 'butterfly view' of the sacroiliac joints, the 'frog-leg view' of the hip joints, the 'sunrise view' of the patella and the 'tunnel view' of the intercondylar notch of the distal femur. For adequate visualization of the hips, knees and fibulas, particularly in children, the feet should be turned inwards with the toes close together (radiographic neutral position or reverse frog-leg view). In general, it is desirable to take two crossing or orthogonal views whenever one finds suspicious lesions on one view.

Planar pinhole scintigraphy can be performed using both a single and a dual head gamma camera system. A pinhole collimator can be aligned to any desired angle, permitting all-angle imaging, a distinct technical advantage. The scan time is usually 15–20 min. Aperture sizes of available pinhole collimators vary from 2 to 6 mm, with 4 mm being the optimal size. A pair of two magnified images can be obtained by dual head pinhole scintigraphy.

Pinhole SPECT can be achieved by simply substituting a multihole collimator, used for planar SPECT, by a pinhole collimator. Acquisition, reconstruction and display are the same as in planar SPECT. At present, this technique is applicable only to the peripheral appendicular bones and joints, such as those of the ankle and wrist, because of the mechanically limited range of the detector's orbit.

Three phase scintigraphy, useful in assessing the vascularity of a bone lesion, can be interpreted in a semi-quantitative way. It can localize bone infection and distinguish it from soft tissue lesions. A recommended protocol is an immediate post-injection angiography (16 consecutive frames of 2–4 s images), blood pool imaging within 10 min of injection and delayed static bone imaging after 1.5–4 hours and eventually 24 hours after injection of ^{99m}Tc-MDP or -HEDP (the later constitutes a fourth phase).

Indium-111 labelled granulocyte scintigraphy is suitable for the diagnosis of infective bone diseases. The specificity is about 90%, but sensitivity is only 50%. This is expensive, and the separation of pure granulocytes, which is necessary to increase sensitivity, demands high technical skills. Formerly, ^{67}Ga was used for bone imaging, but nowadays its use is mostly restricted to osteomyelitis of the spine, where false negative studies have been reported with ^{111}In granulocyte scintigraphy. Technetium-99m labelled anti-granulocyte antibodies, ^{99m}Tc-HMPAO labelled white blood cells (WBCs) and ^{99m}Tc-ciprofloxacin can also be used for diagnosing bone infections.

5.8.1.10. Interventions

The pelvis can be difficult to evaluate when there is tracer activity in the bladder. In patients with pelvic symptoms, one or more of the following additional views are useful:

— A second image taken immediately after voiding.
— An image taken sitting on the detector (oblique or lateral views).
— An image taken after a 24 hour delay.
— A magnified view or SPECT. Single or multiple rapid (acquisition over 5–10 min) SPECT acquisitions are recommended to avoid artefacts caused by changing activity in the bladder.
— Bladder catheterization should be reserved for those patients who are unable to void and for whom visualization of the pelvis is essential.

5.8.1.11. Normal and abnormal bone scintigraphy

It is essential to be thoroughly familiar with normal bone findings in order to accurately recognize pathology. Physiologically, there tends to be a distinct accumulation of tracer in the cranial vault, facial bones around the nasal cavity, shoulders, manubriosternal junction, sternoclavicular joints, spine, sacroiliac joints, pelvis and hips. The larger joints in the extremities may also show prominent accumulations. It is well known that tracer accumulates intensely in the physes of growing bones.

Scintigraphic abnormalities of bones and joints are presented as either increased or decreased uptakes, often described as ‘hot areas’ and ‘cold areas’ respectively. A cold area is also referred to as a photopenic or photon-deficient area. Increased uptake can be graded as mild, moderate and marked. Among a range of parameters that may distort scan findings, the tilting of the body to either side is probably the most critical. Since photon energy diminishes rapidly according to the inverse distance square law, even a slight difference between the target–detector distances results in significant image distortion and asymmetry. Thus, bone structures closest to the detector may appear unusually hot, leading to an erroneous interpretation.

Bone scintigraphic abnormalities can be recognized in three essential ways: morphology, tracer uptake pattern and vascularity. Morphological alterations are expressed in terms of size, shape and position, and radionuclide uptake pattern and vascularity as increased, unaltered or decreased. Most bone lesions present as hot rather than cold areas. Lesions that tend to display cold areas include acute avascular necrosis, lytic metastasis and multiple myeloma.

5.8.1.12. Clinical applications

Scintigraphy is useful for the following diseases and conditions:

(1) Acute infective diseases of bone;
(2) Tuberculosis of bone;
(3) Non-infective inflammations of bone;
(4) Indium-111 and ^{99m}Tc labelled leucocytes and ^{67}Ga scans in bone infections;
(5) Transient synovitis of the hip;
(6) Acute pyogenic arthritis;
(7) Osteoarthritis;
(8) Rheumatoid arthritis;
(9) Ankylosing spondylitis;
(10) Reiter's syndrome;
(11) Reflex sympathetic dystrophy syndrome;
(12) Avascular necrosis of bone;
(13) Osteochondroses;
(14) Traumatic and sports injuries of bone;
(15) Periarticular rheumatism syndromes;
(16) Muscular and musculotendinous rheumatism syndromes;
(17) Metabolic diseases of bone;
(18) Benign and primary malignant bone tumours;
(19) Metastatic bone tumours;
(20) Tumorous conditions of bone.

(1) Acute infective diseases of bone

Acute infective diseases of the bone include osteomyelitis, osteitis, cortical abscesses and periostitis. Acute osteomyelitis typically involves metaphysis of the long bones where the end-arteries are distributed, providing favourable conditions for bacterial embolization. Osteitis, which commonly occurs in association with osteomyelitis, is the infection of compact bone. Cortical abscesses are a special form of acute pyogenic infection in which the infective focus is within the cortex. This occurs either singly or in conjunction with osteomyelitis. Garre's sclerosing osteitis is a variant of chronic osteomyelitis.

Pinhole scintigraphy can distinguish these conditions by specifically locating the anatomic pathological site and assessing the tracer uptake pattern of the individual diseases. For example, the metaphyseal bone marrow is

primarily involved in acute osteomyelitis, the cortical bone in abscesses and the periosteum in periostitis.

Spondylitis is the acute pyogenic infection of the spine. Infection is either blood borne or the direct result of a traumatic wound or surgery. Infective spondylitis, both acute and chronic, produces the characteristic 'sandwich' sign on magnified scintigraphs. This sign consists of intense tracer uptake in two apposing end-plates with narrowed disc space.

(2) Tuberculosis of bone

Tuberculosis can affect the spine, skull and appendicular bones. Tuberculosis is more common in the spine than in other bones. Pathologically, bone tuberculosis is characterized by destruction with relatively mild reactive bone formation. With chronicity, it may form cysts accompanied by sclerosis. A special form of tuberculosis, which involves the finger in infants, is known as spina ventosa.

Planar bone scan findings are usually not specific, but pinhole scintigraphy reveals findings of diagnostic value. The diseased bone shows a localized area of increased tracer uptake, occasionally with associated photopenic area(s). The latter finding denotes either necrosis or cystic changes. In the spine, as in acute infective spondylitis, tuberculosis involves two or more neighbouring vertebrae and intervertebral discs. Extended tracer uptake can be seen deep in the vertebral bodies, confirming that the chronic granulomatous process spreads from the end-plate into the vertebral body.

(3) Non-infective inflammations of bone

Non-infective inflammations of the bone include osteitis condensans ilii, osteitis pubis, condensing osteitis of the clavicle, sternocostoclavicular hyperostosis, infantile cortical hyperostosis and radiation osteitis. Tietze's disease may also be included in this category. Each of these diseases manifests characteristic signs on pinhole images that are comparable to radiographic signs.

(4) Indium-111 and ^{99m}Tc labelled leucocytes and ^{67}Ga scans in bone infections

White blood cells, either granulocytes or lymphocytes, labelled with ^{111}In or ^{99m}Tc and ^{67}Ga, are used for the detection of bone infections. Granulocytes avidly accumulate in acute infective foci while lymphocytes accumulate primarily in chronic foci. The sensitivity and specificity of ^{111}In-leucocyte scans have been variously reported, ranging between 50 and 100%, and 69–100%,

respectively. Technetium-99m HMPAO labelled leucocytes, anti-granulocyte monoclonal antibodies and antibiotics labelled with ^{99m}Tc are used for the same purpose. The image quality of ^{99m}Tc-HMPAO leucocyte scans has been reported to be comparable or superior to that of ^{111}In-leucocyte scans. However, there is much normal uptake of ^{99m}Tc-HMPAO in the axial skeleton, reducing the diagnostic value. A ^{99m}Tc-HMPAO leucocyte bone scan is more suited for the rapid screening of acute infections, whereas ^{111}In labelled leucocytes are preferred for chronic cases. Gallium-67 scans are non-specific, accumulating in both inflammatory and neoplastic lesions.

(5) Transient synovitis of the hip

Transient synovitis, also known as irritable hip syndrome, transient arthritis or transient coxitis, is a self-limited non-specific inflammatory disease of the hip in children. Technetium-99m MDP scintigraphy may demonstrate an ill defined, increased uptake in the hip joint on vascular and static images. In contrast, pinhole scintigraphy precisely localizes tracer uptake to the synovia, which cover the femoral head and acetabular fossa. Such uptake is due to an increase in blood flow through the anastomotic vascular channels in the inflamed synovium. The tracer uptake may be prominent in the active stage but rapidly returns to normal with rest and conservative treatment. It is to be noted that in the early stage with large synovial effusion, tracer uptake may become reduced due to ischaemia of the femoral head created by capsular distension.

(6) Acute pyogenic arthritis

Like acute osteomyelitis, pyogenic arthritis is difficult to diagnose radiographically in its early stages. However, bone scintigraphy reveals an increased blood flow and blood pool in septic joints, and intense tracer uptake in the subchondral bone on static images in the early stages. The ‘wrapped bone’ sign, which indicates diffuse synovitis, is characteristic. The intensity of subchondral tracer uptake in acute pyogenic arthritis has been described as roughly paralleling the intensity of infection. Dual head pinhole scintigraphy produces a pair of either the anterior and posterior, or the medial and lateral, images, permitting a three dimensional analysis of the disease.

(7) Osteoarthritis

Osteoarthritis is the most common disease of the joints. Histologically, it is characterized by the derangement and eventual destruction of the cartilage and subchondral bone without obvious inflammation. Synovitis is not a

constant feature. The involvement is random, pauciarticular and asymmetrical. The knees, hips and spine are the most common sites. Bone scintigraphs may show discrete unifocal or multifocal tracer uptake in subchondral bones, and can be spotty, patchy or segmental in type. When synovitis is present, tracer uptake becomes diffuse.

(8) Rheumatoid arthritis

Rheumatoid arthritis is characterized by diffuse synovitis, pannus formation, and cartilagous and bone destruction. Involvement is symmetrical and polyarticular. Whole body bone scans are the only way to portray symmetric polyarthritis panoramically; spot views can depict characteristic changes in both large and small joints in great detail. Pinhole scintigraphy is useful in delineating many scintigraphic signs of rheumatoid arthritis. Nuclear angiography provides information on lesional vascularity and on the activity of the pathological process. In remission, vascularity reverts to normal.

(9) Ankylosing spondylitis

Ankylosing spondylitis and Reiter's syndrome are the two most common seronegative spondyloarthropathies (SNSAs). Ankylosing spondylitis is a non-specific inflammatory disease of the sacroiliac joints and the spine. The disease primarily involves the synovial components of the sacroiliac joints and the cartilaginous discovertebral junctions as well as the apophyseal, costovertebral and neurocentral joints of the vertebrae. In the late stage of the disease, bony ankylosis of these joints ensues. Planar bone scintigraphy reveals symmetric intense tracer uptake in the sacroiliac joints and/or spine. Pinhole scintigraphy can portray the characteristic ribbon-like tracer uptake in the synovial joints of the spine, producing a 'bamboo spine' appearance. In the late stage, tracer uptake becomes reduced, reflecting a quiescent metabolic state.

(10) Reiter's syndrome

Reiter's syndrome is not, as once considered, a rare disease. The syndrome consists of a triad of urethritis, arthritis and conjunctivitis. The disease mechanism is still obscure, but an interaction between several different infective organisms and a specific genetic background is currently being given serious consideration. Pathologically, the main alterations are present in the enthesis, which is the site of insertion of a tendon, ligament or articular capsule into the bone, creating characteristic inflammatory enthesopathy. Typical clinical manifestations include Achilles tendinitis, calcanean plantar fasciitis

and spurring, 'sausage digit', whiskering of the iliac crest, ischiopubic bone and trochanter, and paravertebral enthesitis. Conspicuous involvement of entheses in this syndrome sharply contrasts with the dominant involvement of the synovium in rheumatoid arthritis. Bone scintigraphy appears to be the diagnostic method of choice. The whole body scan can panoramically reveal characteristic asymmetrical pauciarticular involvement of the spine and appendicular bones and joints. Pinhole scintigraphy often detects characteristic enthesopathy in the pre-radiographic stage, especially in the heel and knee. In addition, pinhole scintigraphy can show specific signs of Reiter's syndrome, namely the 'knuckle bone' sign of the sausage digit, the 'teardrop' sign of paravertebral enthesopathy and the 'whisker' sign of periarticular hyperostosis.

(11) Reflex sympathetic dystrophy syndrome

Reflex sympathetic dystrophy syndrome (RSDS) is a rather common condition. It is a rheumatic disorder of clinical importance and academic interest, often related to previous trauma. The involvement is usually diffuse but can be segmental. The pathogenesis has not yet been clarified, although the theory of the internuncial pool is widely accepted. The identification of the 'sympathetic vasoactive intestinal peptide-containing nerve fibres' at the cortical bone and the bone–periosteal junction has provided a biochemical basis for the theory. Vasoactive intestinal peptides released from sympathetic nerve fibres have been shown to cause hyperaemia and bone resorption, as seen in RSDS. Three phase scintigraphy is useful, revealing increased blood flow and blood pooling, which denotes hyperperfusion. Pinhole scintigraphy and pinhole SPECT show mottled and band-like areas of tracer uptake in the bone peripheries or cortical bones. Involvement of periarticular structures of one or more joints of a limb is characteristic.

(12) Avascular necrosis of bone

Avascular necrosis of bone, or osteonecrosis, results from deprivation of blood flow. The common causes include trauma, embolism, thrombosis, elevated bone marrow pressure, irradiation and vasculitis. Scintigraphically, avascular necrosis presents as a hot area on the planar image, especially in small bones. However, when magnified using pinhole scintigraphy, a photopenic area can be detected within the hot area. Typical examples are avascular osteonecrosis of the femoral head and of the internal femoral condile of the knee.

(13) Osteochondroses

Contrary to the broadly accepted unitary concept of primary avascular osteonecrosis, osteochondroses are now recognized as a group of heterogeneous pathological entities. Common clinical features include a predilection for actively growing bone, chronic exposure to trauma and local pain, and tenderness.

Osteochondroses affect the capital femoral epiphysis (Legg–Calvé–Perthes disease), the tarsal navicular bone (Koehler's disease), the metatarsal head (Freiberg's disease), the medial clavicular end (Friedrich's disease), the secondary ossification centres of the vertebrae (Scheuermann's disease) and the tibial tubercle (Osgood–Schlatter's disease). Bone scintigraphic findings differ according to the type of underlying pathology. Large avascular osteonecrosis produces cold areas, whereas microfractures or bone infraction are represented by hot lesions. Scintigraphy can provide information regarding the size, shape, location, texture and osteochondral junction pattern, frequently leading to specific diagnosis.

(14) Traumatic and sports injuries of bone

Bone scintigraphy is the imaging procedure of choice in the diagnosis of traumatic and sports injuries. In elderly patients, it is useful for the study of contusion and fracture in osteoporotic ribs and spine. Bone scintigraphy is valuable for the detection and differential diagnosis of shin splints and stress fractures. It can be used for the classification of stress fractures, showing the characteristic tracer uptake in the absence of radiographic alteration. Usually, a planar whole body scan and spot images are sufficient for the diagnosis of a fracture. Occasionally, however, magnification is needed for accurate localization of the fracture, differential diagnosis between bruise and fracture, and detection of an occult fracture. The posterior aspect of the cortical tibia is very often affected.

(15) Periarticular rheumatism syndromes

Diseases in this category include bursitis, tenosynovitis and enthesitis. Bone scintigraphy reinforced with pinhole magnification can portray tracer accumulation in sites specific to the individual diseases. For example, in Achilles tendinitis the tracer accumulates in the upper retrocalcaneal surface.

(16) Muscular and musculotendinous rheumatism syndromes

These syndromes include myositis ossificans, rhabdomyolysis and musculotendinous unit injuries. Bone scintigraphy is useful for the demonstration of bone tracer accumulation in denatured or calcified muscle fibres and musculotendinous units. Bone scintigraphy aided by pinhole magnification is useful to delineate the individual structures affected.

(17) Metabolic diseases of bone

Metabolic bone diseases may be caused by a number of conditions, including vitamin deficiency or excess, undernourishment, endocrine disorders, renal failure, and disturbed calcium and phosphorus metabolisms. Clinical entities are diverse and manifestations are complex. This presentation describes involutional osteoporosis, osteomalacia, rickets and renal osteodystrophy, all of which can be diagnosed by scintigraphy.

(a) Involutional osteoporosis

Involutional osteoporosis includes senile osteoporosis and post-menopausal osteoporosis. Osteoporosis causes bone mass reduction, which makes bones brittle and fragile. In post-menopausal osteoporosis, trabecular bone mass is disproportionately reduced in comparison with cortical bone mass. On the other hand, senile osteoporosis is characterized by the proportionate loss of cortical and trabecular bone. Frequent complications are fracture of the spine and Colles's fracture. Other common fracture sites are the femoral neck, proximal humerus, tibia and pelvis. Women suffer twice as frequently as men. The aetiology has not been established, but a generalized decrease in metabolism may be responsible. Bone scintigraphy may show a generalized decrease in tracer uptake. Pinhole scintigraphy reveals characteristic thinning of the cortices of the long bones or sparse end-plates of the vertebrae. When porotic vertebral end-plates are fractured they display an intense concentration of tracer. Compressed porotic end-plates give rise to the 'fish vertebra' appearance.

(b) Hyperparathyroidism

Hyperparathyroidism is either primary, secondary or tertiary and is associated with increased parathormone production with consequent excessive bone calcium mobilization. Scintigraphically, diffusely increased tracer uptake can be observed in the calvarium, mandible, sternum and shoulder bones.

Pinhole scintigraphy can portray linear tracer uptake in the subperiosteal bone resorption in the phalangeal shafts and acrolysis of the tufts. In the calvarium, pinhole scintigraphy shows a 'salt and pepper' pattern of diffusely increased tracer uptake.

(c) Renal osteodystrophy

Renal osteodystrophy is caused by hyperparathyroidism secondary to chronic renal insufficiency. The bone scintigraphic features of renal osteodystrophy include the 'tie sternum' sign, 'striped tie' sign and costochondral beading or 'rosary' sign. The so-called 'hot patella' sign is not specific for metabolic bone diseases since it is also observed in chondromalacia patellae, metastases and disuse osteoporosis or as a normal variant. Pinhole scintigraphy is useful in the study and documentation of stimulated bone turnover, either focally in Looser's infraction or diffusely in the malacic skeleton. It can also be used for the detection of subperiosteal bone resorption, cystic change and osteosclerosis in renal osteodystrophy.

(d) Rickets and osteomalacia

Rickets and osteomalacia are characterized by deficient mineralization of the osteoid. The basic difference between the two conditions is that the former disease occurs in actively growing bones and the latter in mature bones. The aetiology includes a deficiency of vitamin D and its active hormonal form (1,25-dihydroxyvitamin D3) and a disturbed calcium–phosphorus metabolism. The scintigraphic manifestations of rickets and osteomalacia can be divided into systemic and local. For the study of systemic changes a whole body bone scan is advantageous, and for the portrayal of local changes pinhole scintigraphy is suitable. Whole body scintigraphy may show a generalized increase in tracer uptake in the entire skeleton, producing a 'superscan' sign. The phenomenon occurs more typically in the osteomalacia related to renal osteodystrophy. Small, spotty, hot areas in cortical bones of the skeleton with a 'superscan' represent infractions, a pathognomonic sign of osteomalacia. Such hot spots are mostly found in the lower rib cage, pubic bone and proximal femur, which are easily subjected to external trauma or stress. In rickets, pinhole scintigraphy may show very intense tracer uptake in the flared metaphyses and ossification centres of the long bones, creating the 'chicken bone' sign. The joint spaces appear spuriously widened as a result of small dystrophic ossification centres and the bulky cartilaginous zone.

(18) Benign and primary malignant bone tumours

Planar bone scintigraphy is less specific than radiography in the diagnosis of primary bone tumours. The use of pinhole scintigraphy, however, is changing that perception. With magnification, bone scintigraphy is capable of making an accurate diagnosis of many tumours and tumorous conditions of the bone such as bone cysts, giant cell tumours, osteochondroma, osteoid osteoma, Paget's bone disease, fibrous dysplasia and primary malignant bone tumours such as osteosarcoma. It can also be used for the detection of soft tissue invasion of osteosarcoma and bone-to-bone metastasis. Bone scintigraphy is particularly helpful in the diagnosis of pathological fractures.

(19) Metastatic bone tumours

Evaluation of malignant bone metastases is one of the most widely accepted indications for bone scintigraphy. It facilitates the early detection and assessment of disseminated areas of metastasis, provides assistance about future therapy and is useful for prognosis. Nonetheless, there are still unanswered questions concerning the appropriate use of bone scintigraphy in staging of the disease.

Bone scintigraphy can detect metastases weeks, and often months, before radiography. The large majority of metastases are multiple, with only about 7% presenting as a solitary lesion. Breast and prostatic cancers tend to spread to the spine through the vertebral veins, while lung cancer spreads haematogenously to random sites in the skeleton. Metastases from renal and thyroid cancers are often photopenic. The incidence of photopenic metastasis has been reported to be 2%. Extensive metastases to the axial skeleton may produce the 'superscan' sign. Approximately 5% of metastases with radiographically visible osteolysis may not be visible on a bone scan. Certain scintigraphic features are helpful in distinguishing metastases from benign lesions. Transaxial hot areas in the ribs generally indicate fractures, while longitudinal hot areas are usually metastases. A solitary hot area in the rib is malignant only in 1.4% of cases, 90% being related to a benign aetiology such as trauma or irradiation. In contrast, 68% of solitary lesions in the axial bone are malignant. A solitary hot area in the sternum in patients with known primary cancer indicates metastasis if trauma is excluded. Segmental or spotty hot areas in the vertebral end-plates and diffuse tracer uptake in the vertebral body usually indicate metastases, while tracer uptake involving the whole length of an end-plate is characteristic of compression fracture.

(20) Tumorous conditions of bone

(a) Paget's disease

Paget's disease is relatively common in elderly Caucasians. It was considered rare in Asians but appears to be increasing slowly. The main clinical symptoms are local bone pain and tenderness with bone deformity but these symptoms often represent an incidental finding. Common sites of involvement are the skull, vertebrae, thoracic cage and long bones. Planar bone scintigraphy characteristically shows bone growth with diffuse, intense tracer uptake. Pinhole magnification is useful to delineate the characteristic tracer accumulation pattern in the cortex and peripheries of the skull, vertebrae, sacrum and long bones. Polyostotic Paget's disease can be mistaken for metastasis, although both may coexist.

(b) Fibrous dysplasia

Fibrous dysplasia is an uncommon hamartomatous disease of the bone. Its histology is characterized by metaplastic production of benign fibrous tissue stroma and curled spicules of woven bone formed therefrom. The involvement may be either monostotic or polyostotic and the lesion is a frequent site of pathological fracture. Whole body bone scintigraphy is suitable for the detection and mapping of fibrous dysplasia. Pinhole magnification is used to differentiate between a fibrous and an osseous focus of the disease. In general, an osseous focus is characterized by an intense concentration of tracer compared with the poor concentration in a fibrous focus. When fractured, lesions accumulate tracer very intensely.

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5.9. SPECIAL PROCEDURES IN ONCOLOGY

5.9.1. Gallium-67 citrate imaging

5.9.1.1. Principle

Gallium-67 citrate was one of the earliest radionuclides used in nuclear medicine. Over the years, with the increased use of X rays, CT and MRI in morphological imaging as well as the advent of other specific and non-specific radiopharmaceuticals in tumour and infection imaging, the use of ^{67}Ga-citrate for tumour imaging has become very limited. However, it still plays an important role in malignant lymphoma but is likely to be eclipsed in the near future by the broad use of ^{18}F-FDG PET technology, especially following the introduction of dual head gamma camera coincidence imaging. Other indications for ^{67}Ga include the localization of acute infections, the evaluation of the extent or severity of certain benign diseases such as sarcoidosis and interstitial pulmonary fibrosis, and monitoring the response to therapy. Gallium-67 has also been used in tuberculosis, although clinical and laboratory findings are more cost effective in developing countries, where the incidence of tuberculosis is higher than in industrialized countries.

Gallium-67 has a physical half-life of almost 73 hours, which allows its delivery worldwide, limited shelf-storage and easy scheduling. These characteristics have enabled its price to fall to a reasonable level in most parts of the world. Gallium-67 decays by emission of four gamma rays at 93, 184, 296 and 388 keV; the first three peaks being used for imaging.

5.9.1.2. Clinical indications

(a) Oncological applications

There are applications to the following malignant conditions:

(1) Malignant lymphomas:
- At the time of diagnosis before commencing treatment, in order to determine whether the tumour is gallium-avid. Gallium-67 citrate imaging can then be used to evaluate treatment response. However, it cannot be used to accurately assess the stage of the disease.
- After two or more cycles of chemotherapy in order to determine response and prognosis.
- At the end of treatment, to identify residual disease and characterize the nature of any residual mass revealed by X rays or CT scans in the mediastinum or abdomen.
- Whenever early recurrence is suspected.

(2) Lung carcinomas:
- Evaluation of mediastinal nodal enlargement (if the scan is positive bilaterally, mediastinoscopy could be avoided).
- Evaluation of residual disease in collapsed lungs after radiotherapy, particularly in patients who have previously undergone a lobectomy.

(3) Bone and soft tissue sarcomas:
- Evaluation of pre-operative chemotherapy response, although ^{201}Tl-chloride or ^{18}F-FDG are more appropriate, as is probably ^{99m}Tc-sestamibi.

(b) Benign applications

There are applications to the following benign conditions:

(1) Infection localization:
- In patients with fever of unknown origin or sepsis, gallium is preferred in the chronic phase when the WBC count is not increased. In the acute phase, where there is an increase in the WBC count, it is preferable to use infection localizing radiopharmaceuticals such as ^{111}In- or ^{99m}Tc-HMPAO WBCs.

(2) Sarcoidosis:
- Evaluation of the extent of the disease at the time of initial diagnosis.
- To assess the response to treatment.

(3) Idiopathic interstitial lung disease:
- At the time of the initial diagnosis.
- To verify the presence of disease.
- To establish the severity of lymphocytic infiltration.
- To monitor the response to treatment.

5.9.1.3. Patient preparation

The following procedure should be followed:

(a) Before injection of radiopharmaceuticals:
- A full clinical examination and the information gathered from laboratory tests and other sources of morphological imaging are needed. There should be a valid justification for the test.
- For patients who are receiving chemotherapy, treatment should have been stopped for at least ten days.
- Radiotherapy should cease for a period of two to four weeks before the ^{67}Ga injection depending on the dose of radiation given, the length of time and the volume irradiated. Steroids should be stopped for at least one week. Patients should not have a gadolinium enhanced MRI for several hours before a ^{67}Ga injection;
- Patients who have received blood transfusions or had a haemolytic crisis requiring parenteral iron therapy will have saturated transferrin binding sites. In such cases, gallium will be mainly taken up by the bone marrow, with less uptake in the liver and pathological sites; the sensitivity of the test will be low.
- If the gallium scan is requested in order to localize acute infection, ^{111}In-WBCs are preferred if there is leucocytosis and the duration of fever is less than two weeks.

(b) After injection of the radiopharmaceutical:
- Bowel activity presents a problem for the recognition of abnormal abdominal areas. Bowel cleansing with a mild laxative such as magnesia milk or a washing enema is recommended. When imaging malignant diseases, the problem of bowel activity can be resolved by delayed imaging up to seven days following intravenous injection.

5.9.1.4. Doses and time of imaging

(a) Dose and route of injection

The following doses should be given:

(1) For tumour imaging:
- 296–370 MBq (8–10 mCi) intravenously for adult doses.
- (5–7 MBq)/(kg body weight) ((0.15–0.16 mCi)/kg), with a minimum of 74 MBq (2 mCi) in paediatrics.

(2) To localize infection:
— 185–296 MBq (5–8 mCi) intravenously for adult doses.
— (3–5 MBq)/(kg body weight) ((0.07–0.15 mCi)/kg)), with a minimum of 54 MBq (1.5 mCi) in paediatrics.

(b) Time of imaging

Images should be taken at the following times:

— For acute infections: anterior images of the ROI at 4 hours post-injection using a large FOV gamma camera for 2 million counts with a 128 × 128 matrix.
— For infection localization: a planar whole body image at 24–48 hours, and SPECT if required for the chest and/or abdomen.
— For other benign applications: a planar whole body image at 48 hours, and SPECT if needed for the chest and/or abdomen.
— For oncological applications: a planar whole body image at 48–72 hours, and SPECT if needed for the chest and/or abdomen. Imaging should be delayed 7–10 days if the result is equivocal.

The study may be repeated at variable times, in accordance with department protocol, if there is bowel activity.

5.9.1.5. Procedure and equipment

The following procedures and items of equipment are required:

(a) A medium energy, parallel hole collimator (high energy collimators are also used).
(b) Data acquisition:
(1) Planar acquisition should either be:
— Total body scanning on a 256 × 1024 matrix for at least 40 min with anterior and posterior projections from the head to below the knees (approximately 4 million counts in total).
— Static planar images acquired on a 128 × 128 matrix for:
● 500 000 for head and neck (anterior and posterior);
● 1 000 000 for chest, abdomen, pelvis and upper thighs (anterior and posterior);
● 500 000 for thighs and knees (anterior only, posterior if needed).
— Occasionally lateral views for the axilla of 500 000 counts are needed.
— Arms should be raised above the head when imaging the chest.

— Images acquired at 7 days should have at least half the number of counts of the early images.

(2) SPECT imaging:
— 360° rotation.
— 64 × 64 matrix.
— A minimum of 60–64 projections for single head gamma cameras and 120–125 projections for dual head cameras.
— Step and shoot for 40 s per projection.
— Total counts: 3–8 million.

(c) Image processing:
Data are filtered by back-projection using a Metz or Butterworth filter or other appropriate filters according to the manufacturers' recommendations. The cut-off frequency or power of the filters should be adjusted according to the total counts acquired. Alternatively, an iterative reconstruction method should be used if one is available. An attenuation correction should be considered for deep structures (especially the abdomen).

(d) Image display:
Data should be displayed in coronal, transaxial and sagittal projections, 1–2 pixels thick, with an additional 3-D volume display. A surface 3-D display is not adequate.

(e) Mechanism of uptake:
Gallium-67 binds to plasma proteins, especially transferrin, lactoferrin and ferritin at the iron binding sites, competing with iron. The labelled plasma proteins cross the target cellular membrane to intracytoplasmic liposomes or stick to the binding sites on the cellular membrane. There is no clearance of ^{67}Ga from the cells after intracellular localization. Malignant tumours that are rich in these receptors are gallium-avid.
Conditions that saturate iron binding sites in the plasma interfere with the biodistribution of ^{67}Ga, which remains in the blood pool and has more bone uptake, thus decreasing its sensitivity.

(f) Radiation dose:
The critical organ is the distal colon, which receives 0.43 mGy/MBq (0.9 rad/mCi).

5.9.1.6. Interpretation

(a) Patterns of gallium uptake

Normally, one third of the dose will be in the liver, one third in the bone marrow and spleen, and one third excreted in the urine and by the bowels.

Other sites of normal uptake are the salivary glands, lacrimal glands, nasal mucosa, external genitalia and growth plates in paediatrics, children and adolescents. Renal and bladder activity can be seen up to 48 hours post-injection. Bilateral breast uptake occurs pre-menstrually, during pregnancy and lactation. Hyperplastic breasts, secondary to oral contraceptives, also can have bilateral increased uptake. This finding can also be expected in men following prolonged use of digitalis.

(b) Healing wounds and fractures

The healing time of tissue from the day of an accidental, provoked or surgical wound until complete healing may vary from two weeks to several months, depending on the size of the wound and whether the healing is primary or secondary. In fractures, healing will take several months before completion.

(c) Abnormal patterns of gallium uptake

Abnormal patterns secondary to previous chemotherapy and radiotherapy treatment include the following:

— Bilateral parotid, submaxillary and lacrimal uptake secondary to chemotherapy induced sialedenitis.
— Diffuse bilateral lung uptake due to interstitial pneumonia.
— Gastric uptake due to gastritis.
— Renal uptake, especially with cytoxam therapy.
— Urinary bladder uptake secondary to cystitis induced by chemotherapy.
— Thymic uptake due to thymic hyperplasia and rebound phenomenon requires full clinical data for correct identification, to avoid a biopsy or further chemotherapy. This is a transient phenomenon seen in about 5–10% of patients that disappears after a few weeks.

(d) Special patterns of gallium uptake

Special patterns of gallium uptake with no clinical significance following treatment include the following:

— Mild low-grade bilateral pulmonary uptake.
— Bilateral low-grade hilar uptake.

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5.9.2. Scintimammography and sentinel node localization

Mammography is the current accepted approach for screening women above the age of 40–50 years for the purpose of early detection of breast cancer. It has been successful for diagnosing more than 80% of cases of breast cancer at an early stage. As a consequence, patients are being treated at an earlier phase of their disease and their prognosis has improved. Mammography, more than any other procedure, has contributed to the more successful care of breast cancer and survival rates. Nevertheless, it poses a few problems.

Because of the non-specificity of the findings that differentiate between benign and malignant lesions, many patients are biopsied for benign lesions. The yield of malignant lesions varies between 15–30% according to the population screened and the expertise of the interpreting physician.

Mammography is not sensitive in dense breasts, or in breasts that have been deformed as a result of a previous biopsy, treatment of previous malignancy by lumpectomy, radiotherapy either of the whole breast or locally

in the form of brachytherapy, previous drainage of a breast abscess, or following reconstruction surgery of the breast and mammoplasty. Similar problems are also encountered in patients with fibrocystic disease of the breast.

There have been various attempts to reduce the number of biopsies for benign lesions in order to save costs and to avoid the psychological impact on those patients who are left with a scar following lumpectomy. These attempts involved the use of ^{99m}Tc-sestamibi, ^{201}Tl-chloride and ^{18}F-FDG but none gave convincing results that could substitute for biopsy. All such attempts failed to demonstrate major clinical value because the sensitivity of mammography for small lesions under 2 cm is less than 70%. Therefore, it is very difficult to substitute for a biopsy whenever there is suspicion of malignancy in the mammogram.

Once the diagnosis of malignancy has been established, the next step is determining the stage of the disease in order to decide on the best treatment for the patient. Eighty per cent of breast cancers are discovered at an early state and are operable. The most important staging criterion in these patients is the status of axillary node involvement by malignant cells. Until recently, total axillary node dissection with histological examination was the only way for axillary staging. Axillary dissection requires longer hospitalization and is followed by complications in more than 30% of patients due to infection, pain, oedema of the arm and limitation of movements. This is a high price to pay, since in the majority of patients the pathological examination of the axillary specimen shows no evidence of metastatic spread. In stage T-1 lesions (<2 cm), axillary node involvement varies from 3 to 19%.

The new approach to localize the sentinel node, either by methylene blue or by radionuclide techniques, represents a major development. It is considered the second most important milestone for the treatment of breast cancer following the changes from the mutilating radical or modified radical mastectomy to the more conservative approach of lumpectomy and post-operative radiotherapy. Sentinel node localization is successful and accurate in more than 98% of patients. Those who show no involvement of the sentinel node after microscopic examination of the frozen sections, haematoxylin and eosin staining and special PCR staining do not require further dissection of the axillary nodes. Patients who have metastatic disease to the sentinel nodes require dissection of all the axillary nodes. The significance of a few metastatic microscopic cells detected only by PCR staining is not yet known. Protocols are currently under evaluation in order to determine the prognostic impact of these findings. The sentinel node approach has a negative predictive value of more than 99% in T-1 lesions, which constitutes the most significant feature of this approach.

5.9.3. Procedure recommendations for scintimammography

5.9.3.1. Patient selection

Nothing else is as effective as a biopsy whenever a malignant lesion is suspected. A biopsy, however, is only appropriate for those patients for whom the results of a mammography are inconclusive, namely under the following conditions:

- Dense breasts, when there is clinical suspicion of a mass that cannot be detected in the mammogram. Palpable masses in dense breasts should be always biopsied irrespective of size. Scintimammography might help localize the site of a biopsy.
- A breast with distortion of its architecture by previous surgery, irradiation or reconstructive procedures.
- Fibrocystic disease of the breast, when there is a suspicious mass on the mammogram. Scintimammography helps guide the surgeon to pinpoint the biopsy.

5.9.3.2. Patient preparation

The procedure, its benefits and the time needed to perform it should be explained to the patient when obtaining her consent.

5.9.3.3. Radiopharmaceuticals

Technetium-99m sestamibi is preferred to ^{201}Tl-chloride because of the higher injected dose and greater photon flux, as well as data from tissue culture experimental work that show a higher uptake of ^{99m}Tc-sestamibi than ^{201}Tl-chloride in breast cancer cells. Other ^{99m}Tc compounds such as tetrofosmin can also be used.

(a) Dose and route of administration:
- Technetium-99m sestamibi or other ^{99m}Tc labelled compounds: 555–740 MBq (15–20 mCi).
- Thallium-201 chloride: 111–148 MBq (3.0–4.0 mCi).

(b) Route of injection:
- An intravenous injection should not be given in the arm on the side of the suspicious breast mass. Either the other arm or a foot should be used.

— It is important to inject straight into the vein. Any extravasation will lead to lymphatic permeation and uptake by the lymph nodes in the axilla that could be misleading for the interpretation of axillary metastasis.
— In patients with bilateral breast masses, injection in the dorsal veins of the foot is recommended.

(c) Waiting time:
— Although imaging can be started 15 min after injection, longer periods of up to two hours are indicated in patients with suspected inflammatory lesions. It is known that both thallium (^{201}Tl) and ^{99m}Tc-sestamibi wash out with time in benign lesions. In most malignant lesions, the washout is usually slower than in benign inflammatory lesions.

5.9.3.4. Procedure and equipment

The following procedure and items of equipment are recommended:

(a) Patient positioning and views to be acquired:
— The patient should lie face down with the affected breast resting on a foam lined aperture on the imaging table. The arm on the side to be imaged should be raised above the head. The patient should be in the prone position and with the breast hanging, relaxing the pectoralis muscle and allowing separation of the breast tissue from the chest wall muscles and from cardiac and liver activity.
— Lateral projections should be acquired for each breast.
— A posterior oblique view of the affected breast is recommended.
— Following the two lateral views, an anterior view of the thorax with the arms raised above the head is needed for axillary evaluation.

(b) Recommended gamma camera:
— Single or double head.
— Large FOV.
— The collimator should be of low energy and of the parallel hole, high resolution type.
— Matrix size of $128 \times 128 \times 16$.
— The zoom factor should be sufficient for the breast to cover two thirds of the FOV of the gamma camera in the lateral and posterior oblique projections. In the anterior projections, both breasts, the axillae and supraclavicular regions should be included so magnification is not critical.
— Acquisition time of 10 min per projection.

5.9.3.5. Interpretation

The interpretation should be made in steps, firstly blind to other data and then with all the information available from the clinical examination, mammography and any previous interventions.

Malignant tumours show up as areas of focal increased uptake that can be graded in different ways according to the intensity and distribution of the uptake. Difficulties usually occur with small lesions. Large lesions are generally very well evaluated.

Lymph node metastases should be checked in the axillae, supraclavicular, infraclavicular and internal mammary regions.

Although no special processing is needed, reporting directly from the computer screen with threshold enhancement and background subtraction is recommended.

5.9.3.6. Reporting

Details should be provided on the radiopharmaceutical used, the site of injection, waiting period, projections acquired and the intensity of uptake, as well as the confidence in the interpretation.

5.9.4. Procedure recommended for lymphoscintigraphy for axillary sentinel node localization in breast carcinoma

5.9.4.1. Patient preparation

No special preparation is required for patients undergoing surgery the same day. At least three hours should be allowed for the usual pre-surgical preparation. Patients whose surgery is scheduled for the next morning can be injected and imaged late in the afternoon. The patient should be made aware of the procedure and provide her consent for the study.

5.9.4.2. Patient selection

Preference is given to:

— Patients with primary breast lesions that have been proven malignant by a needle or core biopsy;
— Patients where the lesion is up to 3 cm in size;
— Patients who have not undergone a previous lumpectomy.

5.9.4.3. Patient exclusion

Usually excluded from the procedure are:

— Patients with a breast lesion over 4 cm in diameter;
— Patients showing signs of advanced stage of the disease such as retracted nipples, oedema and orange skin, bleeding from the nipple, or palpable lymph nodes in the axilla or supraclavicular region;
— Patients with local recurrence;
— Patients with multicentre malignant lesions;
— Patients where the margins are ill defined at the time of surgery or if recognized clinically.

5.9.4.4. Radiopharmaceuticals and procedure

Technetium-99m labelled radiopharmaceuticals with reproducible 200 nm particle size are used such as:

— Technetium-99m antimony SC;
— Unfiltered ^{99m}Tc-SC;
— Filtered (0.22 μm) ^{99m}Tc-SC.

Technetium-99m HSA and ^{99m}Tc-dextran have been mentioned. They travel faster through the lymphatic system and allow visualization of proximal lymph nodes in addition to the sentinel node, although this can be disadvantageous.

5.9.4.5. Site of injection

(a) Intratumoral injection

Intratumoral injection should only be used in conjunction with ultrasound guidance because of difficulty in precisely locating the mass in certain patients. Lack of lymphatic system in the tumour may produce late or no migration of particles.

(b) Injection around the tumour

A 24 gauge needle, 2 in (51.2 mm) long should be inserted at four points around the tumour, with the injection site less than one centimetre from the edge of the tumour at four quadrants, 90° apart. This presents difficulties in

dense large breasts. After lumpectomy, injection around the tumour leads to reduced visualization of the sentinel node in a large percentage of patients as a result of distortion of the lymphatic vessels. It is imperative to avoid injecting into the wall or into the cavity of the post-surgical lumpectomy site.

(c) Subcutaneous or intradermal injection

The lymphatic system of the breasts converge from all sectors towards the areola and then intradermally towards the draining lymph node. Subcutaneous injection is slower than intradermal injection. In order to achieve the desired results, an intradermal injection of 0.3–0.4 mL should be given using a TB needle. By inducing a bleb in the skin to increase intracapillary pressure, the injected particles will be forced into the lymphatics. After the injection, the site should be massaged by applying pressure with a finger for 10 s. Using this technique, the sentinel node can be visualized in less than 5 min in most patients.

Intradermal injection is preferred in patients who have previously had a lumpectomy. The radiopharmaceutical should be injected in these patients on both sides of the centre of the scar, in the section of the breast overlying the breast mass. In non-palpable masses, localization by ultrasonography is recommended and the ultrasonographer should mark the location of the breast mass. Internal mammary lymph nodes, however, have less chance of being visualized with intradermal injection.

5.9.4.6. Dose and volume injected

The following procedure should be applied:

(a) Injection around the tumour:
- —Patients imaged on the day of surgery require 18.5 MBq (0.5 mCi) in 4 mL saline. Inject 1.0 mL at four quadrants, 90° apart.
- —Patients undergoing surgery the following day have an increased total dose of 37 MBq (1.0 mCi) in 4 mL saline. Warming the saline to body temperature helps reduce the pain at the injection site that is frequently experienced by patients.

(b) Intradermal injection:
- —Patients imaged on the day of surgery require 8–12 MBq (0.2–0.3 mCi) in 0.3–0.4 mL saline.
- —Patients undergoing surgery the following day have an increased dose of 37 MBq (1.0 mCi) in 0.3–0.4 mL saline.

5.9.4.7. Mode of acquisition

Dynamic images of 30–60 s each should be acquired in a 128×128 matrix in a lateral projection to include the breast and the axilla, starting immediately after the injection, for a total of 30–45 min. This is followed by static acquisition for 5 min in the lateral and anterior projections. If the nodes are still not seen, static images should be repeated after two hours. A transmission scan is recommended using either ^{99m}Tc or ^{57}Co flood sources to outline the body contours.

5.9.4.8. Display of data

Attention should be paid to the following points:

— Dynamic images are summed and displayed representing 1 or 2 min each.
— Static images with transmission are used to provide anatomical references.
— The upper threshold should be adjusted for optimal visualization as well as masking of the injection site.

5.9.4.9. Reporting

The report should include:

— Clinical history;
— Radiopharmaceutical used, dose, volume and route of injection;
— Whether the study succeeded in 'localizing' the sentinel node;
— Whether the sentinel node localized is in the lower or middle axilla (upper axilla is rare);
— Whether internal mammary and/or other (intrapectoral or supraclavicular) lymph nodes are visualized alone, or in association with axillary nodes.

5.9.4.10. Intra-operative procedures

The intra-operative procedures are summarized below:

(a) The surgeon injects Methylene Blue (Blue Patent V) around the breast mass.

(b) The surgeon should excise the breast mass first, which will also include the radioactivity injected around the tumour or intradermally. This will lower the background activity.
(c) Using a surgical probe (radiation detection), the surgeon locates the sentinel node in the axilla and determines that it is the same node visualized with the Methylene Blue technique to trace the lymphatic system. In the case of a discrepancy, both nodes should be excised.
(d) Using the surgical probe, the background activity in the area is defined. Any node with activity higher than twice the background activity should be excised.
(e) If the primary tumour was not removed and the purpose of surgery is only to excise the sentinel node, the site of injection should be shielded with lead in order to decrease the background activity and to avoid saturation and electronic jamming of the detector.
(f) The axilla should be scanned carefully using the surgical probe.
(g) If an internal mammary node is the only one detected or if it is visualized in addition to the axillary node, the institution's own procedures should be followed. Internal mammary lymph nodes can be excised from the third and fourth intercostal space next to the outer border of the sternum.

5.9.5. Procedure manual for sentinel node localization in malignant melanomas

5.9.5.1. Patient selection

Only cases with the following characteristics should be investigated:

— Early stage malignant melanomas of the skin of no more than 3 mm in thickness;
— No invasion of the subcutaneous tissue and no clinically palpable regional lymph nodes present;
— Referral usually after an excisional biopsy or a wide surgical excision of the lesion.

5.9.5.2. Radiopharmaceuticals

The following radiopharmaceuticals are used for sentinel node localization:

— Technetium-99m antimony tin colloid;

— Technetium-99m filtered (0.22 μm) or unfiltered SC (filtered SC is preferred, although both work well);
— Technetium-99m HSA or dextran.

These can be used for fast visualization of the lymphatic channels and sentinel nodes provided the patient is due to enter the operating room soon. The first node seen after injection is the sentinel node and this should be properly marked on the skin.

The following procedure should be observed:

(a) Route of injection:
— Intradermal injections, within 1 cm of the edge of the lesion or the scar at four corners and 90° apart, can be used.
— For larger lesions or scars, multiple injections around the lesion can be used, provided they are not more than 1 in (25.4 mm) apart.

(b) Dose and volume injected:
— Patients undergoing same-day surgery require 9–12 MBq (0.3–0.4 mCi).
— Patients undergoing surgery the following day require 37 MBq (1.0 mCi).
— The volume used is 1.0 mL injected at multiple sites, for each site about 0.2 mL intradermally and enough to form a bleb in the skin and induce pressure inside the lymphatic system.

(c) Acquisition:
— A single head, large FOV gamma camera is recommended.
— Collimator: low energy, high resolution.
— Matrix size: 128×128.

(d) Mode of acquisition:
— An anterior dynamic image should be taken every 30 s for 45 min, followed by static images for 5 min in the anterior and lateral projections.
— Transmission images should be acquired for both dynamic and static images to outline the body contours.

(e) Region to be imaged:
Depending on the location of the lesion, clinical judgement should be used in identifying the region to be imaged. These regions are:
— For lesions near the midline of the trunk, both the inguinal and axillary regions;
— For the axilla, the supraclavicular region;
— For the inguinal region, the iliac and pelvic nodes;

— For the head and neck, the supraclavicular regions;
— For the lower limbs, the corresponding inguinal region;
— For the upper limbs, the corresponding axillary region.

Markers over the site of the sentinel node should be attempted using a point ^{57}Co source, and an ink mark on the skin should be performed.

(f) Reporting:
Details should be included on the site of injection and the radiopharmaceutical used, as well as on the location and number of sentinel nodes visualized.

(g) Preparation for the surgical probe:
— The battery of the probe should be fully charged.
— A quality control has to be performed before using a calibration source.
— Adjustments should be made to the window and the energy range according to the radionuclide used.
— The amplification and the time window should be adjusted according to the level of radioactivity.
— The probe has to be sterilized according to the manufacturer's recommendation. Most probes are currently covered by disposable, sterile plastic tubing for use in the operating room, usually supplied by the manufacturer or obtained commercially.

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5.9.6. Radioimmunoscintigraphy

5.9.6.1. Principle

Radioimmunodetection or radioimmunoscintigraphy uses tumour targeting antibodies or antibody fragments, labelled with a radionuclide suitable for external imaging, for the detection of specific cancers.

Monoclonal antibodies have been developed against a variety of antigens associated with tumours and have been shown to target tumours with minimal side effects. Numerous radionuclides suitable for external imaging have been conjugated to antibodies, or antibody fragments, and the radioimmunoconjugates have been shown to be stable in vivo.

Antibody fragments clear the circulation more rapidly than intact immunoglobulin. Antibody fragments have been conjugated with ^{99m}Tc, allowing same or next day imaging. Intact immunoglobulin conjugated with ^{111}In permits imaging as late as a week after administration.

5.9.6.2. Clinical indications

Radioimmunoscintigraphy has been shown to be of benefit in the detection of occult disease, in the management of patients with potentially resectable disease, and for the evaluation of lesion recurrence and therapeutic response. Radiolabelled antibody imaging in prostate cancer has been shown to be useful in risk stratification and in patient selection for loco-regional therapy.

5.9.6.3. Contraindications

The following points should be borne in mind:

— Pregnancy and/or lactation is an absolute contraindication.
— A known hypersensitivity to foreign proteins is a relative contraindication.
— The safety of these agents in children has not been conclusively demonstrated.

5.9.6.4. Equipment

The following items of equipment are required:

— A SPECT gamma camera;
— A low energy collimator for ^{99m}Tc (or ^{123}I) labelled antibodies;
— A medium energy collimator for ^{111}In labelled antibodies.

5.9.6.5. Radiopharmaceuticals

Currently approved antibodies for imaging are conjugated with ^{99m}Tc and ^{111}In. Both ^{99m}Tc and ^{111}In have been labelled to immunoglobulins, while ^{99m}Tc has also been labelled to Fab´ fragments. The route of administration is intravenous and the doses (for an adult of 70 kg) are 750–1000 MBq (15–25 mCi) ^{99m}Tc and 185–220 MBq (5–6 mCi) ^{111}In labelled antibodies.

The nuclide characteristics are as follows:

Tc-99m: $T_{1/2}$ = 6 hours (140 keV gamma photons),
In-111: $T_{1/2}$ = 2.8 days (178 and 245 keV photons).

5.9.6.6. Protocols

It is important to obtain at least two, and preferably three, sets of images. For ^{99m}Tc labelled antibodies these are usually obtained at 10 min, 4 hours and 24 hours following administration. The time interval between image sets is longer for ^{111}In labelled antibodies, typically from the day of administration to 4 days after.

To evaluate the abdomen optimally, it is advisable to clear the bowel, usually by administration of 10 mg of bisacodyl taken orally, four times a day, but this may increase non-specific intestinal uptake. An enema on the day of delayed imaging is useful for ^{111}In labelled antibody imaging.

Whole body images at 8 cm/min with a high resolution acquisition matrix are optimal for the early image sets; delayed images should be acquired at a slower speed, typically of 6 cm/min. Spot images of at least 1 000 000 counts are also useful, in addition to whole body images. Serial squat views are essential for colorectal and prostate cancer.

SPECT images of the abdomen and pelvis are particularly useful. For ^{99m}Tc labelled antibodies, these are carried out on the day of administration and at 24 hours. For colorectal and prostate cancer, serial SPECT images of the pelvis are recommended. Indium-111 labelled antibody SPECT may be carried out at multiple time points. These should be acquired in a matrix of 64×64, for 40 seconds per angle for a minimum of 64 angles over 360°.

5.9.6.7. Interpretation

Specific uptake increases with time over 24 hours, whereas non-specific uptake after the initial distribution decreases with time as the antibody or fragment clears from the blood. Thus, the image at 10 min acts as a reference with which to compare later images. The use of change detection analysis, comparing the early and late images as a probability map of significant changes, allows the detection of lesions down to 3.5 mm size in normal nodes (1 cm or less) found radiologically.

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5.9.7. Peptide receptor targeted scintigraphy

5.9.7.1. Background information

The high level expression of peptide receptors on various tumour cells as compared with normal tissues or normal blood cells has provided the molecular basis for the clinical use of radiolabelled peptides as tumour tracers in nuclear medicine. In particular, binding sites for members of the somatostatin (SST) receptor family (hSSTR1–5) are frequently found, and their expression has led to therapeutic and diagnostic attempts to target these receptors specifically. SST receptor (SSTR) scintigraphy using ^{111}In-DTPA-(D)Phe^{1}-octreotide has a high positive predictive value for the vast majority of neuroendocrine tumours and has gained its place in the diagnostic work-up as well as follow-up of patients with these tumours. Clear evidence suggests that scintigraphy with SST receptor imaging agents is superior to CT, MRI or FDG PET in the primary detection of neuroendocrine tumour sites. In a large number of patients, additional (previously unknown) tumour sites are identified by SSTR scintigraphy, which leads to a change in patient management in about 10% of patients. In recent years, technetium based radiopeptide ligands have been implemented to study SSTR expressing tumours. One of these, ^{99m}Tc-NEOTECT, has been approved for non-small-cell lung cancer.

5.9.7.2. Radiopharmaceuticals

Native SST exists in two forms (with 14 or 28 amino acids), but it is readily attacked by aminopeptidases and endopeptidases, and has a short in vivo half-life. Consequently, synthetic SST analogues, which incorporate a Phe-(D)Trp-Lys-Thr (or similar sequence) and which are metabolically stabilized at both the N and C terminals, were developed for clinical applications. So far, three commercially available SST analogues (i.e. octreotide ((D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr(ol)), lanreotide ((D)β-Nal-Cys-Tyr-(D)Trp-Lys-Val-Cys-Thr-NH_2) and vapreotide ((D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-Trp-NH_2)) have been shown to be effective in controlling the growth of some human tumours. These three SST analogues have similar binding profiles for four of the five hSSTR subtypes (i.e. a high affinity for hSSTR2 and hSSTR5, a moderate affinity for hSSTR3 and a very low affinity for hSSTR1), but lanreotide and vapreotide also have a moderate affinity for hSSTR4, whereas octreotide has little or no affinity for this hSSTR.

(a) Iodine-123-Tyr^3-octreotide

This was the first radiopeptide tracer for which proof of principle was obtained. It is no longer frequently used but may be produced in a functional radiopharmacy laboratory. Clinical results are not as good in the abdomen as those with the ^{111}In labelled compound, due to higher hepatobiliary clearance.

(b) Indium-111-DTPA-(D)Phe^1-octreotide

This is the commercially available radiopeptide tracer of choice for the detection and follow-up of neuroendocrine tumours (OctreoScan®, Tyco). It can be conveniently prepared using a kit: 10 μg DTPA-(D)Phe^1-octreotide are labelled with approximately 120–150 MBq ^{111}In-Cl_3. No further purification steps are necessary.

(c) Indium-111-DOTA-lanreotide

This is synthesized using the commercially available lanreotide (Somatuline®) and 1,4,7,10-tetraazacyclo-dodecane-N,N´,N´´,N´´´-tetraacetic acid (DOTA) as starting materials. It can be obtained from the University of Vienna. Results are similar to those of the octreotide based peptide tracers.

(d) Indium-111-DOTA-(D)Phe-Tyr^3-octreotide

This is currently under investigation at several sites with results even better than those of ^{111}In-DTPA-(D)Phe^1-octreotide.

(e) Technetium-99m-NEOTECT (^{99m}Tc-P829)

This is an SSTR receptor based radiopharmaceutical which, in contrast to octreotide based ligands, also binds to hSSTR3 with high affinity. It is available from GE-Amersham in Europe and Schering in the USA.

5.9.7.3. Clinical indications and patient selection

Indium-111-DTPA-(D)Phe^1-octreotide is used in patients clinically suspected to bear an SSTR expressing tumour such as a carcinoid, insulinoma, glucagonoma or paraganglioma (primary tumour localization) and ^{99m}Tc-NEOTECT in patients with non-small-cell lung cancer in order to evaluate the solitary pulmonary module and to assess the extent of the disease. It should also be used in the follow-up of cancer patients known to bear a tumour which

expresses SSTR. Such follow-up scintigraphies should be performed every six months.

A scintigraphic evaluation should be performed before primary surgery.

Patients refractory to conventional treatment strategies may be referred for potential treatment evaluation with ^{90}Y-DOTA-lanreotide/octreotide (see also Section 6.12).

5.9.7.4. Patient preparation

In all patients, diagnosis and disease staging should be established according to WHO criteria. The location and size of primary tumours and/or spread of metastases should be investigated by conventional CT or MRI, radiography, colonoscopy or surgery.

Patients receiving treatment with long acting SST analogues should stop treatment for at least three days prior to scintigraphy.

Patients should be informed that they will have to come for the scintigraphic acquisitions at several time points, usually at 4–8 and 24 hours post-injection. When abdominal activity is present, acquisitions may also become necessary after 48 hours.

If there is marked intestinal activity, the patient may be asked to take laxatives. No additional special preparation is necessary.

5.9.7.5. Gamma camera imaging and analysis

(a) Indium-111-DTPA-(D)Phe^1-octreotide scintigraphy

The peptide tracer should be injected as a bolus, usually in the morning. The patient is then asked to come for acquisition in the afternoon. The peptide tracer can also be injected in the afternoon, and acquisitions performed the next morning. For planar and SPECT studies, a large FOV gamma camera equipped with a medium energy, general purpose collimator is required. There is no need to perform sequential images.

Planar images should be obtained at two time points:

— Early acquisition at 4–8 hours post-injection;
— Late acquisition at 24–48 hours post-injection.

Planar images (thorax and abdomen) should be gathered in the anterior, posterior and lateral views (matrix at least 128 × 128 pixels, (150 000–300 000 counts, scanning time 10–20 min). Both energy peaks are used for scanning (set at 173 and 247 keV) with a 20% window.

When searching for a neuroendocrine tumour, it is absolutely mandatory that at least one abdominal SPECT acquisition be performed. This should be either early or delayed, at 6 or 24 hours post-injection, respectively. A chest SPECT is indicated occasionally if the patient is clinically suspected to have a tumour and the abdominal images are negative.

SPECT imaging should always be done in a 360° orbit, using 6° steps with 30″ per step.

The scintigraphic data should be filtered with a Wiener filter and reconstructed in three planes (with a slice thickness of about 7 mm).

(b) Technetium-99m-NEOTECT (^{99m}Tc-P829) scintigraphy

The primary indication for NEOTECT scintigraphy is non-small-cell lung cancer. Other indications such as endocrine orbitopathy associated with the thyroid are under investigation.

The radiotracer should be injected as a bolus.

For planar and SPECT studies, a large FOV gamma camera equipped with a low energy, general purpose or high resolution collimator is required.

Planar images can be obtained as early as 15 min post-injection. It is recommended that acquisition should start not earlier than 1 hour post-injection and should be completed within 3 hours post-injection. In all patients, chest SPECT images are required.

Planar images (thorax and abdomen) should be gathered in the anterior, posterior and lateral views (a matrix of at least 128 × 128 pixels, 300 000 counts, scanning time 10 min).

SPECT imaging should always be done in a 360° orbit, using 6° steps with 30″ per step.

The scintigraphic data should be filtered with a Wiener filter and reconstructed in three planes (with a slice thickness of about 7 mm).

5.9.7.6. Side effects

No acute or chronic side effects have been reported so far. In a few patients, however, antibodies have been demonstrated which may interfere with octreotide scintigraphy.

5.9.7.7. Comments

Other peptide tracers, such as vasoactive intestinal peptide (VIP) based peptide tracers, may become available in the near future. These may even gain

a broader clinical application as VIP receptors are expressed on more common tumours such as colorectal cancer and breast cancer.

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5.10. HAEMATOLOGY

5.10.1. Introduction

The role of nuclear medicine in haematology covers the following:

(a) Determination of blood volume, both red cell volume and plasma volume;
(b) Mean red cell lifespan;
(c) Sites of red cell destruction;
(d) Megaloblastic anaemias, especially the vitamin B_{12} absorption test (Schilling test);
(e) Iron metabolism;
(f) Radiolabelled platelets;
(g) Radiolabelled granulocytes;
(h) Splenic function;
(i) Bone marrow imaging.

5.10.2. Blood volume

5.10.2.1. Principle

Total blood volume consists of separate plasma and cellular compartments. Knowing the haematocrit, blood volume can be calculated from either the plasma volume (PV) or the red cell volume (RCV). However, more accurate results are obtained if the total blood volume is determined by separate measurements of plasma and red cell volume. In clinical situations, the ratio between total body haematocrit and peripheral haematocrit often varies widely. A separate determination of plasma and red cell volume is thus strongly advised.

Plasma and red cell volumes are determined using the dilution principle, where the volume in question is calculated from the concentration of a tracer added in an accurately measured amount, mixed homogeneously within the compartment to be measured, using the following formula:

$$V = Q/C$$

where

V is the volume of the compartment;
Q is the quantity of tracer added;

and C is the concentration of diluted tracer after equilibrium.

The following conditions must be fulfilled for the formula to be valid:

— The tracer must be homogeneously distributed within the compartment.
— There must be no loss of the tracer during the study period, unless the loss can be accurately compensated for.
— The tracer distribution must not change at equilibrium.
— The tracer must not affect the compartment in any way.
— The tracer must be easily and accurately measurable.

5.10.2.2. Clinical indications

The main indications for the test are in the diagnosis of polycythaemia (erythrocytosis). This condition is diagnosed by finding elevated haemoglobin, haematocrit and red cell counts, and may be absolute (increased red cell volume) or relative (haemoconcentration).

The classification of polycythaemias is as follows:

(a) Relative polycythaemia (haemoconcentration: RCV normal, PV reduced):
  (i) Dehydration:
    — Reduced water intake;
    — Vomiting;
    — Diarrhoea;
    — Alcohol;
    — Diuretics;
    — Burns;
  (ii) Stress polycythaemia:
    — Relative erythrocytosis;
    — Pseudopolycythaemia;
    — Gaisböck's syndrome.

(b) Absolute polycythaemia:
  (i) Primary (low erythropoietin):
    — Polycythaemia vera;
    — Erythraemia (pure erythrocytosis);
  (ii) Secondary (high erythropoietin):
    — Appropriate (O_2 saturation decreased):
      ● chronic lung diseases,
      ● living at high altitudes,
      ● smoking,
      ● cyanotic heart disease,
      ● massive obesity,
      ● certain haemoglobinopathies.
    — Inappropriate:
      ● bronchial or renal cell carcinoma,
      ● hepatoma,
      ● cerebellar haemangioblastoma,
      ● uterine fibroids,
      ● phaeochromocytoma,
      ● renal ischaemia,
      ● adrenal cortical hyperplasia or adenoma,
      ● essential erythrocytosis.

(c) Pseudo-anaemia (normal RCV, raised PV), may occur in:
  — cirrhosis;
  — nephritis;
  — congestive cardiac failure;

— marked splenomegaly;
— pregnancy.

Differentiating between these conditions has important therapeutic implications.

The availability of erythropoietin determinations has decreased the use of blood volume determinations, perhaps because of the uncertain accuracy of the latter. To improve the reliability of blood volume determinations, it is imperative to pay attention to technical details, including adjustment of normal and/or reference values for the patient's body build, especially in obese patients.

5.10.2.3. Radiopharmaceuticals

Although other labels may be used, the most common are:

— For RCV, ^{51}Cr and ^{99m}Tc labelled red cells;
— For PV, ^{125}I radioiodinated human serum albumin (RISA).

For RBC labelling, ^{51}Cr offers the advantage of negligible elution loss compared with ^{99m}Tc. The use of ^{51}Cr becomes essential when RCV determination is carried out in conjunction with red cell survival studies. In vivo ^{99m}Tc labelling of red cells is a practical alternative if only RCV needs to be determined.

5.10.2.4. Equipment

Although dedicated semi-automated counters can be used for ^{125}I-RISA PV determinations, a simple well counter will normally suffice.

5.10.2.5. Patient preparation

Since radioactive iodine is taken up by the thyroid, 200 mg of potassium iodide should be given orally per day for two days before and eight days afterwards, in order to block thyroid uptake.

Owing to the postural effects on PV, the patient should be in the supine position for about 30 min prior to the study and remain so throughout the entire study.

5.10.2.6. Procedure

(a) Plasma volume (RISA)

The plasma volume can be found by the following procedure:

(1) Measure the patient's weight and height.
(2) Draw 5 mL of blood (EDTA) for a 2 mL plasma background sample.
(3) Carefully inject a known volume containing 100–200 kBq of ^{125}I-RISA intravenously. Care must be taken not to extravasate the injected dose. Timed blood samples should be drawn from the opposite arm at exactly 10, 20 and 30 min post-injection. Centrifuge and pipette 2 mL of plasma for gamma counting.
(4) Standard solution: dilute a known volume of ^{125}I-RISA with water to a known and exact volume of 1000 mL. Use an identical injection-flushing technique to that used for patient injection. Since albumin can adhere to glass, siliconized glassware is recommended. Alternatively, carrier albumin or detergent can be added before addition of the RISA and mixed well. Pipette 2 mL for gamma counting.
(5) Count the patient and standard specimens in a well counter at ^{125}I settings (20–80 keV), subtracting background counts to obtain net counts/min.
(6) Use a semilogarithmic plot of count rate of the serial 10, 20 and 30 min plasma samples and extrapolate to time zero to obtain the count rate before removal of the labelled protein from the circulation occurred. This count rate (at $t = 0$) is used in calculating the PV value:

PV = (Net counts/min standard × 1000)/(net counts/min plasma sample).

(b) Red cell volume

The red cell volume can be found by the following procedure:

(1) Measure the patient's weight and height.
(2) Under sterile conditions, draw approximately 10 mL of blood. Add 6 mL of blood to 2 mL ACD in a sterile tube and mix well. Anticoagulate the remaining 4 mL with EDTA and pipette a 2 mL aliquot for background counting.
(3) Aseptically add 1–2 MBq ^{51}Cr sodium chromate to the ACD blood tube. Incubate for 45 min at room temperature with continuous gentle rotation.
(4) Centrifuge at 1000*g* for 10 min. Remove the supernatant with a fine needle and discard as radioactive waste.

(5) Resuspend the red cells gently in sterile saline, repeat step (4) and resuspend to approximately the original volume.
(6) Measure exactly 1 mL of the labelled cells, dilute to 1000 mL with distilled water and pipette two 2 mL aliquots for gamma counting.
(7) With the patient in the basal resting state for at least 30 min, inject exactly 5 mL of labelled cells. With the patient still in the supine position, draw 5 mL EDTA blood samples at 10 and 40 min from the opposite arm.
(8) Check the injection site with a hand radiation monitor to ensure that there is no extravasated radioactivity compared with the opposite arm.
(9) From each blood sample, pipette 2 mL for gamma counting and perform a haematocrit measurement on the remainder.
(10) Count the patient and standard specimens in a well counter at ^{51}Cr settings (280–360 keV), subtracting background counts to obtain the net counts/min. Use an adequate time to obtain valid statistics. All specimens are counted for the same time (approximately 10 min).
(11) RCV for each sample can be calculated from:

RCV (mL) = [(net counts/min)/mL std] × Hct × 1000 × 5 / [(net counts/min)/mL blood sample)]

where Hct stands for haematocrit.

(12) Total blood volume (TBV) can be calculated from:

TBV (mL) = (RCV/Hct) × 0.91.

(13) Plasma volume (PV) = TBV – RCV.
(14) If the 40 min RCV is significantly higher than the 10 min value and the patient does not have active bleeding or haemolysis, the 40 min value is considered to be more accurate — especially if the patient has marked splenomegaly causing delayed equilibrium.

If repeat measurements of RCV are necessary, labelling with ^{99m}Tc can be used with the in vitro technique. The disadvantage of ^{99m}Tc is its fairly high elution from red cells, making this method unsuitable for delayed sampling as in splenomegaly or congestive cardiac failure.

Simultaneous RCV and PV determinations can be done using dual channel counting. Corrections have to be made for ^{51}Cr scatter into the ^{125}I channel.

5.10.2.7. Interpretation

Normal blood volume values (mL/kg) are given in Table 5.19.

Using a fixed reference range in mL/kg does not take into account the fact that obese individuals will have relatively lower values when expressed in mL/kg. It is more accurate to use individualized reference values for each patient, using tables based on the patient's weight and height or body surface.

TABLE 5.19. NORMAL BLOOD VOLUME VALUES AND RANGES *(in mL/kg)*

	Males	Females
RCV	30 (25–35)	25 (20–30)
PV	40 (35–45)	40 (35–45)
TBV	70 (60–80)	65 (55–75)

5.10.3. Red blood cell survival

5.10.3.1. Principle

A test is done to determine whether anaemia is caused by decreased survival of RBCs.

5.10.3.2. Technique

The following technique is used to measure RBC survival:

(1) Collect 10 mL of blood with 1.5 mL ACD as anticoagulant.
(2) Centrifuge the blood and discard the plasma.
(3) Label the remaining packed cells with 0.5 μCi/kg ^{51}Cr-chromate.
(4) Wash the cells and recentrifuge them to remove any unbound ^{51}Cr.
(5) Restore the volume by adding isotonic saline. Re-inject the patient.
(6) Withdraw samples every other day for 21 days, beginning 24 hours later. The sample taken at time zero cannot be obtained earlier than 24 hours, because approximately 10% of the label is lost on the first day. This may be due to normal accelerated elution and/or damage during labelling.
(7) Determine the microhaematocrit for each sample. The counting is performed in a well counter.

(8) Plot blood counts on semilogarithmic paper.
(9) Dividing the whole blood cell counts by the sample haematocrit improves accuracy and corrects for daily fluctuations of haematocrit.

5.10.3.3. Interpretation

(a) Normal findings

The half-life of RBCs is 25–35 days. Normal RBCs have a 120 day lifespan. Thus, 0.8% of the RBCs are lost each day through senescence. The measured ^{51}Cr-RBC survival half-life is not 60 days as predicted, because of an additional ~1% elution loss of the ^{51}Cr from the RBCs.

(b) Abnormal findings

An RBC survival time of less than 25 days is abnormal.

The patient must be in a steady state. Recent haemorrhage or transfusion affects accuracy. If the haematocrit varies significantly (where the patient is not in a steady state), a closer approximation of RBC survival is obtained by plotting whole blood cell counts without haematocrit correction.

5.10.3.4. Protocol

(a) Red cell labelling procedure with ^{51}Cr (ICSH, 1980)

The following procedure should be used:

(1) Collect 10 mL of the patient's blood in a sterile tube or plastic labelling bag that contains 1.5 mL of ACD (NIHA) solution. Centrifuge the blood, withdraw the plasma and add 0.5 μCi/kg (20 kBq/kg) of high specific activity ^{51}Cr sodium chromate.
(2) Incubate the blood at room temperature for 15 min with occasional gentle mixing.
(3) Wash the cells twice with isotonic saline to remove unbound ^{51}Cr.
(4) After the second centrifugation and decantation, restore the original volume with isotonic saline.
(5) A measured aliquot of the labelled RBCs can be used for red cell survival and sequestration studies.

(b) In vitro ^{99m}Tc red blood cell labelling (Brookhaven National Laboratories method)

The following procedure should be used:

(1) Draw 4 mL of the patient's whole blood into a heparinized syringe and transfer this to a Vacutainer kit with a lyophilized stannous citrate mixture containing 2 mg of tin. The blood is incubated with these reagents for 5 min.
(2) Add 1 mL of 4.4% EDTA (disodium or calcium disodium salt), mix and centrifuge the tube upside down at 1300*g* for 5 min.
(3) Withdraw 1.25 mL of the packed RBCs, transfer to a vial containing 1–3 mL ^{99m}Tc-pertechnetate and incubate with gentle mixing for 10 min.
(4) The red cells are now ready for injection. Alternatively, they can be heated at 49°C for 15 min and used for spleen scintigraphy.

(c) In vivo ^{99m}Tc-RBC labelling

The following procedure should be used:

(1) Constitute commercial stannous pyrophosphate containing 2–4 mg of stannous ions with normal saline, and inject an aliquot, containing 10–20 μg of tin per kg body weight, into the patient.
(2) After 30 min, inject the required quantity of ^{99m}Tc-pertechnetate (usually 10–25 mCi or 370–925 MBq). RBC labelling occurs almost immediately.
(3) It is important to inject the stannous pyrophosphate shortly after reconstitution to avoid oxidation of the tin.

(d) In vivo ^{99m}Tc-RBC labelling

The following procedure should be used:

(1) Inject 0.5 mg of stannous ions intravenously from a reconstituted commercial stannous pyrophosphate kit.
(2) Insert a 19 gauge butterfly infusion set into an appropriate vein. Attach a four way stopcock and flush from a syringe containing ACD.
(3) Approximately 20 min following injection of the tin, withdraw 3 mL of blood into a 5 mL shielded syringe containing 370–925 MBq (10–25 mCi) ^{99m}Tc-pertechnetate.
(4) Flush the tubing with ACD solution. After 10 min of incubation with gentle agitation at room temperature, the labelled RBCs are re-injected through the infusion set.

5.10.4. Splenic sequestration

A test is performed to determine if destruction of RBCs, which shortens RBC survival, is caused by splenic destruction of RBCs.

5.10.4.1. Technique

The following procedure is used:

— Usually performed in conjunction with an RBC survival study.
— Imaging or regional counting (using non-imaging detectors) should be performed on the same every-other-day schedule as the RBC survival study.
— Activity is measured over the precordium, anterior liver and posterior spleen.

5.10.4.2. Interpretation

Normal and abnormal findings can be characterized as follows:

(a) Normal findings:
— The spleen-to-liver ratio is 1:1.
— This ratio remains approximately 1:1 during the course of the study.
(b) Abnormal findings:
— Increased spleen-to-liver ratio.
— The spleen-to-liver ratio increases over the course of the study. Splenomegaly itself, without pathological sequestration, can yield spleen-to-liver ratios of between 2:1 and 4:1. For this reason, a rising ratio is the best evidence of significant sequestration.

5.10.5. Schilling's test

5.10.5.1. Physiology

Vitamin B_{12} is not synthesized by plants or animals, but is produced by microorganisms found in the soil and in the intestines and rumens of animals. Dietary vitamin B_{12} comes from meat and dairy products.

Vitamin B_{12} is primarily stored in the liver. Total body stores are high and daily excretion low. It takes three to five years to develop vitamin B_{12} deficiency if dietary intake is halted or malabsorption occurs. Thus, vitamin B_{12}

deficiency caused by diet is rare (unlike a deficiency of folate), occurring only in strict vegetarians.

For absorption, vitamin B_{12} must be complexed with intrinsic factor (IF), which is a protein secreted by parietal cells in the gastric fundus. Vitamin B_{12}-IF complex moves down the bowel and is absorbed in the terminal ileum. Its absorption requires an alkaline pH and calcium. The vitamin B_{12}-IF complex dissociates and vitamin B_{12} diffuses or is actively transported across mucosa. Vitamin B_{12} enters the portal vein, bound to transcobalamin-II transport protein and is delivered to the liver. Over the next 8–12 hours, a portion re-enters the circulation, binding to a larger transport protein, transcobalamin-I. When the storage capacity of transcobalamin-I is exceeded, vitamin B_{12} is excreted.

Vitamin B_{12} deficiency is caused by several mechanisms:

(a) Decreased intrinsic factor:
  — Pernicious anaemia (usually caused by autoimmune disease);
  — Gastrectomy.
(b) Intestinal malabsorption in the terminal ileum:
  — Short bowel syndrome;
  — Sprue;
  — Regional enteritis;
  — Lymphoma.
(c) Pancreatic conditions:
  — Chronic pancreatitis;
  — Cystic fibrosis.
(d) Fish tapeworm infestation.

5.10.5.2. Background

The following conditions are clinical manifestations of vitamin B_{12} deficiency:

(a) Megaloblastic anaemia – this may be absent early in the disease. Because of the close metabolic relationship of vitamin B_{12} and folate, folate administration can correct anaemia. Unfortunately, the neurological changes are unaffected and can progress. For this reason, it is important to differentiate folate from vitamin B_{12} deficiency.
(b) Thrombocytopenia.
(c) Leukopenia.
(d) Subacute combined degeneration of the spinal cord.

It should be noted that although the haematological changes are reversible, neurological damage may not be.

5.10.5.3. Radiopharmaceuticals

Vitamin B_{12} (cyanocobalamin) has cobalt as a central metal atom. Radioactive isotopes of cobalt can substitute the 'cold' atom, producing the tagged form.

The following radionuclides are available:

(a) Cobalt-57: physical half-life, 270 days;
photon energy, 122 keV.
(b) Cobalt-58: physical half-life, 71 days;
photon energy, 810 keV.

5.10.5.4. Technique

The following technique is used:

(1) Ensure the patient has nothing to eat or drink after midnight.
(2) Prepare dose standards of 2 mL.
(3) Administer 18.5 kBq (0.5 μCi) of ^{57}Co labelled vitamin B_{12} in 0.5 mg vitamin B_{12} orally, together with a capsule of 18.5 kBq (0.5 μCi) of ^{58}Co complexed with IF.
(4) Two hours later administer 1000 μg of cold vitamin B_{12} intramuscularly or subcutaneously. This flushing dose is administered to saturate the transport proteins.
(5) Make two consecutive 24 hour urine collections. Cobalt-57 vitamin B_{12} absorbed through the gastrointestinal tract will not be bound by saturated transport proteins and will thus be excreted in urine.
(6) Measure volume of the 24 hour collection.
(7) Pipette and count 2 mL aliquots of urine and standards.
(8) Calculate percentage of administered dose excreted over each 24 hour period.

5.10.5.5. Interpretation

Normal and abnormal findings can be characterized as follows:

(a) Normal findings:
— More than 10% of the administered dose is excreted in the urine in the first 24 hours. Values between 6 and 10% are considered to be indeterminate.

(b) Abnormal findings:
(i) Pernicious anaemia – less than 6% excreted (usually in the range of 1–3%).
(ii) Intestinal malabsorption – less than 6% excreted – abnormality not corrected by IF.
(iii) Chronic vitamin B_{12} deficiency from pernicious anaemia can produce atrophy of the ileal mucosa. This, in turn, causes a decreased intestinal absorption of vitamin B_{12}.
(iv) False positive results may occur in patients with diminished renal function or obstruction. In patients with extremely poor renal function, a collection should be performed over three days.
(v) False positive results may occur if a portion of the urine volume is lost. Maximum excretion occurs 8–12 hours after administration. Check for loss by:
— Measuring urine specific gravity;
— Measuring creatinine – normally greater than 1 g;
— Differences in volume between the 24 and 48 hour collections.
(vi) False negative results may occur if faecal contamination of urine occurs, invalidating the test.

Although less readily available, a whole body counter can be used for vitamin B_{12} absorption studies. The main advantage of this technique is that a flushing dose of non-radioactive vitamin B_{12} is not needed, thus leaving vitamin B_{12} determinations, the bone marrow and haematological changes unaltered. The results are also not dependent on renal function.

5.10.6. Radiolabelled platelets

5.10.6.1. Anatomy and physiology

(a) Platelets

Platelets are formed in the bone marrow by megakaryocytes. Platelets are small disc shaped cells and do not have a nucleus. They have the ability to change shape on contact with foreign materials or subendothelial surfaces, stimulating the release of substances involved in haemostasis.

(b) Haemostasis

Haemostasis is characterized by:

— Platelet adhesion to the periphery of the wound;
— Localized vasoconstriction;
— Platelet plug formation;
— Reinforcement of plugs with fibrin;
— Removal of clots by the fibrinolytic system.

(c) Prostaglandins

When platelets are activated, thromboxane A2 is released. This is one of the most potent vasoconstrictors known and also promotes platelet aggregation. Prostacyclin, produced in the vessel wall, inhibits the effects of thromboxane. Aspirin and other drugs that decrease platelet aggregation do so by inhibiting cyclo-oxygenases. This blocks the conversion of arachidonic acid to peroxidase, reducing thromboxane A2 levels.

5.10.6.2. Technique

Two types of platelet labels are used:

(1) Cohort (pulse) labels – taken up by megakaryocytes and incorporated into the components of forming platelets. With these labels, only freshly released platelets of uniform age are labelled. These labels are currently not used in clinical practice.
(2) Random labels – these label platelets of all ages. ^{51}Cr-chromate and ^{111}In-oxine are examples.

(a) Collection of blood

The anticoagulant used in the blood collecting syringe is critical. ACD has a less deleterious effect on platelet function than other anticoagulants.

(b) Centrifugation

There are two steps in the technique of centrifugation:

(1) First, centrifugation at 1750–2700g min^{-1} produces platelet-rich plasma (PRP). If too high centrifugal forces are used, too many platelets will be

lost. Lower forces cause excessive RBC contamination. The duration of centrifugation is also important. With increased time, younger platelets, which are more adhesive, tend to sediment out.

(2) Second, centrifugation (at 10 000–20 000g min^{-1}) of PRP forms platelet pellets to remove plasma.

(c) Addition of ^{111}In-oxine

Because of its physical characteristics, ^{111}In is superior to ^{51}Cr as a platelet label. Labelling in plasma, although reducing labelling efficiencies, may improve platelet function.

(d) Other chelates

Other agents that may have less toxic effect on platelets include Tropolone.

(e) Radiation effects

Individual platelets incubated with 37 MBq (1 mCi) of ^{111}In may be exposed to radiation doses of up to 129 Gy (12 900 rad). This high value is due to their relatively small size and long biological lifespan. Since they lack nuclei they are highly radioresistant. Normal survival times and function have been reported at radiation doses of 500–700 Gy.

5.10.6.3. Clinical uses

Radiolabelled platelets have various uses:

(a) One of the most common uses is measurement of platelet lifespan:
  (i) Survival curves are normally linear. Disappearance of platelets from the circulation is related to age dependent destruction by the reticuloendothelial system (RES).
  (ii) Lifespan is normally about nine days.
  (iii) Platelet survival is diminished in a number of conditions and by some surgical treatments including:
    —Idiopathic thrombocytopenic purpura (IT);
    —Prosthetic heart valves;
    —Arterial grafts;
    —Peripheral vascular disease;
    —Atherosclerotic coronary heart disease;

— Eisenmenger's syndrome;
— Primary pulmonary hypertension;
— Diabetes mellitus;
— Hepatic cirrhosis;
— Hyperlipidaemia;
— Renal transplantation.

(b) Deep venous thrombosis:
  (i) In animals, heparin prevents visualization of thrombi. In humans, however, this effect does not appear as significant.
  (ii) The degree of uptake is dependent on the age of the thrombus. Less localization occurs in older thrombi.
  (iii) Delayed imaging at 24–48 hours is often necessary, especially in older thrombi.

(c) Pulmonary embolism:
  (i) Heparin appears to prevent embolus visualization.
  (ii) The length of time a thrombus is present in the lung is more important for sensitivity than the age of the thrombus itself. With time, in situ adherence of platelets to emboli diminishes.
  (iii) Large emboli that completely occlude an artery may cause a false negative study because emboli are not exposed to the labelled platelets.

5.10.6.4. Interpretation

Labelled platelets are rarely used for the diagnosis of pulmonary embolism because of the complexity of their preparation. Their main use is to aid a decision on splenectomy in patients with idiopathic thrombocytopenic purpura. High splenic uptake as determined by external counting is taken as an indication for splenectomy.

5.10.7. Bone marrow imaging

5.10.7.1. Anatomy and physiology

The functional red marrow approximately equals the liver in total size, with a total mass of about 1.5 kg. In adults, active marrow is found primarily in the axial skeleton including the vertebral bodies, pelvis, sternum, scapula, skull and in the appendicular skeleton, generally in the proximal third of the femora and humeri. In children, the volume of active marrow depends on age, while in newborns it extends the full length of the extremities. As the child grows, the marrow gradually retracts until an adult pattern is reached at the age of 10.

5.10.7.2. Radiopharmaceuticals

(a) Radioiron

Radioiron and its analogues bind to transferrin and are incorporated into active erythroid precursors in the bone marrow. Iron-2 would be the most physiological to use, but it requires a cyclotron for its production and has a half-life of only eight hours; it normally requires high quality images produced with a positron camera.

(b) Colloid imaging

Colloids are removed from the blood by the phagocytic cells of RES that line bone marrow sinusoids. Normally, the erythroid and RES distributions are parallel; thus RES scintigraphy shows an identical marrow distribution to that with radioiron. About 92% of an injectable dose of ^{99m}Tc-SC is removed by phagocytes in the liver and spleen; only the remaining 8% localizes in bone marrow. With the usual dose of SC used for liver-spleen imaging and the relatively short imaging times, activity in the bone marrow is not normally seen. Higher doses and enhanced display techniques such as thresholding and masking allow the bone marrow to be visualized.

5.10.7.3. Technique

The following technique is used:

(a) Inject the patient with 12–15 mCi of ^{99m}Tc-SC (intravenously).
(b) Image with a large FOV camera using a low energy, all purpose (LEAP) collimator.
(c) Image 30 min to 1 hour after injection.
(d) Obtain 50 000–200 000 counts per image.
(e) Image the entire body, both anteriorly and posteriorly.

5.10.7.4. Interpretation

Normal and abnormal characterizations are as follows:

(a) Normal findings
  (i) Technetium-99m SC:
    —The majority of the uptake is in the axial skeleton.

—The activity in the appendicular skeleton should not extend beyond the proximal one third.
—The skull has a variable degree of uptake.
—The sternum and ribs are often difficult to visualize although they contain active marrow.
—The lower thoracic and upper lumbar regions of the spine are not well seen because of intense uptake in the liver and spleen.

(ii) Radioiron:
—Activity is confined primarily to the axial skeleton.
—Not normally accumulated in the spleen.

(b) Abnormal findings:
(i) Decreased central marrow.
(ii) Peripheral extension.
(iii) Focal defects.
(iv) Increased liver and spleen size.
(v) Increased uptake outside normal regions of the extramedullary haematopoiesis.

5.10.7.5. Clinical applications

There are clinical applications of bone marrow imaging in the following areas:

(a) Avascular necrosis, especially of the femoral head;
(b) Extramedullary haematopoiesis;
(c) Determination of presence and contribution of splenic erythrocytosis in patients being considered for splenectomy in myeloproliferative disorders such as myeloid metaplasia;
(d) Evaluation of any disparity between the patient's marrow histology and peripheral blood smear;
(e) Diagnosis of bone marrow infarcts and haemolytic anaemias;
(f) Detection of metastases.

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5.11. INFLAMMATION AND INFECTION

5.11.1. Introduction

The choice of imaging agents depends on the biological processes of inflammation, whether it is acute or chronic, and the cause and site as well as the clinical problem to be addressed. The nuclear medicine imaging of inflammatory processes and infection is a form of tissue characterization which has moved from the generally sensitive, but non-specific or context specific, agents, to more disease specific agents.

5.11.2. Inflammation

Acute inflammation is typically initiated by trauma, burns or infective agents resulting in tissue injury and tissue necrosis. These factors initiate defence mechanisms, such as the release of cytokines, complements and antibodies, which are associated with vasodilation and increased capillary permeability resulting in extravasation of proteins and cells to the affected area. Leucocytes are recruited by adhesion molecules on the endothelium which are activated and bind to white cells such as E-selectin, ICAM-1 and

V-CAM, so that cells roll and adhere to the endothelium and diapedesis. They migrate down the chemoattractant gradient and lead to the classical combination of swelling, redness, pain and protective loss of function. Labelled leucocytes are appropriate for imaging of acute inflammation.

Chronic inflammation is characterized by a reduction in vasodilation and capillary permeability, a reduction in leucocyte activity, and an increase in monocytes and macrophages with lymphocytic infiltration. It is perpetuated by continuing necrosis, followed by activation of dendritic cells and appearance of antigen presenting cells, with possible formation of autoimmune disease granuloma. Clearly, radiolabelled white cells and agents that depend on vascular permeability will be less effective in this situation.

5.11.2.1. Clinical indications

Clinical problems may range from fever of unknown origin (FUO) to localization of the site of inflammation, as in Crohn's disease, to localization of the site of infection when a blood culture for bacteria is positive.

An FUO requires a catch-all approach since there may be non-infective causes of fever, including cancer, granuloma and vasculitis, making a sensitive, non-specific agent such as ^{67}Ga-citrate, ^{99m}Tc labelled MDP used in bone scans or even ^{18}FDG appropriate.

In determining the site of an inflammation, a more specific agent is preferred such as ^{111}In, ^{99m}Tc labelled white cells or human immunoglobulin (HIG), commonly referred to as IgG, or additionally ^{99m}Tc small molecular weight dextran.

In the case of infection, a yet more specific agent is preferable, such as one binding only to bacteria, for example ^{99m}Tc-ciprofloxacin (Infecton).

5.11.2.2. Inflammation and infection imaging agents and their indications

(a) Imaging inflammation

The choice of imaging agent depends on the biological processes, as outlined above. Advantage can be taken of increased vascular permeability by using ^{67}Ga citrate transferrin complex; polyclonal human immunoglobulin; liposomes (100 μm), particularly if pegylated; nanocolloids; and dextrans. A second approach is through direct targeting of inflammation by radiolabelled leucocytes, either labelled ex vivo using ^{99m}Tc-HMPAO or ^{111}In-oxine, or labelled in vivo using monoclonal antibody fragments with ^{99m}Tc. More recently, similarly labelled peptides have come into use.

For chronic inflammation, direct targeting of lymphocytes using ^{99m}Tc Interleukin 2 will demonstrate activated T cells in, for example, autoimmune diseases. Macrophages and monocytes have been imaged using ^{99m}Tc-J001X. The endothelium in vasculitis may be shown with E-selectin antibodies and Amyloid by using ^{123}I serum amyloid protein or ^{99m}Tc-aprotonin.

(b) Imaging infection

The key questions to be asked are:

— Is there infection or not?
— If so, where is it?
— What is the cause?
— Has it responded to therapy?

The physical approach is to use ultrasound, local X rays, CT or MRI; however, all these depend on the infection becoming loculated, as for example in an abscess in the liver, which is easily demonstrable by ultrasound. Prior to abscess formation it is difficult for radiology to demonstrate soft tissue infection.

Nuclear medicine techniques depend on radiolabelling elements of the inflammatory process or bacteria directly. The US Center for Disease Control (CDC) has defined virtually all bacterial infections, with the exception of tuberculosis, which has been defined by the WHO, and endocarditis, which has been defined by Duke's criteria. Evaluation as to whether a patient has an infection or not should be made against these established systematized criteria. They include clinical evidence and laboratory results that are not confined to microbiology, diagnostic tests incorporating radiology and nuclear medicine, and further supporting evidence such as response to antibiotics.

It can be seen that the imaging of inflammatory processes and infection is a form of tissue characterization by nuclear medicine. This has moved away from the use of more non-specific techniques that react with inflammation, infection, granuloma and tumours, such as the conventional three phase bone scan, with ^{67}Ga-citrate and ^{18}F-deoxyglucose, towards agents specific to inflammation such as radiolabelled white cells or human immune globulin, and to agents specific to a particular disease (Table 5.20).

The three phase bone scan (as described in Section 5.7) is still the basis for the diagnosis of osteomyelitis, but is relatively unreliable in cases of fracture, joint prosthesis, recent surgery and in newborns. For ^{67}Ga imaging of infection, see Section 5.8.1.

TABLE 5.20. TISSUE CHARACTERIZATION OF INFLAMMATORY DISEASE

Non-specific	Inflammation specific	Disease specific
Bone scans	In-111 WBC	Tc-99m Infecton
Ga-67	Tc-99m WBC	Tc-99m Interleukin 2
F-18 DG	Tc-99m MoAb WBC	I-123 Interleukin 1
	Tc-99m peptide WBC	I-123 Interleukin 8
	In-111 HIG	Tc-99m anti-CD3
	Tc-99m HIG	Tc-99m anti-CD4
	Avidin In-111 biotin	Tc-99m anti-E-selectin
	Tc-99m nanocolloids	Tc-99m anti-VAP1
	Tc-99m liposomes	I-123 serum amyloid
	Tc-99m J001X	Tc-99m aprotinin
	Tc-99m dextran	

(c) White cell imaging

The major indication for white cell imaging is inflammatory bowel disease. Although ^{111}In-oxine labelled white cells can be employed, ^{99m}Tc-HMPAO white cell labelling is more commonly used. The disadvantages of these techniques are that blood has to be collected and labelled ex vivo, with increasing risk of AIDS related and hepatitis related needle stick injuries, as well as deterioration of the white cells themselves. The labelling is labour intensive and should eventually be replaced by in vivo labelling methods.

In inflammatory bowel disease imaging using ^{99m}Tc-HMPAO labelled white cells, serial imaging at 5 min, 1, 2 and 3 hours can demonstrate the site or sites of bowel inflammation due to Crohn's disease or ulcerative colitis. Furthermore, the rate of appearance of a positive uptake gives some indication of the inflammation activity.

On later images, white cell uptake may be seen in the region of the caecum as a non-specific effect and movement of white cells along the bowel is to be expected in inflammatory bowel disease. White cell uptake will also be evident in infective enteritis, in focal lymphadenitis and in appendicitis. It is not possible to distinguish bacterial enteritis from inflammatory enterocolitis. Another key indication is fever and pain following abdominal surgery, in order to identify a subphrenic abscess, pelvic abscess or focal peritonitis.

Bone infections are more difficult to evaluate. In the spine, destruction of bone marrow may cause a focal defect in a vertebra, instead of a focal increase. In hip prostheses, the extent of the functional marrow may need to be determined by a colloidal scan to demonstrate whether the white cell uptake is due to marrow (physiological) uptake or to true inflammation at the prosthesis. In neonates, the identification of osteomyelitis with white cells may be unreliable. In cases of fracture, either of the stress or traumatic variants, white cell uptake is non-specific.

(i) Human immune globulin (HIG)

HIG may be labelled with ^{111}In, in which case the tracer tends to be deposited at the site of the inflammation, or with ^{99m}Tc, in which case the uptake may be transient. The mechanism of uptake of HIG is mainly due to leakage through capillaries at the site of inflammation. However, there is a small uptake component by white cells due to the Fc portion of the gamma globulin, and an even smaller proportion of bacterial binding, depending on the source of the pooled HIG.

The best use of ^{111}In-HIG is in patients with fever and neutropenia of whatever cause, pulmonary infections in immunocompromised patients, pneumocystic and fungal infections of the lungs, joint prostheses, inflammatory arthritis, vascular prostheses and diabetic feet. However, the procedure is unable to distinguish infection from inflammation. Technetium-99m HIG appears similar to, but is less reliable than, ^{111}In-HIG. Imaging with ^{99m}Tc labelled small molecular weight dextran is an alternative.

(ii) Monoclonal antibodies

Monoclonal antibodies may be used to label white cells. The advantages are the lack of a need to obtain blood, since they can be given by direct intravenous injection, and their selectivity for particular white cells or inflammatory elements. The disadvantages include a high molecular weight, which may give poor tissue penetration, although fragmentation helps to improve this; a longer blood residence time; high liver and bone marrow uptakes; potential development of human antimouse antibodies (which is, however, much overstated and not a problem for imaging); and a potential alteration of target cell function. Leucocyte antigens have been utilized. Non-cross-reacting antigen (NCA) 95, with the antibody BW 250/183, is an IgG ^{99m}Tc labelled antibody given at a dose of 100 μg. Bone marrow uptake is typically 50%, liver uptake 10%, spleen uptake 5% and circulating activity 30%, half of which is

free and half of which is bound to white cells. A ^{99m}Tc-Fab fragment is commercially available as Leucoscan.

5.11.2.3. Radiopharmaceuticals

The preparation of three reagents is outlined in the following sections. These are:

— Tc-99m leucocytes;
— Tc-99m IgG;
— Tc-99m Infecton (ciprofloxacin).

5.11.3. Preparation of ^{99m}Tc-leucocytes

5.11.3.1. Equipment and materials

The following equipment and materials are needed:

— A vertical laminar flow cabinet;
— 30 or 50 mL sterile syringes;
— ACD solution and Formula A as defined in the British Pharmacopoeia (BP);
— Hetastarch solution;
— A syringe stopper;
— A 20–22 gauge butterfly needle;
— A 20–22 gauge needle;
— A centrifuge tube;
— A Whatman No. 1 strip;
— Two TB syringes;
— Ether;
— A small vial for TLC;
— HMPAO (Ceretec);
— $^{99m}TcO_4$;
— A gamma counter, with a single channel or a multichannel analyser;
— A centrifuge;
— Sets of balance tubes;
— Lead shields and containers;
— A clamp and stand;
— Methylated spirit;
— Scissors;
— A syringe marker;
— A dose calibrator.

5.11.3.2. Procedure

The following procedure should be adopted:

(1) Clean the vertical laminar flow cabinet with 70% industrial methylated spirit (IMS); the cabinet must be turned on at least 30–60 min before use.
(2) Draw up 4 mL of ACD into a 30 mL syringe and swirl to cover the entire surface.
(3) Change the needle to a 22–20 gauge needle and collect approximately 20 mL of blood using the syringe containing the ACD, then replace the needle with a syringe stopper. Write patient details on the barrel of the syringe.
(4) Remove the syringe stopper and add 4–5 mL of 6% hetastarch to the blood, replace the stopper and mix gently.
(5) Clamp the syringe into an upright position (with the syringe stopper facing upwards) inside the laminar flow cabinet and allow the blood to settle for approximately 45–60 min.
(6) Replace the syringe stopper with a 20 or 22 gauge butterfly needle and carefully push the supernatant leucocyte rich plasma (LRP) into the 10 mL blood collection tube.
(7) Centrifuge the LRP at 150*g* for 10 min.
(8) While the centrifuging is taking place, prepare the ^{99m}Tc-HMPAO, by:
    (a) Placing an HMPAO (Ceretec) vial into a suitable lead container.
    (b) Adding 1110 MBq (30 mCi) $^{99m}TcO_4$ from a previously eluted generator (within 24 hours) into the Ceretec vial; the $^{99m}TcO_4$ should not be older than 2 hours.
    (c) After 10 min, perform a quality control by placing a spot on a Whatman No. 1 strip using a TB syringe; develop the strip using ether as the mobile phase.
    (d) After development, cut the strip into two, and count each half in a gamma counter; the ^{99m} Tc-HMPAO migrates to the solvent front, as shown:

Percentage of radiochemical purity

$$= \frac{\text{counts of upper half}}{\text{counts of upper half} + \text{lower half}} \times 100\%.$$

    (e) The radiochemical purity should be more than 90%.
    (f) Use the HMPAO within 30 min of preparation.

(9) After centrifugation, remove the supernatant (PRP) using a spinal needle (after carefully removing the inner needle) and a suitably sized syringe, taking care not to disturb the leucocyte pellet at the bottom. Label the syringe "PRP".
(10) Draw up the ^{99m}Tc-HMPAO and transfer it to the LRP tube, suspend the leucocytes gently and allow them to incubate for 20 min in a lead container at room temperature.
(11) After incubation, add 3 mL PRP (of step (9)) and centrifuge at 150*g* for 5 min; measure the radioactivity of the centrifuge tube in a dose calibrator.
(12) Using a spinal needle and a suitably sized syringe, remove as much supernatant as possible without disturbing the leucocyte pellet.
(13) Measure the radioactivity of the leucocyte pellet in a dose calibrator:

Labelling efficiency

$$= \frac{\text{radioactivity of leucocyte (see step (12))}}{\text{radioactivity of leucocyte + PRP (see (step 11))}} \times 100\%$$

(14) Resuspend the leucocyte pellet with 3–5 mL PRP and draw into a 5 mL syringe using a spinal needle.
(15) Spot the end of the spinal needle on a Whatman No. 1 strip. Develop the strip in ether.
(16) Change to a 22 gauge needle for injection, label the syringe '~^{99m}Tc-leucocyte' and place in a suitable lead container. The dose is now ready for injection.
(17) After development, cut the strip into two and count each half, i.e.:

Radiochemical purity of ^{99m}Tc-leucocyte

$$= \frac{\text{counts of lower half}}{\text{counts of lower half + upper half}} \times 100\%$$

(18) Dispose of the syringe and radioactive waste in a Sharp's container and radioactive waste bag, and disinfect the laminar flow cabinet with 70% IMS and hydrogen peroxide solution.

5.11.3.3. Quality assurance log

The following forms should be completed:

Material	Supplier & log No.	Expiry date	Quantity
ACD	_____	_____	_____
Hetastarch	_____	_____	_____
Ceretec	_____	_____	_____
Tc-99mO_4	_____	_____	_____

(a) Quality control of ^{99m}Tc-HMPAO

— Stationary phase: Whatman No. 1;
— Mobile phase: ether;
— Counts of upper half = _____;
— Counts of lower half = _____;
— Percentage of radiochemical purity = _____ × 100% = _____%.

(b) Quality control of ^{99m}Tc-leucocyte

— Radioactivity of leucocyte (see Step (12)) = _____;
— Radioactivity of leucocyte + PRP (see Step (11)) = _____;
— Percentage of labelling efficiency = _____ × 100% = _____%.
— Stationary phase: Whatman No. 1;
— Mobile phase: ether;
— Counts of upper half = _____;
— Counts of lower half = _____;
— Percentage of radiochemical purity = _____ × 100% = _____%.

Prepared by _____ Date _____

5.11.4. Preparation of ^{99m}Tc-IgG

5.11.4.1. Equipment and materials

The following equipment and materials are required:

— A vertical laminar flow cabinet;
— Mercaptoethanol;

— Sephadex G-50 (3 g in 50 mL of water, soaked overnight);
— $^{99m}TcO_4$;
— NaCl;
— An MDP vial;
— A μm filter;
— $NaH_2PO_4H_2O$;
— Na_2HPO_4;
— H_3PO_4;
— Water for injection;
— Melolein dressings;
— Test tubes and a test tube rack;
— Micropipettes (10–100 μL) and tips;
— A 1 mL pipette;
— A cuvette;
— Lead shields and containers;
— A clamp and stand;
— A gamma counter, single channel analyser or multichannel analyser;
— Instant thin layer chromatography (ITLC) equipment;
— A silica gel strip (ITLC-SG strip) or a Whatman No. 1 strip;
— Methyl-ethyl-ketone (MEK) and small vials for TLC;
— A three way stopper;
— A dose calibrator;
— A pH meter;
— Nitrogen;
— A spectrophotometer;
— Scissors;
— A syringe marker.

5.11.4.2. Procedure

The following procedure should be adopted:

(1) Weigh out the following chemicals:
— 0.61 g $NaH_2PO_4H_2O$;
— 0.87 g Na_2HPO_4;
— 0.9 g NaCl.
(2) Prepare phosphate buffered saline (PBS) by dissolving these chemicals in 100 mL water for injection and adjusting the pH to 6.7–6.9 using H_3PO_4 and a micropipette. Purge the PBS on ice with nitrogen for 30 min.

(3) Prepare a gel filtration column in the following way:
   (a) Remove the barrel from a 30 mL syringe. On a melolein dressing pack, mark a circle with a diameter equal to that of the inside of the syringe. Cut out this circle, remove and wet the dressing thoroughly with PBS, then place the dressing on the bottom of the syringe.
   (b) Clamp the syringe vertically and close it with a three way stopper. Shake a bottle of swollen Sephadex G-50 and carefully transfer the Sephadex G-50 to a 30 mL syringe, right to the top. Allow a few minutes for the Sephadex to settle.
   (c) Place a liquid waste container under the syringe and open the three way stopper to allow the water to drain out.
   (d) When the water has run through the column, gently add the nitrogen purged PBS to the top of the column, taking care not to disturb the gel. Continue until 50 mL of PBS has passed through the column.
   (e) Close the three way stopper to allow approximately 1–2 mL of PBS to remain above the gel.
(4) Using a micropipette, add 5 μL of mercaptoethanol to 10 mg human immunoglobulin (IgG, Sigma 10 mg/2 mL), cap the vial and immediately mix well. As mercaptoethanol emits a foul odour, this step should be performed quickly. Do not shake the IgG.
(5) Allow the vial to incubate at room temperature for 30 min.
(6) During the incubation period, mark 11 test tubes (one tube marked 'blank' and the others '1' to '10').
(7) Add 2 mL PBS to the blank test tube and have a 3 mL syringe ready.
(8) After the 30 min incubation period, open the three way stopper of the column to drain the PBS. Gently, layer the reduced IgG onto the gel and allow it to run into the column. Add ten consecutive 1 mL aliquots of PBS to the top of the column and collect 1 mL fractions into the test tubes from the bottom. Add 1 mL PBS to each test tube and close the tubes with parafilm.
(9) Transfer the IgG fractions from test tubes into cuvettes and measure the absorbance of each fraction using a spectrophotometer. Make any necessary dilution if the reading is out of range. Pool all fractions with a concentration of 1 mg/mL (absorbance of 1 mg/mL IgG = 1.4/cm light path).
(10) Divide the pooled IgG into 500 μg aliquots.
(11) Pass the IgG aliquots through 0.22 μm millipore filters into sterile vacuum vials. Fill the vials with sterile nitrogen and freeze immediately.
(12) To label the IgG, reconstitute an MDP vial with 5 mL of 0.9% NaCl, add 50–100 μL to the IgG and mix gently. Add 30 mCi of $^{99m}TcO_4$ to the IgG vial and allow to incubate at room temperature for 15 min.

(13) Mark a syringe '^{99m}Tc-IgG', draw a dose using the syringe, measure the dose in a dose calibrator and place this inside a lead syringe shield for injection.

(14) Apply a spot on a Whatman No. 1 strip with a TB syringe and develop the strip in MEK. Cut the strip into two and count each half, the labelled IgG remaining at the origin, while the free $^{99m}TcO_4$ will migrate to the solvent front, i.e.:

$$\text{Percentage of radiochemical purity} = \frac{\text{counts of the lower half}}{\text{counts of the upper half + lower half}} \times 100\%.$$

5.11.4.3. Quality assurance log

The following forms should be completed:

Material	Supplier & log No.	Expiry date	Quantity
IgG	_____	_____	_____
Mercaptoethanol	_____	_____	_____
Sephadex G-50	_____	_____	_____
$^{99m}TcO_4$	_____	_____	_____
0.9% NaCl	_____	_____	_____
MDP vial	_____	_____	_____
0.22 μm filter	_____	_____	_____
$NaH_2PO_4H_2O$	_____	_____	_____
Na_2HPO_4	_____	_____	_____
Water for injection	_____	_____	_____

Quality control of ^{99m}Tc-IgG

— Stationary phase: ITLC-SG;
— Mobile phase: MEK;
— Counts of upper half = _____;
— Counts of lower half = _____;
— Percentage of radiochemical purity = _____ × 100% = _____%.

Prepared by _____ Date_____

5.11.5. Preparation of ^{99m}Tc-Infecton (ciprofloxacin)

5.11.5.1. Principle

Technetium-99m Infecton is a 4-fluoro-quinolone that is able to chelate metals and bind to DNA gyrase, the enzyme that helps DNA to unwind when bacteria divide. This enzyme is common to all bacteria, and Infecton appears to bind to DNA gyrase in all bacteria including those of tuberculosis and, with the radiolabel, allows imaging of sites of active bacterial infection. It does require dividing bacteria and does not bind to dead bacteria.

Infecton is prepared under aseptic conditions by adding 1 mL of NaCl 0.9% solution to a sterile vial of special reducing agents including 0.5 mg of stannous tartrate so that it can chelate the ^{99m}Tc; 370 MBq (10 mCi) of generator eluted sodium pertechnetate ^{99m}Tc of high specific activity (>1000 MBq/mL) is added, immediately followed by an ampoule of 2 mg of ciprofloxacin. This mixture is shaken and allowed to stand for 10 min. Quality control on a 3 cm Whatman No. 1 chromatography strip is developed in butanone, which takes 30 min. Usually 95% of the ciprofloxacin is labelled with ^{99m}Tc. The solution appears clear and colourless and is stable over eight hours.

5.11.5.2. Equipment and materials

The following equipment and materials are required:

— A vertical laminar flow cabinet;
— A syringe (5 or 2 mL TB syringe or insulin needle);
— A needle (22 or 25 gauge);
— An Infecton ampoule;
— A stannous tartrate vial;
— NaCl;
— $^{99m}TcO_4$;
— A lead syringe shield;
— A syringe marker;
— A gamma counter, single channel analyser or multichannel analyser;
— An instant thin layer chromatography silica gel (ITLC-SG) strip;
— MEK and small vials for ITLC;
— Scissors and test tubes;
— A dose calibrator.

5.11.5.3. Procedure

The following procedure should be adopted:

(1) Add 1 mL of 0.9% NaCl to the stannous tartrate vial and shake well, to dissolve the powder.
(2) Aseptically draw up 20–25 mCi of $^{99m}TcO_4$, ideally from a previously eluted generator (within 24 hours).
(3) Carefully open the Infecton vial and draw up the contents into a 3 mL syringe with a 25 gauge needle or insulin syringe to minimize the formation of metal complex.
(4) Add the Infecton to the stannous tartrate vial and place the vial in a suitable lead shield.
(5) Immediately add the $^{99m}TcO_4$ to the Infecton through a 25 gauge needle. Shake the contents of the vial.
(6) Leave the contents of the vial to incubate at room temperature for 10–15 min.
(7) Mark a syringe '^{99m}Tc-Infecton' and draw the dose (370 MBq (10 mCi)) with a 25 gauge needle. Measure the dose in a dose calibrator and place the dose inside a lead syringe shield for injection.
(8) Using an insulin needle or a TB syringe with a 25 gauge needle, apply a spot on a Whatman No. 1 strip. Develop the strip in MEK.
(9) Cut the strip into two at two thirds of the length from the origin and count each half. The ^{99m}Tc-Infecton remains at the origin, while the free $^{99m}TcO_4$ moves to the solvent front, i.e.:

$$\text{Percentage of radiochemical purity} = \frac{\text{counts of the lower half}}{\text{counts of the upper half + lower half}} \times 100\%.$$

5.11.5.4. Quality assurance log

The following forms should be completed:

Material	Supplier & log No.	Expiry date	Quantity
Infecton	_____	_____	_____
Stannous tartrate	_____	_____	_____
$^{99m}TcO_4$	_____	_____	_____
0.9% NaCl	_____	_____	_____

Quality control of ^{99m}Tc-Infecton

— Stationary phase: ITLC-SG;
— Mobile phase: MEK;
— Counts of upper half = _____;
— Counts of lower half = _____;
— Percentage of radiochemical purity = _____ × 100% = _____%.

Prepared by _____ Date_____

5.11.6. Interpretation

(a) Interpretation of white cell images

Normal white cell images show transient lung uptake, persistent normally increased spleen uptake with some uniform liver uptake and widespread red bone marrow uptake. Four hours after injection there is evidence of some urinary and caecal activity. A poor white cell preparation may show persistent lung uptake and a more equal spleen-to-liver uptake ratio. The thyroid does not need to be blocked, but some thyroid uptake may be seen if there is a delayed injection. Blood pool activity is persistent and decreases slowly with time.

White cell uptake will persist during the healing phase of osteomyelitis even when the bacterial infection has been treated successfully. Lack of white cell uptake may occur in chronic inflammation when a more monocytic or lymphocytic infiltration is present or when there has been prolonged antibiotic therapy for resistant infection. This may be due to loss of leucotaxin and related cytokine stimulation. Technetium-99m HIG or ^{67}Ga-citrate uptake may persist in such situations.

(b) Interpretation of HIG images

HIG images show a generalized high blood pool and marrow distribution in normal individuals. Liver uptake may be more marked than spleen uptake and later images may show renal uptake, urinary excretion and gut activity.

(c) Interpretation of ^{99m}Tc-Infecton images

Technetium-99m Infecton accumulates at the sites of acute bacterial infection in sufficient amounts to be imaged. No thyroid blockade is required.

This procedure is contraindicated in patients who are sensitive to ciprofloxacin, pregnant or suspected to be pregnant, lactating, have renal failure or are under the age of 14. About 370 MBq (10 mCi) are injected intravenously. Anterior and posterior regional or whole body images are acquired at 1 and 3–4 hours, and in some patients at 24 hours after injection, using a large FOV gamma camera set with a low energy, general purpose, parallel hole collimator, peaked to 140 keV with a 15% window. Images with 500 000 counts are collected.

Infecton is a small highly diffusible molecule that enters sites of inflammation non-specifically and leaves progressively as blood level falls through renal clearance. Sites of acute inflammation, such as an active rheumatoid joint, will show initial uptake that may be maintained for the first four hours or fade. A 24 hour image will show fading in a rheumatoid joint or other active inflammatory arthropathy but there will be persistence of uptake in septic arthritis, since binding to the dividing bacteria persists. A 24 hour image is also helpful in suspected endocarditis, vascular or orthopaedic prosthesis and fracture. One is generally not required when the site of uptake is obvious on the early images or when chest or abdominal infection is suspected.

Technetium-99m Infecton is excreted into the bladder, where activity increases with time so that voiding should be undertaken between sets of images. Initial blood pool activity in the liver and spleen decreases in the later images. No activity is seen in normal bone marrow, bone, muscle or soft tissue. Exceptions are bone epiphyses in children, where symmetrical uptake is seen. Occasionally, scrotal activity may be observed in adults as a result of vascularity. Biological barriers prevent access of intravenously injected agents, while bacterial flora does not usually divide actively. As a result, normal gastrointestinal flora does not take up Infecton. There is, however, occasional weak biliary excretion with some caecal activity seen after 4 hours. Definite biliary or intestinal uptake after 1 hour is pathological. Uptake in the gut after 4 hours is considered as a normal variant in Asians, probably as a result of active ‘normal’ flora.

(d) Abnormal Infecton images

There is focal uptake on the first image around the site of an abscess (but not in its centre where the pus contains dead bacteria) and at other infection sites containing actively dividing bacteria. There may be 10^9 bacteria in 1 mL of infected material, giving a great number of binding sites for ciprofloxacin.

The great advantage of ^{99m}Tc-Infecton imaging is the lack of normal bone marrow uptake, so that sites of infection in the spine are positive even when a white cell scan reveals a ‘cold’ defect. When infected, hip and knee prostheses show uptake and beading around the prosthesis much more clearly than with

white cells or ^{67}Ga. This is partly due to the easy penetration of the small molecules of ciprofloxacin.

In the bowels, inflammatory but non-infective diseases such as Crohn's disease and ulcerative colitis show negative scan findings, but an associated abscess may yield a positive image. Enteritis due to clostridia or salmonella will usually be positive. It is interesting to note that gut bacterial infections tend to be segmental rather than diffuse in parts of either the small or large intestine. It has been found that active tuberculosis takes up Infecton.

Renal abscesses may be detected, provided enough time is allowed for the renal excretion of the agent to be completed. Single photon emission tomography may be helpful in hip prostheses. In diabetic feet, it can distinguish skin from bone infections. In the heart, serial images will show persistent uptake in valve infections as the blood pool clears. The technique is particularly useful in demonstrating whether infection is present around a pacemaker, as well as in the sternal split after open chest cardiac surgery. Given the normal lack of marrow uptake, persistent sternal uptake indicates infection rather than a response to surgery.

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5.12. RADIOIMMUNOASSAY PROTOCOLS

Protocol 1a: Preparation of hormone (T4/T3) free serum

The following procedure is used:

(a) Collect a pool of human serum, preferably from a range of donors. Each donation must be individually tested for hepatitis B markers and anti-

HIV antibodies (the antibodies to the AIDS virus). Sera found positive should not be used. Note that serum pools may yield false results when tested for viruses. Alternatively, donor horse serum may be used. This avoids the potential infection risk.

(b) Filter the serum through a coarse filter (e.g. glass wool) to remove coarse particles.

(c) Add ^{125}I-T4 to give approximately 2000 counts/(min·mL). Allow time for equilibration and remove 1 mL for counting. This yields the initial counts needed to monitor the removal of endogenous hormone.

(d) Assemble a 50 mL disposable syringe to serve as a column as illustrated in Fig. 5.2.

(e) Weigh out Celite (Celite Hyflo-Super-Cel, Koch Light) and charcoal (Norit PN5) in the ratio 1:4. Typically, using a 50 mL syringe, weights would be 2 g Celite and 8 g charcoal.

(f) Mix the charcoal and Celite in a covered beaker or screw cap bottle and add approximately 25 mL of distilled water. Continue to add water in small amounts until a thick slurry is obtained that can be poured into the column.

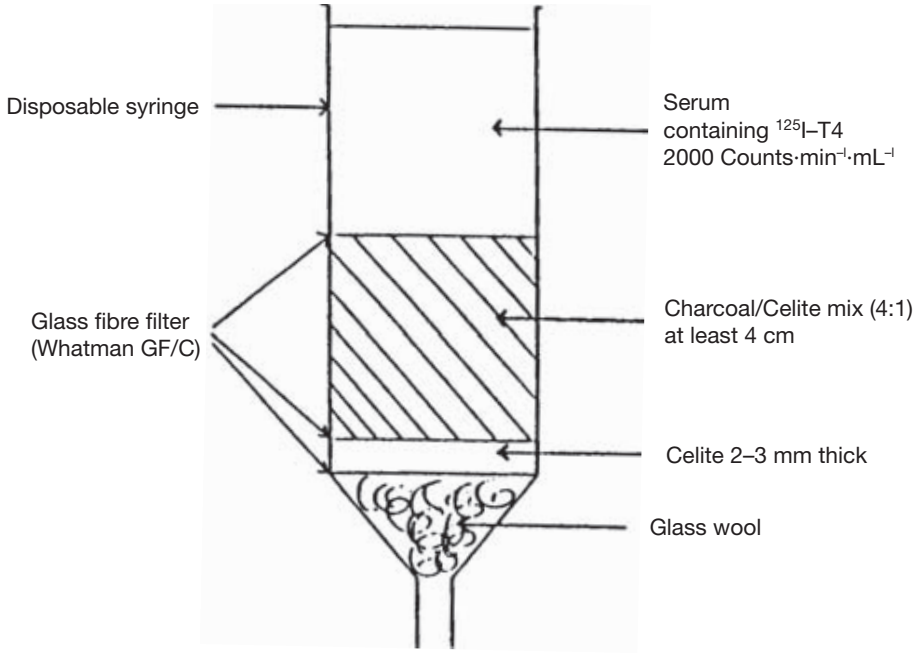

FIG. 5.2. Column used for preparation of hormone (T4/T3)-free serum.

(g) Using a Pasteur pipette, add the charcoal–Celite mixture to the column and allow it to settle until the water is just visible at the top. Do not allow to run dry.
(h) Gradually add approximately 50 mL of the serum to the column.
(i) Allow the column to run under gravity and discard the water void volume. The first 1 mL of the eluted serum may also need to be discarded.
(j) Collect the serum that has passed through the column. When all the serum has entered the column, more water may be added to the top, to facilitate the passage of the serum down the column.
(k) Count a 1 mL aliquot of the serum to monitor the removal of T4 and T3. Normally, about 98% or more of endogenous hormone is removed by this process.

Protocol 1b: Preparation of hormone-free serum

Preparation is by absorption by anion exchange resin stripping:

(a) Add ^{125}I-T3/T4 1000–2000 counts/(min·100 μL) of serum; incubate for 30 min for equilibration. Count an aliquot before addition of resin.
(b) Add AGI-X8 200–400 mesh Cl anion exchange resin (BioRad No. 140-1451) in the labelled serum (300 mg/mL); stir at 37ºC for three hours.
(c) Filter the serum (coarse glass microfibre filter paper GF/D).
(d) Count an aliquot of serum to monitor the stripping efficiency.
(e) Add further resin in the same proportions and repeat the operation for another three hours.
(f) Filter the serum after 2 days of incubation with resin, followed by a coarse filter (GF/D) and a finer filter (GF/B).
(g) Add 1:100 dilution of 10% sodium acid, store at –20ºC.
(h) Wash the used resin in 2 L of double distilled H_2O; soak the washed resin in double distilled H_2O.
(i) Check the stripping efficiency and count 100 μL serum for 1 min; repeat the procedure if more than 5% of radioactivity is shown; the stripping efficiency should be more than 95%.

Protocol 2: Preparation of T3/T4 standard

(a) Preparation

The following procedure is used:

— Weigh: 3.255 mg (tri-iodothyronine, free acid, sigma) for T3
2.223 mg (L-thyroxine free acid, sigma) for T4.
— Dissolve with a small amount of ammonical (2N) ethanol (<0.5 mL) and dilute in a 10 mL volumetric flask with a 50% propylene glycol aqueous spectrophotometric check.
— Dilute stock standard with propylene glycol aqueous solution in a ratio of 1:10.
— Check that absorbency is: 320 nm for T3
325 nm for T4.
— Check that molar concentration (μm) is: OD/4658 for T3
OD/6210 for T4.
— Dilute the T3 solution to 1:20 with 0.05M phosphate buffer and 1:50 in hormone-free serum. Finally, dilute the working solution to 1, 2, 4, 6 and 10nM.
— Dilute the T4 solution to 1:100 with 0.05M phosphate buffer and 1:10 in hormone-free serum. Finally, dilute the working solution to 10, 50, 100, 150 and 300nM.

(b) Regeneration of resin

The following procedure is used:

— Rinse the resin with double distilled water.
— Stir with 1M NaOH (1 L NaOH = 300 g resin) for 2 hours at room temperature.
— Filter and discard NaOH; rinse resin until the pH of the water returns to 7.0.
— Stir with 1M HCl (1 L HCl = 300 g resin) for two hours at room temperature.
— Filter, discard HCl and rinse until the pH of the water returns to 7.0.
— Store the resin in a suitable dry container.

Protocol 3: Typical immunization schedule for production of polyclonal antibodies to antigens in animals

(a) Preparation

The antigen should be as pure as possible since any impurities present may also produce antibodies. This is especially important if the impurities are structurally similar to the primary antigen (e.g. many steroids) or if the tracer or standard used for the assay is likely to contain the same impurities.

The high doses of antigen used for immunization may induce tolerance and a poor antibody response. Do not exceed 100 μg/kg body weight of the animal.

The first, and sometimes the second, immunization is given in Freund's complete adjuvant. Subsequent secondary immunizations are given in Freund's incomplete adjuvant. The complete adjuvant contains inactive TB and other bacteria that are highly immunogenic. To make up the suspension, mix 1 mL of adjuvant and antigen solution in a buffer in a syringe. Connect this syringe to another syringe via a three way tap and pass the mixture several times from one syringe to the other until it is completely emulsified. Good emulsion formation is seen when a drop 'floats', rather than disperses, on the surface of the water.

Immunogens are always administered subcutaneously or intradermally, never intravenously. The multisite injection technique is preferred. For subcutaneous injection, 1 mL of emulsion at four to six sites along the back and neck is used. Abscesses may form, and if these are causing great inconvenience, the animal may need to be sacrificed. Where the intradermal route is used, the back of the animal is shaved and 25–50 μL per injection of emulsion injected at multiple (10–100) sites.

The more animals immunized, the greater the chances of obtaining antisera. Generally, where rabbits are used, immunizing four to six animals may yield at least two good antibody producers.

A small test bleed (about 2 mL from an ear vein in the case of rabbits or guinea pigs) is taken two to four weeks after the first injection of immunogen. If three test bleeds between four to twelve weeks do not provide any evidence of antibody formation, it may not be worthwhile to continue and the animal may be discarded.

Animals producing good antisera may be bled regularly, at twice weekly intervals. About 20 mL can be collected from the ear vein of a rabbit, but with guinea pigs, cardiac puncture will be necessary. The characteristics of antibodies can change with time and each bleed needs to be tested separately.

If an animal producing good antibodies becomes ill, it should be carefully observed and, if there is any likelihood of it dying, it should be sacrificed by exsanguination under anaesthesia.

At each bleed, blood should be collected in glass tubes and allowed to clot for one to two hours at room temperature and two to six hours at 4ºC. The tubes should not be disturbed during the clotting process as haemolysis may result. Centrifuge at 1500*g* for 15 min, preferably at 4ºC. If the clot is left for a further period (e.g. overnight) at 4ºC, it may further retract, releasing more antiserum.

(b) Immunization protocol

The following protocol is used:

(1) For primary immunization, use 50–100 μg/kg antigen in Freund's complete adjuvant. Inject subcutaneously or intradermally as described above.
(2) Wait for two to four weeks.
(3) First make a test bleed, taking 2 mL of blood and testing for its binding activity to the antigen.
(4) Then perform a secondary immunization using the same quantity of antigen in Freund's complete adjuvant.
(5) Bleed every two to four weeks, test the serum for binding activity; store the serum as indicated in the next section.
(6) Repeat the secondary immunization every six to eight weeks as in step (4), but using Freund's incomplete adjuvant.
(7) Collect, test and store the serum as in step (5).

(c) Storage of antisera

If a preservative such as 0.1% sodium acid (but not Merthiolate) is added, antisera may be stored at 4ºC for several years.

Antisera are also stable for many years stored frozen at –20 or –40ºC. Rapid (snap) freezing using a mixture of CO_2 and acetone is preferred. The storage should be in aliquots that can be reconstituted just prior to use. Where storage at –20ºC is convenient, the antiserum may be diluted in the ratio 1:10 in buffer containing 0.1% sodium acid before aliquotting. At this temperature, diluted antiserum is stable.

If freeze drying equipment is available, antisera may be lyophylized and stored in aliquots for reconstitution immediately before use.

Protocol 4: Production of immunogens from haptens using the mixed anhydride reaction

(a) Activation of hapten (steroid)

The following procedure is used:

— Add 40 μmol (5 mL) of N-methylmorpholine to 40 μmol of the steroid derivate in 250 μL non-aqueous solvent (e.g. N,N-dimethylformamide (DMF) or dioxane).

— Cool to –15° C (+10°C if using dioxane).
— Add 40 μmol (6 μL) isobutylchloroformate.
— React for 3 min at –15°C.

(b) Conjugation to bovine serum albumin (BSA)

The following procedure is used:

— Add the activated steroid prepared as above slowly to 20 mg (0.3 μmol) BSA in 800 μL DMF.
— React at –15°C for one hour and then at 0°C for three hours.
— Dialyse the product.

Protocol 5: Conjugation of T4 to BSA, using carbodiimide

The following procedure is used:

— Dissolve 20 mg of T4 in 5 mL of DMF.
— Dissolve 50 mg of BSA in 25 mL of distilled water.
— Add 30 mg of 1,1′ carbonyl-di-imidazole (CDI) to the BSA solution and adjust the pH to 5.5 with NaOH.
— Allow to react for 10 min.
— Add 10 mg more of CDI and adjust the pH to 5.5, if necessary.
— React overnight at room temperature in the dark.
— Dialyse and lyophylize the product.

Protocol 6: Antibody purification methods

(a) Preparation with ammonium sulphate

The following procedure is used:

— Dilute 3 mL of antiserum to 10 mL with 0.9% saline.
— Add 2.7 g of ammonium sulphate and stir mixture gradually (45% saturation).
— Mix for one hour at room temperature.
— Centrifuge at 2000 rev./min for 30 min at room temperature.
— Re-dissolve the pellet in the minimum amount of phosphate buffer, pH7.4 (or other buffer as may be used for the antibody coating).
— Dialyse against phosphate (or other coating) buffer.

(b) With caprylic acid (n-octanoic acid)

The following procedure is used:

— Dilute 2 mL of antiserum to 6 mL with 60mM acetate buffer, pH4.
— Adjust the pH to 4.8 using NaOH/HCl.
— Add 1 mL caprylic acid (= 136 mg) and stir continuously for 30 min at room temperature.
— Centrifuge at 3000 rev./min for 45 min at 20ºC.
— Adjust the pH of the supernatant to 5.7 using NaOH.
— Dialyse against three changes of 15mM acetate buffer of pH5.7 to remove the caprylic acid.

(c) Preparation with diethylaminoethyl (DEAE) cellulose chromatography

The following procedure is used:

— First prepare purified antibody using ammonium sulphate precipitation as described in (a) above.
— Load the DEAE cellulose column with phosphate buffer of pH6.
— Add 2 mL of antibody solution in phosphate buffer of pH6.
— Run column with phosphate buffer of pH6 and collect 1 mL fractions.
— The fractions containing purified antibodies are located by UV spectroscopy at 280nM.

Protocol 7: Direct iodination of protein using chloramine T

(a) Preparation of ^{125}I-T4 and ^{125}I-T3

The following procedure is used:

(1) Suspend 2 mg of T3 in a few millilitres of phosphate buffer of pH7.4 and add (one molar) N.NaOH dropwise until the T3 is dissolved. Transfer to a 20 mL flask and make up to volume with phosphate buffer.
(2) For iodination, aliquot in 15 mL (1.5 mg) volumes and store at –20ºC, in polypropylene vials.
(3) To a vial containing 15 mL of T3, add:
—20 μL 0.5M of phosphate buffer of pH7.4;
—10 μL (1 μCi) of sodium ^{125}I-iodine;
—10 μL of chloramine T solution in 50mM phosphate buffer of pH7.4.
(4) Mix for 20 s.

(5) Add 10 μL (10 μg) of sodium metabisulphite in 50mM phosphate of pH7.4 and 100 μL potassium iodide (10 mg/mL) containing 1% BSA.
(6) Vortex mix and count vial with contents.
(7) Transfer contents to column and count empty vial.
(8) The separation column is Sephadex G-25 Fine (approximately 2 g Sephadex) in a 15 cm × 0.9 cm column. Equilibrate and elute with 50mM $NaHCO_3$ of pH9.0 at a flow rate of 10–15 mL/hour.
(9) Collect 10 min fractions. Count each fraction and plot the counts against fraction number, to derive the chromatographic profile. Calculate the proportion of radioactivity in each peak eluted (see the examples shown in Fig. 5.3).
(10) Pool the desired fractions containing T3 and T4 and adjust the pH of each of them to 7.5 by dropwise addition of NaHCl. Dilute each to a radioactive concentration of 5–10 μCi/mL, adding also phosphate buffer (pH7.4), cysteine hydrochloride and mannitol to give a final concentration of 50mM phosphate buffer, 4% (wt/vol.) mannitol and 0.1% cysteine.
(11) Aliquot in 0.5 mL volumes and freeze dry.
(12) Store at 4°C. The product is stable for at least four weeks.

The procedure described above incorporates 40–60% of the initial ^{125}I into T4 and 25–40% into T3, with only about 5% of the ^{125}I remaining unreacted. Specific activities are about 600–1000 μCi/μg for T4 and 200–400 μCi/μg for T3.

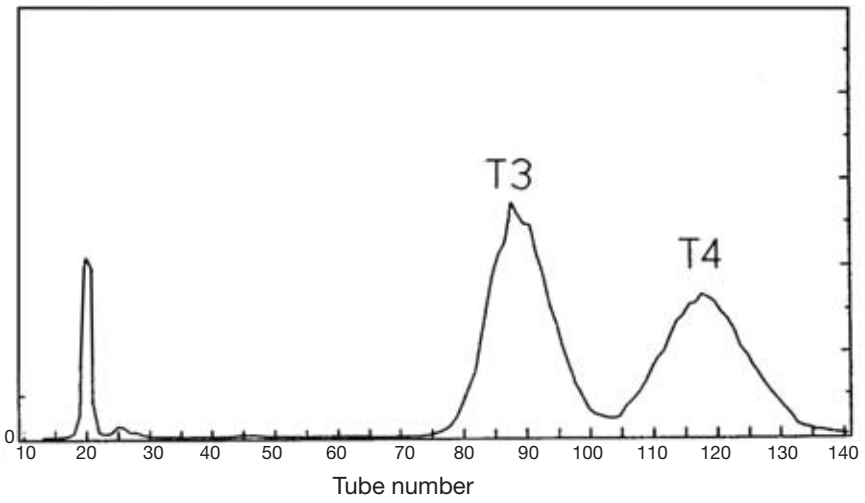

FIG. 5.3. Typical result from a Sephadex G-25 column.

(b) Elution patterns of iodinated T3 and T4

The results are given in Table 5.21.

Protocol 8: Radioiodination using solid phase lactoperoxidase

The following procedure is used:

(a) Add to 10 mg antigen in an iodination vial:
—10 μL 0.5M phosphate buffer of pH7.4;
—1 μCi sodium ^{125}I-iodide.
(b) Add 10 mL (10–20 ng) solid phase lactoperoxidase and 5 μL H_2O_2 (0.5nM).
(c) React for 10 min.
(d) Add 5 μL more of H_2O_2 (0.5nM).
(e) React for 20 min.
(f) Add 100 μL of 0.1% sodium acid phosphate.
(g) Purify the products as described in Annex VII.

TABLE 5.21. ELUTION PATTERNS OF IODINATED T3 AND T4

Tube no. (rows of ten)	(Number of counts)/10 s									
	1	2	3	4	5	6	7	8	9	10s
1–10										
11–20	124	111	133	212	283	549	2 819	47 929	4 248	678
21–30	413	607	444	1 249	728	707	378	246	188	200
31–40	197	215	223	149	175	178	158	169	156	188
41–50	174	241	360	320	335	288	209	191	199	175
51–60	179	168	148	174	166	181	181	212	188	182
61–70	155	191	178	137	143	145	152	177	172	186
71–80	205	257	309	524	824	1 312	2 314	3 881	5 730	7 541
81–90	11 352	15 306	19 015	21 577	22 831	27 370	25 986	24 897	24 763	21 496
91–100	19 154	15 487	13 337	10 527	8 359	6 172	4 827	3 304	2 687	2 442
101–110	2 193	2 182	2 405	3 533	4 254	5 068	6 289	7 969	9 257	10 065
111–120	11 860	13 550	14 171	15 590	15 707	16 780	16 368	15 852	14 720	13 920
121–130	12 503	10 503	9 677	8 327	7 216	6 088	5 145	4 233	3 291	2 574
131–140	2 004	1 480	1 181	1 074	806	745	545	489	419	341

Protocol 9: Iodination of peptides by the iodogen method

(a) Preparation of Ultrogel column

The following procedure is used:

— Fill the column with water or buffer.
— Pour in the correct amount of swollen Ultrogel using the reservoir.
— Allow the column to settle whilst running with water or buffer.
— Equilibrate column with buffer using a high enough reservoir for it to compact.
— After use store in buffer or water with bacteriostat. The solution is stable for at least six months.

(b) Preparation of iodogen coated tube

The following procedure is used:

— Make up a 20 μL solution of iodogen in chloroform or dichloromethane (e.g. by making up a 1 mg/mL solution and diluting in the ratio 1:50).
— Pipette 100 μL of the iodogen solution into the bottom of a glass tube (e.g. a glass LP4 tube cut to a depth of approximately 4.5 cm).
— Allow to dry in a fume cupboard. This takes approximately two hours.

(c) Iodination

The following procedure is used:

— Mark the test tubes for collection of 400 μL fractions. Number the tubes.
— Set the Ultrogel column reservoir at a height that gives flow rates of 6 mL/hour.
— Add 20 μL of peptide solution in 0.05M phosphate buffer of pH7.2 to the iodogen coated tube.
— Add 10 μL of low specific activity $Na^{125}I$.
— Leave to react for 20 min with occasional gentle shaking.
— Transfer the reaction mixture into another tube containing 200 μL phosphate buffer of pH7.2 and leave for 5 min.
— Place diluted reaction mixture into a 60 cm × 0.9 cm column of Ultrogel ACA 54 and run column slowly (6 mL/hour) using phosphate buffer containing BSA. Collect about 50 fractions of about 400 μL each.
— Count the radioactivities of the fractions and plot the elution profile.

Protocol 10: Typical protocol for conjugate iodination of steroids

(a) Activation of steroid derivative

The following procedure is used:

— Take 2.4 mg steroid in 50 μL dioxane.
— Add 10 μL of a 1/5 solution of tri-n-butylamine in dioxane.
— Add 10 μL of a 1/10 solution of isobutylchloroformate in dioxane.
— React for 20 min at 10ºC.
— Add 3.5 mL dioxane to stop the reaction.

(b) Iodination of histamine

The following procedure is used:

— Take 220 ng of histamine in 10 μL phosphate buffer.
— Add 0.5 μCi of $Na^{125}I$ (5 μL).
— Add 50 μg of chloramine-T in 10 μL phosphate buffer.
— React for 30 s.
— Add 300 μg sodium metabisulphite to stop the reaction.

(c) Conjugation

The following procedure is used:

— Add 50 μL activated steroid to iodinated histamine.
— Add 10 μL of 0.1M NaOH.
— React on ice for one hour.
— Add 10 mL of 0.1M NaOH.
— React for 1 hour on ice.
— Acidify with 1 mL of 0.1M HCI.
— Extract excess unconjugated histamine and related products with 1 mL toluene/ethyl acetate.
— Neutralize with 1 mL of 0.1M NaOH.
— Add 1 mL of phosphate buffer.
— Extract product with 1 mL toluene/ethyl acetate.

(d) Purify on TLC

The following procedure is used:

— Develop with chloroform/methanol/acetic acid (90/10/1).
— Locate by autoradiography and scrape off product band.
— Dissolve in ethanol.

Protocol 11: Iodination of antibodies (rabbit IgG) by the N-bromosuccinimide method

The antibodies must be pure for iodination. Purification can be done by ammonium sulphate or caprylic acid precipitation, followed by isolation of IgG using a DEAE or protein A sepharose chromatography.

The optimal specific activity for iodinated IgG is approximately 12 μCi/μg. The final specific activity of the product can be altered by adjusting the amount of protein added, the amount of $Na^{125}I$ added, the amount of N-bromosuccinimide added and the reaction time:

(a) Equilibrate a small Sephadex G-25 column with 0.05M phosphate buffer.
(b) Make up a solution of N-bromosuccinimide (200 μg/mL) in 0.05M phosphate buffer.
(c) To a small tube (e.g. an Eppendorf tube):
— Add 10 g IgG (e.g. 10 μL of a 1 mg/mL solution).
— Add 10 μL of 0.5M phosphate buffer.
— Add 10 μL of the low activity $Na^{125}I$ provided.
— Add 5 μL of N-bromosuccinimide solution, mix and react for 20 s.
— Add 200 μL of 0.05M phosphate buffer to dilute the reaction mixture (some authors add excess tyrosine to, in effect, stop the reaction).
— Immediately apply the mixture to the chromatography column and run with 0.05M phosphate buffer. Collect about 30 fractions of ten drops each.
— Count each fraction for 1 s. Plot the elution profile.
— Add about 5–10 mg of BSA to the fractions saved (e.g. 20 μL of 30% BSA).

Calculate the specific activity of the label:

$$\text{Specific activity} = \frac{\text{labelled counts} \times \mu\text{Ci } Na^{125}I}{(\text{labelled} + \text{free counts}) \times \text{mass IgG}(\mu\text{g})} = \mu\text{Ci}/\mu\text{g}.$$

Protocol 12: Preparation of ^{125}I-thyroxine with product separation by HPLC

(a) Apparatus and procedure

The procedures described in the following are used with the equipment noted:

(1) The following equipment is required: A high pressure pump, for example an Altex model 110A, a Rheodyne No. 7125 syringe loading injector, a flow-through radioactivity detector and recorder, a fraction collector, a column, an ultrasphere 5 m ODS, 4.6 mm × 4.5 cm (Beckman), 1.5 mL conical microfuge tubes, snap-top volumetric glassware, pipettes and tips, and disposable plastic ware.
(2) A manual counter (e.g. mini-assay type 6-20) is used for radioactive counting; the bottom of the sample tube holder should be 17 cm above the bottom of the counting well.
(3) Assemble the column and detector into the HPLC system, pump water through the system for 15 min at 1 mL/min and open the sample loop so this is also washed through.
(4) Note that this *washing with water* is most *important*.
(5) Transfer to pumping the eluent for a further 10 min through the column, including the sample loop, then reduce the flow to 0.5 mL/min and leave it running into the sink.
(6) Load the fraction collector with tubes, set to collect 30 drop fractions, turn on and bring the arm to the start position, set the ratemeter to 3×10^4 counts/s, linear with a time constant of 3.3 s, and the recorder to 15 cm/hour.

(b) Iodination

Perform the iodination (using chloramine-T) as described in Annex VII, using T3, free acid, as the starting material.

(c) Purification

The following procedure is used:

(1) Insert the column outlet into the fraction collector drophead, start the eluent flow at 0.5 mL/min and observe the fraction collector to see if it is operating correctly.
(2) Switch on the chart recorder and check that it is operating.

(3) Load approximately 0.2 mL of eluent buffer into a 1 mL disposable syringe fitted with the injection needle, followed by the iodination mixture and another 0.2 mL (approximately) of eluent.
(4) With the sample loop injector in the inject position, slowly load the sample from the syringe into the sample loop.
(5) Count the vial again to check the residual activity, and record the counts.
(6) Mark the chart recorder and turn the injection valve to the load position, watch for the first peak to appear on the chart recorder, then turn the loop back to the inject position and leave the pump running.
(7) Using a Pasteur pipette, rinse the vial with water into the disposal sink and again count the vial to record the solid waste activity.
(8) After 20–30 min when all the product peaks should have been eluted, stop the pump. Transfer the column outlet back to the wastewater outlet and the eluent back to water, continue washing with water for at least 30 min, and open the sample loop so that this is also washed.
(9) Remove the syringe and injection needle, and wash these with water; also wash through the channels of the injection valve.
(10) Wash the column with methanol for 15 min before turning off.

(d) Dispensing and drying

The following procedure is used:

(1) Count each of the collected fractions for 1 s in the holder of the mini-assay, and calculate the percentage of radioactivity in the iodine, T3 and T4 peaks.
(2) For pooling, use the fractions corresponding to T4 but omit one fraction from the beginning and one from the end of the peak. Calculate the total activity.
(3) Pool the selected T4 fractions into diluent buffer and dilute to give a radioactive concentration of 10 μCi/mL.
(4) Count 10 mL of the diluted T4 solution for 10 s in the well of the mini-assay. There should be approximately (25 000 counts)/(10 s $\cdot$ 10 mL) (= 10 μCi/mL at 70% efficiency) but no less than 20 000 counts.

(e) Aliquot

Aliquot into 0.5 or 1.0 mL fractions (5–10 μCi) and freeze dry.

This is a typical protocol, obtained through the courtesy of Dr. R. Edwards, Director, NETRIA, St. Bartholomew's Hospital, London, UK, which

describes the preparation of ^{125}I-thyroxine, including details of the HPLC procedure.

Protocol 13: Antibody coated tubes and wells

The following procedure is used:

— Dispense 300 μL per tube or 200 μL per well of a 1, 10 and 100 μg/mL IgG solution in phosphate buffer of pH7.4. For blanks dispense 300 μL per tube or 200 μL per well of buffer.
— Enclose in a container in a humid atmosphere and leave at 4ºC overnight.
— Aspirate IgG solution from the tubes and wells.
— Dispense 500 μL per tube or 250 μL per well wash buffer, and aspirate again.
— Dispense 500 μL per tube or 250 μL per well 1% BSA solution to block remaining binding sites. Two hours at room temperature is sufficient time for blocking, but for convenience leave the tubes and wells containing 1% BSA overnight at 4ºC.
— Aspirate or decant the 1% BSA solution.
— Add 300 μL per tube or 200 μL per well iodinated rabbit IgG in assay buffer and incubate at room temperature for two hours.
— Aspirate and wash with 1 mL assay buffer.
— Count.

Protocol 14: Antibody coated cellulose

Activation procedure

Five grams of Sigmacell are weighed into a 50 mL conical flask fitted with a ground glass stopper. Following which 0.61 g CDI and 25 mL acetone are added and the mixture left to react for one hour at room temperature with shaking. The activated imidazole-carbamate, cellulose, is recovered by filtration over a glass microfibre filter, washed with three 100 mL aliquots of acetone and allowed to air dry. The cellulose may be used immediately or stored dry at –20ºC.

The procedure is as follows:

— Weigh 200 mg of activated cellulose into a polystyrene tube.
— Add 1 mL of 10 mg/mL IgG solution in barbitone buffer of pH8 and vortex briefly to form a slurry.
— Leave the tube rotating overnight at room temperature.

— Centrifuge at 2500 rev./min for 5 min at room temperature.
— Retain the supernatant for use again and to test for the protein concentration.
— Resuspend the cellulose in 10 mL 0.5M bicarbonate buffer of pH8 and rotate for 20 min.
— Centrifuge at 2500 rev./min for 5 min at room temperature.
— Resuspend the cellulose in 10 mL 0.5M bicarbonate buffer of pH8 and rotate for 20 min.
— Centrifuge at 2500 rev./min for 5 min at room temperature.
— Resuspend the cellulose in 10 mL 0.1M acetate buffer of pH4 and rotate for 60 min.
— Centrifuge at 2500 rev./min for 5 min at room temperature.
— Resuspend the cellulose in 10 mL 0.1M acetate buffer of pH4, sonicate for 30 s and rotate overnight at room temperature.
— Centrifuge at 2500 rev./min for 5 min at room temperature (adding assay buffer to maintain constant volume throughout the procedure).
— Resuspend the cellulose in a 5 mL assay buffer.
— Pipette 5, 10, 20, 50, 100 and 200 μL cellulose in duplicate into assay tubes.
— Add 100 μL labelled rabbit IgG solution and leave at room temperature for 2 hours with occasional shaking to keep the cellulose in suspension.
— Add 1 mL of wash solution, centrifuge at 2500 rev./min for 5 min at room temperature, decant the supernatant and count the cellulose pellets.
— Plot a dilution curve for the solid phase antibodies.

Protocol 15: Antibody coated magnetic particles

The activation procedure is as follows:

— Roll the bottle containing the magnetic particles for 30 min at room temperature at 30 rev./min.
— Pipette or pour out the required amount (20 mL = 1 g = sufficient for IgG isolated from 1 mL serum).
— Sediment the particles on a magnetic block and aspirate the supernatant. Wash the particles three times with 20 mL water, by mixing gently with water, sedimenting and aspirating the supernatant. Wash the particles five times with acetone.
— Adjust the volume to 10 mL with acetone and add 0.12 g CDI.
— Mix gently by rolling for one hour at room temperature.
— Sediment the particles and wash four times with 40 mL of acetone, four times with 40 mL of water and four times with 40 mL bicarbonate buffer of pH8.

Day 1:

— Dispense 0.5 g of magnetic cellulose into a polystyrene tube.
— Add 0.5 mL of 10 mg/mL IgG solution in a barbitone buffer of pH8 and adjust the volume to 10 mL with a bicarbonate buffer of pH8.
— Leave the tube rolling overnight at room temperature.

Day 2:

— Sediment the particles and wash twice with 20 mL bicarbonate buffer.
— Sediment the particles and wash with 20 mL bicarbonate buffer containing 3 mL/L ethanolamine.
— Sediment the particles and resuspend in 20 mL of bicarbonate buffer containing 3 mL/L ethanolamine and roll for 30 min at room temperature.
— Sediment the particles and resuspend in 20 mL acetate buffer of pH4 and roll for 30 min at room temperature.
— Sediment the particles and wash twice with 20 mL assay buffer.

Day 3:

— Pipette 5, 10, 20, 50, 100 and 200 mL magnetic cellulose suspensions in duplicate into assay tubes (adding assay buffer for constant volume).
— Add 100 mL labelled rabbit IgG solution and leave at room temperature for two hours, with occasional shaking to keep the particles in suspension.
— Add 1 mL wash solution; sediment the particles and aspirate the supernatant. Wash the particles with a further 1 mL wash solution and count the magnetic pellets.
— Plot a dilution curve for the solid phase antibodies.

5.13. MOLECULAR METHODS — USE OF RADIONUCLIDES IN MOLECULAR BIOLOGY

5.13.1. Introduction

Cell anatomy can be studied by several distinct methods using microscopy but, although revealing cellular architecture, very little information is provided about cell physiology. The biochemistry and molecular biology perspective is to identify molecules involved in a specific pathway and to determine where and why the pathway occurs. To address these points, it is

necessary to deal with a spectrum of different molecules, ranging from inorganic ions to large macromolecules such as nucleic acids and proteins.

In the 1970s, several technical approaches were described bringing sensitive methodological advantages that drastically altered the amount of data generated in this field of research. To monitor the molecular aspects of cell physiology, radioisotopes are routinely used, for example, in tracing chemical pathways, evaluating the dynamic behaviour of compounds in cytosol and nucleus, and identifying and typing molecules. Radioisotopic methods are specific and sensitive, capable of detecting a particular molecule sample present in small amounts in complex mixtures.

In human health, molecular biology has several applications. The most developed are in the molecular diagnosis of infections, genetic diseases and cancer. Molecular techniques can be applied to the diagnosis of diseases such as human papilloma virus infection and Chagas' disease (infective diseases), Fragile X syndrome (a genetic disease) and the mutated p53 gene (cancer).

Besides improving diagnosis, molecular methods can also be used to address the control of disease through:

— Identification of common transmission sources;
— Assessment of drug resistance;
— Follow-up of treatment efficacy;
— Strain typing to distinguish more pathogenic organisms.

5.13.1.1. Molecular diagnosis

The polymerase chain reaction (PCR) is an in vitro method of nucleic acid amplification by which a particular segment of DNA can be specifically replicated. It involves two oligonucleotide primers that flank the DNA fragment to be amplified, repeated cycles of heat denaturation of the DNA, annealing of the primers to their complementary sequences and extension of the annealed primers with DNA polymerase. The combination of the four bases that constitute the DNA (adenine, thymine, cytosine and guanine) in an approximately 20 base segment, which corresponds to the oligonucleotide primers, is unique enough to be specific to a single microorganism or a mutation. This specificity is combined with the sensitivity of the PCR due to the exponential amplification of the target. Specificity is further enhanced by molecular hybridization using probes, making these approaches ideal tools for diagnostic purposes.

5.13.2. Molecular diagnosis of infective diseases using molecular techniques (PCR coupled with hybridization with radioactive probes)

Molecular approaches allow ready detection (in one or two days) of single pathogenic organisms, an accomplishment provided before by in vitro culturing (requiring weeks) of such pathogens. Furthermore, they allow detection of pathogens, such as the human papilloma virus, that are refractory to in vitro propagation. Antibodies used in a direct search for a given pathogen typically recognize antigens found in multiple copies on the microorganism and thus circumvent the need to replicate the agent. Unfortunately, the cross-reactivity of these antibodies with host antigens and other pathogens has compromised the convenient and broad use of these diagnostic reagents for some pathogens. In addition, some viruses establish latent infections in which active viral replication is substantially attenuated, thereby preventing detection by antigen based methods.

Molecular diagnostic methods can provide non-invasive alternatives. For example, in visceral Leishmaniasis, the parasitological diagnosis can be performed using peripheral blood instead of bone marrow or spleen aspirates. Similarly, in chlamydia infections, urine can be used instead of urethral scrapings.

In Chagas' disease, parasitological diagnosis can be performed by PCR and molecular hybridization using blood. This approach is in contrast to the traditional xenodiagnosis method, where 40 insects starving for 45 days are applied to the patient's arms. In cutaneous Leishmaniasis, the invasiveness is reduced from regular biopsies to needle aspirates.

Examples of pathogens that can be detected using these approaches are listed below. Pathogens with an asterisk represent those for which molecular based methods are the gold standard:

Adenovirus
Bartonella henselae and Bartonella quintana
Borrelia burgdorferi
* Chlamydia pneumoniae
* Chlamydia trachomatis
Cytomegalovirus
Epstein–Barr virus
Helicobacter pylori
Hepatitis B virus
* Hepatitis C virus
Hepatitis G virus
Herpes viruses 6 and 8
Legionella pneumophila
* Leishmania sp.
Microsporidia
Mycobacterium avium
Mycobacterium bovis
Mycobacterium leprae
Mycobacterium tuberculosis
Mycobacterium ulcerans
Mycoplasma pneumoniae
* *Neisseria gonorrhoeae*
Onchocerca volvulus
Parvovirus B19

Herpes simplex types 1 and 2
Histoplasma capsulatum
* HIV
HTLV I/II
* human papilloma virus
Plasmodium sp.
Pneumocystis carinii
Toxoplasma gondii
Trypanosoma cruzi
Varicella–Zoster virus.

In this section, two distinct models are chosen for more detailed consideration: human papilloma virus (HPV) infection and Chagas' disease.

5.13.2.1. Human papilloma virus

HPV is the most common genital virus, with transmission mediated by direct contact. More than 70 genotypes based on DNA sequence have been described and clustered into subtypes. The virus site of latency is the epithelium, where most of the clinical presentations are encountered. The distinct virus genetic groups present different cellular tropisms and therefore present distinct clinical features (skin warts, benign head and neck tumours, genital warts and cervical carcinoma). For example, HPV 1 produces plantar warts, HPV 2 and 4 cause common warts on the hands, HPV 6 and 11 are involved with genital warts and HPV 16 and 18 are the causative agent of genital warts and cervical carcinoma.

The possibility of detecting HPV in a clinical sample depends on the sensitivity of the assay. No culturing system is available. Histopathological or cytological analyses are indirect methods detecting morphological alterations produced by HPV. The presence of 'HPV changes' or koilocytes is neither sensitive nor specific to the presence of the virus and cannot differentiate between high and low risk subtypes. Identifying the distinct HPV subtypes is possible due to genetic differences that are present in the different viruses. Molecular methods have been developed in order to detect and type the virus in clinical specimens. This is achieved by molecular hybridization with radiolabelled probes in the Southern Blot procedures.

Ninety-five per cent of viruses that belong in the low risk group are typed as HPV 6, 11, 42, 43 and 44 and cause anogenital warts. The high risk group is associated with the development of cervical intraepithelial neoplasia (CIN). Ninety-nine per cent of the viruses of this group can be typed using probes to HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. HPV DNA is detected in 95–100% of cervical tumour samples and is considered as the major risk factor for cervical cancer. Fifteen–28% of HPV positive women will develop neoplasia within 2 years in contrast to 1–3% of HPV negative women. Therefore, the relative risk of developing CIN is 11 times greater in HPV positive women.

HPV interaction with host tumour suppressor proteins, such as p53, is the oncogenic basis for the development of cancer. Polymorphism in the host p53 gene has prognostic significance, defining groups that are more susceptible to cancer development and therefore requiring closer follow-up, leading to early detection and treatment of cervical cancer.

Considering of the high prevalence of cervical cancer and its association with HPV, a molecular approach that detects and types the virus, determines the viral load and analyses the oncoprotein involvement is helpful in identifying the risk group of women who need close follow-up. These molecular methods are complementary to conventional investigations such as cervical smears and histopathological analyses (see the chronology of investigation in Fig. 5.4).

5.13.2.2. Trypanosoma cruzi and Chagas' disease

Chagas' disease is caused by the protozoan *T. cruzi*, which is mainly transmitted by an insect vector, the triatomine bug. The infective stages of the parasite are present in insect faeces and can penetrate through the epithelium into host cells. During the acute phase, which can last up to 60 days, a large number of circulating parasites are observed in the bloodstream. Patients may develop different clinical presentations, varying from oligosymptomatic to the development of myocarditis and meningoencephalitis. Following the acute phase, a chronic stage develops in which the level of circulating parasites is far below the threshold for microscopic detection. Patients are symptomless for several years with the only marker for the *T. cruzi* infection being the high titre of anti-*T. cruzi* antibodies. Approximately 10–20% of infected individuals will develop a symptomatic chronic disease hallmarked by cardiovascular–gastrointestinal involvement.

In addition to transmission through the insect vector, *T. cruzi* may also be transmitted congenitally or by blood transfusion from asymptomatic carriers. The infected newborn may show a clinical picture characterized by prematurity, hepatomegaly, splenomegaly, jaundice, anaemia and alterations in the CNS, resulting in a high mortality. Chagas' disease due to blood transfusion is a problem in Latin America since in some endemic areas a significant percentage of potential blood donors (2–63%) may be infected. In the last few years, reactivation of Chagas' disease has been reported, mainly associated with immunosuppression in hosts with HIV infection and individuals undergoing organ transplantation.

Etiological therapy for Chagas' disease is indicated in either acute or recent chronic infections of less than ten years that seem to respond to treatment. In practice, it is recommended that all children with positive

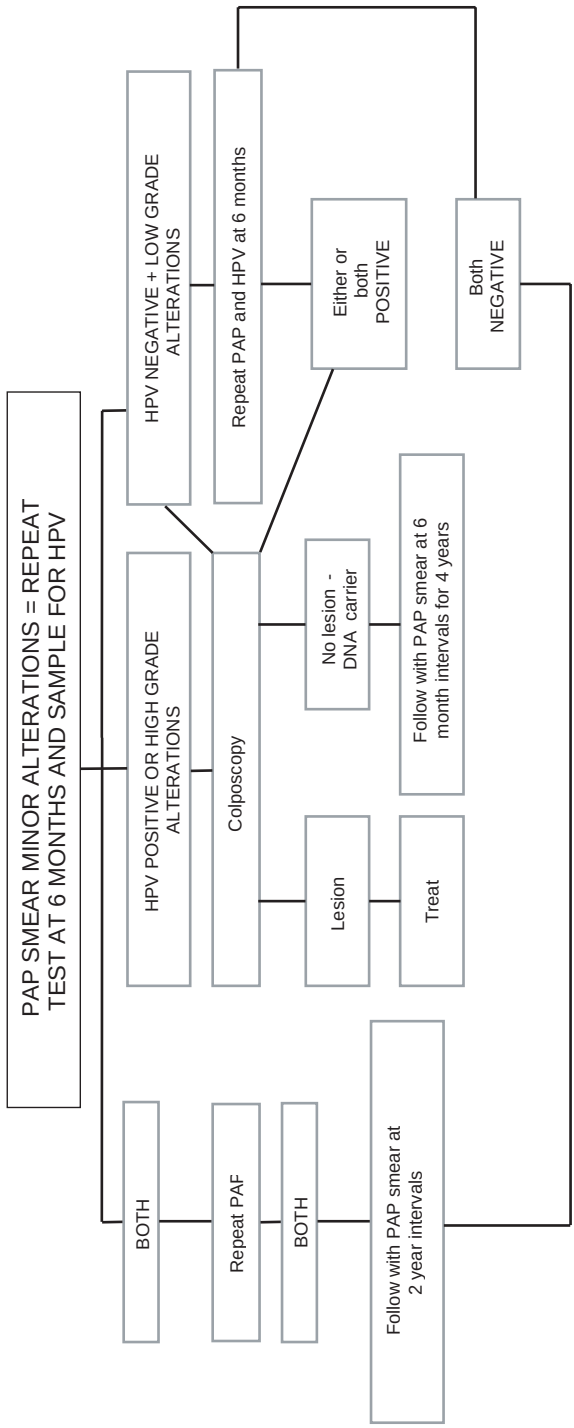

FIG. 5.4. Flow chart showing the chronology of an investigation for HPV.

serological reactions be submitted for specific therapy. Furthermore, patients with the chronic indeterminate form of the disease, the slight cardiac–digestive form, should also be treated.

The diagnosis of Chagas' disease can be made by different means depending on the phase of the disease. In the initial phase of *T. cruzi* infection, large numbers of parasites circulate in the blood, and diagnosis is primarily by direct microscopy. By contrast, in the chronic phase, circulating levels of parasites are low and therefore diagnosis depends on detection of the host serological response or on in vitro amplification of the parasites, such as xenodiagnosis or haemoculture. In the former, 40 uninfected triatomine bugs are allowed to feed on the patient and a month later the intestinal contents of the insects are examined for the presence of *T. cruzi*. However, both parasitological methods lack sensitivity, and positive findings are achieved in less than 50% of seropositive chronically ill patients. In addition, these methods may select parasite subpopulations, distorting the typing of the involved parasite and epidemiological data.

Although conventional serological assays can offer fast and fairly reliable diagnosis, they lack specificity, giving rise to false positive results that need confirmation by a parasitological test. Moreover, in congenital infection, serology is precluded by the circulation of maternal IgG antibodies during the first six months of life. The early diagnosis of congenital transmission is essential because treatment is more efficient when given closer to the time of delivery. Thus, a highly sensitive parasitological assay is needed for the diagnosis of an infected newborn of a Chagasic mother or for monitoring the presence of the parasites in the chronic phase of the disease.

Oligonucleotides derived from the conserved region of mitochondrial DNA have been used in PCR based assays to detect this parasite in human blood samples. The amplified products are detected by gel electrophoresis and hybridized with a radiolabelled molecular probe. The PCR assay can attain a better sensitivity and specificity than combined serology and clinical diagnosis. Furthermore, PCR positivity is much better than any other direct parasitological method, such as xenodiagnosis and haemoculture. Details of the appropriate use of the molecular diagnosis of Chagas' disease are given in Fig. 5.5.

5.13.3. Molecular diagnosis of genetic diseases using radioactive labelling

It is known that several human diseases are caused by defective genes, but until very recently very few had been identified. The identification of such genes for a number of important diseases, such as cystic fibrosis, Huntington's disease, Fragile X syndrome and haematological disorders, has led to the

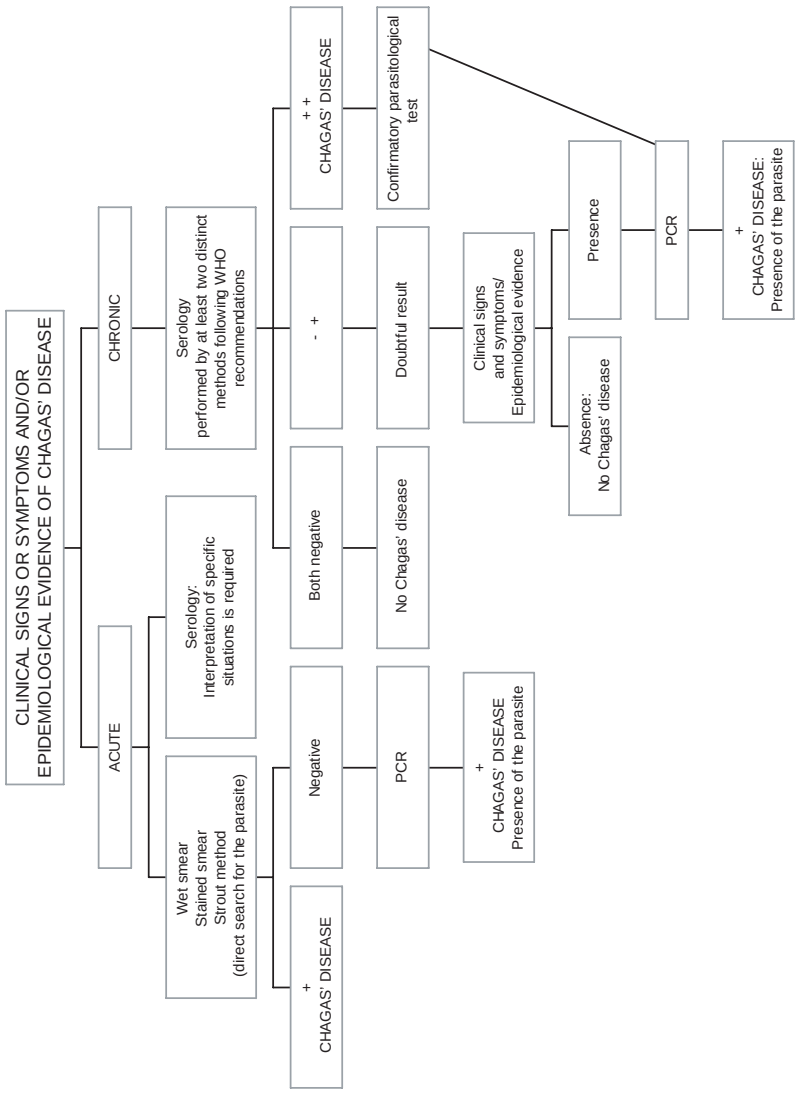

FIG. 5.5. Flow chart showing chronology of investigation for molecular diagnosis of Chagas' disease.

application of molecular methods to the diagnosis of such diseases. Hundreds of mutations can cause the same genetic disease. For example in β-thalassaemia approximately 200 mutations are implicated. However, for any particular ethnic group, about ten mutations will cover 90% of the genotype spectrum, simplifying screening strategies in genetic programmes. PCR technology has further simplified prenatal diagnosis and carrier testing.

Molecular genetics laboratories now offer tests for a large number of diseases, such as:

α- and β-Thalassaemia
Alport's syndrome
Alzheimer's disease
Androgen's insensitivity
Angelman's syndrome
Antitrypsin deficiency
Charcot–Marie–Tooth's disease
Choroideraemia
Cystic fibrosis
Fragile X syndrome
Haemophilia A
Hereditary neuropathies
Huntington's disease
Myotonic dystrophy
Niemann–Pick's disease
Ornithine transcarbamylase deficiency
Prader–Willi's disease
Retinitis pigmentosa
Sickle cell anaemia
Spinal muscular atrophy
Tay–Sach's disease
Von Hippel–Lindau's disease
Von Willebrand's disease
X-linked muscular dystrophy.

In this section, a model of a genetic disease, Fragile X syndrome, in which the molecular diagnosis has had an impact in prevention, is given in more detail.

5.13.3.1. Fragile X syndrome

Fragile X syndrome (also termed FRAXA) is the most common form of inherited mental retardation in humans, and is associated with a wide range of behavioural and physical features, affecting both males and females, with a prevalence of 1 in 4000 males and 1 in 6000 females. The cloning of the FMR1 (Fragile X mental retardation 1) gene and the identification of the mutational mechanism underlying the Fragile X syndrome, a large expansion of a trinucleotide repeat $(CGG)_n$ in the first exon of the FMR1 gene, has allowed the development of accurate molecular diagnostic tests, which have replaced cytogenetic marker analysis (of a fragile site at Xq 27.3) as the procedure of choice and dramatically improved diagnosis of the disease.

The standard diagnostic procedure used is to measure the CGG repeat amplification in patients. More than 99% of Fragile X syndrome cases identified have resulted from CGG expansions, with only a few cases derived

from FMR1 point mutation or deletions. Since the great majority of Fragile X patients share a mutation at precisely the same site in the gene, as opposed to a range of mutations scattered along the length of a gene, the genetic diagnosis (or exclusion) of Fragile X syndrome is remarkably reliable.

Southern Blot analysis is the procedure of choice for medical diagnosis of Fragile X syndrome. The use of this methodology is essential to characterize the Fragile X mutation in males and females, to distinguish premutation from full mutation and to detect methylation.

The probes frequently used to identify CGG amplification are StB12.3, Pfxa3 and Ox1.9. For Southern Blot analysis, different restriction enzymes may be used, such as *Pst*I and *Eco*RI. A double digest, including a methylation sensitive restriction enzyme (*Eag*I, *Sac*II and *Bss*HII), enables detection of the methylation of the CGG repeats and associated CpG island. The double digest may also be used in distinguishing between an unmethylated large premutation and a small methylated full mutation. The *Eco*RI/*Eag*I protocol using the Stb12.3 probe is a standard diagnostic procedure. The digestion of genomic DNA with *Eco*RI (insensitive) and *Eag*I (sensitive), after hybridization with the StB12.3 probe radiolabelled with [α-P 32]dNTP, generates a 2.8 kb *Eco*RI–*Eag*I fragment from a normal unmethylated allele (representing an inactive X chromosome). Southern Blot methods allow the molecular classification of alleles by estimation of the approximate size of the Fragile X expansion. Premutation alleles are detected as 5.3–5.7 kb *Eco*RI–*Eag*I fragments, whereas full mutation alleles, which are consistently methylated and thus not cleaved by *Eag*I, are detected as *Eco*RI fragments larger than 5.8 kb in size. The ability to discriminate between a premutation and a full mutation in the FMR1 gene for the use of this methodology provides a much improved prediction of the risk of mental retardation.

Because of the prevalence, underdiagnosis and high risk of recurrence of Fragile X syndrome, direct DNA analysis of the FMR1 gene is extremely important in providing correct diagnosis of the disease and may be used in the differential diagnosis of individuals with mental retardation of unknown aetiology and in the genetic counselling of families with Fragile X syndrome.

5.13.4. Molecular diagnosis of cancer

Cancer is due to genetic alterations that affect cell growth and differentiation. The molecular approach to cancer aims at three distinct goals:

(1) Identification of mutations that predispose an individual to cancer development (BRCA1 in breast cancer);
(2) Detection of minimal residual disease (following leukaemia treatment);

(3) Detection of mutations that are diagnostic for neoplasms (leukaemia), which define prognosis (p53) and which are resistance indicators (p53).

The most common molecular approach can be used for the following cancers:

— Bladder cancer;
— Breast cancer;
— Colon cancer;
— Leukaemia;
— Liver cancer;
— Lymphoma;
— Melanoma;
— Multiple endocrine neoplasia;
— Neuroblastoma;
— Ovarian cancer;
— Prostate cancer;
— Thyroid cancer.

5.13.4.1. The p53 genetic marker

The most common genetic tumour marker is p53. Mutations in this gene and loss of the normal allele is the most common alteration that leads to the progression of cancer. These mutations can be determined by PCR and sequencing as well as by other techniques, such as single strand conformation polymorphism (SSCP), which are directed towards the detection of new mutations. The p53 gene is involved in distinct neoplasms, and alterations in this gene are considered as diagnostic mutations, prognostic markers, a susceptibility indicator or a resistance indicator.

Neoplasms in which p53 is involved include:

— Bladder tumours;
— Breast tumours;
— Colorectal tumours;
— Head and neck tumours;
— Leukaemia;
— Liver tumours;
— Lymphoma;
— Ovarian tumours;
— Prostate tumours.

The early appearance of breast cancer (at a mean age of 37 years) is highly associated with p53 mutations. Fifty per cent of Li–Fraumeni syndrome cases, a predominantly inherited syndrome associated with multiple primary neoplasms of children and young adults, have a germ line p53 mutation and 49% of families with germ line p53 mutations meet the criteria for Li–Fraumeni syndrome: occurrence of sarcoma before 45 years of age and at least one first degree relative with a tumour before 45 years of age or a sarcoma at any age.

Inherited germ line p53 mutations are associated with breast cancer (24%), bone sarcomas (13%), brain tumours (12%) and soft tissue sarcomas (12%). Adrenocortical carcinoma is not common (4%) but in children is almost always associated with a germ line p53 mutation.

Another interesting feature is found in some geographical areas where an overlapping of hepatitis B infection and exposure to the mould toxin (aflatoxin B) leads to mutation in the p53 gene. This can be reflected in the future appearance of an aggressive hepatocellular carcinoma.

The p53 gene can be considered a prognostic marker for several tumours. In colorectal carcinoma p53 mutations are present in 75% of cases. In lymph node negative breast cancer the 8 year survival rate in p53 negative women is 82%, whereas in p53 positive cases it is 66%. Furthermore, in lymph node positive cases, p53 positive cases are associated with a 20% 8 year survival rate and p53 negative cases present a 56% survival, leading to the conclusion that the presence of mutations in the gene corresponds to a poor overall survival. In prostate cancer, p53 is considered to be a marker of tumour progression. In lymphoma, the survival is lower in patients bearing mutations in the p53 gene than in those patients with wild type p53. Considering colorectal carcinoma, the 5 year survival rate in p53 positive patients is 18% lower than that of p53 negative patients.

Aggressive myeloid chronic leukaemia presents mutations in p53 in 29–50% of cases. These values contrast to those found in typical cases (6–9%). Furthermore, the survival time in p53 positive patients with that kind of leukaemia is 12–18 months, while in p53 negative patients the survival period extends to 60–117 months.

Resistance to a specific therapeutic scheme can also be affected by mutations in the p53 gene. In breast cancer, p53 mutations increase resistance of tumours to ionizing radiation. In B cell chronic leukaemia, the mutations in the gene are translated as a resistance to chemotherapy. The same phenomenon is observed in ovarian cancer, where wild type p53 tumours are more sensitive to chemotherapy.

5.13.5. Basic protocols in molecular biology

5.13.5.1. Isolation of DNA

DNA is usually isolated by digestion of cells with proteinase K in the presence of EDTA and a detergent such as SDS, followed by extraction with phenol. This method yields DNA whose size (100–150 kb) is adequate for Southern Blot analysis.

DNA from biopsies (tissue) should be immediately processed or placed at –20°C. Commercial kits for purifying genomic DNA from fresh tissue biopsies or from archival material are available. Special protocols for paraffin embedded tissue are provided and should be strictly followed. Removal of the paraffin is recommended before extraction of the DNA.

If it is necessary to extract DNA from blood or buffy coat, the use of commercial kits is also recommended. For this specific procedure, the vast majority of available kits require the use of 500 μL of whole blood/EDTA or buffy coat. The results of manipulations of DNA extracted with commercial kits are usually better than those with home-made traditional protocols.

In field work where biopsies are taken, one of the simplest and perhaps best methods of preserving the DNA is to place the sample in 50% ethanol. Although DNA can be extracted from formalin fixed tissues, the least degraded DNA and the highest yield of DNA can be isolated from ethanol fixed samples. The fixed cells are suitable for DNA extraction for many days when left at room temperature and for more than 6 years at 4ºC. Thus, this protocol is ideal for sample preservation by field molecular biologists. In animal blood samples, citrated blood can be used for DNA analysis after 1 or 2 days at room temperature, perhaps longer. A good way to preserve blood samples for DNA analysis is to simply make air-dried blood smears on slides or use filter paper.

5.13.6. Polymerase chain reaction (PCR) technology

5.13.6.1. Optimization of the PCR method

The primers used in the PCR hybridize to opposite strands of the target sequence and are oriented so that DNA synthesis by the polymerase takes place across the region between them. Since the extension products are also complementary to and capable of binding primers, successive cycles of amplification essentially double the amount of the target DNA synthesized in the previous cycle. The result is an exponential accumulation of the specific target fragment by a factor of approximately 2^n, where n is the number of cycles of amplifications performed.

The PCR is so sensitive that a single DNA molecule can be amplified, and single copy genes are routinely extracted out of complex mixtures of genomic sequences and visualized as distinct bands on agarose gels. The use of thermostable DNA polymerases and automation of the method invented by K. Mullis in 1987 have led to the development of diverse PCR applications. Each new PCR is likely to require optimization, especially when used for routine diagnostic or analytical procedures in which optimal performance is necessary. Problems that can be encountered are:

— No detectable product or a low yield of the desired product;
— The presence of non-specific background bands due to mispriming or misextension of the primers;
— The formation of 'primer-dimers', which compete for amplification with the desired product;
— Mutations or heterogeneity due to misincorporation.

5.13.6.2. Standard PCR amplification protocol

Standard conditions will amplify most target sequences and are presented to provide starting conditions for designing new PCR applications.

First of all a 50 μL reaction is set up in a microfuge tube (adequate for the available thermocycler). If the PCR machine is not supplied with a hot bonnet, a mineral oil overlay will be necessary to prevent evaporation:

(a) 20 pmol of each primer.
(b) For the buffer 20mM Tris-HCl (pH8.3), 1.5mM $MgCl_2$, 25mM KCl, 0.05% Tween 20 and 100 μg/mL of BSA is used.
(c) To 200μM each dNTP.
(d) Two units of Taq DNA polymerase.
(e) A DNA template (10^5–10^6 target molecules).
(f) Perform 25–35 cycles of PCR using the following temperature profile:
    — Denaturation: 96ºC for 15–30 s (a longer initial time of 4 min is usually desirable).
    — Primer annealing: 55ºC for 30 s.
    — Primer extension: 72ºC for 30 s to 1 min.

Cycling should conclude with a final extension at 72ºC for 5 min.

(a) Enzyme concentration

The recommended concentration range for *Taq* DNA polymerase (PerkinElmer Cetus) is between 1 and 2.5 units per 100 μL reaction when the other parameters are optimum. However, enzyme requirements may vary with respect to individual target templates or primers. When optimizing a PCR, it is recommended to test enzyme concentrations ranging from 0.5 to 5 units per 100 μL and to assay the results by gel electrophoresis. If the enzyme concentration is too high, spurious non-specific background products may accumulate, and if it is too low, a low yield of products will be in evidence. As soon as the enzyme is received it should be aliquoted into 10 μL samples and stored at –20°C in Area 1 (Section 3.8).

(b) Deoxynucleotide triphosphates

Stock dNTP solutions should be 10mM, aliquoted and stored at –20ºC in Area 1 (Section 3.8). Working stock solutions each containing 2mM dNTP are recommended. Deoxynucleotide concentrations between 50 and 200μM each result in an optimal balance of the yield, specificity and fidelity. The four dNTPs should be used at equivalent concentrations to minimize misincorporation errors. Low dNTP concentrations minimize mispriming at non-target sites and reduce the likelihood of extending misincorporated nucleotides. The lowest dNTP concentration appropriate for the length and composition of the target sequence should be decided upon.

(c) Magnesium concentration

It is beneficial to optimize the magnesium ion concentration. The magnesium concentration may affect all of the following: primer annealing, strand dissociation temperatures of both template and PCR product, formation of primer-dimer artefacts, and enzyme activity and fidelity. *Taq* DNA polymerase requires in addition free magnesium bound by template DNA, primers and dNTPs. Accordingly, PCRs should contain 1.0–5mM $MgCl_2$.

(d) Other reaction components

A recommended buffer for PCR is 10–50mM Tris-HCl (between pH8.3 and 8.8). Up to 50mM of KCl can be included in the reaction mixture to facilitate primer annealing. However, values above 50mM inhibit *Taq* DNA polymerase activity. Bovine serum albumin (100 μg/mL) and non-ionic

detergents such as Tween 20 are included to help stabilize the enzyme, although many protocols work well without added protein.

(e) Primer annealing

The temperature and time required for primer annealing depend upon the base composition, length and concentration of the amplification primers. An applicable annealing temperature is 5ºC below the true melting temperature (T_m) of the amplification primers. Because *Taq* DNA polymerase is active over a broad range of temperatures, primer extension will occur at low temperatures, including the annealing step. The range of enzyme activity varies by two orders of magnitude between 20 and 85ºC. Annealing temperatures in the range of 55–72ºC generally yield the best results. At typical primer concentrations (0.2μM), annealing will require only a few seconds. Increasing the annealing temperature enhances discrimination against incorrectly annealed primers and reduces mis-extension of incorrect nucleotides at the 3′ end of primers.

(f) Primer extension

Extension time depends upon the length and concentration of the target sequence and upon temperature. Primer extensions are traditionally performed at 72°C because this temperature is near optimal for extending primers. Estimates for the rate of nucleotide incorporation at 72ºC vary from 35 to 100 nucleotides per second, depending upon the buffer, pH, salt concentration and nature of the DNA template. An extension time of 1 min at 72°C is considered sufficient for products up to 2 kb in length.

(g) Denaturation time and temperature

The most likely cause for failure of a PCR is uncompleted denaturation of the target template and/or the PCR product. Typical denaturation conditions are 95°C for 30 s, but higher temperatures may be appropriate, especially for G+C-rich genomes. It only takes a few seconds to denature DNA at its strand separation temperature; however, there may be a lag time involved in reaching the desired temperature inside the reaction tube. Denaturation steps that are too high and/or too long lead to unnecessary loss of enzyme activity. The half-life of *Taq* DNA polymerase activity is more than 2 hours, 40 min and 5 min at 92.5, 95 and 97.5ºC, respectively.

(h) Cycle number

The optimum number of cycles will depend mainly upon the starting concentration of target DNA when other parameters are optimized. A common mistake is to execute too many cycles, which can increase the amount and complexity of non-specific background products. Of course, too few cycles give a low product yield. Thirty cycles seems to be a good choice for a PCR protocol.

(i) Primers

Primer concentrations between 0.1 and 0.3μM are generally optimal. Higher primer concentrations may promote mispriming and accumulation of non-specific product and may increase the probability of generating a template independent artefact termed a primer-dimer. Non-specific products and primer-dimer artefacts are themselves substrates for PCRs and compete with the desired product for enzymes, dNTPs and primers, resulting in a lower yield of the desired product.

(j) The Southern Blot technique

This technique refers to the transfer of DNA to fixed supports, such as membranes. It was first developed by E.M. Southern and thus bears his name. A specimen of DNA is digested with restriction enzymes, resulting in DNA fragments that are then submitted to agarose gel electrophoresis. The double stranded DNA present in the agarose gel is denatured and the electrophoretically separated fragments immobilized on an inert support (a nylon membrane) in such a way that self-annealing is prevented and yet bound sequences are available for hybridization with an added nucleic acid probe. An adaptation of the method can be used for amplified products from PCR where the simple transfer of the PCR products from an agarose gel to a nylon membrane is also named a Southern Blot experiment.

The transfer is mediated by soaking gel in NaOH 0.5N/NaCl 1.5M for 30 min, then in Tris-HCl 0.5M pH8.0/NaCl 1.5M for 30 min and transferring by capillarity overnight in 20X SSC (a sodium chloride–sodium citrate buffer).

(k) Dot blot

Dot blot is a rapid technique used to detect the presence of a specific DNA in a specimen. Dots or spots of the DNA containing sample are placed onto a nylon membrane and fixed. Dot blot hybridization is ideally suited to

the analysis of multiple samples. The technique has the added advantage that it is easy to prepare replicate filters, allowing many filter-bound sequences to be analysed at the same time, for example with different probes or under different hybridization and washing conditions. Multiple DNA samples are spotted next to each other on a single filter in dots of uniform diameter. For quantitative analysis, known amounts of DNA are applied. To evaluate the extent of hybridization of the probe, a standard consisting of a dilution series of DNA dots is applied in an identical way to the same filter. The procedure binds samples quickly so that many samples can be handled at once. As little as 1–3 pg of a hybridizing DNA sequence can be detected. Dot blots do not distinguish between the number and size of the molecules hybridizing, so the hybridization 'signal' is the sum of all sequences hybridizing to the probe under the conditions used. Commercial apparatus has been developed for binding multiple samples of DNA to filters.

The DNAs (100 μL = 10 μL of DNA + 90 μL of double distilled water) are denatured by heating for 5 min at 95°C and put on ice. Ten microliters of NaOH 4N are added and the mixture applied to a dot blot apparatus following the manufacturer's instructions.

(l) Binding of nucleic acid to filters

There are several types of filter currently in use for the immobilization of DNA and ribonucleic acid (RNA), for example nitrocellulose, nylon and chemically activated papers. The material of choice depends on the purpose of the experiment. Nylon membranes, due to their higher resistance, are now the most commonly used type for Southern and dot blots. For DNA dot blots, filters with a pore size of 0.45 μm are used for large nucleic acid molecules and 0.22 μm for molecules of less than 500 nucleotides.

Ideally the binding of nucleic acids to membranes should be covalent mediated. This will prevent the gradual leaching-off of the nucleic acids from the surface when filters are hybridized for long periods, particularly at high temperature. The most commonly used technique for binding of nucleic acid to filters is that with UV light. The same goal can be achieved by baking the membrane at 80°C for 2 hours.

5.13.6.3. Hybridization strategy

Nucleic acid hybridization, the formation of a duplex between two complementary nucleotide sequences, is the basis for a range of techniques now in widespread use in modern biology. Optimal use of this molecular method requires a clear understanding of its principles so that the basic

techniques can be modified to suit the particular purposes and conditions of a desired experiment.

The process which underlies all of the methods based on molecular hybridization relies on the complementarity of the two sequences involved in duplex formation: the target DNA and the probe used. The specificity of a hybridization assay depends on the stringency that usually is mediated by the temperature and sodium concentration (SSC) of the hybridization and washing solutions. The higher the temperature the more stringent is the hybridization condition. On the other hand, as the salt concentration is decreased, the stringency increases.

There are various types of hybridization commonly in use, such as filter hybridization and in situ hybridization. The membrane containing the denatured DNA is hybridized with an added nucleic acid probe. To facilitate analysis, the probe is labelled, often with ^{32}P. Hybridization is followed by extensive washing of the filter to remove unreacted probe. Detection of hybrids is usually by autoradiography or a phosphoimager analyser. The procedure is widely applicable, being used for Southern Blot and dot blot hybridization for example.

(a) Types of nucleic acid probes for hybridization

In theory, any nucleic acid can be used as a probe provided that it can be labelled with a marker that allows identification of the hybrids formed. In practice, double and single stranded DNAs are mainly used as probes. The choice of probe depends on three factors: the hybridization strategy, the availability or source of material for use as a probe and the degree to which it can be labelled. Probes can be made by cloning, PCR or using a DNA synthesizer.

(b) Labelling nucleic acids for use as probes

(i) Choice of radionuclide

Traditionally, filter hybridizations have been carried out with radioactively labelled probes, and several radionuclides can be used in molecular biology experiments (Table 5.22). However, for nucleic acid hybridization, ^{32}P is the isotope of choice since its high energy results in short scintillation counting times and short autoradiographic exposures. Each of the four deoxyribonucleotides is available in an α-^{32}P labelled form, of high or low specific radioactivity, suitable for incorporation into DNA using one of the polymerase reactions, replacing a proportion of the nucleotides in a nucleic acid with ^{32}P derivatives. In addition, γ-[^{32}P]–ATP is also available for 5′ end

TABLE 5.22. ISOTOPE, AVAILABLE REAGENT AND ASSAY IN WHICH RADIONUCLIDES CAN BE USED

Isotope	Reagent	Assay
^{32}P	α-^{32}P–dNTP	Double strand DNA labelling
	γ-^{32}P–dNTP	5′ end-labelling of single strand DNA (oligonucleotides — probes and primers)
^{33}P	α-^{33}P–dNTP	Double strand DNA labelling

labelling DNA using polynucleotide kinase, which adds ^{32}P to the end of the molecule. Phosphorus-32 has the advantage over other radionuclides in that high specific activities can be readily attained. Much of the technology of filter hybridization has been developed with it. However, precautions must be taken when handling ^{32}P because of the radiation emitted (Chapter 2). Detection by autoradiography, while sensitive, may take a long time if there are few counts in the hybrids. Alternatively, phosphoimagers may be used. Furthermore, since ^{32}P has a half-life of 14.3 days, experiments should be completed within one half-life.

(ii) Radionuclide labelling methods

There are three radionuclide labelling methods:

(1) *End labelling with T4 polynucleotide kinase.* Polynucleotide kinase is mostly used to label probes for hybridizations and primers for PCR reactions, transferring the γ phosphate of ATP to a free 5´ OH group in the DNA. Specific activities of 5×10^5 counts/(min·pmol) can be achieved. This method is suitable for probes and primers that are single stranded and short (20–30 bases).

(2) *Synthesis of labelled DNA probes using random oligonucleotide primers.* DNA probes can be radiolabelled to very high specific activities (5×10^8–4×10^9 counts/(min·μg)) synthesized in this way, by using α-^{32}P–dNTP and three unlabelled dNTPs as precursors. Commercial kits of random primers are available by synthesizing on an automated DNA synthesizer, a population of octamers that contains all four bases in every position.

The most widely used DNA polymerase for this purpose is the Klenow fragment of *E. coli* DNA polymerase. This method is used for labelling PCR products, cloned DNA molecules that are double stranded and mostly for products larger than 100–1000 bp.

(3) *Hybridization and washing procedure.* The membrane containing the denatured DNAs should be prehybridized in a solution for at least two hours. The most commonly used solution is composed of SSC, SDS and bovine lacto transfer technique optimizer (BLOTTO). After prehybridization the membrane is hybridized with radiolabelled single stranded DNA fragments with sequences complementary to those being sought. The probes should be boiled prior to addition to the hybridization solution in order to be single stranded. The hybridizations are usually carried out at 37°C for oligoprobes and at 60°C for regular cloned molecules or PCR products. The resulting double stranded DNA bearing the radiolabel is then, if present, detected by autoradiography.

The stringency of the assay is mediated mainly by the concentration of the SSC in the washing procedure and also the temperature used.

(c) In situ hybridization

The previous sections describe techniques in which DNA removed from clinical samples is separated and placed on nylon membranes and then probed for target sequences. In some cases, it is preferable to apply these labelled probes directly to cells and tissues to localize the source of the signal. This technique is known as in situ hybridization. Using this method the cells are previously digested with proteinase K while DNA within the native tissues is denatured by heating, followed by application of a labelled probe that binds to the complementary sequence of interest. Following several washing steps, a detection method is used to localize the signal indicating areas in which the probe has bound to the tissue. When radioactive probes are used, the tissue sections on microscope slides are dipped in a silver emulsion similar to an X ray film. These slides are kept in the dark for a period of time (from days to weeks) after which the emulsion is developed in a fashion similar to developing a film.

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Chapter 6

RADIONUCLIDE THERAPY

6.1. SETTING UP A UNIT

6.1.1. Introduction

The therapeutic use of radionuclides may be a potential radiation risk for both family members and individuals close to the patient, as well as health workers and the environment. Radionuclides must be used in strict accordance with safety measures and any special instructions, and all precautions must be taken to avoid unnecessary exposure to radiation. This chapter summarizes the steps to be taken before commencing therapy procedures.

6.1.2. Licensing

The administration of therapeutic doses of radionuclides must be under the responsibility of a physician who is licensed under national regulations to administer radioactive materials to humans. Radioactive material for diagnosis or therapy should only be used and stored at medical institutions which have purpose designed facilities. Technical staff, physicists and nurses may also be subject to licensing.

Licensing requirements vary from country to country and may even include minimum design and construction requirements for the facility. Local requirements must be established at the start of a project.

6.1.3. Facility design and construction

The general principles for the design of nuclear medicine units are discussed in Chapter 3, and further information regarding therapy units is provided in Section 6.2.

When designing therapy units, it is important to bear in mind the following:

(a) Patients must be housed in a separate room, with dedicated bathroom and toilet.
(b) Access to the treatment room must be controllable.
(c) Any required shielding must be designed for the proposed floor plan in the eventuality of pregnant patients in adjacent rooms.

It is both easier and less costly to design a unit correctly from the start than to modify it later. Close cooperation between the nuclear medicine staff and architects and builders is vital. If an existing space is to be modified, it may be necessary to determine experimentally the adequacy of walls and floors as radiation shields.

If any building work is to be performed, a regular inspection of work in progress is advisable to ensure adherence to agreed plans and specifications. In particular, it should be noted that:

— Brick walls often have inadequate mortar joints, which can be a shielding problem.
— Flooring should be free of open joints and sealed to the walls, a fact often forgotten by builders.

6.1.4. Responsibilities

The physician administering the therapeutic radionuclide dose is ultimately responsible for taking every precaution to avoid unnecessary radiation to staff, other patients, visitors and the general public.

Before commencing therapy, agreement should be reached on medical and radiation safety protocols. This chapter is intended to provide assistance in determining these protocols.

6.1.5. Records

A record keeping system must be in place before treatment commences. In addition to normal medical records, a logbook should be kept, listing the patient's name, the radiopharmaceutical and radioactive quantities administered, and the administration date.

6.1.6. Training

Radionuclide therapy may involve staff outside the nuclear medicine department, especially nurses and medical staff. A little effort devoted to familiarization and training in the medical and safety aspects of radionuclide therapy can avoid potentially serious problems later.

6.2. RADIONUCLIDE THERAPY — SAFETY PRINCIPLES

6.2.1. General principles

Radionuclide therapy presents relatively few hazards to staff and patients, but there are a number of common principles of radiation safety that have to be observed. This section will consider the requirements for patient accommodation (design requirements including shielding), as well as radiation safety procedures necessary for safe practice.

For safety purposes, each therapy can be divided into different stages, with specific safety issues that may need to be considered (Table 6.1).

All of the above may not be necessary for every type of therapy. The specific radiation safety issue for each of the common therapies is discussed later in this section.

6.2.2. Discharge limits

Patients may be discharged only when the remaining activity is less than that prescribed by the local regulatory authority. This can be estimated using a simple ratio of dose rates at a standard distance referenced to the dose rate immediately following dose administration, or by measurement of a dose rate alone. In the absence of local regulations, the limits in Table 6.2 (NRC Guide 8.39, see Bibliography to Section 6.2) can be used.

TABLE 6.1. SAFETY ISSUES

Stage	Typical issues
Pre-therapy	Possible pregnancy, including advice to patient by physician, and pregnancy testing
Day of therapy	Physician's guidelines, administration protocol, advice to nursing staff and preparation of patient room (if an inpatient)
During therapy	Nursing care, control of radioactive waste including urine, faeces, syringes, swabs and other items that may be contaminated, control of visitors, and monitoring of both patient and the local environment
Discharge	Discharge limits and information given to the patient
Post-discharge	Advice on future pregnancies

TABLE 6.2. RADIOACTIVE PATIENT DISCHARGE LIMITS

Radionuclide	Activity remaining (GBq (mCi))	Dose rate at 1 m (mGy/hour) (mrad/hour)
I-131	1.2 (33)	0.07 (7)
P-32	No practical limit	Not applicable
Re-186	28 (770)	0.15 (15)
Re-188	29 (790)	0.2 (20)
Sm-153	26 (700)	0.3 (30)
Sr-89	No practical limit	Not applicable
Y-90	No practical limit	Not applicable

On discharge, patients must be given information regarding contact with children and adults, toilet use, etc., for the period following discharge. This information is often modified to take into account the specific circumstances of each patient.

6.2.3. Design of therapy areas

There are two types of therapy areas – inpatient areas and areas where outpatient therapies are administered. The factors to be considered are:

— Types of radiation emitted (photon or particle, or mixed);
— The potential for contamination and the degree of the hazard;
— The type of waste products generated – human excreta, biological waste and general waste – and the way they should be handled;
— The role of nursing and medical staff in the care of the patient (high or low level of care).

General building requirements have been discussed in Section 6.1. Normally, the only difference between therapy areas is in the degree of any shielding required and the issues involved in integrating inpatient areas into a ward, such as access control and toilet facilities.

(a) Inpatient therapy areas

It is necessary to designate a *single* bedroom in the ward that meets the general radiation protection requirements. These include:

— A non-porous, easily decontaminated floor and wall surfaces with coved junctions to make cleaning easier;
— A minimum of projections, to prevent dust collection;
— A dedicated shower and toilet, the toilet draining directly to the main sewer or to a system of radiation waste disposal, depending on local regulatory requirements;
— A physical barrier to entry: a simple door may be sufficient;
— Moveable lead shields to minimize nursing exposure;
— The possible installation of a remote patient monitoring system (video);
— Door signs prohibiting entry by pregnant women, children and other persons without permission, giving a time limit for approved visitors, prohibiting removal of anything from the room without clearance, and requiring use of protective clothing in the room;
— A place to keep supplies of disposable gloves and gowns, and possibly overshoes, outside the room;
— Storage within the room for collection and temporary storage of waste.

Patient comfort should be catered for by radio, music, television and/or videotape facilities as well as a comfortable (but easily decontaminated) chair. Disposable sheets, blankets and eating utensils should be provided.

(b) Outpatient therapy areas

Outpatient areas are usually part of the nuclear medicine department, located at some distance from imaging equipment and public areas; the construction requirements for these are the same as for imaging rooms and the radiopharmacy. A floor drain is advisable in case of spillage of the therapy radiopharmaceutical. A trolley or folding steel bench is useful to hold the items used in the therapy.

6.2.4. General inpatient therapy guidelines

Most inpatient therapies involve ^{131}I, as reflected in the guidelines given below. If radiopharmaceuticals with a low risk of contamination are involved, the guidelines may be suitably modified.

6.2.5. Guidelines for ward staff

The entrance to the radionuclide therapy room must be clearly labelled with:

— A radiation warning sign;
— A sign prohibiting entry by pregnant women and children;
— A notice giving the visiting time limit;
— A notice, if necessary, to require use of protective clothing;
— A notice to the effect that nothing should be removed from the room until it has been declared free of radioactive contamination.

No member of staff should enter the therapy room without wearing a radiation monitor. Where digital dosimeters are in use, a record of the dose and the name of the staff member should be kept with the monitors outside the treatment suites. A record of all doses should be maintained.

Only essential nursing procedures may be performed during the treatment period. No blood samples, urine or faecal samples should be collected without nuclear medicine approval.

There must be no rubbish disposal from the room during the course of treatment.

Persons entering the room should put on plastic aprons, gloves and shoes. As the barrier is crossed on leaving the room, this protective clothing must be removed and placed in the disposal bag provided. (Note: This protective clothing only protects against radioactive contamination but NOT against a radiation hazard within the room.)

6.2.6. Guidelines relating to the patient

The following guidelines apply:

(a) The patient must be aware of the basic regulations listed below before the administration of a radionuclide. Before therapy, the patient should be given a booklet of common questions and answers.
(b) Any patient who is very ill and is considered incapable of a reasonable level of self-caring should NOT be given a therapy dose.
(c) Incontinent patients may need to be catheterized prior to the administration of therapy.
(d) Patients should be offered disposable hospital nightwear and towels to prevent their own becoming contaminated. If they wish to wear their own clothes, they must be advised on what should be done with garments on discharge.
(e) Patients may only use dedicated toilets, showers and washing facilities.
(f) Meals must be supplied on disposable plates, and eaten with disposable cutlery.

(g) Adult patients should normally be supplied with an electric kettle, plus coffee powder, tea bags, sugar and milk. Ideally, there should be a refrigerator to keep milk fresh, and to store cold drinks if required. This encourages the patient to drink freely and reduces the radiation exposure to nursing staff.
(h) Patients should use disposable tissues rather than handkerchiefs, since nasal mucosa tends to have a high radioactive content.
(i) Should sheets and/or pillow covers require changing, used linen should be placed in a bag and left within the suite. Under no condition should it be sent to the laundry until checked for contamination. Disposable linen may be preferred.
(j) Rubbish must be kept within the suite until dealt with by a physicist. This may involve storage prior to incineration in a licensed incinerator or storage until complete decay of the contamination.

Patients should only leave the therapy room for the purpose of a scan or in an emergency, in which case protective clothing (i.e. plastic aprons, shoes and gloves) must be worn. Unless an emergency precludes this, protective clothing should be put on upon leaving the room and removed on re-entry to the suite. When the patient is ready for discharge, all the patient's belongings must be checked for radioactive contamination and stored or washed separately as necessary. Any other belongings that may have become contaminated must be stored for a suitable length of time to allow the radioactivity to decay. The patient should be given a discharge card listing the radionuclide and activity administered, the activity on discharge and any necessary precautions.

6.2.7. Contamination

With any radionuclide therapy, there is a high potential for contamination. Decontamination procedures are covered in Chapter 8. It is, however, strongly advisable to keep a small decontamination kit in or near the therapy area (inpatient or outpatient) for immediate access if required.

6.2.8. Radioiodine therapy

(a) Pre-therapy

It is imperative that a doctor explain to female patients that therapy cannot be given to pregnant patients. If there is any chance that a patient may have become pregnant by the time the therapy administration is to commence, she must report this to a nuclear medicine doctor or technologist.

Female and male patients should be told what precautions, if any, are necessary if they wish to conceive, or father, a child subsequent to the therapy. A minimum of six months should be observed before conception.

(b) Therapy procedure — low doses (below discharge level)

Iodine-131 therapy doses are obtainable in liquid form or in capsules. Because of the significantly greater radiation hazards from liquid sources, the comments below assume the use of capsules. In addition to the general advice given above, the following points should be considered when designing the treatment protocol:

- Patients should be given written information about the therapy, and in particular instructions for when they return home.
- When patients are first brought in, they should be asked if they fully understand the procedure. If not, the procedure should be explained to them in simple terms.
- Patients should be asked to wear gowns and gloves (in case of contamination).
- The procedure should be explained carefully to the patient.
- The capsule should be placed in a plastic cup to avoid handling.
- The patient is required to swallow the capsule without chewing, followed by a drink of water.
- The patient should place the plastic cup and gloves in a waste bag.

The patient may then leave, after any subsequent restrictions are clearly understood. These restrictions may include:

- Flushing the toilet twice after urinating, for the first 72 hours after therapy;
- Maintaining a safe distance (1 m) from children or pregnant women for a few days.

(c) Therapy procedure — high doses (above discharge level)

The procedure for administering high doses is the same as for low doses except that the therapy should be administered in the patient's room.

Patients with thyroid cancer will have a very low iodine uptake, and a high proportion (often more than 95%) of the dose will be excreted, generally in the 72 hours following administration. While most excretion occurs in the urine, significant contamination can occur in saliva, with less in sweat and

faeces. Until the dose is fully absorbed from the gut, vomiting can cause a major contamination problem. To deal with these problems, the following measures can be considered:

(1) A prophylactic anti-emetic should be given prior to, or immediately after, the dose is administered.
(2) All linen, mattresses and pillows used by the patient must remain in the patient's room until checked for contamination; pillows in particular may be significantly contaminated.
(3) The therapist should check with the local regulatory authority for any requirements relating to discharge of radioactive human waste in the sewer. The simplest precaution is to tell the patient to flush the toilet at least twice after urinating. Even then there may still be a requirement (in some countries) to connect the toilet to a storage tank, where the waste may decay for some weeks before discharge to the sewer.
(4) If the patient is incontinent, or confused, a bladder catheter should be inserted prior to dose administration.
(5) To stimulate excretion, patients should be advised to drink freely, and void frequently.
(6) Salivary excretion can be stimulated by sucking or chewing confectionary.
(7) The patient's food must be served on disposable plates, and with disposable cutlery because of the potential for high salivary contamination.

These precautions may usually be discontinued after 72 hours.

(d) Inpatient therapy procedures (MIBG)

The above precautions also apply to MIBG therapy, although there are some extra issues to be considered and the rate of excretion will vary according to the degree of tumour retention. The precautionary period for excreta control might need to be extended.

The patient information should also be varied, with an example given below.

SPECIMEN PATIENT INFORMATION SHEET

Introduction

1. This is a short information sheet to help you understand the restrictions that will be placed on you after undergoing treatment using radioactive iodine.

2. There are several precautions that you and your family must observe both during the time you are in hospital and after you have been discharged. These precautions must be discussed fully with you; they are outlined below to ensure that they are clear. The radioactive treatment cannot be administered unless you understand these restrictions and sign a consent form by which you agree to adhere to them.

3. There will be a telephone within the room that may be used for incoming calls. In an emergency, the nursing staff can arrange for you to make an outside call.

Radioactivity and radiation

4. The treatment involves the administration of radioactive iodine, which will be attached to a non-radioactive label called MIBG specifically designed to adhere to the appropriate tumour.

5. Since you will become radioactive and will emit radiation after the treatment, you will be required to remain within the radionuclide treatment room until you are advised that it is safe to leave. This consists of a side room, shower/bath and toilet. You will excrete a considerable amount of radioactive iodine in urine, faeces, sweat, saliva and nasal mucous. It is very important that these substances are not allowed to contaminate other people, or areas outside the room.

6. You may ONLY use the toilet, shower and washing facilities within the radionuclide room. **You are not allowed out of the room, except for a scan.**

Meals

7. Your meals will be supplied on disposable plates and with disposable cutlery. Waste may not be thrown away by anyone on the ward. You will be provided with an electric kettle, coffee powder and tea bags so that you may make your own drinks. There is also a small refrigerator in the room to keep

milk or other cold drinks that you may like. If you have a preference for a particular drink, you should bring it with you.

Personal belongings

8. ***Money:*** It is not advisable to bring more money than you think you will require into the ward. If you would like the nursing staff to buy you daily newspapers, please give them some cash prior to the initiation of your treatment. If you are likely to have much excess money, it is wise to ask the nursing staff to lock it away until it is time for you to go home. The nurses will discuss this with you.

9. ***Clothes:*** Any clothes that you wear may become contaminated with radioactive iodine. Ideally, clothes worn while in the ward should be suitable for laundering in a washing machine; they should be taken home in a polythene bag and washed in a machine. Towels and flannels may also become contaminated and must be machine washed.

10. ***Other personnel belongings:*** It is advisable not to bring too many personal belongings into hospital, since anything you handle could become contaminated. Books, CDs and DVDs generally remain free of contamination.

General precautions

11. You will be required to start taking some tablets prior to the administration of the radioactive iodine-MIBG treatment. If you are on any medications, including nose drops, eye, ear, throat or cough drops or tablets, you must inform your doctor since they could prevent the radioactive treatment from acting efficiently.

12. It is important for you to drink as much fluid as possible, as this helps to keep the radiation dose to the bladder to a minimum, thus preventing a possible cystitis. It is also important not to be constipated, since this will lead to the stomach and bowel becoming unnecessarily irradiated. As a result the residual whole body activity will remain high for a longer period, possibly delaying your discharge.

13. You should use disposable tissues rather than handkerchiefs, if possible, since nasal mucous tends to have a high radioactive content. These tissues *must* be disposed of in the macerator.

14. ***Bed linen***: Should this require changing, the used linen should be placed in a bag and left within the suite. It must *not* be sent to the laundry. Any radioactive contamination can normally be washed out in one washing machine cycle, but this must be done under the control of a physicist.

15. ***Rubbish*** must be kept within the suite until checked for contamination. This may involve storage prior to incineration in a licensed incinerator or storage until 'complete' decay of the contamination.

16. You may only leave the suite for the purpose of a scan or in an emergency. On leaving the room you should put on protective clothing (i.e. plastic aprons, shoes and gloves) and remove it on re-entry to the suite. In an emergency this may not be feasible if time is crucial.

Visitors

17. It is permitted for you to have visitors, provided that they comply with the regulations and that neither children nor pregnant women visit you at any time.

18. Visitors should respect the maximum visiting time as displayed on the door. They should keep as far away from you as possible. **Under no circumstances** should visitors eat, drink, smoke or use the toilet facilities in the treatment room.

Discharge

19. There are different levels of remaining activity at which the hospital is allowed to discharge you depending on your home circumstances and on your means of transport home. After two or three days (possibly longer, depending on the estimate of residual activity), the activity remaining within you will be measured and you will then be advised as to how many more days it may be necessary for you to stay. Once you have been discharged, we may have to request you to observe certain restrictions such as not going to the cinema or mixing with children. These restrictions are dependent on the level and rate at which the radioactive iodine-MIBG has been passing out of you.

20. In the event of an emergency, the nuclear medicine department should be contacted immediately (be sure to include contact information).

6.2.9. Pure beta emitters and mixed beta/gamma emitters

6.2.9.1. Pure beta emitters

Beta emitters such as ^{186}Re, ^{169}Er, ^{166}Ho, ^{90}Y, ^{89}Sr and ^{32}P generally require consideration only at the time of administration, with little if any hazard afterwards, although higher energy beta emissions can cause measurable bremsstrahlung X ray radiation exterior to the patient.

Extravasation and surface contamination of beta emitters must be avoided. Administration must only be performed in a room designed for unsealed radionuclide use; in particular, all surfaces should be free of gaps and easily washable. Following administration the injection site must be checked for spilt or leaked radionuclides by swabbing and checking the swab with a beta detector.

In the case of ^{89}Sr, most of the unbound strontium will be excreted in the urine, normally within 48 hours of administration. Patients should be advised to flush the toilet twice after voiding. Precautions must be taken if staff or relatives come into contact with urine, for example if the patient is incontinent or catheterized, in which case gloves must be worn and properly disposed of.

Similarly, ^{32}P is largely excreted in the urine, with some in the faeces. Patients should again be advised to flush the toilet twice after voiding for the first 48 hours.

The patient must be given written instructions covering the need to wash hands following toilet use, cleaning up any spilt urine, flushing toilets and the washing of any clothing that may be urine contaminated. The instructions should also include contact names and phone numbers in case of an emergency.

6.2.9.2. Emitters of both beta and gamma radiations

Samarium-153 and ^{188}Re produce some gamma emission but this is not normally a significant safety problem. Staff and patient should be advised to remain at a distance of 1 m until discharge.

6.2.10. Precautions following death of a therapy patient

Procedures should be put in place for the safe disposal of the bodies of patients who have received therapeutic doses. This may include labelling, contamination avoidance and notification of the staff who may have to handle the body. These procedures will obviously depend on the radiopharmaceutical involved, the dose and the time since administration.

Cremation may usually be undertaken with negligible hazard to crematorium operators or members of the public at the normal doses used for therapy, although some radionuclides may be present in significant quantities in the ashes. The local regulatory authority should be contacted for advice.

If a post-mortem or embalming is to be performed, then advice from the radiation safety officer *must* be sought, as there could be a significant contamination and radiation hazard, especially with ^{131}I.

Ward staff in particular should be provided with instructions for dealing with the death of a radionuclide therapy patient.

6.2.10.1. Procedures

The nuclear medicine department must be notified as soon as possible after a death. Only a minimum of laying out procedures should be attempted (e.g. replacement of false teeth) and the sheet in which the body is wrapped should be clearly labelled as containing a radioactive body. The label should be clearly visible to all those handling the body. The body should be removed from the ward to the mortuary as soon as possible after death, without attempting to remove any of the radioactive material, and placed in, if possible, the centre section of the body storage refrigerator. This is to minimize any radiation exposure to staff who may be working in the mortuary.

6.2.10.2. Mortuary procedures

Exposure of individuals to radiation emitted by radioactive materials retained in or on a corpse can be reduced by:

(a) Working quickly to reduce the time of exposure;
(b) Working, where necessary, behind adequate shielding;
(c) Consulting with nuclear medicine staff for advice on radiation safety and removal of highly contaminated tissues such as the thyroid.

6.2.10.3. Autopsies: General comments

If a corpse contains less than 150 MBq (4 mCi) of colloidal ^{90}Y, 300 MBq (8 mCi) of ^{32}P or 450 MBq (12 mCi) of ^{131}I, the procedures normally observed during autopsy are adequate for the examination unless such examinations are carried out frequently.

If, however, a corpse contains radioactivity in excess of the levels given above, the pathologist should be informed of the radiation levels likely to be

encountered and of the hazards involved. The methods employed and the precautions adopted should be chosen accordingly.

Occasionally corpses are assigned to medical schools for dissection or are to be transported overseas. Any hazards to persons involved in these operations or the need for compliance with international transport regulations depend on several factors relating to the nature of the radioactive sources. In most instances the issue is resolved by keeping the corpse in appropriate cold storage until twenty half-lives of radioactive decay have passed.

If it is known that the radioactive material used for treatment will be selectively absorbed in a particular organ, for example ^{131}I in the thyroid, the organ should be excised before the examination proceeds and removed from the work area. It may later be disposed of with the body.

If it is known that radioactive material used for treatment will be distributed in particular body fluids, these should be drained off, using suitable equipment, before the examination proceeds. These fluids may often be safely disposed of via the sewerage system. The equipment should later be decontaminated by thorough rinsing in a detergent solution followed by washing in running water.

6.2.10.4. Cremation

No special precautions are necessary for the cremation of corpses containing not more than 1000 MBq (30 mCi) of ^{90}Y, ^{131}I, ^{89}Sr or 400 MBq (10 mCi) of ^{32}P.

Transport of a corpse containing radioactive materials should be considered in accordance with the requirements of local legislation covering the transport of radioactive materials.

6.2.11. Cardiac or respiratory arrest, or transfer of a therapy patient for medical reasons

Resuscitation of patients containing radioactive material for radiotherapy or therapeutic nuclear medicine purposes poses special problems.

The general principles to be followed in all cases are that the patient's welfare is paramount and possible radiation exposure to staff should not be considered a barrier to resuscitation, as, for the short times involved, the potential radiation exposure will be small.

6.2.11.1. Patients treated with ^{131}I

Attention should be paid to the following points:

(a) Do NOT apply direct mouth-to-mouth resuscitation.
(b) Staff involved in resuscitation should wear disposable gloves.
(c) Materials that have come into direct contact with the patient should, as far as is practicable, be kept to one side for examination by nuclear medicine staff. This particularly applies to airways, masks, endotracheal tubes, etc.
(d) Notify the Department of Nuclear Medicine immediately.

6.2.11.2. Transfer to intensive care or the coronary care unit

Attention should be paid to the following points:

(a) If a transfer is required, the fact that the patient may still contain radioactive material should not interfere with the management of the case.
(b) In the case of patients treated with ^{131}I for whom intubation, catheterization or use of a nasogastric tube may be necessary, staff should wear gowns and gloves when handling the patient.
(c) Urine, gastric contents or other body fluids should be contained as far as possible by means of absorbent pads, and the pads held in a contaminated waste bag for examination by nuclear medicine staff.
(d) Any suction bottles or urine bags used must not be discarded until checked for contamination.

6.2.11.3. Examination of staff involved in resuscitation or handling of the patient

Staff who have been directly involved with the patient will need, for their own safety and peace of mind, to be assessed as to their potential radiation exposure, however small. This will include the best possible estimation of radiation exposure, and, where ^{131}I is involved, administration of Lugol's iodine as soon as possible to block thyroid uptake of any absorbed contaminant, if necessary, and subsequent measurement of any thyroidal accumulation of ^{131}I.

BIBLIOGRAPHY TO SECTION 6.2

UNITED STATES NUCLEAR REGULATORY COMMISSION, Release of Patients Administered Radioactive Materials, NRC Regulatory Guide No. 39, USNRC, Washington, DC (1997).

6.3. DOSIMETRY AND MATHEMATICAL MODELS IN RADIOPHARMACEUTICAL THERAPY

6.3.1. Introduction

Most radiopharmaceutical therapies are based on the amounts of radioactivity given, with adjustments made for body weight or surface area. However, radiopharmaceutical toxicity is dependent upon the radiation absorbed dose to critical normal organs; measurement of the radiation absorbed dose provides an optimal estimation of potential toxicity. This section will provide an overview of the methods used to estimate radiation absorbed dose. In most cases, the critical normal organ is the haematopoietic system.

In order to evaluate potential toxicity to other organs, mathematical models to describe biodistribution are important.

6.3.2. Rationale

Calculation of the radiation absorbed dose to organs permits a more accurate prediction of toxicity and side effects than assessments of toxicity based on the amounts of radioactivity administered. It therefore follows that maximization of the dose delivery to tumours may be achieved by the accurate calculation of the radiation dose to critical organs (usually the haematopoietic system).

Dosimetry is carried out in deciding the maximum safe amount of ^{131}I that can be administered to patients with thyroid carcinoma. In order to determine the radiation absorbed dose to the haematopoietic system, serum and whole body measurements are typically carried out.

6.3.3. Indications

Dosimetry is carried out to permit determination of the radiation absorbed dose to critical normal organs, calculation of the safe amount of radioactivity that may be administered and calculation of the radiation absorbed dose to the tumour.

6.3.4. Procedure

For all calculations, it is mandatory to measure a known amount of radioactivity in a manner identical to that used for patient or sample measurement so that estimates of counts per unit radioactivity may be made.

6.3.4.1. Calculation of whole body and/or red marrow radiation absorbed dose

For radionuclides that emit photons, estimates of whole body radiation absorbed dose are made over a period of time, using whole body imaging or counting.

For radionuclides that are pure β^- emitters, whole body estimates cannot be made. Red marrow radiation absorbed dose is usually calculated by measuring the radioactivity of serum samples obtained over time with a scintillation counter (a well counter for photon emitting nuclides and a liquid scintillation counter for pure β^- emitters).

6.3.4.2. Calculation of radiation absorbed dose to tumour

Estimates of tumour volume are critical and may be obtained by appropriate radiological procedures. The amount of radioactivity in the tumour is estimated by serial gamma camera imaging with semi-quantitation usually carried out by application of conjugate view methodology.

6.3.4.3. Calculation of radiation absorbed dose to other organs

Conjugate view imaging over time is necessary to determine the radioactivity in normal organs. Organ mass estimates may be made using CT or other appropriate imaging methods, including transmission gamma camera imaging.

The radioactivity–time data are entered into a mathematical program that permits measurement of residence time, i.e. the time during which the radiopharmaceutical is present in the given 'compartment' (e.g. serum or whole body). Once the residence time has been calculated, the radiation absorbed dose may be estimated.

The simplest compartmental model is the exponential clearance model, which assumes that the radiopharmaceutical leaves the compartment at a constant exponential rate, which is then used to fit the data to an exponential curve. More complex models assign rate constants to the transfer of the radiopharmaceutical between compartments. These are then used to obtain the residence time in the given compartment. The better the fit, the more valid the model.

Most radiation absorbed dose estimates may be made using simple two-compartment models whereby one compartment is the serum and the other the urine or the rest of the body. Such models are adequate for most biodistribution estimates. In some cases, notably with radiopharmaceuticals that target normal bone, it may be necessary to define more compartments.

6.4. RADIOIODINE THERAPY FOR THYROTOXICOSIS

6.4.1. Principles for radioiodine therapy in thyrotoxicosis

The most common form of hyperthyroidism is Graves' disease, which accounts for 60–90% of all cases of thyrotoxicosis. Other causes of thyrotoxicosis include toxic adenoma and toxic multinodular goitre. Elevated T4 and suppressed TSH are the biochemical hallmarks of thyrotoxicosis. Less frequently, T3 alone may be elevated (T3 toxicosis).

6.4.2. Clinical indications and contraindications

The following points should be noted:

— Iodine-131 is the treatment of choice for hyperthyroidism.
— Pregnancy and lactation are absolute contraindications.
— Incontinence is a relative contraindication.

6.4.3. Dose and administration

In all cases, ^{131}I therapy may be repeated after a six month interval if the patient remains biochemically thyrotoxic.

6.4.3.1. Graves' disease

Iodine-131 uptake at 24 hours must be estimated to ensure that the thyroid will receive the desired dose and also to rule out a silent thyroiditis. The following methods are commonly used:

(a) Calculation of the dose to render the patient euthyroid

It is believed that calculation of the dose to the gland results in a greater proportion of euthyroid patients. Dose estimates may be made based upon radioactivity retained (3–5 MBq/g ((0.08–0.12 mCi)/g) at 24 hours) in the thyroid or upon radiation dose (80–120 Gy) to the thyroid.

(b) Administration of an ablative dose higher than 555 MBq (15 mCi)

The rationale is to suppress hyperthyroidism as speedily as possible and prevent recurrence. Ablative doses quickly induce hypothyroidism; this may be

diagnosed early and treatment with thyroid hormone may be instituted before the development of symptoms.

The incidence of hypothyroidism following either treatment option has been shown to be comparable. For the above reasons, some prefer the use of an ablative dose, starting early with thyroid hormone substitution, which is a simple treatment with no contraindications and low cost.

(c) Administration of a treatment dose

Treatment doses of 200–400 MBq (5–10 mCi) render 50% of patients euthyroid and reduce the incidence of hypothyroidism. The dose may be repeated if required.

6.4.3.2. Toxic adenoma

The dose of ^{131}I administered to patients with toxic nodules differs widely. Between 740 and 2220 MBq (20–60 mCi) may be administered depending on the size and the percentage ^{131}I uptake by the nodule. The suppressed normal thyroid tissue should recover and the patient should become euthyroid without the requirement for thyroid replacement.

6.4.3.3. Toxic multinodular goitre

Multinodular glands, whether toxic or not, are relatively resistant to ^{131}I. For this reason, some physicians increase the standard dose by 20–50%. Frequently, doses are between 555 and 1850 MBq (15 and 50 mCi). It is frequently found that areas of low functional activity in the thyroid at the time of therapy may become activated after destruction of the hyperfunctioning areas.

6.4.4. Patient preparation

Iodine-containing contrast media and other substances should be avoided or discontinued as shown in Table 6.3. Although patients may be treated as outpatients, some countries may require inpatient therapy for higher doses of ^{131}I.

Patients should be rendered euthyroid prior to ^{131}I therapy and should discontinue anti-thyroid medication for 2–8 days prior to therapy, to be resumed if necessary no earlier than 5 days after ^{131}I therapy. Propranolol may be continued.

TABLE 6.3. DRUG INTERACTIONS

Type of medication	Mechanism of interference	Recommended time of withdrawal
Anti-thyroid medications (carbimazole and propylthiouracil)	Interference with iodination process within the thyroid	2–8 days
Natural and synthetic thyroid preparations (thyroxine and tri-iodothyronine)	Blocking uptake	2–4 weeks (4 weeks – thyroxine) (2 weeks – tri-iodothyronine)
Expectorants, vitamins and health food preparations	High iodine content; results in competitive blockade	Dependent on iodine content, 1–6 months
Iodine containing medications (amiodarone)	Competitive blockade of iodine uptake	Variable, 1–6 months
Topical iodine (surgical skin preparation agents)	Competitive inhibition of thyroid uptake	1–9 months
Radiographic contrast agents Intravenous Oral, e.g. cholecystographic Oil based, e.g. bronchographic and myelographic	Competitive blockade of iodine uptake	1–2 months 6–9/12 months 6–12 months 2–10 years
Phenylbutazone		1–2 weeks
Salicylates		1 week
Steroids		1 week
Sodium nitroprusside		1 week
Benzodiazepines		4 weeks
Miscellaneous agents: Anticoagulants Antihistamines Antiparasitics Penicillins Sulphonamides Tolbutamide Thiopental		1 week

Female patients should be advised not to conceive for at least six months following therapy.

On the day of ^{131}I administration and throughout the following day, patients should be encouraged to drink large volumes of fluid, to micturate frequently in order to minimize the radiation dose to the bladder and to suck sweets to reduce salivary gland doses. Patients do not have to be on a low iodine diet as the overstimulation of the thyroid gland makes the eventual amount of iodine in the diet irrelevant.

6.4.5. Immediate side effects of ^{131}I therapy

The immediate side effects of ^{131}I therapy are typically minimal. Transient exacerbation of thyrotoxicosis and apparent thyroid storm may occur within days of ^{131}I therapy in patients who were not made euthyroid before therapy. A few patients develop mild pain and tenderness over the thyroid or salivary glands and, rarely, dysphagia. These inflammatory effects tend to appear within days of administration and are short lived, often lasting less than a week.

In some cases, exophthalmos may be worsened. Pretreatment with anti-thyroid drugs may prevent this complication, as may administration of prednisone. Steroid pretreatment is now advised for any patient with exophthalmos. Steroid administration should likewise be considered if pressure symptoms to the trachea are anticipated or have set in.

6.4.6. Patient follow-up

Follow-up should include the following:

— Serum T4 (or T3 in the case of T3 toxicosis) and TSH one month after therapy;
— Quarterly clinical examination;
— Evaluation of treatment response with serum T3/T4 and TSH six months after therapy;
— If permanent hypothyroidism develops, hormone replacement therapy for life.

6.4.7. Radioiodine treatment in children and adolescents

There is no formal contraindication for the use of radioiodine in children. Nevertheless, caution is recommended and ^{131}I therapy is restricted to those for whom other treatments have failed or in whom surgery is not advised.

6.4.8. Radiation safety considerations

There are no reports of an increased risk of neoplasms, genetic damage or infertility with the doses used in hyperthyroidism.

6.5. IODINE-131 THERAPY IN THYROID CANCER

6.5.1. Clinical benefits

Iodine-131 therapy is beneficial in the therapy of thyroid remnants or of metastatic thyroid cancer.

The primary treatment for thyroid carcinoma is thyroidectomy. Following thyroidectomy, almost all patients have functioning (iodine avid) thyroid tissue in the neck. It is impossible to distinguish, except by histopathological examination, between normal and malignant thyroid tissue. Eradication of all thyroid tissue is essential, and since both normal and malignant thyroid tissue produce thyroglobulin – a marker for thyroid cancer – only eradication of all thyroid tissue will permit accurate evaluation of disease status. Finally, eradication of normal thyroid tissue will permit uptake of therapeutic radioiodine by malignant tissue, maximizing the therapeutic benefit.

6.5.2. Physiological basis

Radioiodine, in a manner identical to iodine, is concentrated in functioning thyroid tissue, either normal thyroid tissue or thyroid carcinoma. Most differentiated thyroid cancers concentrate iodine to a variable extent; papillary and follicular cancers invariably concentrate iodine, while many Hürthle cell and other ‘tall cell’ variants of differentiated thyroid cancer may not concentrate iodine. Iodine uptake has been shown to be mediated by an iodide symporter.

6.5.3. Indications

The indications are iodine-avid thyroid remnants or metastatic disease in patients with thyroid carcinoma, usually papillary or follicular.

6.5.4. Contraindications

The contraindications are:

(a) Absolute: pregnancy and/or lactation;
(b) Relative: patient on thyroid hormone replacement therapy (patient may be treated with stimulation by recombinant human TSH (rhTSH)).

6.5.5. Equipment

Iodine-131 therapy is sometimes carried out, especially in patients suspected to have metastatic cancer, after demonstration of iodine-avid thyroid tissue (normal or malignant) by a gamma camera or whole body counter. Most centres carry out gamma camera imaging using a high energy, general purpose collimator. Most centres also carry out imaging with comparable imaging methods, to demonstrate targeting of therapeutic ^{131}I to thyroid tissue.

No special equipment is required for outpatient therapy, apart from adequate shielding of the ^{131}I and appropriate monitoring of patients to ensure adherence to radiation safety criteria for outpatient therapy.

High doses of ^{131}I should be administered within areas that meet radiation protection requirements. These conditions have been detailed above.

6.5.6. Radiopharmaceuticals

Iodine-131, in the form of sodium iodide, is administered orally.

6.5.7. Action prior to ^{131}I therapy

Patients at intermediate or high risk of thyroid cancer usually receive ^{131}I therapy after definitive thyroid surgery (usually total or radical thyroidectomy, with recurrent laryngeal nerve and parathyroid preservation). Skin sterilization for thyroid surgery must not use an iodine containing compound.

Patients must not receive thyroid hormone replacement for at least four weeks prior to ^{131}I therapy. Patients who tolerate hormone withdrawal poorly may receive tri-iodothyronine (T3) until two weeks prior to therapy.

No intravenous contrast should be administered for at least two months prior to planned evaluation and therapy.

Patients should be encouraged to reduce the iodine content in their diet to optimize uptake of ^{131}I by thyroid tissue.

Serum thyroglobulin estimations are usually carried out immediately prior to administration of ^{131}I tracer.

A tracer study may be carried out prior to administration of ^{131}I therapy, to ensure ^{131}I uptake in thyroid tissue and/or in metastatically diseased tissue. Smaller doses of ^{131}I (20–80 MBq) are given to assess for residual thyroid tissue, while larger doses (80–200 MBq) are given to evaluate metastatic disease.

Iodine-131 is given orally; neck uptake and imaging is carried out between 24 and 72 hours after administration. Whole body imaging at 72 hours should also be carried out, especially when the results of neck imaging are negative. Many centres, however, proceed directly to ablative therapy.

A form signed by the patient giving their informed consent for therapy is required.

6.5.8. Therapy

Ablative therapy is defined as that given immediately following definitive surgery. In most centres, a fixed dose of between 1 and 4 GBq (25–100 mCi) ^{131}I is given. When the mass of thyroid remnant can be estimated, for example using ultrasound, a dose of ^{131}I calculated to deliver 30–50 Gy to the thyroid remnant may also be used. Ablative therapy should be given to all patients with iodine-avid thyroid/malignant tissue in the neck or elsewhere, or in those patients who, immediately after surgery, have no evidence of iodine-avid thyroid tissue 72 hours after oral administration of ^{131}I tracer but who have elevated serum thyroglobulin levels.

Patients should be evaluated not earlier than six months after ablative ^{131}I therapy for evidence of residual or recurrent disease. This evaluation is carried out not less than four weeks after cessation of thyroid hormone replacement or, if the patient cannot tolerate hormone withdrawal, by the following regimen:

— Stop levothyroxine and substitute with a comparable dose of T3 for two weeks.
— Stop T3 for at least two weeks prior to ^{131}I.

Where available, patients may be evaluated using rhTSH, which is given as an intramuscular injection of not more than 0.9 mg daily for two days, followed a day later by not more than 185 MBq ^{131}I taken orally.

Therapy for recurrent or metastatic disease is given to patients who show evidence of uptake of a tracer amount (80–185 MBq) of ^{131}I in such sites. Anterior and posterior whole body imaging should be carried out at least 72 hours after administration of the tracer, using high energy collimation. An alternative to whole body imaging is static anterior and posterior imaging of the relevant areas (head, neck, chest, abdomen, pelvis and lower extremities), taken for at least 10 min each.

If there is evidence of iodine-avid disease from scintigraphy and/or if the serum thyroglobulin level is elevated, the patient should be treated with ^{131}I. The dose of ^{131}I is usually between 5 and 7 GBq. The maximum safe dose of ^{131}I may also be calculated using clearance data, obtained by measurement of

radioactivity in serum and in the whole body. More details about dosimetric evaluation may be found in Section 6.3. The maximum safe dose of ^{131}I has been found to be that which delivers no more than 2 Gy to the blood. In patients who have diffuse lung involvement, the whole body retention of ^{131}I should be no more than 3 GBq at 48 hours.

6.5.8.1. Post-therapy follow-up

Hormone replacement may be resumed two days after treatment.

In most centres, anterior and posterior images of the body are obtained a week to 10 days after ^{131}I therapy to ensure targeting.

Patients are followed up with thyroid function tests carried out every quarter, to ensure adequate suppression of TSH by thyroid hormone. Estimation of serum thyroglobulin should also be carried out during this time. This can be done most reliably when the patient is no longer on T4 or T3 treatment. When patients are treated at the maximum safe dose, haematological evaluation should be carried out between four and six weeks after therapy, to ensure lack of haematopoietic toxicity.

Patients are usually not re-treated earlier than six months after therapy, unless there is evidence of rapidly progressive disease as evidenced by a progressive rise in serum thyroglobulin and/or radiographic evidence of progressive disease. Pretreatment is carried out in a manner identical to the initial therapy: adequate hormone withdrawal, evidence of ^{131}I-avid disease on a tracer (185 MBq ^{131}I) study and/or demonstration of elevated serum thyroglobulin levels.

Six months to a year after treatment, efficacy should be evaluated by carrying out a whole body survey with 185 MBq ^{131}I after hormone withdrawal. Two successive negative whole body studies, with concurrent non-measurable serum thyroglobulin levels, separated by intervals of at least six months, indicate successful therapy. The patient may then be managed by serum thyroglobulin estimations twice yearly for five years and then annually for at least another five years.

6.5.9. Suggestions for a written instruction sheet for patients

Why are you going to receive radioactive treatment?

You are going to receive radioactive iodine treatment because your doctors have decided that this is the best option for your disease. Most of the radiation emitted by the iodine will be absorbed by thyroid tissue. This radiation damages the tissue, producing the desired beneficial effect for your

disease. However, small quantities of the radiation present in your body may reach people close to you, exposing them to this radiation unnecessarily. Although there is no evidence that this radiation exposure has damaged other individuals, you should avoid exposing others to any unnecessary radiation.

How is radioactive iodine administered and what sort of preparation is required?

Radioactive iodine is given in a capsule or liquid form by mouth in variable quantities according to the type of your disease. Your treating doctor and the physician who will actually administer the treatment determine the dose. According to the administered dose and your condition, it is possible that you may be hospitalized for some days. Women must be absolutely sure that they are not pregnant at the time they receive the treatment and should not be breast feeding. Food should not be ingested in the two hours before treatment and, in some cases, a low iodine diet will be recommended for a few days. You should talk to your doctor to clear up any doubts you may have.

How long does iodine remain in my body?

Radioactive iodine remains in your body for just a few days. Most of the iodine not retained in thyroid tissue is eliminated through the urine within 48 hours. A small quantity will be present in the saliva, sweat and stools. Radioactive iodine that remains in your thyroid tissue also decreases quickly. This means that the possibility of unnecessary radiation exposure to other people also decreases in a matter of days.

In what ways may other people be exposed to my body's radiation?

Radiation emitted by the radioactive iodine in your body is very similar to the X rays used in radiological examinations. For this reason, people who remain close to you for prolonged times may be exposed to unnecessary and avoidable radiation.

Besides the above mentioned radiation, there is the possibility that other people close to you may directly ingest small quantities of radioactive iodine eliminated by your body in the saliva or sweat. Your urine is also radioactive.

In what ways can I reduce the risk of radiation exposure to other people?

Even though the amount of radioactive iodine present in your body is small, and there is no evidence that the radiation emitted by it may cause

problems, it is advisable to reduce the exposure to other individuals as much as possible. The three principles to avoid unnecessary radiation exposure are:

(1) **Distance:** Do not get too close to any other person. Radiation decreases significantly with increasing distance.
(2) **Time:** Radiation exposure to other people depends on how long they remain near you. Therefore, avoid prolonged contact with other people.
(3) **Hygiene:** Good hygiene minimizes the possibilities of direct contamination with radioactive iodine. Because most of the iodine is excreted in the urine it is very important that you wash your hands thoroughly after going to the toilet.

PRACTICAL ADVICE:

Ask your doctor for detailed guidance in order to avoid exposing people who are around you to unnecessary radiation. Be quite clear about the situation and do not be afraid to ask further questions.

Sleep alone during the first days after the treatment. During this period, avoid kissing and sexual intercourse. Avoid close and prolonged contact with other people, especially children and pregnant women, who are more sensitive to radiation than the rest of the population.

If you have a small child or you are in charge of one, request special instructions from your doctor. Do not hold the child on your lap, feed him or her, or change nappies. If you are breast feeding, you must stop before therapy begins because the iodine is excreted into breast milk. You must switch to other types of milk.

It is crucial to wash your hands thoroughly after going to the toilet. Flush the toilet two or three times after using it. Men are advised to urinate sitting down to avoid splashing urine outside the toilet bowl or in its borders.

Drink large amounts of fluid to eliminate as much urine as possible. Eat sweets or drink lemon juice to produce more saliva and in this way prevent iodine retention within your salivary glands. Keep your toothbrush separate from those belonging to the rest of the family.

Do not bite your nails or put objects in your mouth such as pencils or necklaces.

Reserve a separate towel for your own exclusive use. Wash your underwear and bed linen separately from those of the rest of the family and rinse several times.

6.6. PALLIATIVE TREATMENT OF METASTATIC BONE PAIN

6.6.1. Clinical benefits

The aim of radionuclide therapy for metastatic bone pain is to ameliorate pain, reduce the intake of analgesics and improve quality of life. The requirement for such treatment is the demonstration of good focal uptake of ^{99m}Tc bone-seeking radiopharmaceuticals in bone scintigraphy at sites corresponding to the bone pain. The main primary tumours are carcinomas of the prostate, breast and lungs. Similar results have been obtained using ^{32}P (as the orthophosphate), ^{89}Sr (as the chloride) and ^{153}Sm (as ethylene-diamine-tetramethylene-phosphonate (EDTMP)). Between 60 and 75% of patients normally show a good response to such treatments; the duration of response lasts between 6 and 24 weeks (with a mean of 12 weeks) and is independent of the radioisotope used. A significant proportion (40–50%) of responders do not require analgesics, while the rest require only mild doses of oral analgesics in order to remain free of pain. Studies have also demonstrated that there is significantly delayed onset of new bone pain following therapy.

Mild to moderate myelosuppression (thrombocytopenia, leucopoenia and rarely anaemia) is sometimes observed. Haematological toxicity with ^{32}P may be minimally greater than that with ^{89}Sr or ^{153}Sm-EDTMP, but rescue strategies are rarely required.

6.6.2. Physiological basis

Bone metastases have local effects resulting in increased bone destruction (osteolysis), increased bone formation (osteosclerosis) or both. Osteolytic metastases are the predominant types of lesions in most cancers, but a sclerotic appearance is seen in the majority of metastases from prostate cancer, in about 10% of metastases from breast cancer, as well as in those from other cancers. In the majority of skeletal metastases, new bone formation develops simultaneously with bone destruction, and the radiological appearance reflects the process that predominates.

Bone seeking, beta emitting radiopharmaceuticals such as ^{32}P-orthophosphate, ^{89}Sr-chloride, ^{188}Re-HEDP and ^{153}Sm-EDTMP demonstrate a rapid blood clearance with prompt localization in areas of bony repair. Systemic administration provides a means of delivering radiation systemically to the sites of disseminated bone metastases. Several other beta or conversion electron emitting radiopharmaceuticals (^{117m}Sn-DTPA and ^{166}Ho-(1,4,7,10-tetra-azacyclododecane-1,4,7,10-tetramethylene phosphonic acid) (DOTMP)) are also being evaluated and have shown promise.

6.6.3. Indications

Phosphorus-32-, ^{89}Sr- and ^{153}Sm-EDTMP are indicated for the treatment of bone pain due to osseous metastases that demonstrate increased uptake of ^{99m}Tc in bone scintigraphy. They may be used as adjuncts and/or alternatives to external beam radiotherapy for the palliation of metastatic bone pain.

6.6.4. Contraindications

Absolute: Pregnancy or lactation.

Relative: Impending cord compression may require concurrent external beam therapy. Significant myelocompromise (platelet count less than 60 000 mm^{-3} or absolute granulocyte count less than 2000 mm^{-3}); active disseminated intravascular coagulation (DIC) mandates careful monitoring.

6.6.5. Procedure

The regulations and guidelines for the therapeutic administration of radiopharmaceuticals described in Sections 6.1 and 6.2 should be strictly followed. All patients receiving therapy should submit their signed, informed consent.

6.6.6. Pretreatment preparation

6.6.6.1. Pretreatment investigations

The following pretreatment investigations are carried out:

(a) Haematological screening, to ensure adequacy of platelets and granulocytes;
(b) Bone scans, to ensure that skeletal lesions are positive on scintigraphy;
(c) Radiographs of skeletal lesions, when necessary, to rule out impending cord compression or fracture.

6.6.6.2. Patient information

Before administering therapy, the patients should be informed that:

(a) The treatment has an 80% probability of reducing their bone pain, although the chance of complete pain relief is low.

(b) The treatment is not a curative for cancer, but a palliation for pain, even though some cancer cells will be killed in the process.
(c) There may be a significant increase in bone pain (a so-called 'flare') within a few days of injection which may last for two to five days; and also there is a possibility of a drop in leukocyte and platelet counts.
(d) Chemotherapy and external beam radiotherapy have additive effects on myelosuppression; hormone therapy need not be discontinued.
(e) Use of narcotic analgesics for control of symptoms should be continued if necessary.

6.6.7. Treatment procedure and amount

A 185–370 MBq (5–10 mCi) dose of ^{32}P is administered intravenously or a 370–444 MBq (10–12 mCi) dose orally.

Strontium-89 is administered intravenously as the soluble salt strontium chloride. The activity administered is usually 1.5–2.2 MBq/kg (40–60 μCi/kg), to a maximum of 148 MBq.

Samarium-153 is administered intravenously as ^{153}Sm-EDTMP. The usual administered activity in adults is about 18.5–37 MBq/kg (0.5–1.0 mCi/kg). Higher doses may increase side effects without any significant gain in pain palliation.

6.6.8. Evaluation of palliative efficacy

On average, all the three above cited radiopharmaceuticals produce pain relief in between 60 and 75% of patients suffering from painful bone metastases. The effect usually shows between one and three weeks after dose administration and generally lasts between 6 and 24 weeks. The response starts with a slight improvement, increases with time to a plateau, then slowly declines with the recurrence of pain. About 25–35% of patients may have a complete pain free phase for a certain period of time, but most patients experience a varying effect day-to-day throughout the course.

Samarium-153 EDTMP, ^{32}P and ^{89}Sr are reported to accumulate in the trabecula of bones, while ^{32}P additionally binds to red marrow. The major toxicity is myelosuppression, which is relatively greater in the group of patients treated with ^{32}P in comparison with those treated with ^{89}Sr or ^{153}Sm-EDTMP. The platelet and white cell counts may drop by 30–50% of the baseline values one to four weeks after treatment. The side effects are not usually severe, and patients will recover spontaneously in most instances. These effects seem positively related to the amount of administered dose.

6.6.9. Follow-up

All patients should be followed up for at least five or six weeks with weekly or bi-weekly clinical, biochemical and haematological examinations. It is believed that 50% of patients who fail the first dose may benefit from another dose. A repeat dose may be considered if haematological parameters permit.

6.7. IODINE-131 META IODOBENZYLGUANIDINE THERAPY

6.7.1. Physiological basis

Iodine-131 MIBG is concentrated by an active mechanism into the storage granules of chromaffin cells in neural crest tumours, mimicking presynaptic re-uptake of noradrenalin. This uptake is blocked competitively by noradrenalin analogues including Ephedrine and pseudoephedrine, which occur frequently in cough lozenges and drops, some antidepressants and related compounds. Passive uptake by diffusion is also seen.

6.7.2. Indications

Treatment is indicated for neural crest tumours, typically carcinoids, malignant phaeochromocytomas, malignant paragangliomas, neuroblastomas and medullary thyroid carcinomas, which show uptake (greater than 1%) of radioiodinated MIBG tracer.

6.7.3. Contraindications

Absolute: pregnancy, continued breast feeding, severe myelosuppression, severe renal failure;

Relative: unstable patient condition not allowing isolation therapy.

6.7.4. Clinical benefits

The requirement for ^{131}I-MIBG therapy is a demonstration of good radioiodinated MIBG uptake by the tumour. The mean survival time of these patients is increased by MIBG therapy, but the main benefit is palliation and control of symptoms as well as correction of elevated serum and urinary markers. A reduction of tumour mass may also be seen.

About 50% of abdominal and 30% of bronchial carcinoids concentrate MIBG, as do 90% of malignant phaeochromocytomas and paragangliomas, 30% of medullary thyroid carcinomas and 90% of neuroblastomas. A partial response by radiological criteria is seen in about 10% of carcinoid tumours that concentrate MIBG, with stable disease in about 70% and progression in about 20%. Serum markers return to normal in about 10%, with a reduction of more than 50% in a further 30% and no change in about 45%. About 15% of carcinoids are non-secretory. Symptoms are relieved completely in about 15% of patients, partly in about 45% with no change in 20%; some 20% have no symptoms. The mean survival time is about 30 months (with a range of 10–70 months) in patients coming for therapy with advanced disease.

A comparable benefit with a better mean survival time has been reported in malignant phaeochromocytomas and paragangliomas.

European data suggest that medullary thyroid cancer is relatively unresponsive to MIBG.

In neuroblastomas, palliative responses are common but complete responses are rare. While ^{131}I-MIBG is typically used last in the sequence of therapy, the benefits of its use preoperatively are to make otherwise inoperable tumours operable, allowing children to continue to grow and not to lose hair, unlike with chemotherapy. Tumours are also less vascular and easier to operate on.

6.7.5. Equipment

Facilities that comply with radiation safety requirements are described in Section 6.2. A method of infusion using a pump within a lead shielded system for radiation protection during the administration of ^{131}I-MIBG is required. The components are as follows:

— An infusion pump.
— A ‘Y’ intravenous giving set tubing.
— A spinal needle to reach the bottom of the bottle of MIBG.
— An ordinary needle for bleeding air to the bottle.
— A two way connector (the ‘Y’ giving set may have to be cut to put the spinal needle into one arm). A pair of Hellman pliers may be required to expand tubing to fit the connector to the needle.
— Saline for the inactive arm.

6.7.6. Radiopharmaceuticals

Iodine-131 MIBG is used for intravenous infusion. The agent should be stored in a shielded container in dry ice (solid carbon dioxide) until an hour before use. It should be thawed preferably in a water bath at not more than 50ºC about an hour before use. It must not be refrozen.

6.7.6.1. Dose

The following points should be observed:

— The dose for adults is in the range 3.7–11.1 GBq (100–300 mCi).
— Children may receive the same dose as adults.
— The dose should be reduced if more than one third of the bone marrow is involved.
— The dose should also be reduced in patients with renal failure.

6.7.6.2. Action prior to ^{131}I-MIBG therapy

The following steps should be taken and warning signs noted:

(a) Check for evidence of ^{131}I-MIBG or ^{123}I-MIBG uptake.
(b) Loss of salivary gland uptake on imaging suggests the patient is taking an interfering medication.
(c) Check on possible interfering drugs being taken by the patient: these should be stopped at least two days before therapy.

6.7.6.3. Interference of other drugs with MIBG uptake

Drugs that interfere with MIBG uptake include the following:

— Most drugs ending in the letters ‘ine’;
— Nose drops, eye drops, ear drops, throat drops, cough drops, pastilles and tablets, which often contain Ephedrine or analogues of Ephedrine which compete with MIBG;
— Certain tricyclic antidepressants, such as Amytriptiline;
— Certain hypotensive agents, such as Reserpine, Labetalol and some calcium channel blockers;
— Certain tranquillizers, particularly phenothiazines.

6.7.6.4. Administration time

Therapy infusion takes:

— Approximately 30 min in adults, after which time the cannula is removed;
— Approximately one to one and a half hours in children, who should remain relatively dehydrated for 24 hours before the cannula is removed.

6.7.7. Patient preparation

6.7.7.1. Pre-therapy requirements

To assess the response to therapy and/or its potential side effects, careful staging, determination of the extent of the disease and identification of volumetric and biochemical parameters should be performed. A preceding ^{123}I-MIBG or ^{131}I-MIBG scintigraphy, indicating sufficient MIBG uptake, is mandatory. Loss of salivary gland MIBG uptake on imaging suggests an interfering medication is being taken.

Haematological parameters, renal function and bone marrow condition have to be evaluated. If patients are taking drugs that may interfere with the uptake and/or retention of ^{131}I-MIBG, they should be taken off these drugs for at least two weeks prior to therapy. Only propranolol as a beta blocker and dibenylene as an alpha blocker to control hypertension may be used without problems.

Thyroid blockade by daily oral administration of potassium iodine (KI), over two weeks starting one day prior to therapy, is indicated (up to 60 mg/day for children and 120 mg/day for adults). It is important that patients, or in the case of children their parents, should have been instructed on the issue of radiation protection.

To prevent side effects of excess catecholamines in circulation during or shortly after ^{131}I-MIBG infusion, blood pressure should be monitored and controlled, if needed, by an infusion of dibenilene and/or propranolol;

In the case of high dose ^{131}I-MIBG therapy, the harvesting of bone marrow prior to therapy should be considered in children with neuroblastomas.

Patients should receive advice on the following:

— In order to eliminate the radiopharmaceutical more rapidly, patients should be encouraged to drink a large quantity of liquid after the infusion.
— Parents should be involved in their child's patient care and receive instructions on radiation protection (Section 6.2.3).

— Female patients should be advised not to become pregnant for at least six months following therapy.
— Patients can eat and drink normally; ingestion of fluids is encouraged to increase the elimination of unbound ^{131}I-MIBG.

6.7.7.2. Follow-up

Follow-up should comprise the following:

— Imaging of the therapy dose after three and/or five days.
— Advice against conception for six months after therapy.
— Blood count at least weekly for up to eight weeks, or until return to baseline.
— Blood pressure measurement at least weekly, with appropriate titration of anti-hypertensive medication, until stable.
— Body weight control, particularly in children.
— Serum and urinary markers of response, four and eight weeks after therapy. Any increase should lead to further radioiodine MIBG imaging.
— CT and other radiological studies to evaluate response, eight weeks after therapy.

6.7.7.3. Re-treatment

The following guidelines about re-treatment apply:

— Patients with evidence of disease should have diagnostic MIBG (usually with ^{123}I-MIBG) studies prior to all therapy, to establish the presence of MIBG-avid disease.
— Treatment is carried out semi-annually in adults, and more frequently in children, after recovery from all toxicity.
— Patients with negative diagnostic MIBG studies should not be treated.

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6.8. PHOSPHORUS-32 THERAPY IN POLYCYTHEMIA RUBRA VERA

6.8.1. Definition and diagnosis

Polycythaemia rubra vera (PRV) is a chronic haematological disorder characterized by increased proliferative activity of the erythroid, myeloid, megakaryocytic and fibroblast cell lines.

Elevated haematocrit and haemoglobin levels or RBC counts are insufficient for a diagnosis of PRV. An absolute increase in red cell mass must be shown by measurement using ^{51}Cr labelled autologous erythrocytes.

The following are treated as confirmatory evidence of PRV:

— Splenomegaly;
— Leucocytosis (>12 000/1);
— Thrombocytosis (>400 000/1);
— Elevated leucocyte alkaline phosphatase (LAP);
— Elevated serum unsaturated vitamin B_{12} binding capacity.

The following signs exclude PRV:

— Compensatory erythrocytosis;
— Abnormal haemoglobin;

— Erythropoietin secreting tumours.

6.8.2. Treatment

6.8.2.1. Radiopharmaceuticals

Sodium ^{32}P-phosphate is used for treatment of PRV; this is supplied as the orthophosphate, which is provided as a sterile non-pyrogenic solution of NaH_2PO_4.

6.8.2.2. Contraindications

Absolute: pregnancy, breast feeding;

Relative: women in their child bearing years.

6.8.2.3. Patient preparation

Patients to be considered for treatment should have failed treatment with venesection alone. Patients with PRV should be pretreated with venesection in order to reduce the haematocrit level to 42–47%. Chemotherapy must be discontinued.

6.8.2.4. Administration

Phosphorus-32 is administered by intravenous injection using a cannula; care should be taken to avoid extravasation. The administration should be performed in accordance with local regulations.

6.8.2.5. Dose

Two approaches are used:

(1) *Fixed approach*: Using a fixed dose method 2–3 mCi (74–111 MBq)/m^2 body surface area is administered, with an upper limit of 5 mCi (185 MBq). This dose can be repeated at intervals of three months.
(2) *Sliding scale approach*: Using this approach, a fixed dose of 3 mCi is first administered. If there is no response a second treatment may be given after three months, with a 25% increment in dose. Treatment may be repeated with continuing dose increments until an adequate response is obtained. The upper limit for a single treatment dose is 7 mCi.

6.8.3. Special precautions

Phosphorus-32 is excreted predominantly in the urine, although some faecal excretion does occur. Patients should be advised to observe rigorous hygiene for the first two days after administration, to avoid contaminating others using the same toilet.

6.8.4. Follow-up

Haematological profiles should be obtained at monthly intervals to assess the response. Phosphorus-32 is generally reserved for patients who cannot be relied on to take hydroxyurea according to instructions, and for the elderly. The increased risk of the development of acute myelogenous leukaemia in ^{32}P treated patients should be taken into consideration during follow-up.

6.9. RADIOSYNOVECTOMY

6.9.1. Clinical benefits

Radiation synovectomy, also known as synoviorthesis or synoviolysis, has become a well established method in the local therapy of inflammatory joint disorders. Many patients with chronic synovitis refractory to medical treatment respond to intra-articular radionuclide therapy. Primary treatment failures or relapses may be successfully treated by re-injection. Patients with less destructive radiographic changes, joint disease of shorter duration and localized disease tend to respond more favourably.

6.9.2. Physiological basis

The use of intra-articular radiocolloids to treat inflammatory arthritis was first reported as early as the 1950s using ^{198}Au-colloid. Normally, the synovial membrane is only a few cell layers thick. The villi have a secretory function and determine the amount and content of the synovial fluid that lubricates the joint. In inflammatory arthritis and the rheumatoid variants, inflammatory changes develop that increase vascularity and result in synovial layer proliferation, lymphocytic infiltration, effusions, fibrosis and pannus formation. The goal of the technique is to destroy the diseased pannus and inflamed synovium by direct irradiation, with the expectation that, following destruction, the regenerated synovium will be free of disease. Histological changes include reduction of cellular infiltrations and, eventually, sclerosis of the synovium.

While the initial results were not convincing, subsequent studies using ^{198}Au-colloid have confirmed the potential value of radiation synovectomy. In the last thirty years, several other radiocolloids have been developed using ^{90}Y, ^{32}P, ^{165}Dy, ^{166}Ho and ^{186}Re as radionuclides.

6.9.3. Indications and contraindications

The indications for radiosynovectomy are:

— Rheumatoid arthritis (with persistent effusions);
— Inflammatory joint diseases other than rheumatoid arthritis;
— Pigmental villonodular synovitis;
— Haemophilic joint disease;
— Chronic pyrophosphate arthropathy;
— Persistent effusion after knee prosthesis;
— Baker's cyst;
— Activated arthropathy;
— Polyarthrosis of finger joints.

The absolute contraindications for radiosynovectomy are:

— Pregnancy;
— Continued breast feeding.

The relative contraindications for radiosynovectomy are:

— Periarticular sepsis;
— Overlying cellulitis;
— Bacteraemia;
— An unstable joint;
— Intra-articular fracture;
— A septic joint.

6.9.4. Patient selection

Patients are eligible if there is inadequate relief after six months of conservative treatment with corticosteroids.

6.9.5. Patient preparation

Preparation should include the following:

(a) The signed informed consent of the patient is obligatory.
(b) The patient should undergo ultrasound (arthrosonography, 7.5 MHz) or MRI to evaluate the joint space as well as the structure of the synovia.
(c) A three phase bone (^{99m}Tc-MDP) scintigraphy must be performed to assess the degree of inflammation. Radiosynovectomy has been demonstrated to be successful only if a clear synovitis is indicated by three phase bone scintigraphy, especially in patients with arthrosis (or arthrosis–arthritis).

6.9.6. Radiopharmaceuticals

6.9.6.1. Colloids

Because of its deep tissue penetration, ^{90}Y-colloid is suitable for the knee and in joints with greatly thickened synovium. For joints of intermediate size (wrist, elbow, shoulder and hip) ^{186}Re-colloid has been successfully used and for the smallest joints (phalanges) ^{169}Er-colloid.

Yttrium-90 has been bound to silicate, citrate and ferric hydroxide compounds as colloids. Currently, it is most frequently used as ^{90}Y-citrate, which ranges in particle size from 10 to 100 nm. Leakage estimates for ^{90}Y-citrate range from 5 to 10% after 24 hours and from 15 to 25% after 4 days. Extra-articular radiation absorbed doses for liver (2.7 mGy/MBq) and regional lymph nodes are, therefore, quite high (270 mGy/MBq). Owing to its small particle size, and thus higher leakage, ^{198}Au is no longer recommended.

6.9.6.2. Dysprosium-165 macroaggregates

In order to reduce leakage from the synovial space, ^{165}Dy-ferric hydroxide macroaggregates have been applied for joint therapy. The particle size averages 5 μm and the activity that does leak from the joint quickly decays (with a half-life of ^{165}Dy of 139 min), thus reducing extraneous organ irradiation.

6.9.7. Dose and route of administration

It is assumed that intra-articular colloids are uniformly distributed over the joint surfaces. The most apparent problem is leakage from the joint space, primarily by lymphatic clearance, which depends largely on particle size. Leakage is reduced by a flushing injection of a long acting steroid (such as prednisolone acetate) after radiopharmaceutical injection. Table 6.4 provides an overview of the doses used successfully for radiosynovectomy.

TABLE 6.4. DOSES USED SUCCESSFULLY FOR RADIOSYNOVECTOMY[a]
(in units of MBq unless stated otherwise)

	Y-90	Re-186	Er-169
Half-life (days)	2.7	3.7	9.5
Maximum β energy (eV)	2.26	0.98	0.34
Mean distance (nm)	3.6	1.2	0.3
Shoulders	—	74	—
Elbows	—	55.5–74	—
Wrists	—	55.5–74	—
Thumbs	—	—	30
MCP[b]	—	—	22
PIP[c]	—	—	18.5
DIP[d]	—	—	15
Hips	—	185	—
Knees	185	—	—
Upper ankles	—	74	—
Lower ankles	—	37	—
Cuneonavicular joints	—	—	37
Tarsometatarsal joints	—	—	22
MTPI[e]	—	—	30
MTP[f] (others)	—	—	22

[a] Modified after G. Möder, Der Nuklearmediziner (1995).
[b] MCP, metacarpophalangeal joint.
[c] PIP, proximal interphalangeal joint.
[d] DIP, distal interphalangeal joint.
[e] MTPI, metatarsointerphalangeal joint.
[f] MTP, metatarsophalangeal joint.

Aseptic precautions must be followed. Biplanar radiographs with the joint positioned at the injection angle are mandatory to correlate palpable bone landmarks as a guide for needle placement. The needle is then inserted, taking care to avoid touching the cartilage. Following injection, the needle position is checked fluoroscopically using a few millilitres of contrast material. Alternatively, 40 MBq of ^{99m}Tc-SC can be injected prior to injection of the therapeutic dose. The joint is then imaged to ensure distribution throughout the joint space.

Following therapeutic injection, the needle is flushed with steroid or lignocaine. The joint is then manipulated through as full an arc as is possible of extension and flexion to distribute the particles throughout the joint space, following which it is splinted to minimize leakage.

6.9.8. Complications

Complications are rare.

6.9.8.1. Early complications

Early complications include:

— Transient increase in pain;
— Radiodermatitis at the injection site (best prevented by flushing with a steroid);
— Septic arthritis;
— Acute crystal synovitis;
— Transient lymphoedaema.

6.9.8.2. Long term complications

Long term complications include:

— Chromosomal aberrations in circulating lymphocytes;
— Chronic myeloid leukaemia (a single case);
— No cancers were found in any of the joints treated. One reason for this low incidence of cancers may be the short follow-up periods.

6.9.8.3. Treatment of side effects

Local side effects can be treated with corticosteroids. If pain increases during the first days after dose administration, local application of ice can be very helpful.

6.9.9. Requirements for a therapy ward

Therapy is usually carried out on an outpatient basis. Further details may be found in Sections 6.1 and 6.2.

6.9.10. Special precautions

Leakage through the needle tract and lymphatic clearance are the major mechanisms whereby radiolabelled colloids escape from joint spaces.

Special precautions are:

— Flushing the needle with a small volume of steroid or lignocaine;
— Prior injection of radiocontrast material or ^{99m}Tc-SC, to confirm correct needle placement;
— Immobilization of the joint for 48–72 hours;
— Reduction of lymphatic clearance by simultaneous injection of steroid;
— Use of short lived radionuclides (e.g. ^{165}Dy), to decrease the radiation burden to extrasynovial tissues.

6.9.11. Therapy control and follow-up

Six to eight weeks after radiosynovectomy a clinical evaluation should be conducted including a physical examination, measurement of haematological and biochemical parameters, a scintigraphic assessment of inflammation, and ultrasound and/or MRI examinations. Ultrasound investigations should be repeated at 4–6 month intervals.

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6.10. IODINE-131 LIPIODOL

6.10.1. Introduction

Iodinized (^{131}I) poppy seed oil (Lipiodol) is used as a treatment for hepatocellular cancer (HCC). This cancer is most common in South and South East Asia, although there are other areas with a high incidence including Mongolia and Latin America. The most commonly identified cause is chronic infection with hepatitis B or hepatitis C. Other contributing factors include alcohol abuse or other causes of cirrhotic liver disease.

Treatment options ideally include complete surgical resection and, if the tumour is large, liver transplantation. However, once the tumour is greater than 5 cm and if it is multifocal, the probability of a surgical cure is reduced. A compounding factor is related to the aetiology of HCC. In most cases this results in the liver around the HCC being abnormal, often cirrhotic, perhaps with a poor synthetic function. It is this combination of growing tumour and failure of the remaining liver that tends to kill the patient.

There are various series of clinical grades that have been used to determine the likelihood of a patient dying from HCC. Details of one of the easiest, that of Okuda, which dates back to the middle 1980s, are given in Tables 6.5 and 6.6.

TABLE 6.5. FACTORS USED TO ASSESS OKUDA GRADING

Tumour > 50% liver mass
Presence of ascites
Jaundice (bilirubin > 50 μmol/L)
Low albumin level (<30 g/dL)

TABLE 6.6. HOW FACTORS ARE USED TO GRADE PATIENTS
(after Okuda)

Number of factors	Okuda grading
None	1
1 or 2	2
3 or 4	3

Those patients with Okuda grade 1 disease have the best prognosis, many surviving for years, especially if given resection. Those with grade 2 disease tend to survive only if their liver disease is stable and if they have a complete surgical resection. The outcome for those with grade 3 disease is poor, with many surviving only a few weeks or months.

It is clear that patients in stage 1 may be resectable if they have no impairment of liver synthetic function, and those with grade 3 will not survive even with treatment. Therefore most effort in terms of treatment should be concentrated on patients with stage 1 and stage 2 disease. Radionuclide or other treatment should be offered if the patient is unresectable or if there is residual and/or recurrent disease after resection.

The traditional means of treating cancer does not appear to work well with HCC, and both chemotherapy and radiotherapy by external beams have been shown not to aid in the treatment of this disease. Tamoxifen was once held to reduce the rate of recurrence after surgery but once it was tested in a placebo controlled trial there was little evidence to support this view.

Lipiodol has been used for many years to image HCCs. Unlike normal healthy livers, most HCCs are supplied by the hepatic artery. It is therefore possible to cannulate the hepatic artery of a patient with a suspected HCC and infuse about 6–10 mL of Lipiodol, which will be taken up by any vascular tumour such as an HCC. (Note that this does not occur with metastases from carcinomas of the colon, which tend to be relatively avascular.) Such HCCs can be imaged by planar radiology or by CT. The cannulation does not need to be precise since the origin of the right hepatic artery will feed the right lobe and likewise the left will feed the left lobe. Many HCCs have daughters seeded throughout the liver that may be missed by a more highly selective method.

6.10.2. Targeting treatment with Lipiodol

As there is preferential accumulation of Lipiodol within HCC cells, it may be possible to use the Lipiodol to carry a lipophilic drug to the HCC cells. Two possibilities are Cisplatin and Epirubicin, both of which show in vitro activity against HCCs. Placebo trials have demonstrated an improvement in survival time if patients with unresectable HCC are treated with chemolipiodol. Nevertheless, there are significant side effects to the treatment that can last for about 10 days after treatment, namely pain, often requiring infusion of opioids, severe nausea and jaundice. Despite these problems, this remains the only form of treatment that can be offered to a wide range of patients. The chemolipiodol must be delivered into the artery feeding the HCC, a procedure that can only be performed in those centres having expertise in liver interventional radiology. It is possible to combine chemolipiodol with tumour embolism.

6.10.3. Radioactive treatments for HCC

A variety of isotopes have been suggested for the treatment of patients with unresectable HCC. Standard nuclear techniques include the use of radioimmunotherapy directed against the alpha-feto protein (AFP) expressed by the HCC cells. This approach has not reached clinical practice but may be a possibility in patients with disease outside the liver.

The other treatments including ^{131}I-Lipiodol require local delivery of the radiopharmaceutical into the cancer via an angiographic catheter. The main agents used are listed in Table 6.7.

Of these the potentially most useful may be the ^{188}Re based pharmaceuticals. Rhenium-188 is produced from a ^{188}W generator with a shelf life of six months; this means that theoretically it is possible to have an almost daily supply of ^{188}Re for use not only in HCC but also in bone metastases and in

TABLE 6.7. RADIONUCLIDES FOR LIVER CANCER THERAPY

Isotope	$T_{1/2}$	Maximum beta energies (MeV)	Carrier
Y-90	64 hours	2.03	Microspheres
I-131	8.04 days	0.28	Lipiodol
Re-188	16.9 hours	2.12	Lipiodol
Re-188	16.9 hours	2.12	Microspheres
Ho-166	23 hours	1.7	Chitosan

intravascular radiotherapy. Clinical trials are under way; 200 patients have received treatment, which is under review. The two commercially available products are ^{90}Y-microspheres and ^{131}I-Lipiodol.

6.10.3.1. General rules for treatment of HCC with radiotargeted therapy

Whatever agent is used, there are general rules and guidelines relating to the minimum and recommended requirements for any department wishing to pursue the treatment of HCC with ^{131}I-Lipiodol or a similar substance. *This treatment can only be administered by a dedicated team of doctors.* The most important prerequisite is the availability of clinicians with expertise in assessing HCC and its consequences; this is normally the case in a large university or regional hospital with experienced cancer department personnel. It is also essential to decide who (the first key team member) will deal with the patient after treatment and tackle any potential problems that may arise. These occur most commonly because of the condition of the liver around the tumour; in a patient with poor liver function a significant degree of liver failure, requiring expert supportive therapy, may occur during the treatment.

The second key team member is a competent radiologist with experience in identifying and cannulating the right and left hepatic arteries. This should be performed with a catheter of a reasonably wide bore such as a 5 French catheter. The type of catheter used will depend on local requirements but should have a Luer lock to enable connection of the syringes carrying the Lipiodol.

The third key team member is the nuclear medicine physician. This person should have experience in therapy and be able to obtain the required national or local approvals before starting treatment of patients with HCC. The present manual merely serves as a guide, and any physician performing these studies *should receive specific training* in this technique. Advice on training centres can be obtained from the Nuclear Medicine Section of the IAEA.

The fourth key team member is the physicist responsible for the safe handling of the product, monitoring the patient on the ward and calculating the dosimetry.

The physical requirements for the administration of ^{131}I-Lipiodol include:

(a) A radiopharmacy with a sterile cabinet or a laminar flow cabinet in which the ^{131}I-Lipiodol is diluted;
(b) A screening X ray room with real time imaging;
(c) A radionuclide therapy room with its own toilet facilities where the patient will need to be isolated for three to six days after treatment;

(d) Sufficient radiation monitors to allow monitoring of facilities and patients;
(e) A gamma camera with a high energy collimator in order to image the patient before discharge.

Once all these are in place, the use of ^{131}I-Lipiodol can commence.

6.10.3.2. Iodine-131 Lipiodol

Development of ^{131}I-Lipiodol started in the 1980s and was pioneered by members of a liver cancer team from Rennes, France. Although they were able to demonstrate the efficacy of the method both in open label trials and in a small trial comparing ^{131}I-Lipiodol and Cisplatin-Lipiodol, the mechanism for its utility was not clearly understood. More recent work on cell cultures of cell lines of normal liver cells and carcinoma of the colon and HCC cell lines showed uptake of the Lipiodol preferentially by the two cancer cell lines. When the cells were bathed in Lipiodol there was a normal cell survival after 24 hours; when bathed in ^{131}I there was again normal survival. However, when bathed in ^{131}I-Lipiodol at three different activities of ^{131}I-Lipiodol, all the cancer cell lines died while the normal hepatocytes had a 90% 24 hour survival. The reason that ^{131}I-Lipiodol does not work in colorectal cancer liver metastases is probably related to the poor blood supply of these metastases in vivo.

When comparing ^{131}I-Lipiodol with chemolipiodol, the Rennes group noted that when 1.8 GBq of ^{131}I-Lipiodol or 70 mg of Cisplatin-Lipiodol was given every four to five months to patients with a patent portal vein, overall survival rates were similar between the two groups. This was also true if the results were compared using the Okuda grading scheme. It was, however, clear that patients in Okuda grade 2 had a very poor prognosis despite treatment. This was confirmed by results from London in which ^{131}I-Lipiodol was compared with Epirubicin-Lipiodol in a total of 70 patients. These patients were given 0.9 GBq of ^{131}I-Lipiodol and had similar survival rates to those of the French patients in the Okuda stage 1 group. In the Okuda stage 2 patients, the survival of the London patients was worse in both treatment groups. There was, however, a significant difference in major side effects, these occurring in 15% of the ^{131}I-Lipiodol group, with discharge after three days related to radiation protection issues. In the chemolipiodol group, 70% had major side effects and discharge was after seven days, related to the need for supportive therapy for the patient. Patients were found to prefer treatment with ^{131}I-Lipiodol.

Recent work has used ^{131}I-Lipiodol in an adjuvant setting to treat patients with 0.9 GBq ^{131}I-Lipiodol six weeks after surgical resection. The theory for this treatment is that, as the liver starts to regenerate after surgery, microscopic daughter tumours can be stimulated. If these were pre-ablated by ^{131}I-Lipiodol, there would be a lower chance of recurrence. A Hong Kong group working on this question has shown that after 24 months there is a significant increase in both the disease-free interval and the overall survival in those receiving ^{131}I-Lipiodol compared with age matched controls. Unfortunately the numbers studied were small, and confirmation in a larger group of patients is required.

6.10.3.3. Patient preparation

Patients being considered for ^{131}I-Lipiodol must have a full understanding of the risks and possible benefits of the procedure, including the angiographic as well as the Lipiodol therapy.

In all patients, a diagnosis of HCC should be established or be strongly suspected. This can be based on the judgement of the hepatologist involved and on information from imaging and a raised AFP level in the presence of evidence for hepatitis B or C.

If a biopsy is required, a laparoscopic rather than a transdermal approach is generally recommended. The patient should not have a blocked portal vein and should have a tumour that is deemed non-resectable by a specialist liver surgeon. There should be no evidence of disease outside the liver on an abdominal and chest CT or in bone scintigraphy.

The patient should be clinically staged using the Okuda staging (or the Child–Pugh staging). Patients should only be treated if they are at Okuda stage 1 or 2.

In patients with a large right lobe tumour that is greater than 50% of the right lobe, evidence should be sought of a shunt, which would allow tracer to pass into the right lung. If there is any doubt, the patient should have a Lipiodol angiogram with non-radioactive Lipiodol, and Lipiodol seepage into the lungs should be sought by a chest radiography or CT. An alternative is to use ^{99m}Tc-MAA injected into the right hepatic artery and, with a gamma camera image, to determine the percentage activity seen in the lungs after two hours. If this is less than 5%, treatment should continue.

The patient should have normal clotting and a platelet count of more than 100 000 mm^{-3}. Platelet infusions can be given but should be discontinued two hours before the angiogram. Since the Lipiodol very rarely leaves the liver, and given the very high ratio of non-radioactive to radioactive Lipiodol, no blockage of the thyroid is required for this treatment.

6.10.4. Pharmaceutical preparation

Although it is possible to produce radioiodinated Lipiodol by passing ^{131}I gas through Lipiodol, it is not without danger as the gas is not only radioactive but highly corrosive. It is recommended that ^{131}I-Lipiodol be acquired from a reputable supplier.

Commercial Lipiodol contains 1.8 GBq in 2 mL and has a two to three day shelf life. This volume is too small for most liver tumours and it is advisable that the ^{131}I-Lipiodol be diluted in non-radioactive Lipiodol, to give a total volume of 6–12 mL depending on tumour size. The recommended activity is 0.9–1.8 GBq ^{131}I-Lipiodol.

If stored in a syringe, a polypropylene variety is recommended since it is important that the syringe does not dissolve in Lipiodol. If in doubt, non-radioactive Lipiodol should be placed in a syringe and the time taken for the plastic to melt measured. To be safe, the syringe should be intact and working after 24 hours. The syringe is capped with a non-dissolvable Luer lock cap.

6.10.5. Administration

The patient should be prepared for angiography in the radiology department. A right groin approach is most widely used. The syringe containing the ^{131}I-Lipiodol is taken to the angiography room in a lead container.

It is advisable to administer analgesia: 50 mg pethidine and 12.5 mg prochlorperazine intravenously before the relevant artery is cannulated. The Lipiodol can then be given over a period of three to five minutes via a non-dissolvable three way tap, attached between the syringe containing the ^{131}I-Lipiodol and the Luer lock of the indwelling catheter. The Lipiodol should be given slowly under fluoroscopic control. The rate should be sufficient to ensure delivery of the dose in five minutes, but not fast enough to cause reflux of the ^{131}I-Lipiodol into the gastroduodenal artery. The substance itself is very viscous and care must be taken during infusion. As it is radiolucent, the distribution of the ^{131}I-Lipiodol can be seen in fluoroscopic examinations. This infusion is performed with a plastic sheet between the syringe and the patient so that any spills will not result in contamination of the patient.

The infusion should be completed within five minutes or there is a danger of the catheter dissolving in the Lipiodol. If this starts to happen at any point during the infusion, the catheter should be removed and the infusion of Lipiodol stopped. When the last Lipiodol has been given, the catheter should be flushed with 10 mL saline and gently removed. All radioactive components (syringe, catheter, three way tap and the operator's gloves) are placed inside a

sealed plastic bag, marked as radioactive and stored in accordance with local requirements.

As is the case with all angiograms, haemostasis is achieved, although the radiologist should not stand close to the liver to do this.

Once the patient is removed from the fluoroscopy room, the drapes used on the patient are collected and put in a sealed plastic bag. This is monitored for contamination; if clear the drapes can be laundered, if not they should be stored until the activity is low enough for them to be cleaned. Monitoring of the room for contamination is also performed and any spills cleaned up.

6.10.6. Post-procedure care

Patients should remain in a supine position for eight hours after an angiogram. Vital signs should be monitored hourly; automatic monitoring devices are ideal for this purpose. After this time, patients may move around, eat and drink normally, and do as they wish within the confines of local radiation protection legislation. There may be some pain and fever 48–72 hours after a procedure, which can be treated with pain relievers and anti-pyrogens such as paracetamol. If fever persists beyond 24 hours, a source of infection must be sought.

Discharge will depend on the radiation levels allowed for discharge of patients who have received ^{131}I. Before discharge, anterior and posterior planar and if required SPECT scans are performed, using a high energy collimator. As activities are highly planar, imaging for 10 min is sufficient and SPECT can be performed in 20 min using a single headed gamma camera. Images should include ones of the liver and the lungs.

If more than 15% of the activity has passed into the lungs, this means that there is a significant shunt and re-treatment is not advised. Unless previously irradiated, the chance of radiation pneumonitis is low even at 1.8 GBq, as the radiation dose to the lungs rarely exceeds 12 cGy. Where there is significant lung uptake, patients should not be re-treated with Lipiodol. If there is any concern about lung radiation pneumonitis, a short two week course of steroids may help.

CT scanning after 10 days is also performed to check for residual uptake in the tumour.

6.10.7. Dosimetry

Dosimetric calculations are rendered difficult by the non-homogeneous nature of the tumour and its uptake of ^{131}I-Lipiodol. Using 0.9 GBq of

^{131}I-Lipiodol, however, the tumour can be expected to receive about 30 Gy with the normal liver receiving about 3 Gy. The bone marrow dose is negligible.

6.10.8. Further treatment

Patients can be treated at intervals of three months until there is no evidence of residual disease (normal CT, or PET if available and normal AFP).

Appendix to Section 6.10

TEAM REQUIREMENTS FOR ^{131}I-LIPIODOL THERAPY

Team member(s)	Minimum/ optimal	Requirements
Physician	Minimum	Interest in liver cancer Experience in treating liver cancer patients
	Optimal	Published research with established research team Knowledge of problems of intra-arterial therapy of the liver
Nursing/ social work/ research doctor	Minimum	Capability to arrange consent Capability to arrange investigations and follow-up (may need to arrange for patient to attend clinic/go to patient's home)
	Optimal	Established research in this field
Radiology	Minimum	A CT scanner and a capability to perform dual phase CT Capability of interventional radiologist to perform fluoroscopic examinations
	Optimal	A spiral CT with capability for triple phase CT A trained interventional radiologist with experience of Lipiodol CT, intra-arterial injection chemotherapy and embolization

Team member(s)	Minimum/ optimal	Requirements
Radiopharmacy	Minimum	Radiopharmacy capability either on-site or one that can be shipped in If radiopharmacy is on-site: A laminar flow cabinet Standard quality control testing (e.g. ITLC) Limulus testing for presence of pyrogens Adequate shielding for high energy or activity Radiopharmaceuticals Dose calibration (+ standards for quality control)
	Optimal	Radiopharmacist
Nuclear medicine	Minimum	A nuclear medicine physician with experience of therapy A gamma camera with regular quality control programme A high energy, general purpose collimator A computer capable of storing images
	Optimal	A medium energy collimator (because of scatter!) SPECT whole body imaging Capability to transfer data from gamma camera via the Internet
Medical physics	Minimum	Medical physicist Capability to coordinate dosimetry Either in-house or with core laboratory Agreement to perform standardized dosimetry A computer with capability to perform dosimetry Capability to collect data and archive in a standardized way Capability to monitor patients on ward and give advice on discharge
	Optimal	Experience in therapy and dosimetry
Patient facilities	Minimum	An individual room or area for radioactive patients Nursing staff trained in radioactive therapy
	Optimal	Own toilet and shower facilities

Team member(s)	Minimum/ optimal	Requirements
Miscellaneous	Minimum	Good data collection facilities Capability to collect data and fill in work sheets (case report form) contemporaneously Provide patient information and consent in patient's language Obtain institutional/national ethical approval for study Obtain required licenses for holding/ administration and storage of radiopharmaceuticals

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6.11. INTRACORONARY RADIONUCLIDE THERAPY USING THE Re-188 DTPA BALLOON SYSTEM

6.11.1. Introduction

Percutaneous coronary angioplasty is an established therapeutic modality in the management of atherosclerotic coronary artery disease, although the high restenosis rate of 30–50% limits its usefulness. In recent years, much has been learned about the mechanism of restenosis. Recoil and remodelling involve the mechanical collapse and constriction of the treated artery. This has

been virtually eliminated by luminal scaffolding using coronary stents. Coronary stenting reduces the likelihood of restenosis by approximately 30%. The principal mechanism of restenosis, intimal hyperplasia, is the proliferative response to injury of a vessel wall, which consists largely of smooth muscle cells.

A large body of animal investigations and a more limited number of clinical studies have established the ability of ionizing radiation to reduce significantly neointimal proliferation and the restenosis rate. It has been reported in human studies that intravascular radiation after first restenosis inhibits a second restenosis.

Various modalities for intravascular radiation based on radiation sources and delivery systems have been proposed. Beta emitters are safe, deposit a large fraction of their energy locally and are preferable to gamma emitters for both operator and patient. Catheter based radiotherapy with beta emitting, nuclide filled balloons provides a safe, technically simple and inexpensive means to deliver therapeutic radiation. The balloon conforms to the vessel geometry in an optimal fashion and naturally locates in the centre of the lumen during inflation.

6.11.2. Clinical indications and contraindications

(a) Indications

Intracoronary radionuclide therapy (ICRNT) is used to prevent coronary artery restenosis. Intracoronary radiation using a ^{188}Re-DTPA filled balloon system is indicated for the treatment of in-stent restenosis, de novo lesions in patients with diabetes mellitus and saphenous vein graft lesions. Possible indications include treatment of long lesions, small vessel lesions and any restenotic lesions.

(b) Contraindications

Acute myocardial infarction within two weeks.

6.11.3. Procedure

6.11.3.1. Medication

Once the patient has been admitted to hospital, the informed consent of the patient must be obtained for administration of the following medications:

— Aspirin, 300 mg orally four times a day;
— Ticlopidine, 250 mg orally twice daily, starting at least three days before the procedure and continuing indefinitely after the procedure;
— Heparin, to maintain an activated clotting time of over 300 s during the procedure;
— Vasodilators such as calcium antagonists or oral nitrates, provided that there is no adverse effect of the drugs.

6.11.3.2. Eligibility criteria

(a) Initial inclusion

Patients must have atherosclerotic coronary artery disease and be eligible for percutaneous transluminal coronary angioplasty (PTCA) as follows:

— Presence of significant stenosis (>75%; reference diameter of arteries: 2.5–4.0 mm) of a de novo lesion, vein graft or restenotic lesion;
— Lesion length of less than 20 mm.

(b) Final inclusion

The eligibility criteria for final inclusion are:

(1) The initial inclusion criteria must be satisfied.
(2) An angiographically successful PTCA is essential, i.e.:
   — Post-PTCA residual stenosis on quantitative coronary angiography (QCA) of less than 30%;
   — No procedure related complications such as a major dissection, rupture or thrombus.

(c) Intervention procedure

The intervention procedure has the following steps:

(1) Coronary angiography and angioplasty are carried out using standard methods.
(2) QCA is carried out two minutes after intracoronary administration of 200 μg of nitroglycerine, before and after ICRT.
(3) An intracoronary ultrasound (ICUS) examination is optional.

Both QCA and ICUS will measure the reference diameter and the minimal luminal diameter; ICUS additionally evaluates the cross-sectional areas (CSAs) of the lumen, internal elastic lamina and external elastic lamina or stent.

The precautions to be taken in handling ^{188}Re are covered in Section 6.2 and preparation of ^{188}Re-DTPA is covered in Section 3.4.

6.11.4. Preparation of brachytherapy devices

A transparent Lucite box is used for shielding the radioactive source during the procedure. The box is wrapped with a transparent vinyl covering and the syringe containing the radioactive source is shielded by a transparent Lucite cylinder. All other unshielded devices containing the radioactive source are manipulated with forceps. The lumen between the radioactive source and the indeflator is filled with mineral oil.

6.11.5. Intracoronary radiation

Brachytherapy immediately follows successful angioplasty. The length of the ICRNT balloon is 10 mm longer than that for the PTCA with the same diameter. The centre of the ICRNT balloon is located at the centre of the successfully dilated lesion, so the irradiated area includes segments 5 mm proximal to 5 mm distal to the injured lesion during PTCA.

After the balloon has been located at the target site, a negative pressure is achieved with the syringe connected to the manifold and containing the liquid Re-DTPA source. The manifold channels are adjusted to communicate from indeflator to balloon. Inflation pressure is limited to 6 atm. The duration of balloon inflation for irradiation is 300–600 s depending on radioactive source activity and the size of the balloon. The session is divided between 1–2 min of inflation and 30 s of deflation for coronary perfusion, adjusted to the tolerance of the patient. The balloon and syringe containing radionuclide are discarded as radioactive waste.

6.11.6. Monitoring of radiation exposure and environmental contamination

Throughout the whole procedure, the radiation exposure to the operator and patient should be monitored with a survey meter. The patient, staff, angiography table and room are investigated for possible residual radioactivity after removal of the radionuclide from the catheterization laboratory.

6.11.7. Follow-up

Patients are evaluated six months after the procedure with quantitative coronary angiography and ICUS where available, to evaluate late effects. The measurements specified above are obtained again and net losses in cross-sectional area and lumen diameter are evaluated.

Further follow-up includes repeated ICRNT. The value of repeat therapy is not known at the current time.

6.12. RADIOPEPTIDE THERAPY FOR CANCER

6.12.1. Clinical indications

Radiolabelled peptides have been used in the therapy of peptide-avid cancers refractory to conventional therapy. These cancers usually express somatostatin receptor subtypes.

6.12.2. Physiological basis

The high expression of peptide receptors on various tumour cells compared with normal tissues or normal blood cells has provided the basis for the clinical use of radiolabelled peptides in the therapy of these cancers. Receptor scintigraphy using radiolabelled peptide ligands, in particular SST and VIP analogues, is established in clinical practice.

Malignant cells of neuroendocrine origin express a high number of receptors for various hormones and peptides. Among these, binding sites for members of the SST family (hSSTR1–5) are frequently found, and their expression has led to attempts to specifically target these receptors. Initial results have indicated the clinical potential for receptor targeted radiotherapy. SSTR scintigraphy using ^{111}In-DTPA-(D)Phe^{1}-octreotide has a high positive predictive value for the vast majority of neuroendocrine tumours and has gained its place in both the diagnosis and the follow-up of patients. A large variety of other tumours have also been found to express an hSSTR subtype.

On the basis of SST receptor (SSTR) recognition, the radiopharmaceuticals ^{111}In/^{90}Y-DOTA-lanreotide, developed at the University of Vienna, and ^{111}In/^{90}Y-DOTA-(D)Phe^{1}-Tyr^{3}-octreotide (Novartis) show promise for the diagnosis and treatment of hSSTR positive tumours. Initial results with ^{90}Y-DOTA-lanreotide and ^{90}Y-DOTA-(D)Phe^{1}-Tyr^{3}-octreotide have pointed out the clinical potential of radionuclide receptor-targeted radiotherapy. Such therapy offers the promise of improved pain palliation and disease control at a

cost much less than that of conventional chemotherapy. Receptor mediated radiotherapy with ^{90}Y-DOTA-lanreotide and ^{90}Y-DOTA-(D)Phe^{1}-Tyr^{3}-octreotide might also be effective in patients refractory to conventional treatment strategies.

6.12.3. Indications and contraindications

(a) Indications

Patients with tumours known to express SSTR, refractory to conventional treatment strategies, are eligible, provided their tumours demonstrate uptake of radiolabelled ligand on scintigraphy. A typical scenario is neuroendocrine tumours that do not, or no longer, take up ^{131}I-MIBG but are hSSTR positive, or non-iodine-avid thyroid cancer that is hSSTR positive.

(b) Contraindications

— Pregnancy or breast feeding;
— Tumours that do not show sufficient sstr ligand uptake.

6.12.4. Patient preparation

The following steps need to be taken:

(a) The patient's informed signed consent should be obtained.
(b) The patient's disease should be staged as per WHO criteria, using conventional CT or MRI, radiography, colonoscopy, or surgery.
(c) Long acting SST analogues should be discontinued for at least three days prior to therapy initiation.
(d) Patients should be evaluated with ^{111}In labelled sstr ligand.
(e) Serial ^{111}In images are used to calculate the residence times of radioligand in the tumour, and organ doses are calculated by standard dosimetry (Section 6.3). Therapy is considered at tumour doses of 10 Gy/GBq or higher, and the amount of ^{90}Y is calculated so as to deliver not more than 30 Gy to the kidneys, the critical normal organ. Organ dose calculation is carried out with MIRDOSE3 software.
(f) Some groups have used lysine infusion starting prior to radiopeptide therapy in order to minimize renal accumulation of radioactivity.

6.12.5. Radiopharmaceuticals

Three commercially available SST analogues (i.e. octreotide {(D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr(ol)}, lanreotide {(D)βNal-Cys-Tyr-(D)Trp-Lys-Val-Cys-Thr-NH_2} and vapreotide {(D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-Trp-NH_2}) have been shown to be effective in controlling the growth of some human tumours. These all have similar binding profiles for four of the five hSSTR subtypes (i.e. a high affinity for hSSTR2 and hSSTR5, a moderate affinity for hSSTR3 and a very low affinity for hSSTR1). Lanreotide and vapreotide have a moderate affinity for hSSTR4, whereas octreotide has little or no affinity for this hSSTR.

Both DOTA-lanreotide and DOTA-(D)Phe^1-Tyr^3-octreotide are research agents currently under investigation.

6.12.6. Dose and route of administration

A 1 GBq therapeutic dose of ^{90}Y-DOTA-lanreotide is administered intravenously over 30 min every four weeks. The cumulative radiation absorbed dose to the kidneys should not exceed 30 Gy, or 6–8 GBq ^{90}Y-DOTA-lanreotide. Patients are monitored for side effects using standard WHO criteria.

The therapeutic dose of ^{90}Y-DOTA-(D)Phe^1-Tyr^3-octreotide is 1–3 GBq/m^2; dose escalation is ongoing.

6.12.7. Side effects

Side effects involve the critical organs, bone marrow and kidneys. Reversible haematopoietic toxicity has been seen at higher cumulative doses; no acute renal dysfunction has been seen. Further investigations into long term follow-up need to be carried out.

6.12.8. Follow-up

Patients are monitored at least weekly for a minimum of eight weeks to evaluate toxicity. Repeat scintigraphy for the evaluation of receptor-positive disease is undertaken at intervals of two months. Response evaluation is carried out at quarterly intervals, using identical parameters to those obtained at baseline.

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6.13. RADIOIMMUNOTHERAPY

6.13.1. Introduction

Radioimmunotherapy is a treatment modality, currently under investigation, which uses radiolabelled antibodies in the therapy of cancer. This section provides an overview of the current status of radioimmunotherapy and outlines the practical considerations.

6.13.2. Physiological basis

Monoclonal antibodies against a variety of tumour associated antigens have been developed and shown to target tumours with minimal side effects. Numerous radionuclides have been conjugated to antibodies and the radioimmunoconjugates have been shown to be stable in vivo.

On the basis of the hypothesis that radiolabelled antibodies will deliver cytotoxic radioactivity selectively to tumours, patients with cancer have been treated with potentially therapeutic quantities of radioactivity conjugated to antibodies. Most studies have used radionuclides emitting β^- particles; a few studies have involved alpha emitters or radionuclides that decay by electron capture.

6.13.3. Indications

Radioimmunotherapy against lymphoma and leukaemia has been shown to result in major responses in the majority of patients treated, even in chemotherapy-refractory disease. There have been few major responses in solid tumours, at least at doses that are non-myeloablative. The dose limiting toxicity has been myelosuppression.

Initial clinical radioimmunotherapy trials were carried out with murine antibodies. Administration of these proteins usually resulted in an immune response, precluding multiple administrations. A significant exception has been radioimmunotherapy using murine antibodies in patients with B cell lymphoma. Developments in genetic engineering have led to the creation of antibody constructs that are less immunogenic, offering the promise of repeated therapy.

6.13.4. Contraindications

Pregnancy and/or lactation are absolute contraindications.

The safety of antibodies in children has not been conclusively demonstrated; the relative risk should be measured against the potential benefit of such a therapy in treating cancer.

6.13.5. Equipment

When radioimmunotherapy is carried out with beta emitting nuclides that also emit photons, demonstration of tumour targeting is carried out by gamma camera scintigraphy. There are no standard protocols for such image acquisition. As a rule, tumour targeting is more evident at later time points: antibodies are large proteins that clear slowly from circulation, and tumour to background ratios are higher at later time points.

No special equipment is required for outpatient therapy, which is usually carried out using pure β^- emitting radionuclides. Most radioimmunotherapy trials have used antibodies conjugated with ^{131}I, and appropriate monitoring of

the patient is mandatory to ensure compliance with radiation safety criteria for outpatient therapy.

Higher doses of radiolabelled antibodies that emit gamma radiation should be administered in areas that meet radiation protection requirements. Details of these are given in Section 6.2.

6.13.6. Radiopharmaceuticals

Antibodies have been conjugated with a variety of radionuclides including ^{131}I, ^{90}Y, ^{186}Re, ^{188}Re, ^{67}Cu, ^{125}I, ^{211}At and ^{213}Bi.

Intact immunoglobulins, usually IgG (K_d ~ 150 000), have been used in most radioimmunotherapy trials.

The route of administration is usually intravenous; a few radiolabelled antibodies have also been administered by the intracavitary (intrapleural or intraperitoneal) route; intralesional injections have been studied, especially in intracranial neoplasms.

Iodine-131 labelled antibodies have been studied extensively. Iodine-131 has a moderate energy beta emission, and its therapeutic efficacy has been well documented in thyroid carcinoma. Its gamma emission of 364 keV also permits external detection, allowing measurement of radiation absorbed dose. Current treatment of ^{131}I labelled anti-CD20 antibodies in B cell lymphomas is based on whole body radiation absorbed dose, with an amount of ^{131}I calculated to deliver no more than 0.75 Gy whole body radiation absorbed dose, in patients with platelet counts of more than 100 000 mm^{-3}.

Yttrium-90 labelled antibodies are usually administered on the basis of body weight or surface area. The energy of the β^- emission of ^{90}Y is three times that of ^{131}I; the lack of a photon permitting external measurement has precluded direct evaluation of the ^{90}Y biodistribution; this is usually carried out using ^{111}In as a surrogate. To reduce the irradiation of normal tissues, three phase radioimmunotherapy has been found to be of benefit in brain tumours and liver metastases.

6.13.7. Therapy

6.13.7.1. Action prior to therapy

The following steps need to be taken:

(a) The patient's informed signed consent for therapy should be obtained.

(b) Patients who are to receive ^{131}I labelled antibodies must receive oral SSKI or Lugol's iodine, up to 200 mg/day in adults and 3 mg/(kg·day) in children. This should be continued for at least two weeks after therapy.

6.13.7.2. Therapy

As radioimmunotherapy is currently experimental, there should be strict adherence to protocol as approved by the hospital ethics or other oversight committee. Radiation safety precautions should be stringently observed, with particular attention paid to the physiological route of excretion of unbound radionuclides. Where applicable, gamma camera imaging to demonstrate tumour targeting must be undertaken.

6.13.7.3. Post-therapy follow-up

Monitoring of the patient for possible side effects, particularly allergic reactions and myelosuppression, should be carried out based on the characteristics of the radioimmunoconjugates under study.

An evaluation of the extent of disease should be carried out prior to therapy, and again following recovery from therapy related toxicity, to assess the response.

Chapter 7

QUALITY ASSURANCE AND QUALITY CONTROL PROTOCOLS FOR RADIOPHARMACEUTICALS

7.1. INTRODUCTION

All radiopharmaceuticals administered to patients must have the safety, quality and efficacy required for their intended use. The employment of short lived radionuclides in radiopharmaceuticals poses problems in quality control testing, since it is not possible to complete the necessary quality control testing before the product's use-by date. This makes it imperative to employ a range of quick validation techniques in order to test the final product; these techniques are outlined in this chapter. A quality assurance programme that takes into account all aspects of preparation is the best way to guarantee a product of the required quality.

7.2. REQUIREMENTS FOR DOCUMENTATION

The way a radiopharmacy functions should be set out in documentation and this documentation should be readily accessible. Three fundamental areas are:

(1) Definition of the standards to which the radiopharmacy operates;
(2) Standard operating procedures that define the methods to be used;
(3) Records of all the work performed.

The standard operating procedures need to cover not only the preparation techniques used but also the required environmental parameters and storage conditions, as well as a definition of the way the facilities should be used and maintained, for example operation and checking of radionuclide assay calibrators and safety cabinets.

This documentation provides evidence that the department has operated according to its defined standards and also permits the reader to trace the history of all the products it has prepared. This is an expected function in good manufacturing practice and is particularly important in departments preparing ^{99m}Tc radiopharmaceuticals where materials are administered to patients very

soon after preparation. In addition, records of receipt and disposal of radioactive materials must be kept in accordance with national legislation.

There should be a regular process of review of the documentation to ensure that it is still appropriate and also that all the necessary records are being maintained. Review of retrospective quality control testing records is critical to ensure that the methods and materials used are consistently producing products of the required standard.

7.3. CONTROL OF STARTING MATERIALS

One of the major aspects of quality is the source and purity of the non-radioactive starting materials. These include: components of kits for technetium radiopharmaceuticals, target materials for use in nuclear reactors or cyclotrons, adsorbents used in columns inside radionuclide generators, and eluents and diluents used in the preparation of the final product. Since these marerials are non-radioactive, it is possible to carry out extensive testing of their quality in the same way as for normal pharmaceutical products. Some of the techniques and equipment required will not be readily available in a hospital radiopharmacy or nuclear medicine department (e.g. mass spectroscopy and nuclear magnetic resonance spectroscopy). It is therefore prudent to purchase materials from radiopharmaceutical manufacturers where possible, since they will have performed quality control procedures on the materials they are supplying. In an increasing number of countries, there is now a mechanism that controls the release of pharmaceutical products, including radiopharmaceuticals and radiopharmaceutical kits, to the market. Such controls will include checking the systems that manufacturers use to ensure the quality of their products. Although this increases the cost to the user at least part of this extra cost is offset by improved quality.

Where the whole manufacturing process is performed in-house, a greater degree of responsibility for quality has to be assumed by the producer, and comprehensive testing of the raw materials is necessary. This is particularly true where the synthesis of non-radioactive components takes place prior to their incorporation into radiopharmaceuticals or radiopharmaceutical kits. Testing may require the use of analytical techniques such as infrared and ultraviolet spectroscopy, mass spectroscopy and nuclear magnetic resonance, and the department should ensure that it has access to such facilities. Details of the synthesis and analysis of certain kits are provided in IAEA-TECDOCs 649 and 805 (included in the bibliography to this chapter). Information on the specifications that radiopharmaceuticals should meet is also available in national and international pharmacopoeias.

Where radiopharmaceuticals are purchased in their final form and the hospital or clinic performs no manipulation, the main determinant of quality is the supplier. If the product has been approved for marketing by an appropriate authority, the user department should have little or no testing to perform on it. Continued satisfactory use of the product enables the user to build up confidence in the quality of the supplier.

7.4. RADIONUCLIDIC ACTIVITY

It is necessary to ensure that the correct activity is administered to the patient. Accurate measurement must take place during the preparation of radiopharmaceuticals and the dispensing of individual doses. There is therefore a requirement for control of the dose calibrator to ensure its correct functioning and accuracy. Details of how this can be achieved are given in Sections 3.4, 4.5.1 and 8.2. In routine use, great reliance is placed on the calibration factors provided by the manufacturer of the calibrator. Particular care is needed when measuring certain nuclides that emit low energy radiations, for example ^{123}I and ^{111}In. Attenuation of these low energy radiations will occur to a greater extent when measuring the activity inside a glass vial than in a plastic syringe. Therefore the activity measured in the syringe may appear to be higher than that present in the vial from which the nuclide was dispensed. In such circumstances it is advisable to measure the vial before and after dispensing the radiopharmaceutical into the syringe. The difference between the readings gives a more reliable indication of the dispensed activity.

7.5. RADIONUCLIDIC PURITY

Radionuclidic purity is defined as the percentage of the activity of the radionuclide concerned to the total activity of the sample.

All radioactive materials are likely to have some radionuclidic impurities, albeit at very low levels, which can make their determination difficult. The situation most relevant to hospitals and clinics is the determination of levels of ^{99}Mo in ^{99m}Tc eluted from a generator. Fortunately, this can readily be determined by a screening method since the principal gamma energy of ^{99}Mo (740 keV) is much higher than that of ^{99m}Tc (140 keV). The total activity of a sample is measured in the normal way in a dose calibrator. The sample is then placed inside a lead pot 6 mm in thickness, which attenuates virtually all the 140 keV gamma rays of technetium but only approximately 50% of the

740 keV gamma rays of ^{99}Mo, and the activity is remeasured using calibration factors supplied with the instrument. It is then possible to calculate the amount of ^{99}Mo present and express this as a percentage of the ^{99m}Tc. Most pharmacopoeias have a limit of 0.1% of Mo at the time of administration, and any eluates that exceed this limit must not be used. The determination should therefore be carried out on the first eluate of a generator and on other eluates as deemed necessary.

Other possible radionuclidic contaminants of the ^{99m}Tc eluate arise from impurities in the ^{99}Mo used and will vary according to the method used in its production. Molybdenum-99 produced by fission of ^{235}U can contain very small amounts of ^{131}I, ^{103}Ru and nuclides of strontium that are not likely to be present in ^{99}Mo which is produced by neutron irradiation of ^{98}Mo. The measurement of these impurities normally has to be performed on an eluate that has been allowed to decay sufficiently, and also requires specialized equipment that is likely to be beyond the means of routine radiopharmacy and nuclear medicine departments.

7.6. RADIOCHEMICAL PURITY

The radiochemical purity is defined as the proportion of the total radioactivity of the nuclide concerned present in the stated chemical form. For many radiopharmaceuticals the radiochemical purity will be expected to be greater than 95%, but this is not universally so. For radiopharmaceuticals purchased in their final form, manufacturers will normally declare the radiochemical purity and the radiopharmacy may not need to perform any further determinations. For materials prepared in-house, either totally from original materials or purchased kits, radiochemical purity determinations are useful to establish the suitability of the final product. Low radiochemical purities may lead to an unintended biodistribution of the radiopharmaceutical. For diagnostic agents, this may lead to confusion in the diagnosis and for therapeutic radiopharmaceuticals it can produce significant dosimetric problems. A range of techniques is available for such determinations, but the techniques must be reliable and simple, and preferably rapid, to perform such that, in an ideal situation, the radiochemical purity of materials containing short lived radionuclides can be established prior to their administration.

The simplest and most widely used technique is that of planar chromatography, using suitable stationary phases (e.g. paper or thin layers of silica gel) and readily available mobile phases (e.g. saline, acetone and butanone). The choice of stationary and mobile phases is determined by the nature of the radiopharmaceutical, and must be such that the various radiochemical species

have different mobilities. Suitable systems for a range of radiopharmaceuticals are given in IAEA-TECDOCs 649 and 805 (included in the bibliography to this chapter). The techniques can be carried out with very simple apparatus, for example with beakers or measuring cylinders as chromatography tanks; in view of the scale of the operation only small volumes of solvent are needed. The levels of each species can be determined by scanning the stationary phase with a suitable detector or cutting it into sections and placing each in a counter. However, the limitations of these simple systems need to be borne in mind, since in many of them only certain impurities (e.g. pertechnetate in Tc radiopharmaceuticals) migrate with the solvent. Most of the activity may remain at the point of application on the chromatography strip and thus be unresolved. The determination is therefore more correctly described as radiochemical impurity determination, since the exact chemical nature of the species remaining at the point of application has not been determined.

Alternative techniques such as electrophoresis or HPLC offer advantages in that they can give more precise information about the radiochemical nature of the species present. Commercial manufacturers of radiopharmaceuticals use HPLC routinely. The technique utilizes the separating power of adsorbent materials packed into stainless steel columns through which a solvent is pumped at high pressure. Different radiochemical species are identified by monitoring the eluate from the column and noting the time at which radioactivity is detected. This technique has limitations in that the apparatus is expensive and may not be routinely available to hospital radiopharmacies. In addition, certain radiochemical species, for example hydrolyzed reduced Tc in Tc radiopharmaceuticals, may be retained on the column used to achieve the separation and may not therefore be accounted for in the analysis.

Recent developments have included the introduction of cartridges containing the same adsorbents used in HPLC, but which can be loaded and eluted with syringes. By using appropriate eluents, different species can be selectively removed from the cartridge and, providing a sufficiently high radioactive concentration is used, activity can be determined with a dose calibrator or other simple scaler. Thus the hospital radiopharmacy can benefit from the resolving power of adsorbents used in HPLC, but without the expense of the equipment required.

7.7. CHEMICAL PURITY

In addition to the problems of ensuring the correct chemical purity of starting materials for radiopharmaceuticals, there are certain situations where the chemical purity of the final material can be affected by the process used in

the preparation. The most likely situation to be met in radiopharmacies is the presence of Al ions in Tc radiopharmaceuticals. These can arise from alumina being washed off the columns used in Tc generators. Very high levels of Al can be toxic to patients, but it is unlikely that such problems will arise from administration of a radiopharmaceutical. However, lower levels can adversely affect radiopharmaceutical formation or stability, for example of colloidal radiopharmaceuticals, where the trivalent Al cation can alter the surface charge of particles and lead to aggregation and hence an altered biodistribution.

Aluminium can be detected by a simple colorimetric limit test, using either a solution or indicator strips containing an Al sensitive marker such as chromazurol S. By comparing the colour obtained with a small volume of the eluate of a Tc generator and that from a solution containing a specified concentration of Al ions (generally 5 or 10 parts per million), it can be determined that the Al content of the eluate is below the specified level and hence suitable for use. Metal impurities may reduce the efficiency of ^{111}In radiolabelling.

7.8. DETERMINATION OF PARTICLE SIZE

Lung imaging agents are normally based on macroaggregates of human albumin. A particle size range of 10–100 μm is generally specified as being optimal. Some pharmacopoeias state that there should be no particles larger than 150 μm. Particle size can be determined by light microscopy, using a graduated slide to ensure that there are no oversize particles and that a suitable range of sizes is present. The limitations of the method are that it is usually only possible to observe a limited number of particles and that prolonged observation subjects the eyes to an increased radiation burden. These limitations can be overcome by reconstituting a macroaggregate kit with saline and observing non-radioactive particles.

Colloidal particles cannot be visualized by normal light microscopy and, in situations where it is important to know the particle size distribution, more elaborate techniques such as light scattering or membrane filtration will have to be used. These may not be readily available in hospital radiopharmacies.

7.9. PARTICULATE CONTAMINATION

Products for parenteral administration should be free from gross particulate contamination. The use of clean glassware, kits, reagents and equipment is the best way to minimize contamination. However, on occasions, particles can be present in the final solution as a result of coring of the rubber

stopper if it is repeatedly punctured. Control can be exercised by visual inspection of the final radiopharmaceutical, while ensuring that adequate measures are taken to protect the eyes. The required level of protection can be achieved by viewing through lead glass screens or by using mirrors to view vials placed behind lead shields. It should be pointed out that such techniques may not detect small amounts of particulate contamination and are not suitable for radiopharmaceuticals which themselves are particulate.

7.10. CONTROL OF pH

For some radiopharmaceuticals, control of pH is essential to ensure they retain their original specification. For example indium (^{111}In) chloride must be maintained at a pH of 1.5. If the pH rises, the material becomes colloidal and unsuitable for labelling reactions. With Tc compounds, the chemical composition and hence biodistribution of DMSA complexes is affected by the final pH of the solution. The normal renal imaging agent must be maintained at a pH below 3.5.

The easiest method of determining pH is to use narrow range pH papers, since only small samples are needed. Papers are readily available from a variety of sources. Assessment of pH is subjective and such papers are normally only accurate to about 0.5 of a pH unit. For the majority of radiopharmaceuticals these limitations are not normally detrimental.

7.11. STERILITY AND APYROGENICITY

Radiopharmaceuticals administered parenterally need to be sterile and apyrogenic. Although these objectives can be achieved by the use of a suitable sterilization technique during preparation of the radiopharmaceutical, it is often necessary to use an aseptic technique to prepare the final radiopharmaceutical, having started with sterile materials (e.g. kits and generator eluate). Control of the environment in which such manipulations take place is important. Sterility testing of radiopharmaceuticals presents difficulties and it is often impracticable to apply tests described in pharmacopoeias; this is not only because of the radioactive nature of the material but also, as is the case with Tc radiopharmaceuticals, because the batch may consist of a single container. This introduces serious problems with sample sizes and makes the test statistically unsatisfactory. In addition, there is evidence that micro-organisms do not survive in Tc radiopharmaceuticals and hence allowing them to decay in order to make testing easier can reduce the value of the test. As a

compromise it is probably better to withdraw a small sample of the radiopharmaceutical whilst it is still active and place it in a suitable culture medium that can be shielded until decay has occurred. It can then be incubated in the normal way.

Alternatively, for Tc radiopharmaceuticals, the culture medium can be added to the remnants of the kit vial at the end of the working day. The vial is kept shielded until inactive and then incubated. Inevitably this means that the result of the test is only obtained retrospectively. In view of these limitations, a more satisfactory technique to ensure sterility of aseptically prepared radiopharmaceuticals involves staff simulating exactly the preparation techniques using culture media. Such tests have the advantages of being more sensitive and of using non-radioactive materials, and can be performed earlier.

Determination of the apyrogenicity of injections is currently only required when the volume administered exceeds 15 mL. This rarely occurs with radiopharmaceuticals and hence the test is not usually performed in hospital radiopharmacies. If a hospital is involved in the development of new agents, it may be prudent to assess the apyrogenicity, particularly if materials of animal origin are used in the preparation. The use of the limulus lysate test for pyrogens is now becoming widely accepted in preference to the rabbit test, but rigorous controls must be used to validate the test. Commercial manufacturers frequently use the limulus lysate test in the control of their materials.

7.12. ONGOING EVALUATION OF PRODUCT PERFORMANCE

Diagnostic radiopharmaceuticals of appropriate quality should have a defined biodistribution within patients. If such observations are made regularly, confidence in the quality of the materials being administered to patients is gained. When nuclear medicine images are reported, unexpected biodistributions are sometimes observed and may result from problems with the radiopharmaceutical, or alternatively may be due to the patient's condition or even the medication the patient may be taking. It is worth trying to determine the cause of the problem. If the problem has occurred with all patients who received that particular batch of radiopharmaceutical, the problem is likely to lie with the product. An example is the visualization of the stomach in patients undergoing bone imaging with a technetium phosphonate complex. This indicates the presence of pertechnetate in the radiopharmaceutical and may have arisen as a result of an incomplete reaction when preparing the kit or of instability after preparation. If this occurs on a regular basis with different batches of the same radiopharmaceutical, action is necessary to eradicate the problem. This may involve review of the methods used in preparation or a

change in purchasing patterns of materials. However, it is not acceptable merely to rely on the biodistribution in patients as the only quality control testing to be performed.

In situations where an unexpected biodistribution is seen in one patient but not in others who received the same product, a patient related cause might be responsible. If this can be identified, it can provide useful information for future reference and to prevent misdiagnosis occurring.

On rare occasions, an adverse reaction may occur in a patient to whom a radiopharmaceutical has been administered. This does not mean that the product is necessarily defective. The prevalence of such reactions has been estimated as 3 per 10^5 administrations and, as such, departments might not encounter a similar situation for many years. Fortunately, adverse reactions that do occur are generally mild and self-limiting and do not require extensive treatment. The adverse reaction most commonly encountered involves the development of skin rashes a few hours after administration of ^{99m}Tc bone imaging agents. Histamine release in the patient is frequently implicated as the cause of the problem, and hence symptomatic treatment with an antihistamine is sometimes beneficial. There are occasions when a severe anaphylactic reaction can occur immediately after administration and prompt action, including administration of adrenalin, may be necessary. Since the occurrence of such events is so low, they should be reported to the manufacturer of the product and, as necessary, to national authorities. In this way a database on the possible reactions that can occur is developed and information can be disseminated. Departments can then be prepared to deal with such events if they occur, thereby enhancing the quality of patient care.

7.13. CONCLUSIONS

Each department needs to have its own quality assurance programme to ensure that the products administered to patients are of the desired quality. This requires the development of appropriate documentation systems, record keeping and quality control testing protocols. These will be influenced by the range of products prepared, the source of the starting materials (e.g. from a commercial manufacturer or prepared in-house) and the facilities used for the preparation. In addition, it is important that the results obtained are reviewed and acted upon where necessary in order to maintain the quality of the products. One vital component in the assurance of quality of products is to have well trained competent staff who have the necessary skills and knowledge to deal with radioactive pharmaceutical products.

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Chapter 8

RADIATION SAFETY PRACTICE IN NUCLEAR MEDICINE

8.1. INTRODUCTION

Good radiation safety practice in nuclear medicine comprises various components:

— Training;
— Design and construction;
— Local radiation safety rules and procedures;
— Preparedness;
— Equipment;
— Monitoring.

This section will concentrate on procedures that have not been covered elsewhere in this manual, and also deal with monitoring.

8.2. LOCAL RULES

Procedures for radiation safety are often called *local rules*. These may include:

— General: departmental radiation safety rules;
— Radiopharmacy: housekeeping, dose dispensing, record keeping, waste management, contamination control and accident procedures;
— Patient studies: activity administration and accident procedures;
— Therapy: administration, waste management, patient advice, discharge and accident procedures (Section 6.2);
— Pregnant patients: dosimetry and advice.

While each department should decide on its own procedures and rules, the following may serve as an example.

(a) Rooms (e.g. scanning rooms and the radiopharmacy) where unsealed or sealed radionuclides are to be used must be labelled at all entry points

with the international radiation warning symbol (trefoil). Such rooms must be regarded as radiation areas.

(b) Radiation monitors are to be worn by staff at all times when in radiation areas.

(c) Eating, drinking, smoking and the use of cosmetics are prohibited in the radiopharmacy and in any room where unsealed radionuclides are used.

(d) All containers used for radioactive materials should be clearly labelled with the radionuclide, form, activity, time, date and, when appropriate, a note as to the sterility or otherwise.

(e) All such containers are to be adequately sealed and shielded at all times. Except for very small activities, containers are not to be handled directly and, if possible, tongs or forceps for vials and syringe shields should be used.

(f) All work surfaces should be covered with absorbent paper.

(g) Pipetting by mouth of any radioactive substance is strictly forbidden.

(h) Staff involved in radiopharmaceutical preparation must check their hands for contamination before leaving the radiopharmacy suite.

(i) All radiopharmaceutical preparation and administration procedures should be carried out behind suitable lead or lead-glass shielding, disposable gloves and a radiation monitor should be worn, and preferably a laboratory coat. Gloves should be removed in the proper surgical manner (with one glove held inside the other) and disposed of correctly as radioactive waste after use.

(j) All radioactive sources are to be returned to safe storage immediately when no longer required.

(k) All operations involving radioactive gases or aerosols should be carried out in a fume hood or similar ventilated device to prevent airborne contamination. Exhaust vents must be situated well away from air intakes.

(l) Glassware and implements for use in the radiopharmacy are to be appropriately marked and under no circumstances removed from that area.

(m) All staff should be familiar with radiation accident and decontamination procedures.

(n) Packaging and containers for radioactive material must be checked for contamination on opening.

(o) Containers, lead pots, etc., that no longer contain radioactive material and require to be disposed of *must* have any radiation warning labels removed or obliterated before disposal.

8.3. RADIATION SAFETY ASPECTS OF RADIOPHARMACEUTICAL PREPARATION

The general rules described in the previous section apply, but because of the large amount of radiopharmaceuticals that is stored and manipulated in the radiopharmacy, some additional rules may be observed. Good housekeeping is important — all work areas should be kept clean and tidy, all radionuclide containers must be safely stored and readily available, adequate supplies of consumables must be available within easy reach of staff performing radiopharmacy work, unnecessary visits to the radiopharmacy should be discouraged and contaminated sharp items such as needles must be safely stored behind shielding.

Records should be kept of:

— Receipt and disposal of radioactive materials;
— All individual preparations for patient administration, including the patient's name, radiopharmaceutical used, activity and date;
— Quality control testing of the radionuclide calibrator.

In addition:

— Regular surveys (preferably weekly) of contamination must be performed.
— A decontamination kit should be held in or near the radiopharmacy.
— A sensitive radiation monitor must be available at all times in the radiopharmacy for contamination checking, not only of surfaces, but also of hands, clothing and disposables.

8.4. SAFETY PRECAUTIONS: WARD AND OTHER NON-NUCLEAR MEDICAL STAFF

8.4.1. Diagnostic studies

In general there are no hazards from patients who have received diagnostic doses. Use of disposable gloves (universal precautions) will provide sufficient protection from excreted radioactive material.

8.4.2. Therapy procedures

Staff caring for or working with patients who have received therapy with radionuclides may be required to follow safe working practices, according to the type of therapy. These are listed in Section 6.2.

8.4.3. Accidental contamination procedures

There are three major causes of spillage of liquid radioactive material:

— From a source container;
— Leakage during an injection procedure;
— From patient excretions such as urine, faeces, sweat, saliva and vomitus.

Spills of radioactive material are not to be regarded as an unavoidable hazard in the day to day operation of the department. Any spill has a level of danger, and acceptance of minor spills will lead to a casual approach to major spills.

A kit of materials used for decontamination should be prepared and kept in an easily accessible location in the department. Additional kits should be kept where needed, for example in the therapy ward.

The contents of a decontamination kit can be decided locally, according to the materials available and the nature of the potential contamination hazards. All kits can be kept inside plastic containers (with a lid), and at a contamination site the container can be emptied and then used to place materials used in the decontamination as well as contaminated items such as clothing. A suggested list of contents for a decontamination kit is:

— Disposable gloves, gowns and overshoes;
— Bottles and/or spray canes of decontaminant (water with detergent and sodium thiosulphate added, at least);
— Small scrubbing brushes;
— Disposable and absorbent towels;
— Felt tip marking pens (water soluble ink) for marking the contaminated area;
— Plastic bags of different sizes;
— Alcohol wipes;
— Radiation warning signs, adhesive tape and labels;
— Absorbent and plastic covered sheets (incontinence sheets);
— Disposable forceps;
— Disposable surgical masks.

In addition, a suitable radiation monitor (Section 4.5.1) should be readily available at all times.

The following procedure should be followed on discovery of a contamination problem:

(a) All persons involved in the incident are to vacate the immediate vicinity but are not to move freely around the department, as this involves a danger of spreading contamination.
(b) Notify IMMEDIATELY the radiation safety officer, or a medical physicist and the senior technologist for the area.
(c) The decontamination kit should be brought to the accident site. Note that any items used from the kit must be replaced as soon as possible.
(d) If the contamination is due to a container spill of liquid and the hands are protected with gloves, right the container, and ensure that it is adequately shielded. If the problem is due to a leaky syringe or other container, place the suspect item in a plastic bag and remove this to a suitable storage area.

The following actions should be performed by a physicist or a senior technologist:

(e) Define the area of contamination using an appropriate survey meter and, if appropriate, mark areas of hot spots with a felt tip pen.
(f) Seal off the area involved and in particular ensure that personnel do not walk on any possibly contaminated floor area. Discard any clothing that is contaminated and place it in a labelled plastic bag. If there is any radioactive material on the skin, flush, in the first instance thoroughly with water.
(g) Do not permit any person to resume work in the area until a survey has been made and decontamination procedures have been satisfactorily carried out.

Decontamination of any contaminated area cannot be performed by a fixed set of rules, but must have regard for the radioisotope form and type of contamination. The following general information can be used in most cases:

(1) In cases of spillage during drawing up or administering a patient injection, a suitably clad (gown, gloves and overshoes) person shall soak up any obvious liquid contamination with absorbent paper, placing such paper into a plastic bag for storage. Once this step has been performed, decontamination of contaminated surfaces can take place.

(2) Swabs or similar absorbent material soaked in decontamination fluid shall be used to swab and scrub contaminated areas until a minimum decontamination effect is noticed. This will in most cases mean that the surface dose rate at the area in question can be reduced to something less than 50 μGy/h (5 mrad/h).
(3) Where items of equipment have been contaminated it may be preferable to store such items until the activity has been reduced to a safe level.
(4) Areas that have been decontaminated and where the dose rate is still at a high level should be avoided until the activity has reached a safe level. Floor surfaces that cannot be completely decontaminated or where it is uncertain if further activity is present should be covered with a plastic sheet until the activity has decreased to a satisfactory level. The covering must be marked with brief details such as the radionuclide, dose rate and date.

8.5. DISPOSAL OF RADIOACTIVE WASTE

Radioactive waste from nuclear medicine procedures can be dealt with either by simply storing the waste safely until radioactive decay has reduced the activity to a safe level or possibly by disposal of low activity waste into the sewage system, if permitted by the local regulatory authority. Long half-life or high activity waste may need long term storage in a suitable storage area.

Waste materials from the drawing up of patient injections can be divided into two groups, those with long and those with short half-lives. Technetium-99m waste normally requires storage for only 48 hours, in a plastic bag inside a shielded container. The container should be labelled with the radionuclide and date. Gallium-67, ^{131}I and other longer half-life materials should be placed in a separate labelled and dated plastic bag and stored safely.

Sharp items, such as needles, should be separated and placed in a shielded plastic container for safety.

When disposing of waste, attention should be paid to the following points:

— Normally once the surface dose rate in any individual bag of waste is below 5 μGy/h it can be disposed of (check with the local regulatory authority).
— Disposable gloves should be worn and caution exercised when handling sharp items.
— Any labels and radiation symbols should be removed.
— Waste should be placed in a locally appropriate waste disposal container, for example a biological waste bag (since waste, once no longer

radioactive, is usually regarded as biological waste). Placement of waste inside two bags is advisable to minimize the risk of spillage.

8.6. ADMINISTRATION OF RADIONUCLIDES TO WOMEN OF CHILD BEARING AGE OR PREGNANT PATIENTS

Patients of child bearing age should be asked if they are, or might be, pregnant. It may be advisable to document the date of the last menstrual period on the nuclear medicine request form.

A sign warning patients to tell staff if they are pregnant should be displayed in the waiting room. Typically, such signs show a drawing of a pregnant woman, with the words "If you are, or think you might be, pregnant, or if you are breast feeding a child, then please let the nuclear medicine staff know BEFORE you are given the radioactive injection, drink or capsule for your nuclear medicine test or therapy". The text should be translated into all languages commonly used at the location.

Pregnancy is not an absolute contraindication to radionuclide studies and in many situations, such as confirmation or exclusion of pulmonary embolus, may provide essential diagnostic information. If a patient is pregnant it is imperative to discuss the indications for the study with a departmental medical officer, and the fact that the patient is pregnant must be clearly marked on the consultation form. A smaller than normal activity of radiopharmaceutical may be administered, thereby minimizing radiation to the foetus. There is little risk involved with the use of ^{99m}Tc radiopharmaceuticals, but studies with other radionuclides should be avoided unless clinically justified. If a pregnant patient does have a nuclear medicine procedure, there are ways of calculating the radiation dose to the foetus, and tables of radiation doses. The foetal dose arises from the mother (usually from bladder activity) and from radionuclides that have crossed the placenta to the foetal circulation.

8.7. BREAST FEEDING PATIENTS

Many radionuclides may be concentrated in breast milk. This may mean that the patient has to stop feeding for a period of time. Table 8.1 gives a guide to the period of time that breast feeding must be interrupted.

TABLE 8.1. ACTIVITIES OF RADIOPHARMACEUTICALS THAT REQUIRE INSTRUCTIONS WHEN ADMINISTERED TO PATIENTS WHO ARE BREAST FEEDING AN INFANT

Radiopharmaceutical	Typical administered activity		Examples of recommended duration of interruption of breast feeding
	(MBq)	(mCi)	
I-131 NaI	120	3	Complete cessation (for this child)
I-123 NaI	50	1.5	9 h
I-123 OIH	20	0.5	Nil
I-123 MIBG	400	10	24 h
I-125 OIH	2	0.05	Nil
I-125 fibrinogen	Any	Any	Complete cessation (for this child)
I-125 HSA	Any	Any	Complete cessation (for this child)
I-131 OIH	2	0.05	Nil
P-32 phosphate	Any	Any	Complete cessation (for this child)
Tc-99m DTPA	800	20	Nil
Tc-99m MAA/microspheres	200	5	Nil
Tc-99m pertechnetate	800	20	40 h
Tc-99m DISIDA	300	8	Nil
Tc-99m glucoheptonate	800	20	Nil
Tc-99m human albumin microspheres	100	2.5	Nil
Tc-99m MIBI	800	20	Nil
Tc-99m MDP	800	20	Nil
Tc-99m pyrophosphate	800	20	Nil
Tc-99m RBCs in vivo labelling	800	20	Nil
Tc-99m RBCs in vitro labelling	800	20	Nil
Tc-99m SC	400	10	5 h
Tc-99m DTPA aerosol	50	1.5	Nil
Tc-99m MAG3	400	10	Nil
In-111 WBCs	40	1	48 h
Ga-67 citrate	400	10	1 month (effectively means cease)
Tl-201 chloride	200	5	30 h

8.8. TYPICAL RADIATION DOSES FROM DIAGNOSTIC STUDIES

Table 8.2 gives typical doses only, and is provided to serve as a guide to the user.

TABLE 8.2. EFFECTIVE DOSES AT VARIOUS AGES FROM A SELECTION OF RADIOPHARMACEUTICALS

	Doses at various ages (mSv/MBq)							
	Effective dose (E)							Effective dose equivalent (H_E)
Radiopharmaceutical	Conceptus (3 months[a])	Newborn	1 y old	5 y old	10 y old	15 y old	Adult (20+)	Adult (20+)
F-18 FDG[b]	0.027	0.43	0.096	0.054	0.035	0.024	0.020	0.027
Ga-67 citrate	0.093	1.16	0.49	0.30	0.20	0.12	0.10	0.10
Tc-99m DTPA aerosol	0.006	0.052	0.023	0.013	0.009	0.008	0.006	0.006
Tc-99m DMSA	0.005	0.086	0.037	0.022	0.015	0.011	0.009	0.016
Tc-99m DTPA	0.012	0.03	0.014	0.012	0.007	0.009	0.007	0.008
Tc-99m HIDA/ DISIDA	0.0013	0.22	0.095	0.054	0.035	0.023	0.018	0.025
Tc-99m HMPAO (Ceretec)	0.0087	0.12	0.054	0.032	0.019	0.014	0.011	0.014
Tc-99m MAA	0.003	0.17	0.068	0.037	0.024	0.017	0.012	0.013
Tc-99m MAG3	0.018	0.027	0.012	0.013	0.009	0.013	0.010	0.012
Tc-99m MIBI	0.015	0.14	0.065	0.042	0.026	0.017	0.013	0.015
Tc-99m MDP	0.0061	0.063	0.026	0.014	0.009	0.0059	0.0048	0.0061
Tc-99m pertechnetate[c]	0.011	0.14	0.062	0.035	0.022	0.016	0.012	0.011
Tc-99m RBC in vivo	0.006	0.070	0.031	0.017	0.012	0.0079	0.0060	0.0072
Tc-99m RBC in vitro	0.007	0.071	0.031	0.017	0.012	0.0080	0.0061	0.0073
Tc-99m SC	0.002	0.093	0.042	0.023	0.016	0.010	0.0080	0.014
Tc-99m leucocytes	0.004	0.20	0.074	0.039	0.025	0.017	0.013	0.020

For footnotes see p. 518

TABLE 8.2. EFFECTIVE DOSES AT VARIOUS AGES FROM A SELECTION OF RADIOPHARMACEUTICALS (cont.)

	Doses at various ages (mSv/MBq)							
	Effective dose (E)							Effective dose equivalent (H_E)
Radiopharmaceutical	Conceptus (3 months[a])	Newborn	1 y old	5 y old	10 y old	15 y old	Adult (20+)	Adult (20+)
In-111 pentetreotide[b,d]	0.082	0.88	0.38	0.21	0.15	0.11	0.08	0.12
I-123 iodide	0.02	2.7	1.9	1.0	0.47	0.32	0.20	0.12
I-123 MIBG	0.018	0.15	0.065	0.039	0.026	0.021	0.017	0.019
I-131 iodide	0.072	283	206	107	47.1	30.4	18.4	10.4
I-131 MIBG	0.11	1.84	0.71	0.34	0.25	0.20	0.15	0.21
Tl-201 chloride	0.097	3.65	2.08	1.34	1.01	0.26	0.16	0.18

Note: In the last two columns, E and H_E, respectively, are given for adults. For the same radiopharmacetical, the ratio of E/H_E does not vary significantly with age.

[a] Effective dose to conceptus per MBq administered to mother.

[b] Effective dose to children calculated from H_E values and adult E/H_E ratio.

[c] With blocking agent.

[d] Estimated using clearance data from ^{111}In-octreoscan (Mallinckrodt Medical, St. Louis, MO).

8.9. MONITORING

Radiation monitoring in nuclear medicine has several forms:

— Personnel (staff) monitoring;
— Routine monitoring (e.g. area and hot laboratory);
— Special monitoring (e.g. pregnant staff and therapy patient staff).

All have their place and all are necessary.

8.9.1. Personnel monitoring

All nuclear medicine staff must be routinely monitored for occupational radiation exposure. This includes nursing staff but may not need to include clerical staff, unless they are involved with patients.

The main points to remember with monitoring are:

(a) Either film badges or TLDs (Section 4.5.1) should be used as these are relatively cheap, passive, rugged and, in the case of TLDs, can be re-used.
(b) Monitors should be worn between waist and chest, and underneath any protective clothing (lead gowns) which might be used.
(c) The monitor should be changed regularly and, in any case, at intervals of no longer than 12 weeks.
(d) Each batch of monitors will come with a control monitor (to correct for natural background radiation and other factors), which *must* be kept in a place where there is no chance of radiation exposure from radionuclides or X rays.
(e) An accurate record must be kept of each person's radiation exposure history. Records must be kept for their working lifetime, including the cumulative (running total) dose. Depending on the local regulatory requirements, it may be convenient to maintain detailed records only for the current year, and to keep yearly totals otherwise.
(f) Staff must be required to wear their monitors at all times whilst working. Under the laws of many countries, the head of nuclear medicine will be held responsible for this, as well as for staff safety.

Monitoring results must be reviewed regularly by an appropriate person, such as a physicist or senior technologist. The basic principle of radiation safety is to aim for the lowest feasible dose, *not* to allow staff to receive any regulatory dose limit. The BSS dose limit for workers is 20 mSv/a, but nuclear medicine staff should generally not receive more than 2–3 mSv/a at the most. A local dose limit of around 5 mSv should be applied to nuclear medicine staff. Staff who exceed this limit, on a pro rata basis (dose multipied by monitoring period in weeks/52), should be checked to ensure that their work practices are safe and that they have not been accidentally or unnecessarily exposed.

8.9.1.1. Special monitoring

There are some occasions where special or additional monitoring of staff may be needed, for example:

(a) Pregnant staff.
(b) Radiopharmacy staff, for whom finger dose monitoring (special small TLD badges that fit onto a finger) may be used. Finger doses will be very much higher than body doses, especially with PET radiopharmaceuticals. Finger badges must of course be worn underneath gloves.
(c) Staff involved with therapy procedures. If nurses are regularly involved, then they should be regularly monitored, otherwise monitoring need only be carried out for each case. This applies especially where the patient needs a higher level of nursing care, such as in MIBG therapy. Here, electronic direct reading dosimeters are advisable to allow continuous knowledge of the total dose. Records must also be kept of these readings.

8.9.1.2. Routine and area monitoring

Routine and area monitoring covers regular surveys of the radiation background in critical areas such as the radiopharmacy. These allow practices and safety measures to be modified before staff doses increase, particularly when new radiopharmaceuticals, radionuclides or increased activities are involved.

The radiopharmacy should have a permanent area monitor (scintillation counter or ionization chamber), with an audible signal for dose rate, to allow staff to know when radioactive sources are exposed.

8.10. RADIATION SAFETY INFRASTRUCTURE

Each hospital or institute with a nuclear medicine facility should have some form of radiation safety infrastructure body, established according to local regulatory requirements. Typically this would be a radiation safety committee with the responsibility for overseeing radiation safety practices in the hospital, and advising the administration on radiation safety issues. Representation from the nuclear medicine section is very important and should be mandatory. Often, the nuclear medicine physician or physicist is the only person who can provide expert advice on internal radionuclide dosimetry, and in investigation of radiation incidents where unsealed radionuclides are involved.

The committee should have among its responsibilities the following:

— Review of staff radiation dose records, especially abnormally high doses;
— Review of radiation safety protocols;
— Approval of applications for licences under radiation legislation;

— Investigation of radiation incidents and accidents;
— Review of applications for research projects where radionuclides are involved, especially where volunteers may receive a radiation exposure.

The hospital should also appoint an appropriately qualified and experienced person as the radiation safety officer. Nuclear medicine physicists, physicians or technologists are usually good candidates for this role.

Chapter 9

NUCLEAR MEDICINE: FUTURE TRENDS

Nuclear medicine is an evolving science. While this is common to all medical specialties, it is particularly true for nuclear medicine because of its relationship to, and dependence on, high technology advances. Rapidly developing areas such as electronics, physics, computer sciences, radiopharmacy and radiochemistry, as well as molecular biology, are closely related to nuclear medicine so that this medical science not only follows developments in such areas but also provides feedback to them. Some particular areas regarding recently achieved advances or future potential ones in nuclear medicine are worth highlighting.

9.1. ELECTRONIC DATA TRANSFER

The delivery of comprehensive nuclear medicine services to patients and referring physicians is increasing around the world. The range and benefits of these procedures, both diagnostic and therapeutic, are gaining in both recognition and appreciation. Their role in medical decision making, as part of standard patient care, helps fulfil an otherwise unmet need. The centralization of nuclear medicine and radiopharmaceutical services is leading to a hub and spoke concept. This means that patients may be studied in a peripheral hospital according to the agreed protocols set out in this manual, and the data transferred to a central point for analysis and reporting. This concept is particularly appropriate in the case of advanced techniques. Just as in the past the success of RIA depended on an efficient postal service, so the future successful distribution of nuclear medicine services may depend on high speed image data transfer.

Nuclear medicine is one of the areas to benefit most from advances in IT, especially through the widespread use of personal computers and the Internet. Many nuclear medicine centres are now fully digitized and electronically connected to permit clinical study file exchanges, teleconsultations, remote reporting, collaborative research and tele-education through PACSs of increasing efficiency. This in turn enables nuclear medicine physicians to assist colleagues who work in new centres or in remote areas. Telenuclear medicine practices have proved to be cost effective and to have a very bright future in promoting the development of the specialty, particularly in developing

countries. The core part of telenuclear medicine is image transfer over the Internet. Simple telenuclear medicine practice requires an image acquisition site coupled with an image interpretation site. In order to provide additional information, a digital film scanner is needed at the acquisition site so that X ray films, CT or MRI images may be used for comparison. In advanced telenuclear medicine networks, different sites should have the same system configurations to ensure basic compatibility and interoperability, enabling image acquisition, data analysis and data interpretation. It is important, however, to ensure the confidentiality of patient data at all times.

The Internet has provided many new opportunities for education in nuclear medicine through distance learning. Universities, scientific societies and international organizations can place a range of teaching resources — slide shows, multimedia teaching packages, relevant textbooks and documents, and digital case study files — on the Internet, for easy access and downloading. Teaching materials on the Internet can be used for both education and on-the-job training in nuclear medicine. Staff members can tailor these materials and design their own purpose made teaching packages. Teaching resources can also be stored on CD-ROM or other universal mass-storage devices so that these resources can be accessed from any conventional computer system. This is particularly useful when there is no Internet connection available or telephone links are too slow for image file transfer.

Advances in telecommunications have opened a new horizon for the promotion of nuclear medicine around the world. Telenuclear medicine will continue to develop quickly once some of the problems, such as the issue of licensing, standards, reimbursement, patient confidentiality, telecommunication infrastructure and costs, have been solved. Ultimately, its cost effectiveness and far reaching impact will make telenuclear medicine an extremely useful tool, particularly for developing countries.

9.2. RADIOIMMUNOASSAY AND MOLECULAR BIOLOGY

The future of RIA will depend on the overall economic development of a country as well as the degree to which the country embraces biotechnology and information technology. In most developing countries, socioeconomic conditions are likely to remain poor. After careful consideration of the local infrastructure, robustness and cost of nuclear and non-nuclear assays, it is likely that bulk reagent methodology will still be the main workhorse of routine diagnostic services. Quality control will remain a key ongoing continuous activity to assure the quality of results.

In economically stronger countries, RIA will be carried out in national reference laboratories and used as the reference method to solve problems generated by non-isotopic immunoassay. With the use of modular robotic systems and improved antibody design for short incubation assays, RIA may be modularly automated to reduce further operating costs. It is well suited to nationwide targeted screening of congenital diseases and other disorders. This can also be implemented in countries with low gross national products.

In more developed countries, the establishment of indigenous immunodiagnostics will become one of the essential components of a comprehensive biotechnological strategic plan. Here RIA will play an important role in early screening of hybridoma clones in the monoclonal antibody production process. It will also be used to set up the first workable immunoassay methodology for new analytes before they are thoroughly evaluated and marketed or transformed into other commercial assay formats.

In the field of research, RIA will continue to be used as the gold standard in the search for novel solid phase, cost effective methods of protein immobilization and cheaper high affinity binders to improve the overall minimal detection limit of immunoassay. Being a reliable methodology, it is an ideal tool for the development of consensus investigative protocols in evidence based diagnostic medicine. The wealth of the knowledge base in RIA should be systematically documented in the format of an interactive multimedia learning resource for self-directed learning, thus further reducing the cost of training.

In the near future, the exact role of thousands of genes will be characterized by the human genome project. Other bacterial, protozoan, helminthic, viral and fungal genomes have already been, or will be, elucidated very soon. The most important application of this variety of sequences will be in diagnostics. Current diagnostic methods can be slow and relatively insensitive, lack specificity, require invasive clinical samples and, moreover, fail to provide quantitative information about the disease. Molecular methods, based on published sequences, will overcome these constraints to a significant extent. Other applications of molecular methods will be as prognostic markers for cancer, drug resistance indicators, predictive markers for malignant and degenerative disorders, models for molecular modelling for drug design, gene therapy, pathogenicity evaluation, detection of minimal residual disease, molecular epidemiological information and control measures, and the detection of new emerging diseases.

Technical trends that will be used in the above applications are quantitative, multiple and in situ PCR, multiple DNA sequencing and diagnosis by hybridization with enriched stable isotope labels using mass spectrometry, peptide nucleic acid probes that provide faster results than traditional DNA probes, DNA biochip technology, where distinct probes can be linked to an

inert support and hybridized with the test DNA from clinical samples, phosphor imagers that detect beta and gamma radiations, as well as X ray techniques with improved sensitivities compared with those of autoradiography, protein tests and functional genomics.

9.3. IMAGING AND THERAPY

9.3.1. Instrumentation and image processing

Magnification scintigraphy enhances the spatial resolution of nuclear medicine images to a level comparable to those of radiography, CT scanning and MRI, and considerably raises the diagnostic accuracy. The dual head mode generates a pair of high resolution images at one time. Pinhole SPECT, a hybrid of the pinhole magnification technique and SPECT, can portray parts of small bones and joints and can detect a subtle increase in bone metabolism at the insertions of tendons and ligaments. Future development of gamma camera systems dedicated to magnification scintigraphy will open new opportunities in nuclear medicine imaging.

The use of multifunctional and hybrid SPECT–PET cameras, as well as dedicated PET cameras and PET–CT systems, will increase and become available to more institutions. More interaction with other imaging modalities is expected in order to use the X ray CT information for rigorous attenuation correction and image registration.

New semiconductor detectors are being developed that allow for the manufacturing of specially dedicated cameras with versatile detector sizes and shapes, exhibiting outstanding sensitivity and resolution never achieved before by a nuclear medicine instrument. These new detectors will also be more portable and will permit bedside examinations, as well as coupling with other non-nuclear devices such as mammographs to obtain high resolution functional imaging with full co-registration to facilitate comparison with structural data and improve interpretation.

Iterative SPECT reconstruction algorithms are now increasingly available in view of the powerful processing capabilities of modern computer systems. These algorithms yield more accurate trans-sectional data, improving tomographic resolution and avoiding some common image artefacts. Attenuation correction with line source devices or transmission–emission hybrid CT–SPECT instruments can significantly improve diagnostic specificity. Gated SPECT will continue to represent a valuable clinical tool for the simultaneous assessment of myocardial perfusion and function.

9.3.2. Clinical applications

The use of sentinel node scintigraphy and other intraoperative applications of probes will increase in their clinical application to a more comprehensive approach to surgical oncology and other non-oncological indications. Imaging probes are also being developed with the use of semiconductor technology, which will aid in intraoperative localization of the target organ or tissue.

PET imaging using ^{18}F-FDG will expand, especially in oncology, and will find new applications even in benign diseases. With the use of special PET tracers it will be possible to image hypoxic cells and apoptotic cell death. Procedure reimbursement will extend to other applications as evidence accumulates in favour of its utilization. Single photon oncological applications of non-specific tumour seeking agents such as ^{67}Ga, ^{201}Tl and ^{99m}Tc-MIBI will be replaced to a great extent by ^{18}F-FDG imaging and by SPECT studies using more specific ^{99m}Tc labelled peptides and genetically engineered monoclonal antibodies. Infection remains a major challenge, although the development of a radiolabelled antibiotic for imaging bacterial infection including tuberculosis through an IAEA Coordinated Research Programme may not only identify the presence and site of infection but also indicate the efficacy of treatment.

The primary basis of nuclear medicine is the tracer technique. Nuclear imaging will become a reference procedure for absolute measurement of physiological and pathophysiological processes for both clinical and research purposes. Receptor tracers are being developed that allow for quantitative and qualitative evaluation of organ functions. Receptor imaging with SPECT and PET will permit advances in the understanding of the complex functions of the brain, aiding in the management of various neurological and neuropsychiatric disorders. Cardiac receptor imaging will be used for more accurate prognosis and management of heart diseases, while other receptor techniques will be employed in oncology for tumour detection and characterization, as well as for selection of the most appropriate therapeutic approach.

Nuclear cardiac testing will consolidate as the non-invasive gold standard procedure for ischaemic heart disease. Furthermore, its applications may expand in view of the possible use of electron beam computed tomography as a screening procedure for the detection of coronary artery calcifications in high risk patients.

9.3.3. Therapy

Radionuclide internal targeted therapy will expand generally. Radiolabelled peptides emitting beta particles and radiolabelled monoclonal

antibody derivatives will be increasingly employed in specialized centres, as well as alpha emitting radionuclides for both palliative and curative purposes in oncology. Other transarterial radioconjugates are being evaluated for liver cancer. These agents will be used in conjunction with tracer doses to evaluate the progress of the treatment, and will constitute a method complementary to other conventional procedures or even become the treatment of choice for some malignant and non-malignant diseases. The radiobiological basis of low dose, long lived radionuclide therapy will be investigated and will probably lead to new therapeutic strategies.

9.4. COMPETENCE AND EDUCATION

The range and quality of services should continue to improve. Departments of nuclear medicine will be expected to meet internationally recognized ISO quality standards for service as well as for the environment. These will in turn lead to accreditation procedures not only for the continuing competence of staff but also for the quality of the facilities and the documentation of patient protocols and procedures. With evolving and more complex techniques available, the challenge is evident for all members of the nuclear medicine team, from physicians to technologists and from physicists to radiopharmacists. Hence, more intensive and extensive training and better continuing education programmes and activities are needed if reliable results are to be obtained and a sustainable growth of the specialty is to be achieved.

Nuclear medicine is underutilized in most countries. As Dr. H.N. Wagner, Jr., likes to say, “Nuclear medicine is the best kept secret in medicine”. That means that nuclear medicine specialists and scientists have to work harder to spread the large amount of information available that favours the use of nuclear techniques for a vast range of clinical applications. Evidence based medicine practice is already becoming standard worldwide, so both individual practitioners and institutions will increasingly include nuclear medicine procedures in their diagnostic and treatment algorithms. Emphasis must be placed, however, on cost effectiveness in order to abolish the argument that procedures are too expensive and to demonstrate the innocuousness of the low dose radiation used in most procedures.

Universities, nuclear medicine societies and associations, commercial companies as well as international institutions like the IAEA are expected to play a major role regarding all the above mentioned aspects. Linking the key professional bodies of nuclear medicine with the IAEA and the use of electronic journals should improve the communication between practitioners of nuclear medicine, especially in developing countries.

For nuclear medicine, the future is bright. It only depends on us to make the future prospects become reality.

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This manual provides comprehensive guidance, at the international level, on many aspects of nuclear medicine practice, including education, training, facilities and equipment, quality systems, and radiopharmacy and clinical practice. It will be of use to those working in both new and more developed nuclear medicine centres.

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