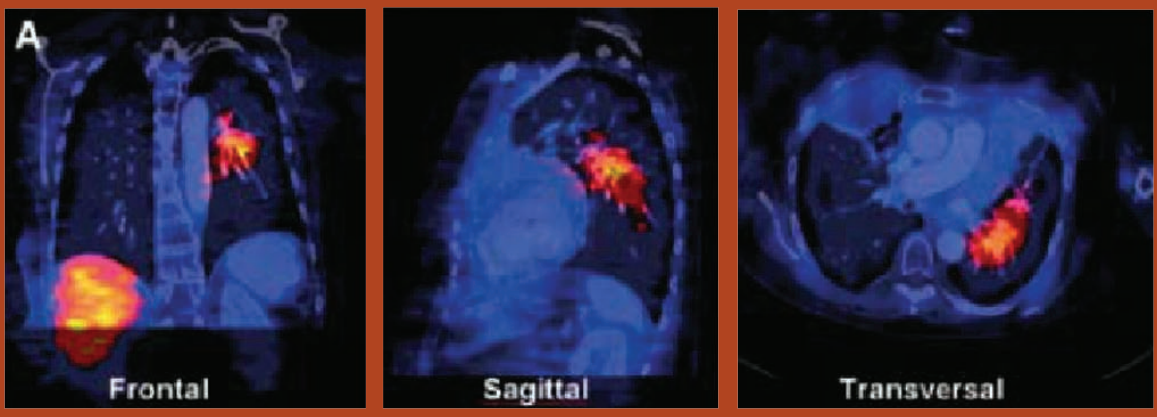

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No. 8

Clinical Translation of Radiolabelled Monoclonal Antibodies and Peptides

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CLINICAL TRANSLATION OF RADIOLABELLED MONOCLONAL ANTIBODIES AND PEPTIDES

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IAEA HUMAN HEALTH SERIES No. 8

CLINICAL TRANSLATION OF RADIOLABELLED MONOCLONAL ANTIBODIES AND PEPTIDES

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 2009

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FOREWORD

Within the context of the IAEA's nuclear medicine activities, a variety of strategies have evolved for radiolabelling biological products, with a view to enhancing diagnosis, palliation and therapy. Currently, more than 350 biological medicinal products aimed at treating over 200 diseases are being tested, including a whole range of monoclonal antibodies (MAbs) and peptides which bind to cell surface receptors and address aberrant cellular functions or those overexpressed in certain diseases. However, very few radiolabelled MAb or peptide products have reached clinical use, and more must be done to enable these products to enter into routine nuclear medicine practice.

This publication provides essential guidance on planning the investigations needed for radiolabelled biologicals to enter into routine use. It addresses the preparation of starting materials, in vitro and in vivo validation, first investigations in humans, and clinical application of radiolabelled biological proteins, peptides and antibodies. As many of these products are likely to be administered periodically for medium to long term follow-up of disease, it is extremely important to document their safety even with repeated exposure.

Written with nuclear medicine clinics in mind, this publication aims to provide guidance on the safe and effective use of radiolabelled biologicals in a busy clinical setting. Compared with traditional radiolabelled chemical complexes, radiolabelled biologicals are more complex and less predictable. Recently reported adverse events associated with biologicals — including one involving a well known technetium radiolabelled antibody used for infection imaging that resulted in a number of fatalities — highlight the complexity of this group of molecules. There is a need for extreme care and well documented follow-up systems to ensure that patient safety remains the highest priority.

Any modification of a biological or generic alternative faces the same challenges. Generic equivalence tests are harder to validate for radiolabelled biologicals than for conventional pharmaceuticals; yet these tests are the main hope for increasing the availability of these valuable tools in many developing countries. Therefore, this guidance will be of importance to many IAEA Member States as they begin acquiring and utilizing radiolabelled biologicals.

Continuous, reliable development and supply of high quality radiopharmaceuticals to patients all over the world is vital for the sustained development of nuclear medicine. In providing guidance on both development and supply of these medicinal products, this publication will be a useful resource for researchers and practitioners alike.

The IAEA is grateful to all those who contributed to and reviewed the manuscript. The IAEA officer responsible for this publication was K.K. Solanki of the Division of Human Health.

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CONTENTS

1. INTRODUCTION

1.1. BACKGROUND INFORMATION

The first biological protein drug manufactured through recombinant DNA (rDNA) techniques entered into clinical practice two decades ago. Recent advances in biotechnology have accelerated the introduction of biological proteins into clinical practice, offering pharmacological specificity, and therefore new treatment options and challenges. Currently, more than 350 biologicals aimed at treating over 200 diseases are being tested, mostly as non-radiolabelled products. They include a whole range of monoclonal antibodies (MAbs) and peptides which bind to cell surface receptors and address aberrant cellular functions or those overexpressed in certain diseases.

This new class of structurally complex drugs is produced by in vitro synthesis or in living systems. Compared with traditional chemical-based radiopharmaceuticals, these drugs require additional care during preparation, purification, evaluation and quality control (QC) analysis. It is important to retain a high level of specificity after radiolabelling. Regulators are keen to see a high level of competence, whether the product is used in clinical trials or is compounded to meet the clinical requirements of a specific patient.

Within the IAEA's nuclear medicine activities, and with a view to enhancing diagnosis, palliation and radiotherapy, a variety of strategies have evolved for radiolabelling MAbs and peptides. However, very few radiolabelled products (e.g. Zevalin, octreotide, capromab pendetide (Prostascint) and radiolabelled antibodies for infection imaging) have reached routine clinical practice. Most remain at the research level, either at universities or at post-graduate research centres. In practice, radiolabelled products are being used 'off label' to meet the needs of specific patients, a situation recognized by the major regulatory bodies around the world. The responsibility for any off label use rests with the physician. Furthermore, reimbursement for off label use of products from health insurance companies or health authorities is difficult to obtain. More must be done to enable these products to enter into routine nuclear medicine practice.

1.1.1. Lessons from recent events

Compared with traditional radiolabelled chemical complexes, radiolabelled biologicals are more complex and less predictable. While there are numerous similarities between traditional radiopharmaceuticals and radiolabelled biologicals, the latter require additional care and particularly

rigorous monitoring. The nuclear medicine community was alarmed by post-marketing reports of serious adverse events in 17 patients using ^{99m}Tc-fanolesomab, a murine M class antigranulocyte antibody for infection imaging [1]. The deaths of two of the patients, both diabetics, were attributed to cardiopulmonary failure. The decision to suspend sales was based on the life-threatening nature of the adverse events and the availability of other methods of diagnosing appendicitis. Among the key lessons from this episode was the need for greater care during development and post-marketing surveillance. Extra care is also required when radiolabelled biologicals are used with specific groups of patients (e.g. diabetics).

Another recent report that illustrates the complexity of this group of molecules involves a Phase 1 clinical trial [2] in which six healthy male volunteers became critically ill and were admitted to intensive care units to receive cardiopulmonary support. These events occurred within 90 minutes of receiving a single dose of a novel 'superagonist' anti-CD28 MAb. Despite evidence of multiple cytokine release syndromes, all six patients survived. One of the main lessons from this episode was the need for additional care in the case of a modified, novel or super biological. Furthermore, it is essential to have available cardiopulmonary support and provisions for intensive care during first clinical trials in humans. Prior to such trials in humans, and in addition to the normal pharmacological and toxicological reviews required before use of these complex molecules, there is a need for critical review not only of quality aspects but also of non-clinical and clinical strategies, including review of the design of the trials. This level of detail is essential for reducing and managing associated risk. It is important to examine the steps to identify potential risks.

1.2. OBJECTIVES

Taking into account the complexity of these peptides and biologicals, it is essential that a set of norms and standards be established; the present publication addresses this need, providing guidance on the safe and effective use of radiolabelled biologicals in a busy clinical setting. It also provides essential information for better understanding and improving research and clinical practices associated with the clinical use of radiolabelled biological proteins, peptides and antibodies.

1.3. SCOPE

This publication describes the specific requirements for clinical application of various radiolabelled biotechnology derived products and chemically synthesized products. It applies to starting materials derived from characterized cells using a variety of expression systems, including bacteria, yeast and mammalian cells. The active substance includes peptides, proteins and antibodies, and can be derived from cell cultures, or produced using rDNA technology or chemical synthesis. Examples include, but are not limited to, cytokines, growth factors, fusion proteins, hormones, receptors, agonist or antagonist peptides, and MAbs and their fragments. The intended indications may include in vivo diagnostic or therapeutic uses.

Aspects covered in this publication include strategies for detection, measurement and characterization of unwanted antibodies induced by biologicals, preparation, processing, standards for starting materials, radiolabelling, analytical tests, in vitro and in vivo specificity assessments, quality controls, sterility tests and bacterial endotoxin tests.

This publication does not cover possible side effects of diagnostic and therapeutic applications of biological radiopharmaceuticals; these are covered in other publications. Nor does it cover endogenous proteins extracted from human tissue, endogenous cells, oligonucleotide drugs, or single amino acids or their variants. A separate IAEA publication on radiolabelled autologous products is under parallel development.

The guidance included here is intended for investigators working in the area of nuclear medicine, including practitioners, radiopharmacists, chemists, biologists and technicians. By establishing an international framework, this publication provides essential guidance related to radiolabelled biologicals, allowing the user to benefit from a more streamlined and standardized development programme. This, in turn, will reduce duplication of work and optimize limited available resources.

In the context of IAEA nuclear medicine activities, this publication also provides the basics for service extensions using radiolabelled biologicals. Continuous, reliable development and supply of high quality radiopharmaceuticals to patients all over the world is vital for the sustained development of nuclear medicine.

1.4. STRUCTURE

This publication covers general operational requirements (Section 2), requirements for starting materials (Section 3), methods of preparing

radiobiologicals (Section 4) and QC of radiolabelled biologicals (Section 5). Guidance is also provided on in vitro testing in cells and tissues (Section 6), tests in animals (Section 7), preclinical safety and toxicity studies (Section 8) and clinical studies (Section 9). Evaluation of the immunogenicity of radiopharmaceuticals is discussed in Section 10. The annexes provide a list of selected abbreviations and definitions of terms; a list of selected guidance materials; a list of selected web sites; and a table of commonly used radionuclides associated with radiolabelled biologicals. Also included is a sample case reporting form for a more structured approach to patient follow-up. As starting materials can be the main cause of side effects, monitoring for these effects is essential during preclinical and clinical investigations. Throughout, there is particular emphasis on quality systems, including quality controls to be applied to starting materials (peptides and antibodies), as well as during operations, various testing stages and, finally, use in humans. This publication will be a useful resource for researchers and practitioners alike.

2. GENERAL OPERATIONAL REQUIREMENTS

2.1. OPERATIONAL GUIDANCE ON HOSPITAL RADIOPHARMACY

The IAEA's Operational Guidance on Hospital Radiopharmacy: A Safe and Effective Approach [3] categorizes hospital radiopharmacy activities according to the following operational levels:

(a) Operational level 1a – Dispensing of ready to use radiopharmaceuticals;
(b) Operational level 1b – Dispensing of radioiodine;
(c) Operational level 2a – Radiopharmaceutical production from kits and generators;
(d) Operational level 2b – Radiolabelling of autologous blood cells;
(e) Operational level 3a – Compounding of radiopharmaceuticals;
(f) Operational level 3b – Compounding of radiopharmaceuticals for therapeutic application;
(g) Operational level 3c – Synthesis of positron emission tomography (PET) radiopharmaceuticals.

This approach takes into account the levels of associated risk and the diversity of radiopharmacy activities. At the international level, the operational guidance on hospital radiopharmacy aims to standardize practices. It takes into consideration the skill mix and the limitations applicable at the clinical practice level, as well as the busy nature of the clinical environment. It also provides guidance for nuclear medicine physicians responsible for routine service at operational levels 1 and 2, and more specifically for the off label uses required in practice, including guidance on training, facilities, equipment, operations and quality systems for safe operation.

In general, it is larger nuclear medicine centres that operate at operational level 3. These centres should have a well qualified chemist and/or their activities should be overseen by a nationally registered pharmacist. Radiolabelled biologicals having regulatory approval should be prepared under operational level 2 conditions; however, most other radiolabelled biologicals are used off label, and therefore are compounded under operational level 3a conditions (for diagnostic uses) or operational level 3b conditions (for therapeutic uses). Only a few kit formulations currently have market authorization, for example, indium labelled octreotide (radiolabelled peptide) and yttrium labelled anti-CD20 MAb (radiolabelled MAbs). Biologicals labelled with radioiodine require additional care, following the principles of operational level 1b. The present publication also contains guidance on quality systems and self-appraisal checklists for operational levels 1 and 2.

2.1.1. Staff and training

Staff should be trained in all aspects of compounding, dispensing and using radiopharmaceuticals, internal dose, and generators in clinical settings. Competence in biochemical methods is essential for preparing and handling radiolabelled biologicals. At operational level 3, radiolabelled biologicals require special quality systems and handling before they are released from the radiopharmacy for patient use. Most national regulations define radiopharmaceuticals as medicines. Preparation of radiopharmaceuticals at operational level 3 should be overseen by either a certified ‘qualified person’ (QP) or a nationally registered pharmacist. Such QPs are responsible for ensuring the safety and quality of radiopharmaceuticals for their intended use. Even in the case of a market authorized therapeutic radiolabelled biological (e.g. anti-CD20 (Zevalin)), additional training and care are required, beyond that required for dispensing traditional radiopharmaceutical kits. A biological labelled with a pure beta emitter presents additional challenges; for example, cross-calibration of the isotope dose calibrator with standardized yttrium and

consideration of geometric differences between different containers (vials, syringes).

2.1.2. Facilities

Preparation of radiolabelled biologicals requires infrastructure in addition to that used for conventional radiopharmacy. The extent of differences in facility requirements will depend on the category of radiolabelled biological (i.e. diagnostic or therapeutic application). The equipment needed for ^{99m}Tc and radioiodine operations is listed in Ref. [3].

Hot cells used for handling radiolabelled biologicals (Fig. 1) should be placed in a European Union (EU) Grade C (ISO 7) environment. If hot cells are employed together with semi-automated modules, the final sterilization of the radiolabelled biologicals should be undertaken in an operational EU Grade A (ISO 5) environment. If a laminar air flow (LAF) workbench (EU Grade A (ISO 5)) is used for dispensing, ideally it should be located in an EU Grade B (ISO 6) environment. When a completely sealed isolated EU Grade A (ISO 5) environment is used, the LAF can be located in a room that meets the EU Grade D level of cleanliness. It is important to periodically demonstrate and document that these standards are being maintained.

FIG. 1. Hot cell suitable for handling radiolabelled biologicals.

For radioiodination, a separate externally ducted fume hood and/or microbiological Class II LAF cabinet/isolator and appropriate radiation safety shielding (for gamma as well as beta radiation) are required. These should be sited in a separate room with at least a European Union Grade C (ISO 7) environment. If large quantities of radioactivity are handled, properly shielded hot cell facilities and semi-automated systems are essential. Again, terminal microfiltration for sterilization should be undertaken only in an EU Grade A (ISO 5) environment.

The QC laboratory should be equipped for routine biological and microbiological testing, including sterility testing, bacterial endotoxin testing and environmental bacteriological analysis. Appropriate storage facilities for biological and radioactive waste disposal systems are essential, primarily owing to the differences in the physical and chemical properties of the radioisotopes, which lead to differences in the chemistries involved in preparing the radiolabelled biological tracers or therapeutic radiolabelled biologicals.

Long lived generators should be housed in a separate Type II LAF/isolator with an operational EU Grade A (ISO 5) environment. Shielding should be appropriate for the energy and type of emission of the radionuclides. All equipment and the hot cells/LAFs/isolators should be regularly validated.

2.1.3. Operations

A well planned quality assurance (QA) programme should be in place when preparing radiolabelled biologicals. Additional quality requirements must be met when preparing therapeutic radiolabelled biologicals for administration in humans. Due care is needed to prevent any cross-contamination when handling MAbs or peptides. Before a radiolabelled biological is used in a patient, the following details should be cross-checked:

(a) Release specifications should be clearly written, taking into consideration both radiochemical and radionuclidic purity.
(b) Records should be kept of the radiation dose measured and the reconciliation of all activities during preparation of the radiolabelled biological.
(c) Checks at the time of release should be formally documented.
(d) The time of release from radiopharmacy and of patient administration should be recorded.
(e) Careful assessment of chemical, biological and radiopharmaceutical stability is required.

(f) There is a high risk to both the operator and the product when open sources of beta emitters are being handled.
(g) Operators should be periodically monitored for ingress and internal exposure to beta radiation. Detailed staff records should be kept.

The radionuclidic purity of the prepared radiopharmaceutical is critical for therapeutic radiolabelled biologicals. The dose calibrator should be routinely monitored and validated to ensure measurement accuracy in both vials and syringes. Annual checks against national or international standards are mandatory. The physical and biological (in vivo) stability of each batch should be carefully assessed.

When preparing PET radiolabelled biologicals, the ultra-short half-lives of PET tracers present additional challenges. Semi-automated systems in hot cells are traditionally used. Installation of the cyclotron and synthesis modules is beyond the scope of this document. Production facility qualifications and staff training are equally important.

2.1.4. Record keeping

Control specification and a record of each batch must be maintained for all radiolabelled biologicals. After each batch run, the specific activity and yield of the radiolabelled biological should be carefully monitored. Reconciliation of radioactivity before the final product is used in patients is important, especially for beta emitters. Critical in-process checks should be documented and cross-checked at the time of final product assessment. Any deviation or change must be taken into account and should be an integral part of the official batch release.

2.1.5. Quality control

The short half-lives of many of the radionuclides used for radiolabelling of biological agents have additional implications for quality systems. Rapid QC methods provide useful details about the radiochemical purity of radiolabelled biologicals; however, where possible, a high performance liquid chromatography (HPLC) system should be used. If the product is transported between institutes, a careful quality assessment should be made following a typical delivery before clinical use. Pre-validation of the whole operation system is essential to ensure consistency. Any change or deviation with respect to the starting material, equipment or process must be validated before clinical use.

Sterility, apyrogenicity and physicochemical purity tests should be routinely performed and closely monitored for all radiolabelled biologicals and long lived generators. If tests cannot be performed prior to the release of the radiolabelled biological, they should be done retrospectively. Ongoing microbiological assessment is essential.

2.2. PATENT ISSUES AND FUNDING

Investigators may start the synthesis of a new radiopharmaceutical from commercially available products (which may or may not be covered by a patent) or from products that are not commercially available. In the latter case, it may be possible to patent the new radiopharmaceutical, provided that the labelling method or the diagnostic or therapeutic application is totally innovative and has not already been published.

An initial screening of registered patents in a specific field can be done via the Internet using a patent search tool (e.g. http://ep.espacenet.com, http://www.delphion.com). Patenting should occur before the publication of any result in the form of a paper, congress abstract or proceedings paper.

From the user point of view, for research purposes (but not for commercial use) a patented product can be used for radiolabelling and in patients.

Regarding research funds, in addition to the expenses of producing or purchasing the starting materials, there are the additional costs of the reagents and animal experiments, before the biological material can be deposited with an International Depository Authority. After this step, the investigator may seek research funds from national or international organizations, or pursue collaboration with a pharmaceutical company. Some research grants have restrictions (e.g. regarding nationality, university), but others are open to all researchers, without restrictions. Funding opportunities may be found on the following web sites:

— The Human Frontier Science Program: http://www.hfsp.org/
— The European Union: http://cordis.europa.eu/
— proposalCENTRAL: https://proposalcentral.altum.com/
— The Weizmann Institute of Science: http://www.weizmann.ac.il/RGP_open/rwebpages.shtml
— The National Institutes of Health (US NIH): http://grants.nih.gov/grants/oer.htm
— The IAEA: http://www-crp.iaea.org/

— The Society of Nuclear Medicine: http://www.uphs.upenn.edu/pet/snmerf/

The costs of production, registration and commercialization of a new radiopharmaceutical currently are extremely high and generally are not recoverable within the first five years after commercialization, owing to the relatively small size of the radiopharmaceuticals market worldwide. As an example, the approximate cost for the development of a new drug for diagnostic imaging by a pharmaceutical company is in the range of US $100–300 million, and the time required is between 8 and 10 years. Well established drug companies have fairly rigorous systems in place to screen out ideas for drugs or targets that do not meet the required financial criteria. This means that compounds that work (scientifically) often are not being developed and suitable targets often are not being addressed. The bar is currently set at peak year sales of at least $100 million for an imaging agent; to meet the $100 million level, 1 million doses must be sold every year at a cost of $100 per dose, or 100 000 doses at a cost of $1000 per dose.

As this publication is aimed at researchers and practitioners alike, the main focus will be on development and preparation centred on clinical needs from the nuclear medicine perspective. The sections in the report start from synthesis, characterization and biological evaluation of radiolabelled biologicals for diagnosis and therapy, mainly of cancer.

2.3. CLINICAL CRITERIA FOR RADIOLABELLED BIOLOGICAL OR PEPTIDE RECEPTOR RADIATION THERAPY

There must be a strong justification and clinical requirement for development of a radiolabelled biological or peptide receptor radiation therapy, including:

(a) Patients with cancer and multiple inoperable metastases;
(b) Palliative therapies (chemotherapy) with little or no success;
(c) A radiolabelled biological or radiopeptide binding specifically to the corresponding peptide receptor;
(d) Internalization and accumulation of the radioactivity.

The key objective would be to develop a receptor selective radiolabelled biological, ideally, radiometallated analogues of the biological or peptides with improved metabolic stability. A common strategy is shown in Fig. 2, many stages of which are described in the various sections of this publication.

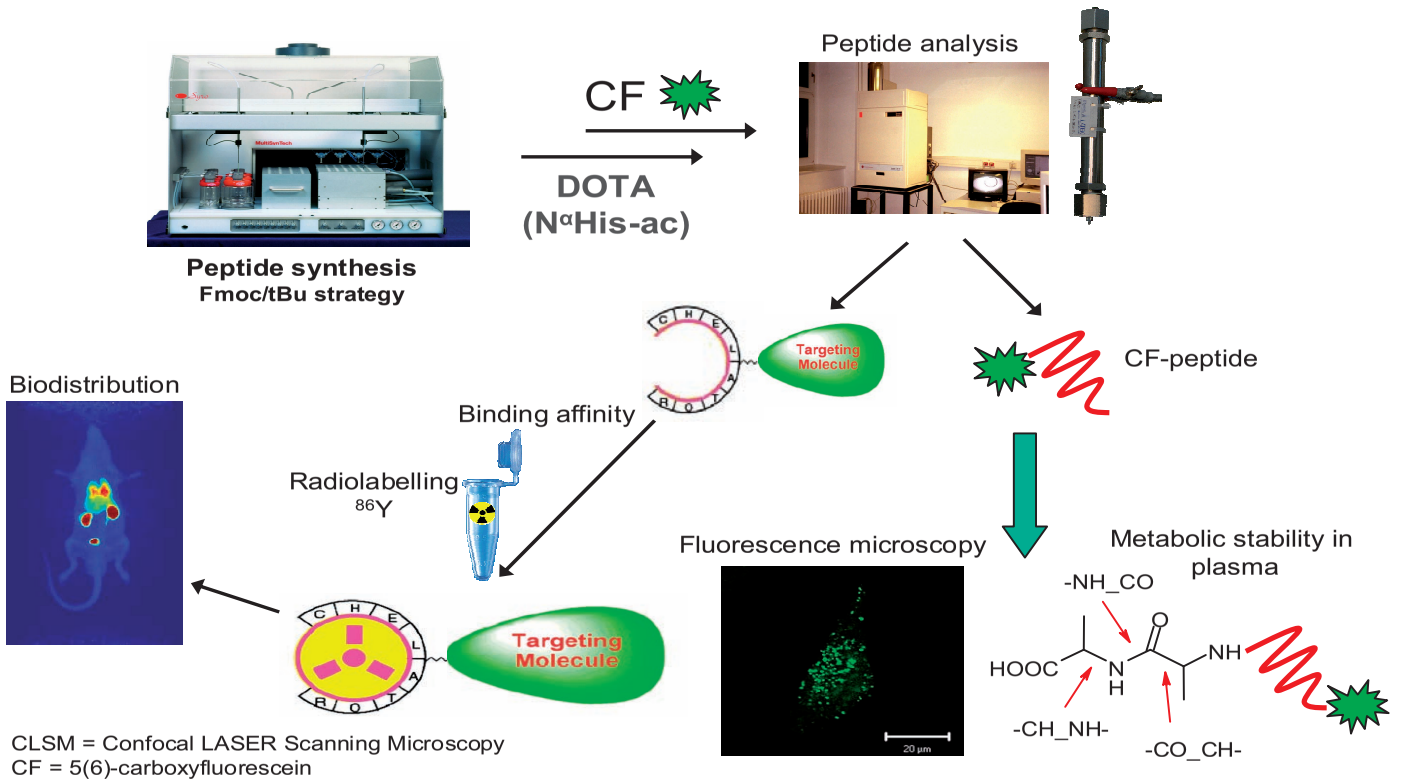

FIG. 2. Common strategy for development of a radiolabelled biological.

2.4. OVERSIGHT COMMITTEES

In countries where the production and use of radiopharmaceuticals is limited to one or a few major hospitals, there may be no local legislative rules or regulations specifically for radiopharmaceuticals, particularly if the number of radiopharmaceuticals produced is limited and the radiopharmaceuticals are based on 'kit' processes. In these cases, the methods and procedures used by the radiopharmacist will be determined by the knowledge base, the training received by personnel (at the facility or elsewhere), and the experience and current compounding practices of the hospital where the radiopharmacy is located.

In such situations, a local Oversight Committee for Radiopharmaceuticals (OCRP) (e.g. the Radiopharmaceutical Committee in India) may perform the role of the Radioactive Drug Research Committee (RDRC), the investigational new drug approval mechanism, and, to some extent, the new drug application, in scrutinizing the standard operating procedures (SOPs), QC protocols, sterility/bacterial endotoxin testing (BET) records, dispensing and various other responsibilities, as appropriate, and in constructing a system that ensures that the manufacturing practices are safe and the radiopharmaceutical is safe for its intended use. The SOPs can be developed taking into account requirements established under good radiopharmacy practice (GRP) and the feasibility of adopting the prescribed guidance within Member States.

Research on and development of radiolabelled biologicals require appropriate investment in radiopharmacy, as additional technologies (including peptide synthesis modules, HPLC and auxiliary equipment), skilled staff and adequate space are essential. Such a radiopharmacy is complex to operate; operational costs are high owing to the basic costs of starting materials, certified precursors and reagents, and spares. The choice of radionuclide is critical, as it has an impact on production, QC and patient scheduling. The use of radiolabelled biologicals requires proper planning and realistic time schedules. Some of the QC is time consuming, and there must be appropriate validation and strict criteria for parametric release of the radiolabelled biological. The stakes are high: failures in production and QC add to operational costs and can damage the radiopharmacy's reputation.

As this implies, radiopharmacy operations involving radiolabelled biologicals must be well planned and expeditiously executed. The SOPs are critical to ensuring that radiolabelled biologicals are produced and delivered on time, and that the QC criteria are consistently met. It is expected that hospital radiopharmacies will use precursors and other critical reagents procured from established and reliable sources for radiolabelled biological compounding, and

that staff will have the requisite training and hands-on experience suitable for the purpose. With the ready availability of much of the equipment and consumables required for satisfactory GRP, there is limited need to look for local alternative equivalents.

The OCRP can oversee compliance with good radiopharmaceutical manufacturing practice through self-inspection in those establishments that lack their own oversight committee. The aim of self-inspection is to monitor the implementation of and compliance with GRP, and to propose necessary corrective measures. This may include, but not be limited to, the following items:

(a) Personnel;
(b) Premises and utilities;
(c) Equipment;
(d) Production and in-process control;
(e) Quality control;
(f) Storage of raw materials and finished products;
(g) Documentation;
(h) Control of printed packaging materials;
(i) Distribution of the products;
(j) Calibration of instruments or of the measurement system;
(k) Validation and revalidation;
(l) Maintenance system;
(m) Sanitation and hygiene;
(n) Complaints management;
(o) Recall procedures;
(p) Results of previous self-inspection and corrective steps taken.

The frequency with which self-inspections are carried out will depend on the size and scope of the facility and on the number of its products. A partial self-inspection can be conducted (e.g. on the production line, facility, SOPs); however, a complete self-inspection should be carried out once a year.

All self-inspections should be recorded. Reports should contain:

(i) Self-inspection results;
(ii) Evaluation and conclusions;
(iii) Recommended corrective actions;
(iv) Implementation and assessment of corrective actions.

Finally, it may be said that OCRPs should periodically carry out a quality audit, consisting of an examination and assessment of all or part of a quality

system by an independent outside agency, with the specific purpose of improving the QA programme.

3. REQUIREMENTS FOR STARTING MATERIALS

All starting materials preferably should be purchased from certified vendors or suppliers. When received, they should be checked against the order to ensure that the delivery is correct. Records should be kept of the batch numbers and quantities received. In addition, a visual inspection should be carried out prior to acceptance. Starting materials with marketing authorization, including those in a kit form, should be used wherever possible. Materials should only be used within their declared shelf life. All starting materials, including biologically active pharmaceutical ingredients (active substances), radionuclides and reagents used as excipients, must be approved before use.

3.1. STARTING MATERIALS

A starting material is any substance, organic or inorganic, that is used as an active or excipient substance for the production of a medicinal product. This includes any substance or radionuclide produced for the radiolabelling process, with a resultant product planned for medicinal use.

MAbs for human use are preparations of immunoglobulin or fragments of an immunoglobulin (e.g. F(ab′)2), with a defined specificity, produced by a single clone of cells. These can be obtained from immortalized B lymphocytes that are cloned and expanded as continuous cell lines or from rDNA engineered cell lines. They may be conjugated with other substances, including by radiolabelling. The following letters are approved by WHO [4] and the United States Adopted Names Council as product source identifiers:

—u: human;
—o: mouse;
—a: rat;
—zu: humanized;
—e: hamster;
—i: primate;

— xi: chimera;
— axo: rat or mouse;
— xizu: combination of humanized and chimeric chains.

These identifiers are used as infixes preceding the –mab suffix stem, thus forming –umab (human), -omab (mouse), -ximab (chimera) and –zumab (humanized). In terms of risk identification, it is also import to note the difference between 'human' and 'humanized' MAbs. The 'human' MAb consists of human derived amino acid residues and is 'fully human', while the humanized antibody has segments of foreign derived amino acid residue. In terms of relative risk and antigenicity, murine ranks the highest and human the lowest.

In general, production is based on a seed lot system using a master cell bank or a working cell bank derived from cloned cells. The production should be validated in order to prevent transmission of infectious agents by the final product. All biological material should be characterized for absence of contamination, for viral safety and for risk of transmission of animal spongiform encephalopathy agents. Therefore, process validation, product characterization, process intermediates, biological assay, reference preparation, and sterility and bacterial endotoxins are important determinants. Peptide mapping is an identity test for proteins, especially those obtained by rDNA technology, and details can be found in major pharmacopoeias, including the European Pharmacopoeia (PhEur).

3.1.1. Precursors for synthesis

Precursors for synthesis are not produced on a large scale, and are accepted for use in a batch using validation data and a certificate of analysis demonstrating consistent reliability.

3.1.2. Related substances

Organic impurities in active substances should be identified, reported and qualified. Those with pharmacological or toxic effects will have specified thresholds. In general, for humans, the maximum dose is <2 g/d, the identification threshold is >0.1%, the reporting threshold is >0.05%, and the qualification threshold is >0.15%.

3.1.3. Certificates of analysis/conformity

Since most radiolabelled biologicals are formulated as injections, the starting materials ideally should be sterile products. Where this is not the case, the responsible radiopharmacist or QP should ensure that the product is of the appropriate quality by means of specifications, certificates of analysis/conformity, quality control testing, bioburden testing, or a combination of these.

3.1.4. Components

Ideally, components (including reconstitution devices, syringes and needles, the product contact parts of filling systems, transfer tubing and final containers) will be purchased presterilized from the manufacturer and should have either CE marking or a documented form of approval.

The synthesis set or filling systems should not be modified. However, if a modification is necessary, it should be validated and demonstrated that the modification does not jeopardize the patient product.

Sterile single use components should not be used beyond one working session. They should be packaged in such a way that they can be passed into the preparation area or final aseptic dispensing environment without increasing the risk of product or environmental contamination. Sterile components should be stored so as to minimize any increase of the bioburden on the surface of the primary packaging.

Any filters used should be preassembled by the manufacturer, and their sterility should be guaranteed. In addition, they should be prevalidated using volumes, pH and pressure as per actual patient batch. Standard bubble point testing following radiolabelled biological filtration should be done with great care, owing to the high radiation risk arising from the destructive nature of the test.

3.1.5. Local sterilization

Local sterilization of non-sterile components and equipment is acceptable, provided that sterility is assured. An audit trail should be available.

3.2. MATERIALS CONTROL PROCEDURES

All raw materials should be obtained from reliable suppliers and should be of pharmaceutical grade, sterile, non-pyrogenic and of good quality. There

has recently been an increased emphasis on ensuring that all starting materials, also referred to as active pharmaceutical ingredients (APIs), are prepared under an appropriate quality management system. APIs can be raw materials or intermediate, isolated or purified substances used in the preparation of radiolabelled biologicals intended for administration in humans. They should conform to API guidelines. Regular quality reviews of APIs should be conducted, with the objective of verifying their consistency. The following guidance applies to certificates of analysis:

(a) A certificate of analysis from a third party can be used to ensure the quality of materials (APIs).
(b) The radiopharmacist or QP must ensure that the certificate of analysis is from a competent organization.
(c) The certificate of analysis must clearly state:
    (i) The name of the issuing organization;
    (ii) The name of the material, the product code and the specification (e.g. International Pharmacopeia (PhInt), PhEur);
    (iii) The API batch number, method of testing, date, result, identified impurities, limitation of the test and conformance;
    (iv) The name of the authorizing person (QP).
(d) Even with a certificate of analysis, identity may need to be confirmed.
(e) The purchaser is responsible for the correctness of the material used in the radiolabelled biological tracer.
(f) It is useful to have knowledge of the certificates of analysis of any intermediates.
(g) Conformance of processing and packaging.
(h) If no expiry date is designated by the manufacturer, an expiry date is to be assigned to the component, material or supply, based on knowledge of its physical and chemical properties and on prior experience with its use.

Materials should be carefully checked using an SOP before being used. In addition to checking the expiry date, the packaging date should also be carefully checked to confirm that the material has been shipped under the required conditions. The certificate of analysis should be checked to confirm the quality and identity of the material (e.g. biological potency, pH, melting point). Approved raw materials should be stored in a clearly identified, secured place away from material not related to production. Only approved raw materials are to be used in the production process. Rejected raw materials should be separated and destroyed or returned to the vendor as soon as possible.

Quality assessment should be conducted of components (e.g. ingredients, reagents, target solutions and gases), containers and closures, and other materials (e.g. transfer lines, purification devices, membrane filters) that come into contact with the final radiolabelled biological.

There should be controlled access to appropriate storage (i.e. based on heat, light and humidity considerations) and checks on the temperature and humidity of storage areas containing components, containers and closures, materials and supplies used for the compounding of radiolabelled biologicals.

3.3. PEPTIDES, PROTEINS AND ANTIBODIES

Assessment of the origin of the product is required directly from the manufacturer or through an authorized dealer. If the product is supplied by the latter, it should be ensured that the dealer is a registered supplier of such starting materials. Preferably, both the peptide compound and the production method will comply with the current national pharmacopoeia monograph (if there is no monograph in the national pharmacopoeia, other internationally established pharmacopoeias apply). If no pharmacopoeia standards are available, the investigators should develop in-house specifications covering all the necessary parameters and test methods. For biological starting materials, it is important that the appropriate storage conditions are assured during both shipment and storage, as specified in the product description, and are respected in compliance with the code of practice of product storage and distribution (e.g. cold chain). Starting materials should be purchased from licensed producers (see Section 3.3.1); if a starting material is not available from a licensed vendor, it can be obtained from research institutions (see Section 3.3.2) or can be manufactured in-house (see Section 3.3.3).

3.3.1. Starting materials with manufacturing authorization

Starting materials with manufacturing authorization, licensed for use in humans, are produced under pharmaceutical good manufacturing practice (GMP). These products are supplied with a QC certificate providing information such as:

(a) Definition.
(b) Character.
(c) Identification.
(d) Test features:
    (i) pH;

(ii) pI;
(iii) Sterility.

(e) Storage conditions.

(f) Expiry date, shelf life after the product is first opened.

(g) For biological starting materials (derived from cell lines, expression systems), specifications should include all of the above as well as details on physicochemical and biological characterization, including:
(i) Expression system;
(ii) Potency;
(iii) Purity of the biological;
(iv) Glycosylation status;
(v) Host cell impurities;
(vi) Biological contaminants (e.g. viral, bacterial and prion agents, mycoplasma, other known biological contaminants such as polynucleotides and endotoxins);
(vii) Expiry date.

3.3.2. Starting materials from recognized institutions

A starting material manufactured by an institution recognized by the local or competent authority (CA) (e.g. small spin-off companies, universities, research centres) can be used. The user should be able to confirm or verify that the centre is recognized or registered by the CA for carrying out this task. If these criteria are not met, the user should categorize the starting material in a manner similar to that discussed in Section 3.3.3.

If the institution is deemed to be acceptable, the user must check whether the starting material is GMP compliant or not. If the starting material is GMP compliant, it should be considered as belonging in the category described in Section 3.3.1.

If the starting material is not GMP compliant, the user should ask the manufacturer for the certificate of analysis of the starting material and check whether the specifications for the starting material and the type of data available are adequate for the starting material's intended use as a radiopharmaceutical for humans.

The user should ensure that the data include information on the identity, content, purity, integrity and sterility of the starting material.

Physicochemical characterization may include the use of a variety of methods (e.g. mass spectrometric analysis, size exclusion chromatography, HPLC).

3.3.3. In-house starting materials

'In-house starting material' refers to a starting material that is manufactured by the user of the material either in-house or elsewhere. It is important to assess the origin of the starting material, as well as the manufacturer and the shipment conditions, as described above.

When using such starting materials, in vitro characterization and preclinical testing in animal models must be performed. These studies are often important for establishing the proof of concept of the different batches of peptides or antibodies. When it has been established that the starting material behaves as expected and has potential as a radiopharmaceutical for studies in humans, a certificate from an authorized contract research organization confirming the analytical status of the starting material, as defined in Sections 3.3.1 and 3.3.2, and proof of its applicability in humans are required.

Some countries are in the process of establishing precise rules and laws for the preparation of starting materials and subsequent radiopharmaceutical preparation. The European Commission (EC) has published a guide to GMP; the Australian and New Zealand Society of Nuclear Medicine (ANZSNM) has published guidelines for GRP; and, following the EC indication of GMP procedures, laws have been passed to standardize all radiopharmaceutical production in Spain (Real Decreto 479 of 12/4/1993), Italy (Gazzetta Ufficiale No. 168 of 21/7/2005) and many other European countries. Many other countries are preparing similar rules and guidelines. Therefore, each user must be informed of local regulations before handling biological materials and producing radiopharmaceuticals for human use.

3.4. REQUIREMENTS FOR ISOTOPES

The radioisotopes used for labelling of biologicals are also considered active pharmaceutical substances, and hence purchasing rules similar to those discussed in Section 3.1 apply. Generally, the source of a radionuclide should be specified (i.e. fission or non-fission), and the decay characteristics of the radionuclide (e.g. half-life, type, energy, probability of its emission) should be stated for its main radiation component. Information on whether the radionuclide is carrier free, carrier added or not carrier added should also be provided, since these carriers may affect the labelling efficiency and final specific activity of the labelled product. In the case of radionuclide generators, details on testing for mother and daughter radionuclides are required.

It is important to ensure the consistency and quality of the isotopes that are used for labelling. Preferably, isotopes with marketing authorization, as

either a radiopharmaceutical or a solution for labelling (precursors for labelling), should be purchased from licensed manufacturers. If a pharmacopoeia monograph exists, the material should comply with it. If no pharmacopoeia monograph is available, the investigators should develop in-house specifications covering all the test requirements, as defined below:

(a) Conformity with pharmacopoeia requirements. The radionuclide ideally should be pharmaceutical grade and should conform to the requirements of the pharmacopoeia (e.g. PhInt, United States Pharmacopeia (USP), PhEur, Japanese Pharmacopoeia (JP)). Identification of the radionuclide should be possible by its main radiation component using spectrometric methods.
(b) Route of manufacture.
(c) Radioactive concentration (and calibration date).
(d) Radionuclidic purity.
(e) Radiochemical purity.
(f) Specific activity.
(g) Chemical purity (special attention should be paid to the impurities that influence the radiochemical purity/labelling yield or biodistribution of the product). Identification of chemical impurities such as metal ions or reducing agents might be necessary.
(h) pH.
(i) Chemical form of the radionuclide and the chemical concentrations of excipients.
(j) Storage conditions.
(k) Expiry date.
(l) Bacterial endotoxin and sterility, if available.

It should be noted that differences are often observed between batches of the same radioisotope from the same supplier, and between isotopes supplied by different manufacturers, and that consistency should be maintained.

In most cases, positron emitting isotopes are produced in-house, and their overall quality and purity need to be verified and certified by local authorized personnel (e.g. hospital pharmacists, radiochemists or QPs) to ensure that they meet the requirements of the pharmacopoeia.

The most commonly used isotope for diagnostic purposes is ^{99m}Tc; ^{111}In, ^{123}I, ^{18}F and ^{68}Ga are also often used for radiolabelling purposes. The most commonly used isotopes for therapeutic purposes are ^{186}Re, ^{188}Re, ^{90}Y, ^{131}I and ^{177}Lu.

Annex IV provides a more complete table of isotopes, including information on diagnostics and therapeutics, energy, half-life, LET and source.

If a radioisotope is not listed in the pharmacopoeia, it is necessary to ensure that the quality of the radionuclide conforms to appropriate specifications, as listed below:

(1) Spectrum identification;
(2) Radioactive concentration (and calibration date);
(3) Radionuclidic purity;
(4) Radiochemical purity;
(5) Specific activity;
(6) Chemical purity (with respect to the impurities that may hinder labelling);
(7) pH;
(8) Chemical composition;
(9) Sterility;
(10) Bacterial endotoxin;
(11) Storage conditions;
(12) Expiry date.

3.5. REQUIREMENTS FOR REAGENTS

As chelators contribute to the final chemical form of the radiopharmaceutical, they should be checked prior to use for their identity and purity in the same way as the biological active pharmaceutical ingredient.

Those reagents that are used as excipients in the radiopharmaceutical preparation process should be purchased from an approved vendor. It is recommended that the QC release certificate issued by the manufacturer be obtained for each batch of reagent purchased, in order to verify the quality of the reagent. Where a relevant pharmacopoeia monograph is available, the reagents should conform to it, and their manufacture method should comply with GMP. When justified, identification of the reagent from each newly opened container may be necessary.

Buffers and water often must be deionized and degassed (usually by nitrogen bubbling for technetium and rhenium chemistries). Nitrogen gas and sterile water for injections (compliant with the national pharmacopoeia or USP/EP monograph) from approved suppliers are often used because of their low contamination and hence low risk of a negative influence on the radiolabelling process.

In the case of PET radiopharmaceuticals, special attention must be paid to ensuring the purity of all starting materials, reactants, chemicals, reagents and solvents used in synthesis and purification.

4. PREPARATION OF RADIOBIOLOGICALS

4.1. ASSESSING THE RISK DURING HANDLING

A search of the literature reveals that, apart from generic statements such as "It is strongly recommended that radiopharmaceuticals should be manufactured in accordance with the basic principle of GMP and with current national regulations", there appears to be no specific guidance on the actual handling and preparation of radiopharmaceuticals. Since GMP statements are mainly for the pharmaceutical industry, such advice is of limited use, especially for individuals handling and preparing radiolabelled biologicals in hospitals. One or two of the available guides emphasize the proteinaceous nature of these products and their potential to cause sensitization in healthcare staff. Very few even comment on the need for good aseptic compounding practices and for the prevention of cross-contamination in pharmacy facilities. If these products are then to be radiolabelled with high specific activities and large amounts of beta emitters, they can present a serious risk to operators. Therefore, proper risk assessment is essential prior to any undertaking. Determining the overall risk for each individual radiolabelled biological involves calculating the combined risk assessment for preparation of intravenous medicines and a health and safety assessment. Any assessment should include the toxicity score reflecting the allergenic potential based on the origins of the protein and radioactive components (see Section 3.1).

Radiation is a general property of all radiopharmaceuticals, and due consideration should be given to national and international radiation safety guidelines. The relevant legislation must be known and complied with.

The recommendations included in this section should therefore be considered as a set of basic standards supporting compliance with general requirements for GMP and GRP that are related specifically to the labelling and QC of peptides, proteins and antibodies.

The quality of radiopharmaceuticals may affect not only the safety of the patient but also the outcome of the diagnostic or therapeutic procedure. A GRP quality management system should therefore be designed and documented to provide the prescribed radiopharmaceutical products of the standard required.

4.2. FACILITIES

Laboratories or facilities should be maintained in a condition adequate for carrying out all operations related to the preparation and control of

radiopharmaceuticals. Laboratories for the handling of radioactive material should be authorized or licensed by the national regulatory authority considering the types of radiation emitted and the half-lives of the radioactive isotopes.

An aseptic work area should be available for the preparation of sterile radiopharmaceuticals. Ideally, this pharmaceutical laminar flow workstation will be situated in a Grade C (or, in some countries, a Grade B) clean room and sanitized at appropriate intervals set by the local authorities. Microbial and particulate contamination should be controlled and monitored by a comprehensive SOP. If the product is to be sterilized by filtration, the sterile filtration should be carried out in this pharmaceutical laminar flow workstation prior to dispensing. The inoculation of product in the growth media for sterility testing also requires a pharmaceutical grade laminar workstation; bacterial endotoxin testing can be carried out in adjacent rooms, under the conditions prescribed for the specific limulus amebocyte lysate (LAL) test used. Preliminary steps before preparation of the bulk solution and final filtration may be performed in other rooms having less strict air flow regimes. Specific requirements for the aseptic work area may vary, depending on national regulations.

Radiation shielding must be designed to minimize the radiation exposure of the operator and to preserve the integrity of the labelling procedure. Air from operations involving radioactivity should be exhausted through appropriate filters, which must be tested on a regular basis. All volatile, gaseous or aerosolized radioactive material must be used in a properly functioning fume hood with a charcoal and/or high efficiency particulate air (HEPA) filter, or in a biological safety cabinet. Labelling with iodine radionuclides and highly radioactive radionuclides (e.g. ^{131}I, ^{90}Y, ^{68}Ga, ^{18}F) should be performed either in a glovebox or in a hot cell, depending on the amount of radioactivity used. Personnel should take precautions to minimize exposure, skin contamination and inhalation.

Because of the physical nature of the radionuclides used and the activities required for therapy, the process of preparing and dispensing radiopharmaceuticals should be performed in a controlled area with entry restricted to essential staff [5]. Cremonesi et al. [5] followed a risk and safety review with a useful list of requirements for any centre that is administering therapy with ^{90}Y compounds. Table 1 lists devices and instruments to be used for radiation protection related to radiopharmaceutical preparation and the hot laboratory.

The radionuclide dose calibrator is the primary instrument for measuring the radioactivity of radiopharmaceuticals during the production process. Standard sources should be used to calibrate the instrument. Sample geometry effects should be established for all radionuclides for all container types.

TABLE 1. DEVICES AND INSTRUMENTS USED FOR RADIATION PROTECTION RELATED TO RADIOPHARMACEUTICAL PREPARATION

Device/instrument	Purpose
Hot cell (see Fig. 1) (COMECER, ELIZAbeta) Observation window: polymethylmetachrylate (PMMA; 10 mm) plus three layers of lead glass Cell walls: wood (10 mm) plus lead (2 mm)	Shielding from ^{90}Y β^- rays and bremsstrahlung
Anti-X gloves (0.1 mm lead equivalent)	Reduction of hand irradiations
Latex gloves	Contamination avoidance
Long tongs	Handling of ^{90}Y vials
PMMA cylindrical case (2 cm wall thickness) Shielded syringes (2 cm)	Shielding of ^{90}Y vials and syringes
Automatic dose fractionation system housed in the hot cell	Fractionation of the radiopharmaceutical
Geiger–Müller probe	Detection of possible contamination

Background activity should be determined every time the dose calibrator is used for measurements.

Proper dose calibrator measurement of pure beta emitting radionuclides is important for the safe and accurate dosing of various radionuclide therapies in nuclear medicine. Accurate measurement of pure beta emitters is a potential problem, as has been well illustrated by Siegel et al. [6], who explored the pitfalls of, and examined the possible solutions for, using dose calibrators for accurate measurement of ^{90}Y-ibritumomab tiuxetan. This applies to all beta emitters, and proper calibrator assessment is vital for dose accuracy.

4.3. PERSONNEL

One requirement for the preparation and quality control of radiopharmaceuticals is that trained personnel should be involved in all operations, under the control, or responsibility, of a professionally qualified person with experience in radiopharmacy and a proven record of academic achievement. All personnel, academic and technical, working in this area should have experience appropriate for their function and should receive additional training specific to this class of products. Aseptic techniques should

be applied when handling radiopharmaceuticals for injection, and personnel must be reliable and must observe the appropriate codes of practice. Personnel should be trained in GMP, the safe handling of radioactive materials and radiation safety procedures. Control of personnel radiation exposure should be performed with approved personnel dosimeters; these should be checked regularly and their readings should be recorded.

4.4. PREPARATIONS

Written SOPs should be prepared for all stages of preparation for all radiopharmaceuticals. These SOPs should be supported by validation studies demonstrating that they lead to radiopharmaceuticals which meet all acceptance criteria.

The name, quality and quantity of each ingredient used during the manufacturing process are to be stated. The written procedure should describe the different steps of the process and the precautions that are of importance to ensure the quality of the finished product.

Materials should be handled and stored according to the in-house SOPs and general quality standards, to prevent degradation and contamination. Weighing and measuring devices should be of an accuracy and a calibration status suitable for the intended use.

All operations involved in the preparation of the radiopharmaceutical should be carried out in a manner that prevents cross-contamination and mix-ups.

4.5. LABELLING METHODS

The utility of radiolabelled MAbs and small peptides in various diagnostic and therapeutic applications has enhanced interest in radiolabelling procedures. Methods have been developed for labelling these biomolecules with a variety of radionuclides having a broad range of chemical and physical properties. There are two main types of method: the direct method, where the radionuclide is bound directly to one or more atoms of the structure, and the indirect method, using bifunctional coupling agents (BFCAs). The indirect method can be performed by preconjugation of the ligand to the biomolecule before labelling, or by prior labelling of the chelating agent. Although direct labelling is the simplest method, indirect labelling using BFCAs generally offers greater control of the structure of the radiopharmaceutical and better in vivo stability.

4.5.1. Direct labelling methods

In the direct radiohalogenation approach, halogen ions are oxidized to become positively charged using oxidants such as iodogen or chloramine T. The positively charged halogen ion then undergoes electrophilic substitution in tyrosine residues. It is possible to obtain a number of different iodinated products; in addition, the radiolabelled tyrosine residue is susceptible to in vivo dehalogenation.

Radioiodination of Tyr^3-octreotide (TOC) resulted in limited in vivo stability of the radiotracer and increased lipophilicity with hepatobiliary clearance [7]. Iodination with ^{125}I, ^{123}I or ^{131}I may require different approaches and different quality controls [8].

The cluster designation 20 (CD20) antigen has become well established as a target for MAbs directed therapy in B cell lymphoma. Rituximab (MabThera) has been labelled with ^{131}I using an optimized chloramine T method [9] followed by purification with anion exchange resin, and optimal radioiodination conditions have been determined in order to preserve the immunoreactivity [10].

The Bexxar therapeutic regimen (tositumomab and ^{131}I-tositumomab) is an anti-neoplastic immunotherapeutic MAb based regimen (Corixa, USA, and GlaxoSmithKline, USA). Encouraging results have been achieved in a series of studies during the past decade [11].

MAbs can be labelled directly with ^{99m}Tc by reducing the disulfide bonds to obtain free thiols, which in turn are able to bind technetium or rhenium. In most cases, the direct method is a combination of two sequential steps: the reduction of disulfide groups in the antibody and the use of a ligand capable of transferring the reduced technetium or rhenium to the sulphydryl groups of the protein [12–14]. Rituximab has also been labelled with ^{188}Re using a direct method, and a freeze-dried kit formulation has been developed [15].

4.5.2. Indirect labelling methods

In indirect labelling methods, the radionuclide is coordinated by a synthetic chelating agent (BFCA), which may be conjugated to the biomolecule either before or after the radiolabelling process. Agents for indirect labelling are usually small molecules with a site for radiolabelling and a site for attachment to the biomolecule.

In indirect halogenation, a precursor molecule is initially halogenated by oxidative electrophilic substitution. The labelled precursor is then coupled to the lysine residues of the biomolecule under slightly basic conditions. Examples of such highly reactive precursors are N-succinimidyl 3-[4-hydroxyphenyl]

propionate (the Bolton–Hunter reagent) [16] and N-succinimidyl meta-(tri-butylstannyl) benzoate [17].

It is possible to radiolabel conjugated peptides and antibodies with radioisotopes of indium, yttrium, lutetium, gallium and copper. Radiolabelling of octreotide and other analogues has been carried out by conjugation of the chelator diethylenetriamine penta-acetic acid (DTPA) to the N-terminal D-Phe [18].

The macrocyclic chelator 1,4,7,10-tetra-azacyclododecane-N,N',N'',N'''-tetra-acetic acid (DOTA) (Fig. 3) ensures high in vivo stability for the corresponding radiometal chelates and therefore is appropriate for single photon emission computerized tomography (SPECT), PET and therapeutic applications [19–21]. Two key advantages of DOTA are:

(a) Kinetically inert and thermodynamically stable chelates with indium, yttrium and lanthanides;
(b) Wide acceptance for in vivo applications with several trivalent radiometals.

S-benzoyl-MAG3 has been used as a BFCA for rhenium. In the preconjugate approach, radiolabelled S-benzoyl-MAG3 is chemically activated by estrification, producing ^{188}Re-MAG3-activated ester, which is coupled to the free amino groups of antibodies (polyclonal or monoclonal), peptides or biotin derivatives, maintaining their biological activity [22, 23].

Peptides labelled with ^{99m}Tc have emerged as an important class of radiopharmaceuticals in diagnostic nuclear medicine during the past decade. In indirect labelling methods, technetium is coordinated by a synthetic chelating agent conjugated to the peptide. The hydrazino nicotinamide system developed by Abrams et al. [24] has been successfully used for preparation of specifically labelled proteins and peptides with high specific activities. For labelling TOC, the chelator is covalently attached and labelling is accomplished by transchelation using tricine and EDDA as coligands [25]. Other examples of biological peptides radiolabelled with ^{99m}Tc have been reported by Chianelli et al. [26] and Rennen et al. [27].

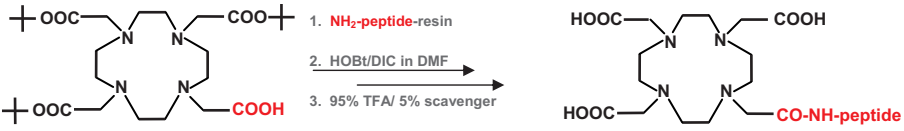

FIG. 3. The DOTA system.

The radiolabelling technique used has an important influence on the physicochemical properties, and consequently on the pattern of biodistribution, of the biomolecule. As long as the radionuclide remains firmly bound to the molecule or conjugate, it is possible to influence the pharmacokinetic parameters. Peptides can be easily modified and stabilized to obtain optimized pharmacokinetics, rather than bigger proteins and antibodies [28].

Stable complexing systems with favourable pharmacokinetics should be obtained through the combination of the radionuclide, labelling technique and chelate structure.

4.6. IN-PROCESS CONTROL AND STERILITY OF THE FINISHED PRODUCT

In-process control should be performed within the preparation area, and written procedures should be established to control processing steps that introduce variability in the quality of the final product. For example, the purification step after conjugation of a biomolecule to a BFCA should be performed using appropriate purification devices (e.g. SepPak columns) following written procedures, and the acceptance criteria should be defined on the basis of the information gained during the development stage.

Final sterilization of the preparation should be performed in a dedicated, clean environment with an as low as achievable risk of microbiological contamination. It is strongly recommended that all filters undergo a filter integrity test. Low protein binding filter membranes with a pore size of 0.22 μm are recommended, since sterility cannot be guaranteed with a larger pore size.

4.7. PHYSICOCHEMICAL/BIOCHEMICAL CHARACTERIZATION OF THE FINISHED PRODUCT

Radiolabelled antibodies and peptides should be prepared in a standardized, well controlled and validated manner. Methods should be developed to estimate the radiochemical purity of each of the three species of concern: free isotope, radiolabelled finished product, and labelled non-antibody or peptide substance. It is recommended that the initial submission for a finished product contain analytical results from an appropriate number of runs demonstrating the preparation of a biologically active, sterile and pyrogen free product. These radiolabelling runs should be performed by two or three of

the staff members who will radiolabel the antibodies or peptides for the study, using the reagents that will be used for the study (see Ref. [29] for details).

4.7.1. Assessment of structural integrity

Both radiolabelling processes and radiation may affect the structural integrity of finished products. In addition, since the finished products consist of a large number of non-radiolabelled antibodies or peptides and a small number of radiolabelled products, both chemical and radiochemical assessment of the structural integrity must be considered before patient use.

4.7.2. Chemical assessment of structural integrity

In the radiolabelling process, both chemical substances and reaction conditions may affect the integrity of the finished products. In direct labelling, the finished products are sometimes exposed to oxidants or to reductants during the radiolabelling process for radioiodination or complexation with ^{99m}Tc or $^{186/188}$Re, respectively. In indirect labelling, on the other hand, chelating agents or radiolabelling reagents (e.g. radioiodinated compounds with active ester groups or preformed radiometal chelates with active ester groups) are incorporated into parental antibodies or peptides prior to complexation reaction or radiolabelling. In this case, the average number of chelating agents or radiolabelling reagents introduced per molecule of antibody or peptide should be determined by well established methods. The effect of the chemical modification procedures (incorporation reaction of chelating agents or radiolabelling reagents) on the structural integrity and potency of parental antibodies or peptides should also be determined.

It is highly recommended that chemical analyses of radiolabelled finished products be undertaken using instant thin layer chromatography (ITLC), sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), isoelectric focusing (IEF), fast protein liquid chromatography (FPLC), size exclusion HPLC, reverse phase HPLC, capillary electrophoresis, UV spectroscopy, mass spectroscopy, nuclear magnetic resonance (NMR) and amino acid analysis, or other appropriate procedures. A combination of more than two analytical methods having different analytical principles should be used. For HPLC analyses, the recovery from the column should be determined by reliable procedures. A full description of the analytical procedures and the apparatuses used for the analyses should be considered before patient use. If difficulties are encountered in performing chemical analyses of radiolabelled finished products, finished products prepared using a non-radioactive isotope

in place of a radioactive one under identical conditions may be used to estimate the chemical assessment of the structural integrity.

Radiation constitutes another factor that may affect the structural integrity of finished products, especially when radiolabelled products of high specific activities are prepared. The chemical analyses of the finished products should also be performed by appropriate methods, as described above.

4.7.3. Radiochemical assessment of structural integrity

The radiochemical identity of the product should be defined (i.e. labelling site, structure of the radiolabelled molecule). The structural integrity of radiolabelled antibodies or peptides in finished products should be determined, to ensure the absence of radiolabelled fragmented, aggregated or otherwise modified antibodies or peptides. For this purpose, the finished products should be analysed by SDS-PAGE, IEF, FPLC, size exclusion HPLC, capillary electrophoresis, cellulose acetate electrophoresis, ITLC and reverse phase HPLC. The radioactivity behaviour of the finished products should be compared with that of parental antibodies or peptides whose structural integrity is confirmed by the chemical methods described above. A combination of more than two analytical methods of different separation principles should be used [8]. When HPLC is applied, the radioactivity recovered from the column should also be determined by reliable methods. A full description of the analytical procedures and apparatuses used for the analyses should be available to the quality controller.

5. QUALITY CONTROL OF RADIOLABELLED BIOLOGICALS

5.1. GENERAL CONSIDERATIONS

The purpose of QC is to identify and control the parameters of the finished product, such as its structural integrity; potency (biological activity); radionuclidic, chemical and radiochemical purity; and specific activity. Radiopharmaceutical grade isotopes should be used when preparing finished products. The purity of the finished products should also be considered from the radionuclidic, chemical and radiochemical points of view.

The sterility and pyrogen free nature of each isotope should be documented by submission of a certificate of analysis and letters of cross-reference for manufacturing information. The concentrations of covalently bound and free isotopes in the finished product, as well as residual levels of labelling reagents and their decomposed products, should be determined during the trial labelling runs. QC tests that will be performed before and/or after each administration to a patient should be described. When appropriate, colloidal formation in the radiolabelled finished products should be determined and limits should be set for it. In the indirect labelling method, it is necessary to assess the quality of the radiolabelling reagents or chelating agents along with the schematic drawing of the procedures. When the products are supplied as a kit formulation, the range of expected labelling efficiencies achieved by the indicated procedures should be submitted to the quality controller, along with the methods to determine the efficiencies. The detection and quantification of chemical substances and additives and potential contaminants in the finished product should be determined and submitted to the quality controller. Sterility and pyrogenicity tests should also be performed and submitted to the quality controller, according to the guideline below.

5.2. RADIONUCLIDIC PURITY

Radionuclidic purity is defined as the proportion or percentage of the total radioactivity that is present as the stated radionuclide in the finished product. Radiopharmaceutical grade isotopes should be used for preparing the finished products. When no pharmaceutical grade isotope is available, appropriate in-house methods should be available to ensure adequate quality. The radionuclidic purity of the radioisotope should be documented by a certificate of analysis from the manufacturer and letters of cross-reference for manufacturing information. If this information is not available, the radionuclidic purity of the stated radionuclide should be determined at the user's laboratory by spectrometric methods. Determination of the radionuclidic purity is recommended when radionuclide generator systems are used. The control of generator eluates should cover all the radionuclidic impurities that are possible due to the mode of manufacture, as well as the breakthrough of the mother radionuclide.

5.3. CHEMICAL PURITY

Chemical purity is defined as the proportion or percentage of the material in the specified chemical form, regardless of isotope substitution or attachment. Use of relevant physicochemical, biochemical and immunochemical analytical methodologies should permit a comprehensive characterization of the finished products and the accurate detection of degradation changes that may result from the radiolabelling process. The chemical purity of the finished products can be determined by SDS-PAGE, IEF, FPLC (size exclusion HPLC), reverse phase HPLC and other analytical methods. Protein staining and/or UV spectroscopy or other chemical methods can be used to identify the position of the finished products, degradation products and impurities. A combination of more than two analytical methods having different separation principles should be used. The recovery from the column must be determined by appropriate methods when HPLC is used to determine the chemical purity. A full description of the analytical procedures and apparatuses should be submitted to the quality controller.

5.4. RADIOCHEMICAL PURITY

Radiochemical purity is defined as the proportion or percentage of the total radioactivity that is present as the stated chemical form in the finished product. The radiochemical purity of the finished product is also required to elucidate the presence of free radionuclide, residual levels of radiolabelling reagent and the decomposed products. The radiochemical purity may change during the shelf life when reoxidation of ^{99m}Tc and $^{186/188}$Re labelled products is involved or when self-radiolysis, especially with finished products of high radioactive concentration, occurs. Thus, since the radiochemical purity may change over time, stability testing of the product is necessary in order to establish its shelf life (i.e. the time of storage during which the radiochemical purity remains within acceptance limits). The radiochemical purity often can be determined using the same methods as for chemical purity assessment, when observing the radioactivity detector trace of the analysed sample. The radiochemical purity can be estimated by comparing the retention times, R_f values or migration distances of the finished products with those of parental antibodies or peptides, free radionuclides, radiolabelling reagents or decomposed products. A combination of more than two analytical methods having different separation principles should be used. The recovery from the column must be determined by an appropriate, reliable method when HPLC is employed for the determination. A full description of analytical procedures

and apparatuses should be submitted. Where appropriate, radioactive impurities such as radiocolloids, free radionuclides or residual radiolabelling reagents in the finished product should be determined and limits should be set.

5.4.1. ITLC

ITLC analysis of finished products is undertaken according to the procedure for determination of the radiochemical purity of Zevalin. Throughout the assessment, proper aseptic techniques and precautions for the handling of radioactive materials should be employed. Waterproof gloves should be used during the determination of the radiochemical purity. Appropriate shielding should be used during the estimation.

At room temperature, a small drop (1–2 μL) of radiolabelled product is placed at the origin of an ITLC strip (Whatman). Usually, a silica gel plate (2 cm × 20 cm, or 2 cm × 10 cm) is used. The finished product is usually spotted 2 cm from the bottom. The drop should not be allowed to dry, and the next step of the process should be started immediately.

The ITLC strip is placed into a chromatography chamber with the origin at the bottom and the solvent front at the top. The solvent (0.9% NaCl) is allowed to migrate at least 8 cm from the bottom of the strip. The strip is then removed from the chamber and is cut in half. Each half of the ITLC strip is counted for 1 min with a well validated counting apparatus. Alternatively, an automatic ITLC reader can be used (see Fig. 4).

The percentage of radiochemical purity (RCP) is calculated as follows:

$$\%\text{RCP} = \frac{\text{CPM bottom half}}{\text{CPM bottom half} + \text{CPM top half}} \times 100 \quad (1)$$

Great care should be taken to assess the radiochemical purity by ITLC, since hydrolysed species of radiometals also remain at the origin. Thus, ITLC should be combined with another analytical method based on a different separation principle.

5.4.2. Size exclusion HPLC

For size exclusion HPLC, TSKgel G3000SW (7.5 mm I.D. × 60 mm) or TSKgel G3000SW$_{XL}$ (7.8 mm I.D. × 30 mm) columns are the most widely applied for the analysis. However, well validated columns that have a resolution comparable with that of TSKgel can also be used.

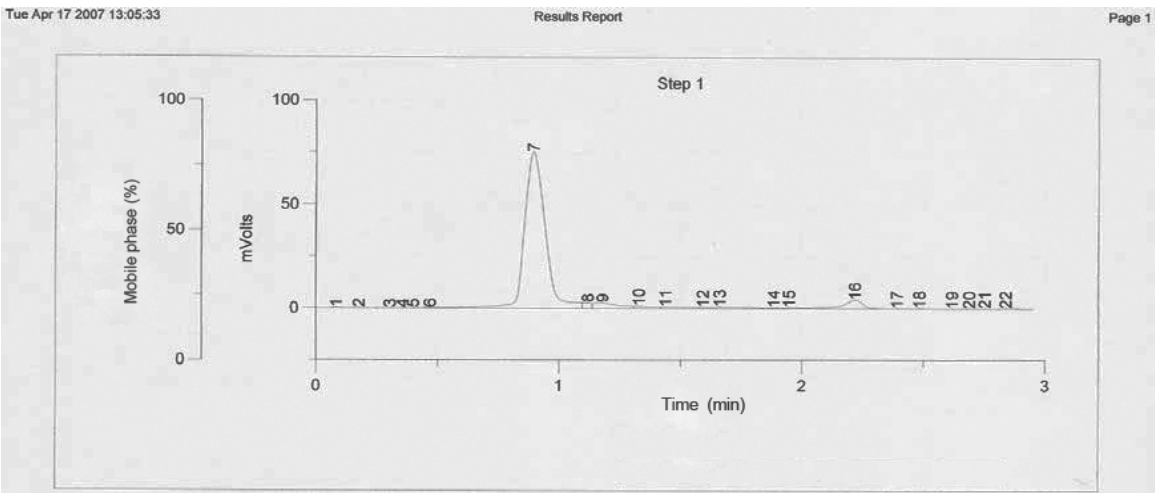

FIG. 4. A typical ITLC graph of a radiolabelled antibody showing that more than 95% of the radioactivity stays at the origin of the strip (peak 7) and less than 5% of the free ^{99m}Tc migrates to the front (peak 16).

The column is usually eluted with phosphate buffer (0.1M, pH7 or 7.4) or phosphate buffered saline (pH7.0 or 7.4) at a flow rate of 0.5–1 mL/min.

The HPLC system should consist of a well validated pump equipped with a radiation detector and a UV detector. Although a fraction collector can be replaced with a radiation detector, care should be taken so as not to overlook shoulder peaks that may appear after a major peak. The fractionation should be performed within a 30 s interval.

The injected radioactivity and the recovered radioactivity must be determined for accurate estimation [30].

5.5. SDS-PAGE

Resolving gels with 10% (wt/vol.) acylamide content and stacking gels with 5% (wt/vol.) acrylamide content can be used for most proteins. Samples are prepared by adding the solution under investigation to equal volumes of sample buffer (25% (wt/vol.) glycerol, 5% (wt/vol.) SDS, 5% (wt/vol.) beta-mercaptoethanol, 0.2% (wt/vol.) bromophenol blue in 100mM Tris HCl at pH6.8) and boiling for 3 min. Aliquots of the sample (10 μL) together with molecular weight standards are loaded onto the gels. Electrophoresis is performed at constant current (15 mA) for 6 h. At the end of the run, the gels are stained in Coomassie stain (0.04% (wt/vol.) Coomassie Brilliant Blue G250, 45% (vol./vol.) methanol, 10% (vol./vol.) acetic acid in water); destained in 45% (vol./vol.) methanol and 10% (vol./vol.) acetic acid; and soaked in 50% (vol./vol.) methanol and 3% (vol./vol.) glycerol, prior to drying.

SDS-PAGE analysis may also be performed under non-reducing conditions to assess the integrity of the disulfide bonds in the antibody molecules [31, 32].

5.6. CELLULOSE ACETATE ELECTROPHORESIS

Cellulose acetate electrophoresis is performed by spotting 1–2 μL of sample solution on presoaked strips of cellulose acetate membrane (e.g. 1 cm × 11 cm) with barbital buffer (0.05M, pH8.6) for 30 min at 300 V or (I = 0.05, pH8.6) at 0.8 mA/cm for 40 min. The cellulose acetate electrophoresis strips are then cut in half, and one half is stained with Coomassie Brilliant Blue (0.025% in water) or Ponceau 3R (5–6% in aqueous trichloroacetic acid). The complementary side is counted for radioactivity distribution by scanning on a

strip scanner or by cutting the strip into 5 mm sections and counting the sections in a well counter [33, 34].

5.7. DETERMINATION OF THE NUMBER OF CHELATING AGENT MOLECULES (OR RADIOLABELS) ATTACHED PER MOLECULE OF ANTIBODY

There is no question that a radiolabelled antibody may be degraded by covalent attachment, of either a chelating group or a radioisotopic atom, and that the extent of degradation increases with increasing substitutions. A representative estimation procedure based on the chemistry used for the conjugation reaction is briefly described here.

5.7.1. Calculation of thiol groups before and after the conjugation reaction

When chelating agents (or radiolabels) are conjugated to the antibody by thiol-maleimide or thiol-haloacetamide chemistry, the antibody molecules are thiolated by reducing their disulfide bonds or by modifying thiolating agents such as 2-iminothiolane. In both cases, the number of thiol groups generated in an antibody molecule can be determined using Ellman's reagent or 2,2'-dithiodipyridine. This leads to the generation of mercaptopyridine derivative (2- or 4-mercaptopyridine) having a high molar absorbance coefficient. After the conjugation reaction, a similar procedure is used to determine the number of residual thiol groups. The conjugation levels (number of chelating agent molecules (or radiolabels) per molecule of antibody) can be determined by subtracting the latter from the former [32, 35].

5.7.2. Calculation of the number of chelating agents by ^{111}In or ^{57}Co titration

The number of attached chelating molecules per antibody was determined by the following two methods before and after purification from free chelate.

5.7.2.1. Direct method

Before removing unconjugated chelate, a small aliquot of the reaction mixture is acidified with 1N HC1 to pH6.0 and reacted with a tracer amount of [^{111}In]acetate for 30 min at room temperature. The mixture is then put on a Sephadex G-50 column (0.7 cm × 20 cm) and eluted with 0.01M acetate buffer at pH6.0. The percentage of radioactivity present at the protein fraction — that

is, the coupling efficiency — is then determined. The number of chelating molecules incorporated per antibody molecule is calculated from the coupling efficiency and the molar ratio of added chelating agent to antibody molecule.

5.7.2.2. Tracer method

A small aliquot of purified chelate–antibody conjugate is incubated with a tracer amount of ^{111}In and stable indium in 0.02N HC1, which contains a two- to fivefold molar excess of the attached chelating agent estimated by the above method. Unconsumed indium ion is then complexed with an excess of ethylenediamine tetra-acetic acid to prevent the formation of insoluble indium hydroxide and the non-specific attachment of indium to the protein. The solution is placed on a Sephadex G-50 column (0.7 cm × 20 cm) and eluted with 0.01M acetate buffer at pH6.0. The number of chelating molecules attached per antibody molecule is estimated from the labelling efficiency and the molar ratio of protein to indium ion added to the solution [36, 37].

The number of chelating agent molecules attached per molecule of antibody is more precisely determined by $^{57}Co^{2+}$ titration of the chelate–antibody conjugate after purification [38].

5.8. USE OF MALDI-TOF-MS

Recently, matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has been applied to estimate the number of chelating agents attached per molecule of antibody [39]. Quality assessment techniques for radiolabelled antibodies have also been reviewed by Hinkle et al. [40].

5.8.1. Consideration of the number of chelating agent molecules attached per molecule of antibody

A chelate–antibody conjugate consists of numerous individual molecular species, including antibody molecules with no chelating molecule, with one chelating molecule, with two chelating molecules and so on. This can be better understood by considering the example of a perfectly random reaction. Since a typical antibody may have perhaps 90 possible conjugating sites, and as experience has shown that only a small number of chelating molecules should be introduced per antibody, the Poisson distribution can be used to predict how the labels will be distributed among the individual protein molecules. As mentioned above, the average number of chelating molecules attached per

molecule of antibody can be determined. Given this number, μ, the Poisson distribution function $P(x)$ tells what portion of the sample consists of unlabelled protein (number of chelating molecules $x = 0$), what portion consists of singly labelled protein ($x = 1$), and so on. The Poisson distribution function is

$$P(x) = \frac{\mu^x}{x!} e^{-\mu} \tag{2}$$

Thus, for a chelate conjugated antibody with an average of 0.3 chelators per molecule ($\mu = 0.3$), the probability that a protein molecule is not conjugated at all is $P(0) = e^{-0.3} = 0.741$; the probability of one chelating molecule per antibody is $P(1) = (0.3)e^{-0.3} = 0.222$; the probability of two chelating molecules per antibody is $P(2) = (1/2)(0.3)^2e^{-0.3} = 0.033$; and so on.

There is another feature that must be considered before the answer is obtained: a protein molecule bearing two chelating molecules will ultimately give twice as many radioactive emissions as a singly conjugated antibody, while an unlabelled antibody molecule will give no emissions at all. That is, the emission intensity derived from each class of labelled antibody is directly proportional to the number of labels per antibody molecule. The fraction of the total emission intensity due to each class of labelled antibody (for $x \geq 1$) is

$$I(x) = \frac{x}{\mu} P(x) = \frac{\mu^{x-1}}{(x-1)!} e^{-\mu} \tag{3}$$

Clearly, there are no emissions from unlabelled proteins, so for $x = 0$, $I(0) = 0$. Again, for a conjugated antibody sample having an average of 0.3 chelating molecules per antibody molecule ($\mu = 0.3$), 74.1% of the emission intensity will come from singly labelled antibody molecules. The remaining 25.9% of the intensity will come from multiply labelled antibody molecules, even though such molecules make up only 3.7% of the total number of antibody molecules [41, 42].

5.9. STERILITY

The sterility of non-radiolabelled precursors must be tested according to the established procedures [43–45]. When another test method is employed, the method's equivalence to the established method should be validated prior to its being performed. If antibiotics are used in product manufacturing, they

should be removed prior to sterility testing. If they cannot be removed, sterility assay using bacteriostasis and fungistasis testing, as described in USP Chapter 71: Sterility Tests [44], is recommended. This assay may be performed to ensure that any residual antibiotics present in the product do not interfere with the results of sterility testing.

5.10. PYROGENICITY/ENDOTOXIN

Endotoxin testing using the LAL assay is acceptable when validated [40]. Comparative testing should be repeated when LAL lots produced by a different manufacturer are used. A description of the protocols and reagents for the tests should be available for review. (For further guidance, see the guideline on Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices in Ref. [46].)

5.11. STABILITY

The stability of the finished products should also be considered from the chemical and radiochemical points of view. A stability test programme should be developed that includes tests for physicochemical integrity (e.g. fragmented or aggregated), potency, sterility and, as appropriate, moisture, pH and preservative stability, at regular intervals throughout the product shelf life. The relevant International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) document [47] contains details that might be applicable for individual situations.

5.11.1. Stability profile

The stability profile provides assurance that any changes in the identity, purity or potency of the finished products will be detected. Testing of both the chemical and radiochemical stability of the finished products should be performed. At the start of clinical research, applicants should have validated the methods that constitute the stability indicating profile, and the data should be available for review. The determination of which tests should be included will be product specific. The items emphasized in the following sections are not intended to be all-inclusive, but represent product characteristics that should

typically be documented to adequately demonstrate product stability. The exact organization of this step will depend on national regulations as well.

5.11.2. Protocol

The dossier should include a protocol for the assessment of the stability of the finished products in support of the proposed storage conditions and expiry dates. The protocol should include information that demonstrates the stability of the finished products throughout the shelf life, including, for example, well defined specifications and test intervals.

5.11.3. Potency (biological activity)

Testing for biological potency of the finished products should be part of the stability tests. For the purpose of the stability testing of the finished products described here, potency is the specific ability of a product to achieve its intended effect. It is based on the measurement of some attribute of the product and is determined by a suitable in vitro or in vivo quantitative method. In general, potencies of the finished products tested by different laboratories can be compared in a meaningful way only if expressed in relation to the potency of an appropriate reference material. For this purpose, a reference material calibrated directly or indirectly with the corresponding national or international reference material should be included in the assay.

5.11.4. Purity

For the purpose of stability testing of the finished products, purity is relative. The radionuclidic, chemical and radiochemical purities of the finished products should be assayed by more than one method (see the discussion of purity determination above).

5.11.5. Plasma stability

The chemical and radiochemical stability of the finished products should be determined in pooled human serum at 37°C under sterile conditions. Plasma may be used in the place of human serum, provided that the anti-coagulant used does not affect the stability of the finished products.

For radiochemical estimation of ^{99m}Tc and $^{186/188}$Re labelled products, stability testing in cysteine solution at 37°C is recommended. When tricarbonyl complexes of ^{99m}Tc(I) or $^{186/188}$Re(I) are used, stability testing in histidine

solution at 37°C is recommended. An alternative stability test for ^{111}In, ^{68}Ga, ^{90}Y or ^{177}Lu (radiometals) is the so-called DTPA challenge [48].

For stability estimation, incubation of radiolabelled antibodies in serum rather than in plasma obviates the need to include an anti-coagulant system, a non-physiologic entity. Blood is collected from volunteers and allowed to clot for 1 h at 37°C in a humidified incubator maintained at 5% CO_2:95% air. The samples are centrifuged at 400g, and the serum is filtered through 0.22 μm filters into sterile plastic tubes. The serum is then either used immediately or stored at –20°C. Radiolabelled antibodies, unattached radiolabels or free radionuclides are incubated for up to 10 d. Each sample consists of serum to which the radiopharmaceutical has been added. The amount of radiolabelled antibody and the molar equivalent of radiolabels or free radionuclides added to the serum should not vary, so that comparisons can be made. When possible, the serum concentration of the radiolabels should be less than 10^{-5}M, to provide a significant challenge from the proteins. The radiopharmaceutical is analysed using an appropriate procedure (as described below) before and after incubation in serum for periods of 1 h and 1, 2, 3, 5, 7 and 10 d, where appropriate [49].

As an alternative to plasma or serum, the stability of a radiolabelled product can be evaluated in phosphate buffered solution at pH7.4 at different time points up to 24–96 h after labelling (depending on the half-life of the chosen isotope).

5.12. STORAGE CONDITIONS

5.12.1. Real time storage (conditions including temperature)

The stability of the finished products should be determined when stored in final packaging and at room temperature.

5.12.2. Humidity and temperature

In some cases, precursors of the finished product may be supplied as a lyophilized kit. In this case, storage conditions such as temperature and humidity should be determined.

5.12.3. Accelerated and stress conditions

Studies under accelerated conditions may provide useful support data for establishing the expiry date, provide product stability information and assist in

the validation of analytical methods for the stability programme. Studies under stress conditions may be useful for determining whether accidental exposures to conditions other than those proposed are deleterious to the product, and for evaluating which specific test parameters may be the best indicators of product stability.

5.12.4. Shelf life

When the products are supplied as a lyophilized kit formulation, the shelf life of the kit should be determined. The shelf life of the radiolabelled product after completing the radiolabelling procedure should be studied, if appropriate, as a function of the specific activities of the finished products.

5.12.5. Specifications

The specification established at the beginning of the stability study is verified after completion of the study with the evidence collected on the maximum acceptable losses of biological activity, limits for physicochemical changes or degradation during the proposed shelf life.

6. IN VITRO TESTS IN CELLS AND TISSUES

6.1. AIM

The aim of in vitro tests is to evaluate the radiolabelled product in appropriate assays (e.g. cell/tissue cultures, antigen coated plates, membranes) and to predict the pharmacological effects of a new biological radiopharmaceutical prior to initiation of in vivo studies.

6.2. DEFINITION

In vitro studies involve not whole organisms, but microorganisms, cells or material isolated from multicellular organisms as test systems.

6.3. RESPONSIBILITIES AND DUTIES

6.3.1. Facility management

The facility management responsibilities are of a general nature and are equally applicable to both in vitro and in vivo studies; they mainly concern the availability of qualified personnel and of appropriate facilities and equipment for the timely and proper conduct of the study. The facility's management ensures that all persons involved in the study clearly understand the functions they are to perform; workers should be provided with specific training for aseptic protocols and for the handling of radioactive materials. This approach helps in the implementation of safe and contamination free test systems. Prior to use, the materials used in the test systems must be checked and their safety, quality and suitability must be ensured by the management, which is responsible for verifying the quality of all types of material before its use in the test system.

6.3.2. Principal investigator

The principal investigator is responsible for the overall conduct and reporting of the study. He or she must emphasize the characterization and justification of the in vivo test system, which may be more difficult to accomplish than for in vitro studies. In some cases this may be straightforward, as in the use of a particular cell line justified on the basis of the characteristics of those cells. In other cases it may be more complicated, as in the justification of QC tests of the performance of the particular test system.

Justification of the test system requires that the test method be validated or be structurally, functionally and/or mechanistically similar to a validated reference test method. With the use of new test methods that are structurally, functionally and/or mechanistically similar to a validated reference test method, the principal investigator should confirm that the new test method has a performance comparable with that of appropriate reference items [50].

6.3.3. Staff

During in vitro test procedures, staff must comply with the requirements of biohazard safety, aseptic conditions and avoidance of pathogen contamination or cross-contamination between the test systems. To minimize risks, staff must be aware of the appropriate precautions and the requirements to isolate test systems involving biohazardous chemicals and materials applied during in vitro studies [51].

6.4. FACILITIES

6.4.1. Basic facilities

Appropriate test facilities should be available, fulfilling all the demands of the particular in vitro test and providing a separate environment dedicated to that study. If more than one study is performed in the same physical atmosphere, an adequate degree of separation between the different in vitro studies should be ensured.

6.4.2. Test study facilities

To ensure the integrity of each in vitro study performed in the same area, and to avoid cross-contamination, an appropriate workplace (i.e. sufficient rooms and work tables) should be available and should be cleaned of any materials used in previous studies. Appropriate precautions must also be taken during the experiment to avoid the possibility of mix-ups, including proper labelling of vials and culture plates, use of separate racks in incubators or freezers, and a suitable time interval between the uses of two different cell lines in the same laminar hood. In the case of special equipment and test systems, a separate area or room should be provided and must be devoted to that particular study.

6.4.3. Facilities for standard and test materials handling

Sterility and test system separation are important aspects of good laboratory practice (GLP)/GMP principles; therefore, separate and careful handling of the standard and test materials in an aseptic condition is required. GLP/GMP principles for handling standard materials (including positive and negative controls) and test materials apply equally to in vitro tests. Thus, to avoid the mixing of standard and test materials, as well as to minimize the possibility of their contamination, aseptic conditions in a separate area should be ensured for standard and test materials preparation.

6.5. LABORATORY DESIGN AND EQUIPMENT FOR THE TISSUE CULTURE

6.5.1. Laboratory design

A good laboratory design for tissue culture facilities includes good quality materials, sterile working conditions and adherence to safety precautions.

The laboratory should be divided into two areas, one for new materials (e.g. new cell lines, culture media) that must be tested for possible contamination (e.g. bacterial, fungal or mycoplasma), and the other for materials that have already been tested for sterility. Considerable planning is required for conducting tissue cultures in a shared facility. All new materials should be handled as 'quarantine materials'. Also, different incubators should be used for the quarantine and tested materials. Ideally, different areas and work facilities should be reserved for the handling of newly received quarantine materials and for materials known to be free of contamination. All GMP principles for sterility and safety must be followed in a cell culture laboratory.

6.5.2. Laminar flow hoods

A good laminar flow hood is the most important requirement of a tissue culture laboratory. The correct method of working in the laminar flow hood must be followed; the laminar flow hood must be cleaned on a priority basis, and regular maintenance is required to maintain the sterile conditions of the in vitro test system. HEPA filters used in laminar hoods protect the user and also the testing materials from the pathogen present in the atmosphere. The laminar hood should be monitored regularly by placing agar plates inside the hood for a minimum of 4–5 h to check the sterility of the internal environment. An appropriate class of laminar hood should be used, depending on the hazard risk and the requirement of higher containment. For all GMP procedures, a class II laminar hood with vertical flow is required.

6.5.3. Incubators

Because different cell lines (e.g. insect, mammalian) require different temperatures and CO_2 levels, a special incubator is required. This incubator should be equipped with a CO_2 supply and should allow control of the exact temperature, degree of humidity and percentage of CO_2 in a stable manner to ensure appropriate growth conditions. The facility for water bath treatment fluid in the incubator water trays will also minimize the risk of microbial (fungal and bacterial) growth in the incubators. However, regular maintenance and cleaning are necessary to avoid contamination.

6.5.4. Centrifuges

The centrifuge is one of the most common and routinely used pieces of equipment in the tissue culture laboratory. A sealed bucket centrifuge with a temperature control facility is the most suitable for tissue culture; it can be of

the fixed angle or free angle type, depending on the specific requirements. The GLP principles require that the centrifuge be calibrated regularly and have a clear lid so that the condition of the load can be observed without opening the lid. This will also protect the user from exposure to hazardous materials.

The load should be balanced carefully before centrifugation is started. The lid must be cleaned before and after centrifugation to avoid cross-contamination.

Most protocols indicate centrifugal forces in gravity (g). However, forces in revolutions per minute (rev./min) can also be found, and must be converted to gravity. This conversion is simple if the radius of the centrifuge, measured from the centre of the rotor to the end of the vial, is known. Figure 5 illustrates the relation between radius, gravity and revolutions per minute.

6.5.5. Plastic and glass consumables

The GLP principles mandate that cell culture facilities should meet the requirements for sterility and avoidance of cross-contamination from different studies performed in the same area. In this respect, the use of plastic consumables (e.g. culture plates, pipettes, centrifuge tubes) is preferable to the use of glassware. These consumables are commercially available as single use, sterile packed plastic ware. If glassware (such as flasks and pipettes) is used, the appropriate cleaning and sterilization procedures should be followed to avoid any possible contamination.

6.5.6. Workplace

The GLP principles require that the workplace, including flooring, walls and working desktops, be waterproof, resistant to chemicals (acids and alkalis), crack resistant (for liquid N_2), smooth and easy to clean, to maintain a sterile and clean working environment. In particular, the tissue culture room should be separated from another smaller room to allow workers to change into sterile gloves, cap and 'sleepers' before entering the main tissue culture laboratory. Moreover, in the main tissue culture laboratory, all windows should be sealed and an air conditioner that is separate from the central air conditioner is required, to avoid any possible contamination by air.

6.5.7. Maintenance of the cell culture laboratory

To maintain an aseptic, clean and safe working environment, routine cleaning, precautions and maintenance are required and must be made a

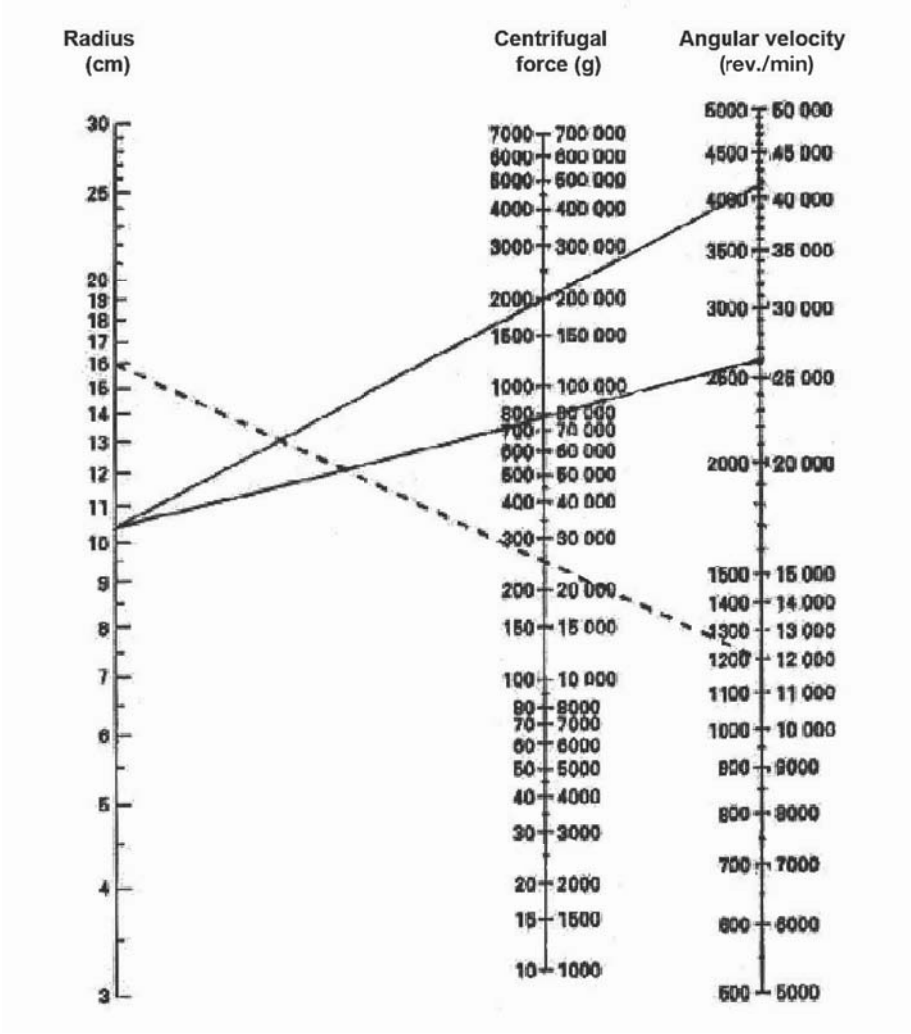

FIG. 5. Relation between radius, gravity and revolutions per minute.

priority. Routine cleaning involves the cleaning of all equipment, including the laminar hood, centrifuge, incubator, workplace and flooring. As a precaution, the water trays inside the incubators and the inner surfaces of the laminar hood should be cleaned, as they are a potential concern with respect to fungal and bacterial growth. All equipment should be maintained and serviced regularly by qualified engineers from the originating companies. For example:

(a) The temperature control of the incubator should be regularly checked with a NAMAS (National Accreditation of Measurement and Sampling (UK)) or equivalent calibrated thermometer.
(b) The laminar hood should be checked every six months to confirm that the airflow is correct and that the HEPA filters are functioning properly, to ensure that the hood is safe to use in terms of product and user protection.
(c) The incubator's CO_2 and O_2 levels should also be regularly checked to ensure that the correct levels are being maintained.

6.6. IN VITRO TEST SYSTEMS

In vitro test systems involve different types of test system, including biological systems (e.g. cell culture), physicochemical test systems (e.g. micro array) and analytical methodologies (e.g. metabolomics). A number of test procedures and kits are available for different systems [52–56].

6.6.1. Test system conditions

To ensure adequate quality of an in vitro test system during storage or during a study, appropriate conditions should be defined and maintained, with monitoring on a regular basis. This may include the viability and tolerance of an in vitro test system, such as recording the cell passage number and population doubling times, etc. Environmental conditions of the test systems, such as the temperature, humidity and CO_2 concentration in incubators, should be recorded both during use and under storage conditions. In the case of specific changes or manipulations, such as subcultivation and antimicrobial treatment, the in vitro test system should be tested for its quality and reliability.

6.6.2. New test systems

All new test systems should be assessed for suitability and reliability, such as morphological and functional status of cells and testing for suspected microbial or viral contamination on the basis of predefined criteria documented by the supplier. These results should be recorded and compared with positive and negative controls; moreover, the data obtained from the supplier of the system should be reviewed. Other important criteria, including origin, age or passage number, and cell doubling time, should also be assessed and records should be maintained.

6.7. QUALITY CONTROL ASPECTS

The quality of the cultures and their products depends on the quality of the materials used in the procedure. Therefore, QC aspects are crucial to achieving a reliable and appropriate tissue culture system. QC is basically focused on the following areas:

(a) Reagents and materials;
(b) Cell line authentication;
(c) Provenance and integrity of cell lines;
(d) Microbial contamination;
(e) Environmental monitoring.

6.7.1. Reagents and materials

The use of quality assured reagents and materials is the key to a reliable and appropriate test system. Although manufacturers supply certificates of analysis and quality checking with their products, retesting and verification are required owing to lot-to-lot variation. Because some basic materials used for the cell culture are a potential source of contamination in the cell lines, regular quality assessment is required before their use in the test system. For example, foetal bovine serum (FBS) is a potential source of bovine viral diarrhoea virus (BVDV), and porcine trypsin is a potential source of *Mycoplasma hyorhinis.* The sterility of disposable plastic wares should also be verified before they are used in tissue culture. All the QC certificates are to be stored for future reference.

6.7.2. Cell line authentication

For a reliable test system, materials must be of an assured quality. Use of the wrong cell line can produce invalid data and thus waste resources. It is advisable to use only cell lines from reliable culture collections whose identity has been certified. To authenticate newly received cell lines, the following methods can be used:

(a) DNA fingerprinting, involving identification of individual cell lines from the same species;
(b) Multilocus DNA fingerprinting, involving multilocus probes, along with Southern blotting technology to produce a complex banding pattern;

(c) Multiplex polymerase chain reaction (PCR) DNA profiling, involving a set of species specific primers recognizing microsatellites in PCR and DNA sequencing techniques;
(d) Iso-enzyme analysis, involving electrophoretic separation of subunits present in the iso-enzyme structure or composition, which differs from species to species.

By using the above methods, along with identification and confirmation of individual cell lines, cross-contamination can also be detected. The routine cell banking procedures of the European Collection of Cell Cultures involve the multiplex-PCR DNA profiling and multilocus DNA fingerprinting methods.

6.7.3. Provenance and integrity of cell lines

The quality of the cell line used directly affects the final results obtained. Newly received cell lines should be checked for provenance and integrity. Because newly received cell lines can be a source of contamination, proper verification of cell line authenticity (as described above) and of freedom from microbial contamination is required. Master and working stocks should be maintained for storage and routine use, respectively. The cell lines should be from a recognized and reliable culture collection, and all QC steps, including routine testing, must be followed. All QC details and the data sheets provided with the cell lines must be stored for future reference.

6.7.4. Microbial contamination

GLP/GMP principles must be applied in routine procedures and in the maintenance of cell stocks and culture materials. Personnel should be properly trained to avoid microbial contamination during routine procedures and to maintain aseptic conditions in the laboratory. Generally, microbial contamination from bacteria, fungi, viruses and mycoplasmas has been seen in cell cultures.

6.7.4.1. Bacterial and fungal contamination

Bacterial and fungal contamination requires immediate action as soon as the first sign of contamination appears. Screening for bacteria and fungi should be a part of routine QC analysis. The following are general characteristics of this type of contamination:

(a) Contamination can be easily seen without microscopy.
(b) There is increased turbidity.
(c) The colour of culture media changes owing to a change in pH.
(d) The number of cell deaths increases.

6.7.4.2. Viral contamination

Special tests are required for the detection of viral contamination of cell lines. BVDV contamination mainly occurs as a result of the use of infected FCS for the preparation of culture media. Although this virus is non-cytopathic and may cause slight changes in the growth rate, major effects are rare. However, some cell lines contain endogenous viruses and secrete virus particles or express viral antigens on their surface (e.g. Epstein–Barr virus transformed lines).

6.7.4.3. Mycoplasma contamination

Mycoplasmas are the smallest free-living self-replicating prokaryotes. They may be of a coccal or filamentous shape. The major species causing cell line contamination are *M. hyorhinis*, *M. arginini*, *M. orale*, *M. fermentans* and *Acholeplasma laidlawii*. A mycoplasma infection requires specific protocols and techniques for detection, as well as removal from the cell culture. This contamination of the cell culture may cause morphological changes in the cells, chromosome aberrations and reduced growth rates. Generally, mycoplasma contamination is not evaluated in laboratories; however, it should be a part of routine QC analysis [57].

6.7.4.4. Responses to contamination

In the case of contamination, it is advisable to discard the culture and generate a new culture from stocks that are free of contaminants. It is possible, although difficult, to remove the contamination (bacterial, fungal or microbial) by centrifugation or by use of antibiotics. Viral infections are intracellular parasites, and it is virtually impossible to remove them from cultures, since they do not respond to antibiotic treatment. The use of antibiotics during routine culture processes should be restricted, as excess use may lead to the development of resistant strains that could mask lower level contamination.

6.7.5. Environmental monitoring

GLP/GMP principles require regular workplace environmental monitoring to ensure an aseptic and clean working environment. The monitoring should include the working desks, laminar hood, incubators and general environment inside the laboratory. For environmental monitoring, the use of agar culture plates is the most suitable practice. In general, settle plates of agar are placed in the open on the laboratory desks or in other places (such as inside the laminar hood or incubator) for a period of 4–5 h, after which they are covered and incubated at 32ºC and 22ºC for up to one week. Finally, the plates are examined for indications of any microbiological contamination, which can be assessed on the basis of the presence or absence of growth on the incubated culture plates. The results obtained after the examination of the incubated cultured plate should be matched with the standard values in order to verify the level of cleanliness and safety, and the values should be recorded and stored for future trend analysis.

6.8. SAFETY ASPECTS

6.8.1. Disinfection

Proper disinfection of the tissue culture workplace, equipment, used materials and waste products is necessary in order to avoid any possible risk of biological harm or contamination [58–60]. The following are some categories of disinfectant commonly used in tissue culture:

(a) Hypochlorites: A freshly prepared solution of hypochlorites is active against viral contamination. It can be used for different purposes, including as a surface disinfectant, a washing reagent for contaminated glass consumables or a discarding solution for culture wastes at concentrations of 1000, 3000 and 10 000 parts per million (ppm), respectively. Example: chloros.
(b) Aldehydes: The use of hypochlorites is avoided in the case of metals, as they are corrosive to metals. Therefore, all metal equipment (e.g. centrifuges, incubators, water baths) should be cleaned using aldehyde chemicals such as glutaraldehyde. Precautions should be required owing to the irritating nature of aldehydes, which may cause sensitization. Examples: glutaraldehyde, formaldehyde.
(c) Alcohol: The most commonly used disinfectant against bacterial, fungal and viral contamination is alcohol, which works by dehydration and

fixation. A 70% concentration may be used as an effective antimicrobial agent. Examples: ethanol, isopropanol.

(d) Phenolics: Disinfectants in this category are not effective against viral contamination, but are active in the presence of organic matter. Examples: sudol, hycolin.

6.8.2. Waste disposal

All biological waste from the tissue culture laboratory must be decontaminated and disposed of according to national requirements in order to maintain a safe environment and to avoid biological hazards. Examples of different biological waste treatments include the following:

(a) Contaminated consumables (such as flasks, measuring cylinders and pipettes) should be placed in a 3000 ppm hypochlorite solution overnight before disposal by autoclaving and incineration.
(b) Tissue culture waste (e.g. degenerated cell lines, culture media) should be inactivated overnight in a 10 000 ppm solution of hypochlorite and then drained with an excess of water before disposal.
(c) Solid waste such as centrifuge tubes, contaminated gloves and tissue paper should be autoclaved prior to incineration.

6.9. PRECAUTIONS IN TISSUE CULTURE LABORATORIES

The following precautions should be taken in tissue culture laboratories:

(a) All personnel involved in a study must wear their own laboratory coats and eye protection during the experiments.
(b) Personal protective equipment (e.g. laboratory coat) used in the tissue culture laboratory should be kept separate from that used in the general laboratory.
(c) Disposable caps and gloves should be used to avoid any possible contamination.
(d) Each culture/reagent bottle or flask must be labelled with the name of the reagent, the date of preparation and the quantity stored.
(e) In the laminar hood, work on different cell lines must be separated by a time interval of 30 min; moreover, the work surface must be cleaned with disinfectant and the UV lamps must be switched on before and after work. This approach will reduce the risk of cross-contamination.

(f) Cell lines should be subcultured at 75–80% confluence, and a frozen stock of the cell line (the master cell line) should be stored in accordance with the cell banking procedure for future renewal.
(g) Culture media must be stored at +4°C and should be used within the shelf life.
(h) The quality and sterility of all media and reagents should be verified before their use.
(i) All culture plates and bottles kept in the incubator should be examined daily for any possible bacterial, fungal or mycoplasma contamination.
(j) Excessive use of antibodies should be avoided in order to prevent the development of antibody resistant strains.
(k) Equipment used in the tissue culture laboratory should be cleaned and calibrated regularly.
(l) The tissue culture laboratory must be separated from the rest of the general laboratory, and frequent entry into the tissue culture laboratory should be avoided.
(m) All national requirements should be met to avoid violation of national standards.

6.10. PROTOCOL FOR CELL BINDING ASSAY WITH RADIOLABELLED ANTIBODIES OR PEPTIDES

In vitro verification of specific and displaceable binding of the radiolabelled peptide is an essential step that must be undertaken before experiments are performed in animals or humans. Several types of binding assay in soluble form or on isolated membranes, cells or tissues have been described. Binding assays and displacement assays can also be performed using autoradiographic methods on cells and tissues.

Here, as an example, a method for cell binding assay is described that can be applied to a variety of radiolabelled peptides or radiolabelled antibodies, providing availability of target cells and biocompatibility of the used reagents.

Assay requirements:

(a) Unlabelled MAb/peptide;
(b) Labelled MAb or peptide (with known specific activity) and its dilutions;
(c) Binding medium: RPMI, 0.1% BSA (add 0.02% EDTA if the cells have grown in adhesion);

(d) Cultured cell suspension of target cells expressing receptors for the labelled product;
(e) Ninety-six well plate;
(f) Ice plate (to maintain a temperature of 4°C) or a basket with ice;
(g) Blackman microtubes and LP3 tubes;
(h) Oil mixture (dibutylphthalate:dioctylphthalate (3:2); to be optimized);
(i) Liquid N_2 in a 0.5 L N_2 container;
(j) Microcentrifuge, gamma well counter, tips, pipette, vial cutter and forceps.

Procedure:

(1) Label the MAb or peptide using a method suitable for high labelling efficiency; carry out QC for labelling. Correct calculation of the specific activity is essential for the Scatchard plot analysis.
(2) Prepare the microtubes filled with oil mixture (150 µL), previously marked. The amount of oil and the ratio of dibutylphthalate to dioctylphthalate must be evaluated in advance by performing centrifugations with the labelled MAb/peptide without cells and with an increasing number of cells. Some peptides can be very lypophilic, and some cells (particularly if they are large or many) may modify the water–oil interface and allow the radiopharmaceutical to reach the pellet in the vial.
(3) Prepare the LP3 tubes, numbered in duplicate for collection of the two portions of the microtube after it is cut (lower part with cell pellet, upper part with supernatant).
(4) Prepare the dilution of cold MAb/peptide using binding medium.
(5) Prepare the appropriate dilutions of hot MAb/peptide in the binding medium.

Note: When dilutions of hot and cold protein are prepared, it must be remembered that the concentration is diluted three times when loaded in the well. One volume should be used for cells, one volume for the hot MAb/peptide and one volume for the cold MAb/peptide or binding medium.

(6) Harvest the cells from the culture plates and collect them in a plastic tube with binding medium; this should be done inside the laminar hood to avoid contamination.
(7) Count the cell suspension using a Neubauer camera, and prepare an appropriate dilution to put into the 96 well plate (2×10^5–10×10^5 cells/well, in 30 µL).

(8) Prepare the ice platform for the experiment (to maintain a temperature of 4°C).
(9) In the 96 well plate, first add the cultured cells in 30 µL per well.
(10) Divide the 96 well plate into two parts, the upper part for the hot MAb/peptide (specific plus non-specific) and the lower part for the cold plus hot MAb/peptide (non-specific). In the hot part, add 30 µL of the labelled antibody (diluted with binding medium) to each well in different concentrations in triplicate and then 30 µL of binding medium. In the cold plus hot part, first add 30 µL of the unlabelled MAb/peptide and then immediately add 30 µL of the labelled antibody in triplicate (diluted with binding medium) to each well in different concentrations, maintaining a constant concentration for the cold MAb/peptide (see Fig. 6).
(11) Incubate the culture plate for 30 min to 2 h at 4°C.
(12) After incubation, collect the cell suspension (90 µL) from each well separately and place it in separate microtubes, above the oil layer in the tube after forced deposition of oil in the bottom of the tube (make sure that no air bubbles are present in the bottom of the microtube).
(13) Centrifuge all microtubes at 9000 rev./min (1350g) for 3 min in a microcentrifuge with a fixed angle rotor.
(14) Quickly freeze all microtubes by briefly dipping them one by one in liquid N_2, for 5–8 s, then remove them using forceps. The cell pellet should be present in the bottom of the tube.
(15) Using a vial cutter, cut the tube above the pellet level and collect the pellet in a different LP3 tube.
(16) Count the radioactivity of cells and supernatants separately in the automatic gamma counter to calculate the specific binding.

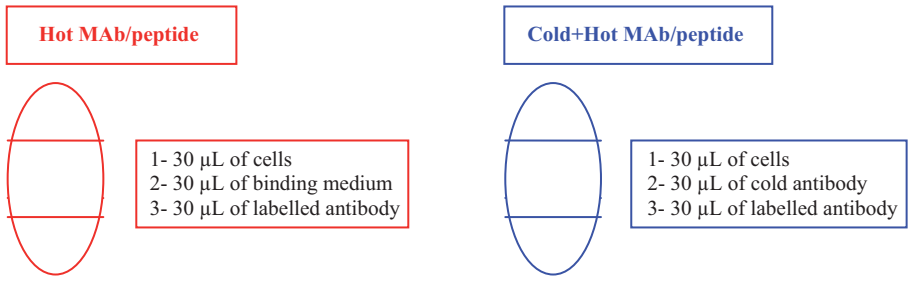

FIG. 6. Schematic illustration of a well plate. Note: The sequence shown in the figure and the pipetting method are important for reproducibility; tips should not be inserted into the well.

General considerations:

(a) EDTA should be mixed in the binding medium to avoid non-specific binding of the cells at the bottom of the 96 well plate.

(b) The dissociation constant, K_d, can be calculated using GraphPad software. It is the mean value of the increasing phase of the 'radioactivity counts' versus the 'hot concentration' graph.

(c) To define optimal radiolabel concentrations, check the binding affinity of the labelled antibody when a competition is performed between labelled and non-labelled antibodies (starting from a 100:1 ratio of cold to hot MAb/peptide).

(d) Prepare the initial solution (mother solution, I); place 500 µL of the solution in the first vial (dilution is one-half of I); place 200 µL from the first vial in a second vial (dilution is one fifth that of the solution in the first vial); place 500 µL from the second vial in a third vial (dilution is one half that of the second vial); place 200 µL from the third vial in a fourth one (dilution is one fifth that of the third vial); place 500 µL from the fourth vial in a fifth vial (dilution is one half that of the fourth vial); place 200 µL from the fifth vial in a sixth vial (dilution is one fifth that of the fifth vial); place 500 µL from the sixth vial in a seventh vial (dilution is one half that of the sixth vial).

(e) After performing these dilutions, it is better to count an aliquot of 30 µL of the 4–5 higher dilutions to obtain the standard curve of the 'total' radioactivity added to each well for each MAb/peptide concentration. These values will then be fitted by linear regression and will serve to calculate the exact 'total' radioactivity for higher concentrations as well. Total radioactivity is an essential measurement for the Scatchard plot analysis.

(f) Expected result: K_d should be approximately the same as that provided for the MAb/peptide by the supplier. This proves the ability of the MAb/peptide to bind the cell after radiolabelling. Data are presented as 'specific binding' over 'concentration of labelled MAb/peptide in µM' or by 'B/T' over 'B in molecules' (Scatchard plot analysis). The 'specific binding' is 'specific plus non-specific' minus 'non-specific'. The calculation of 'B in molecules' is obtained by knowing the specific activity of the labelled antibody and the number of molecules per mole (Avogadro number, 6.022×10^{23} mol^{-1}).

(g) A linear Scatchard plot indicates one type of receptor (or binding site), the K_d of which is the angular coefficient of the interpolating line and for which the total number of receptors per cell (or binding sites) is $y = 0$. A

curved Scatchard plot indicates two or more types of receptor (or binding sites), the K_d of which is obtained by the asymptotes to the curve.

A different type of one-step binding assay for determining the maximum number of bound molecules and the affinity and association rate constants has recently been reported [61]. Other researchers have proposed a displacement binding assay by using a combination of radiolabelled peptides and antagonist fluorescein isothiocyanate (FITC) labelled MAbs, using a fluorescence activated cell sorter (FACS) for data analysis [62].

7. ANIMAL TESTS

7.1. AIM

The objective of the preclinical pharmacodynamic guidance provided here is to assist practitioners in predicting the pharmacological effects of new biological radiopharmaceuticals prior to initiation of studies in humans. Previous in vitro assays (e.g. cell lines and/or primary cell cultures) may be useful for examining the effect in animals.

Although there is no internationally accepted definition, pharmacological studies can be classified as follows:

(a) Primary pharmacodynamic studies: Studies related to the desired diagnostic or therapeutic effect;
(b) Secondary pharmacodynamic studies: Studies not related to the desired diagnostic or therapeutic effect;
(c) Safety pharmacodynamic studies (USA) or general pharmacology studies (Japan, EU): Studies related to potential undesirable pharmacodynamic effects of the test substance on vital physiological functions.

In practice, secondary and safety pharmacodynamic studies can be evaluated independently or as part of toxicological and/or primary pharmacodynamic studies. In this section, discussion is focused on the preclinical diagnostic and therapeutic primary pharmacodynamic effect of radiolabelled peptides, proteins, MAbs and their fragments. Safety pharmacodynamic and toxicity studies are discussed in Section 8. As secondary pharmacological effects (when they exist) may be desirable or undesirable,

further primary or safety studies should be performed, following the guidance provided in Sections 6 and 7 of this report.

Biological radiopharmaceuticals are typically administered into the circulatory system (i.e. intravenously or intra-arterially), and are used for diagnosis, monitoring and therapy. In some special cases, the biological radiopharmaceutical can be administered into a body compartment (e.g. locoregionally into a tumour cavity of a cerebral tumour or intraperitoneally in the case of a peritoneal carcinomatosis) and used for the same purposes. While the diagnostic and monitoring uses apply for different diseases, therapeutic use is practically limited to treating cancer (Fig. 7).

Discussion of radiolabelled peptides is included in this section because of the exponential growth in diagnostic and therapeutic applications of these peptides over the past decade. The automated means of synthesizing these compounds in large quantities and the simplified methods of purifying, characterizing and optimizing them have focused attention on peptides as carrier molecules. These new techniques have accelerated the commercial development of radiolabelled peptides, which has provided additional radiopharmaceuticals for the nuclear medicine community. Peptides have many key properties, including rapid clearance, rapid tissue penetration and low antigenicity, and can be produced easily and inexpensively. However, there may be problems with in vivo catabolism, unwanted physiologic effects, chelate attachment and toxicity because of binding to receptors expressed by non-tumour tissues [63, 64].

7.2. LEGISLATION AND FACILITIES FOR ANIMAL WORK

Depending on the radionuclide used, special considerations must be taken into account regarding the design and performance of preclinical studies of radiopharmaceuticals. Animals, animal waste and materials used during the experiments are radioactive. Conditions in the facilities should be adequate, and investigators should have appropriate experience to protect personnel, the general public and other animals (e.g. control versus treated animals) from any contamination. Facilities and personnel should also be in compliance with GLP for laboratory animals. Where the laboratory animal regulations are in disagreement with the radiological protection regulations, additional considerations may be necessary (e.g. ventilation systems).

Personnel and institutions should be licensed by local authorities to use the specific radionuclide undergoing experimentation. Local regulations should be followed carefully at all times, since animal studies often cannot be

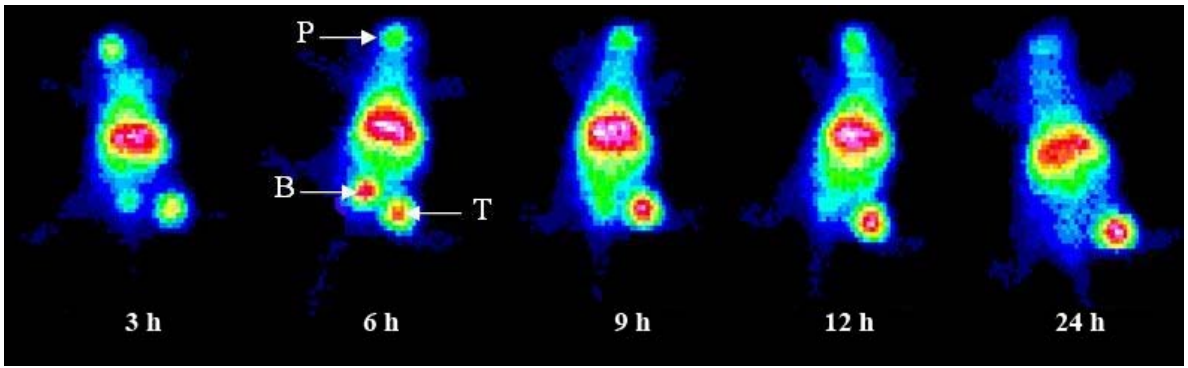

FIG. 7. Gamma camera images obtained from a mouse bearing a tumour (T) overexpressing antigens recognized by a MAb, indicating the tumour uptake kinetics of the ^{99m}Tc labelled MAb administered by an intravenous bolus injection throughout the retro-orbital plexus (P); B = urinary bladder.

replaced by other types of test to predict the pharmacological/toxicological profile of a biological radiopharmaceutical prior to initiating studies in humans.

7.3. GOOD LABORATORY PRACTICE

Ideally, preclinical studies of pharmaceuticals are to be performed in compliance with GLP. Nevertheless, it is recognized that, owing to the specific and unique design frequently used for biopharmaceuticals, and in particular for biological radiopharmaceuticals, it may not be possible to fully comply with GLP.

Primary and secondary pharmacodynamic studies do not necessarily need to be conducted in compliance with GLP [65]. Safety and toxicity studies should be conducted in compliance with GLP to the extent possible.

It is important to emphasize that areas of non-compliance with GLP should be identified. Data quality, documentation of the study and data storage should be ensured throughout and after the study. In these special cases, lack of full GLP compliance does not necessarily mean that the data cannot be used to support clinical trials [65, 66].

7.4. ANIMAL MODELS

The species specificity of many peptides, proteins and MAbs has demanded the determination of species relevance before the initiation of pharmacological/toxicological studies. A relevant species is one in which the test material is pharmacologically active owing to the expression of a receptor or an epitope (in the case of MAbs). Selection of the species is usually accomplished by in vitro comparison of the binding affinity or functional activity of the product in animal and human cells, followed by in vivo demonstration of the pharmacological activity [67].

Absolute equivalence of antigen density or affinity for the biopharmaceutical, however, is not always possible or necessary for an animal model to be useful. Differences in binding, for example, may be compensated for by alterations in dose or dosing frequency [68].

It is important to show that the biological radiopharmaceutical maintains activity and biological properties equivalent to those of the unlabelled material.

In some cases, to study the primary pharmacodynamic properties of biological radiopharmaceuticals, xenograph or transgenic animal models expressing the adequate receptor or epitope can be performed.

In the case of therapeutic biological radiopharmaceuticals, appropriate control groups should be included to distinguish specific radiation effects from potential pharmacological/toxicological effects of the 'cold' non-radioactive labelled material or from the unlabelled peptide, protein or MAb (if their therapeutic profiles are not previously known). Diagnostic biological radiopharmaceuticals typically achieve their intended pharmacological effect owing to the radioactivity administered, and therefore the use of control groups is not necessary. When conducting safety/toxicity studies, appropriate control groups should be included.

7.4.1. Sex of animals

Animals of both sexes should generally be used, or justification should be given for specific omissions (e.g. in studies of ovarian or prostate cancers).

7.4.2. Anaesthesia

When conducting in vivo studies, especially when safety pharmacological studies of vital physiological functions (i.e. central nervous, cardiovascular and respiratory systems) are performed, it is preferable to use unanaesthetized animals. Data from unrestrained animals that are chronically instrumented for telemetry, data gathered using other suitable instrumentation methods for conscious animals or data from animals conditioned to the laboratory environment are preferable to data from restrained or unconditioned animals. In the use of unanaesthetized animals, the foremost considerations are the avoidance of discomfort and pain, as well as of possible radioactive contamination during the injection period, and the radioscintigraphy uptake quality.

The use of unanaesthetized animals is not always possible. When the use of anaesthetized animals is deemed necessary, the appropriate anaesthesia and dose level should be selected according to the animal species.

7.4.3. Administration/dose selection

In general, the expected clinical route of administration should be used in animal studies, where feasible. The use of other routes may be acceptable if the route must be modified owing to limited bioavailability, or to limitations due to the route of administration or the size/physiology of the animal species.

Most biological radiopharmaceuticals in clinical use are administered systemically (e.g. intravenously or intra-arterially for radioimmunotherapy of unresectable hepatocellular carcinoma) [69]. In some cases, the biological

radiopharmaceutical can be administered locoregionally (e.g. into glioma resection cavities [70] or intraperitoneally in advanced ovarian cancer patients [71]), with the objective being to increase the concentration of the biological radiopharmaceutical at the administration site and to decrease the systemic radiotoxicity.

In cases where therapeutic biological radiopharmaceuticals are administered systemically or intraperitoneally, and until there is a better understanding of data extrapolation to humans, the radiation dose should be expressed in terms of body surface (MBq/m^2).

7.4.4. Quality of biological radiopharmaceutical drugs

Biological radiopharmaceuticals used in primary pharmacodynamic studies must have the appropriate chemical, pharmaceutical, radiochemical and radionuclidic standards of identity, strength, quality and purity to be of such uniform and reproducible quality as to give significance to the research conducted. The radiation dose should be sufficient, but not higher than necessary, to obtain valid measurements. It is important to use an acceptable method of radioassay of the biological radiopharmaceutical drug to ensure that the dose calculations actually reflect the administered dose.

Frequently, radionuclides and/or peptides, proteins or MAbs come from different manufacturers, who are independently responsible for the final control of their products.

It is recommended that the formulation used in the primary pharmacodynamic studies be identical to that which will be used in the follow-up preclinical and clinical studies. However, as primary studies are evaluated for establishing the proof of concept, some reasonable changes in manufacturing and/or formulation are expected. In this case, the decision to repeat some or all primary pharmacological studies should depend on an assessment of the impact or likely impact of these changes on the biological radiopharmaceutical properties.

7.5. PHARMACOKINETIC STUDIES

It is difficult to establish uniform guidance on the pharmacokinetics of biological radiopharmaceuticals. Single and multiple dose pharmacokinetic and tissue distribution studies (percentage of injected dose per gram of target tissue and various normal tissues, target to normal tissue ratios) in relevant species and in immunodeficient animals bearing human tumour xenografts are useful. The animal models do not represent an absolutely reliable system for

predicting the behaviour of the biological radiopharmaceutical in humans owing to the biological differences between the animal models and the pathology in humans, and to alterations in the pharmacokinetic profile due to immune mediated clearance mechanisms. Moreover, they are not helpful in identifying areas of normal tissue cross-reactivity. However, the results obtained from these experiments can give important information for the characterization of the compound.

Radiation dosimetry software programs (e.g. Medical Internal Radiation Dose (MIRD) and Organ Level INternal Dose Assessment (OLINDA)) can be used to provide estimates of radiation absorbed doses received by specific organs. Autoradiography (light and/or electron microscopy) and immunohistochemistry studies are useful for determining the histopographic localization of the biological radiopharmaceuticals (see discussion below).

Care should be taken in the interpretation of studies using radioactive tracers incorporated into specific amino acids, because of recycling of amino acids into non-drug-related peptides/proteins.

The pharmacokinetic parameters of biological radiopharmaceuticals should be defined using one or more assay methods (e.g. enzyme linked immunosorbent assay (ELISA)/chromatography and measurement of radioactivity). In general, the expected clinical route of administration should be used, where feasible.

Owing to the mechanism of action of diagnostic biological radiopharmaceuticals, the optimal imaging time is as important as the optimal dose. Organ distribution and washout information will generally establish a theoretically ideal imaging time. The time window of effective imaging (i.e. how soon after administration and for how long) should be established.

The expected consequence of metabolism of radiolabelled peptides, proteins and antibodies is degradation of small peptides and individual amino acids. Therefore, the metabolic pathways are generally understood. Classical biotransformation studies as performed for pharmaceuticals are not needed.

7.6. AUTORADIOGRAPHIC STUDIES

Animal studies can be very useful for performing autoradiographic studies that can provide direct evidence of binding and biodistribution of the radiolabelled product. It is important to emphasize that this type of experiment can also be performed on isolated cells from cell cultures (see Section 6) or, at a later stage, ex vivo on human cells and tissues. In any case, these experiments should always be performed when testing a new radiopharmaceutical, possibly

also including a displacement experiment by using large amounts of unlabelled product, by using a receptor antagonist or by blocking specific binding by other means or drugs. The lack of these types of experiment in many publications describing new radiopharmaceuticals raises doubts concerning these products' in vivo specific binding to the target, thus calling into question all subsequent clinical results.

Several types of autoradiographic study can be performed:

(a) Whole animal section macro-autoradiography on film;
(b) Tissue section macro-autoradiography on film;
(c) Tissue or cell micro-autoradiography at the microscopic level, with or without immunoperoxidase staining of sections using liquid emulsions.

7.7. EVALUATION OF RADIOPHARMACEUTICAL BINDING TO SPECIFIC CELL ASSOCIATED RECEPTORS BY IN VITRO OR EX VIVO MACRO-AUTORADIOGRAPHY

The method set out in the steps below can be applied to small or large tissue sections and uses radiographic film to produce the autoradiographic image (Fig. 8). Thus the overall tissue/organ distribution can be studied, but the

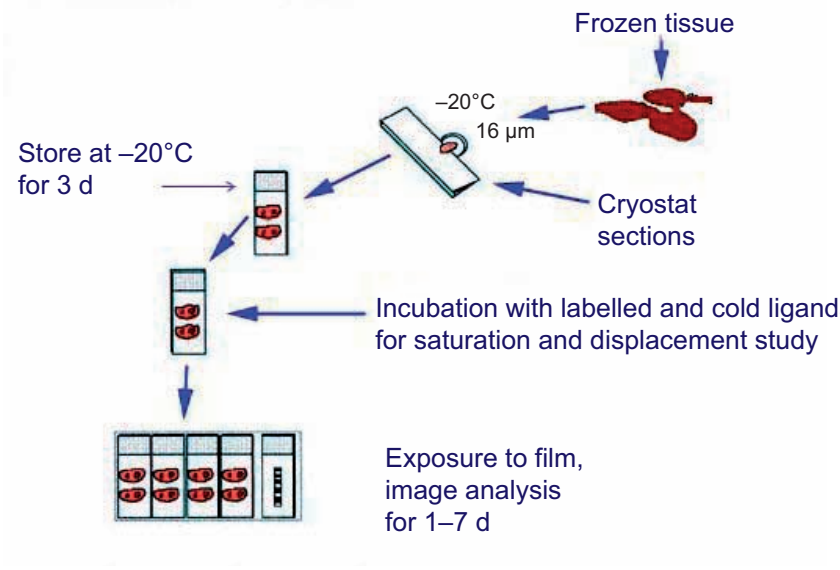

FIG. 8. Autoradiographic slide preparation.

resolution is not high enough to obtain data on single cells. The method can be qualitative, but also semi-quantitative. The radioligand can be applied to the sections in vitro, or can be administered to the animal in vivo, thus obtaining radioactive sections (ex vivo autoradiography) [72–79].

The method is as follows:

(1) For ex vivo experiments, after in vivo injection of the radiopharmaceutical, surgically remove the tissue, immediately freeze it and store it in liquid nitrogen. For in vitro experiments, start with frozen tissue samples.
(2) Cut 10–20 mm thick cryostat sections for the autoradiography.
(3) Mount the sections on glass slides and store them at –20°C for 1–3 d, if isotope decay allows (this step is necessary to increase the adhesion of the section with the glass slide). For ex vivo experiments, go to step 11.
(4) Prepare the incubation medium: Tris-HCl, 50 mmol/L; NaCl, 130 mmol/L; KCl, 4.7 mmol/L; MgCl, 5 mmol/L; ethylene glicolbis (β-aminoethyl ether) N,N,N',N',-tetra-acetic acid, 1 mmol/L; 0.5% bovine serum albumin (BSA).
(5) The pH of the buffer should be 7.4.
(6) Incubate for 30 min at 25°C (room temperature).
(7) Incubate the sections in the same medium for an additional 20 min, but this time without BSA.
(8) Add the radioligand, at binding concentration, to the slides in a solution containing 0.025% bacitracine, 2 μg/mL chimostatine and 4 μg/mL leupeptine, at pH6.5. (Bacitracine and chimostatine have antipeptidase action, and leupeptine is an antiprotease; these compounds are used in order to preserve the structure of the tissue.)
(9) Incubate the slides for 150 min at 4°C.
(10) To generate the displacement assay, using a duplicate set of slides, add to the radioligand an excess of unlabelled protein (usually 100–1000-fold molar excess).
(11) Wash all slides six times in an ice-cold preincubation solution (pH7.4).
(12) Rinse the slides in distilled water twice for 5 s each time.
(13) Dry the slides under cold air flow (4°C).
(14) Put the slides over the ^{3}H hyperfilm and expose them for 1–7 d in an X ray cassette.

7.8. EVALUATION OF RADIOPHARMACEUTICAL BINDING TO SPECIFIC CELL ASSOCIATED RECEPTORS BY IN VITRO OR EX VIVO MICRO-AUTORADIOGRAPHY

The liquid emulsion method was originally described by Belanger and Leblond in 1946 [80] and subsequently developed and improved by Messier and Leblond in 1957 [81] and by Droz and Leblond in 1962 [82]. In it, slides are coated with tissue sections and dipped into a liquid emulsion obtained by mixing commercially available emulsion with distilled water and glycerol (50:50, vol./vol.). The whole procedure must be performed in a darkroom with a security light at 0°C; the environmental temperature should be 20°C and the humidity should be approximately 40–50%.

Tissue sections are obtained from in vitro incubation with radiopharmaceuticals, or ex vivo after in vivo administration of the radiopharmaceutical. They are cut with a cryostat to a thickness of 5–7 μm and fixed to the glass slide. The liquid emulsion system allows sections to be examined under an optical microscope and the possibility for sections to be counter-stained with immunoperoxidase or other histological techniques.

For application of the emulsion, the following equipment is needed:

(a) A regulated thermostatic bath at approximately 43°C (to obtain a thicker emulsion layer, the temperature should be 38–40°C; for a more diluted liquid emulsion, the temperature can be 45°C);
(b) One graded 50 mL cylinder;
(c) One graded 25 mL cylinder;
(d) One pair of plastic forceps or a small plastic spoon;
(e) One glass rod;
(f) One metallic tray to place over a basket of ice;
(g) One small container for dipping the slides (Choplin).

All the above equipment must be properly washed with nitric acid and rinsed with copious amounts of water, and then washed again with soap and rinsed with distilled water.

Procedure:

(1) Mix 25 mL of Kodak or Ilford emulsion for autoradiography with 12 mL of water and glycerol mixture (50:50, vol./vol.) in the dark at 37°C; place the emulsion in a water bath at 43°C in a dipping box.

(2) Gently mix until the emulsion is nicely liquid and without granules (verify by dipping a few slides into it). Place the metal tray on the ice basket to keep it cold.
(3) Dip the slides into the emulsion and remove them immediately with a continuous movement; it should take 2 s to insert the slides and 1 s to remove them. Then blot the back of the slide with some tissue paper and place the slide on the ice tray to cool and dry for 15–30 min.
(4) Place all slides in a slide box for histology, add some silica granules to control humidity, close the box very tightly and keep it in the dark for 15–30 d at 4°C (avoid freezing at –20°C).

Development:

(1) After incubation, develop the slides in the dark with Kodak D19 developer.
(2) Remove the slides from the refrigerator at least three hours before the development process, in order to re-equilibrate their temperature and thus prevent possible thermal shocks.
(3) Prepare the development, washing and fixation solutions according to the manufacturer's instructions and maintain them at 20°C (room temperature). The slides should be dipped in the development solution for 6 min. After development, wash the slides in distilled water and then transfer them to the photographic fixative for 10 min. Wash the slides again under tap water for 10 min, and then dip them in distilled water.
(4) If required, slides can be stained with haematoxilene-eosine, dehydrated in increasing series of alcohol and then mounted in mounting solution for observation under an optical microscope. Slides can also be stained with specific antibodies using the immunoperoxidase technique for further enhancement of antigenic structures [8, 83].

8. PRECLINICAL SAFETY AND TOXICITY STUDIES

8.1. AIM

The objective of this section is to provide guidance to nuclear medicine practitioners on design safety and toxicity studies for determining the potential radiation effect of diagnostic and therapeutic biological radiopharmaceuticals.

Because other guidance is available on preclinical safety/toxicity evaluation of pharmaceuticals [65–67], the focus here is mainly on radiation effects associated with biological radiopharmaceuticals.

It is important to take into account that ionizing radiation causes injury not only to pathological but also to normal cells and tissues by damaging nuclear DNA [84]; this effect is known and accepted as unavoidable. For consideration of the legislation on and facilities for animal work, GLP and animal models, see Sections 7.2, 7.3 and 7.4, respectively.

8.2. SAFETY STUDIES

Safety pharmacology is defined as those studies that investigate potential undesirable pharmacodynamic effects on physiological functions in relation to exposure in the diagnostic or therapeutic range and investigate the mechanism of adverse effects observed and/or suspected [65]. The safety pharmacology study should be designed to identify a dose–response relationship, and doses should elicit moderate to severe adverse effects in this or in other studies of similar route and duration.

The organization of safety pharmacology studies begins with the cardiovascular, respiratory and central (as well as peripheral) nervous systems, which, if acutely affected, can have a significant impact on the ability to sustain life. These three organ systems make up the 'safety pharmacology core battery', studies of which should be completed prior to first administration in humans. Supplemental studies may include, but are not limited to, the renal, gastrointestinal, endocrine or immune systems [65, 85].

8.3. TOXICITY STUDIES

The number and type(s) of toxicity studies recommended depend in part on the phase of development of the biological radiopharmaceutical, what is known about the agent or its pharmacologic class, its proposed use, and the indicated patient population. Owing to the inherent toxic effects of biological radiopharmaceuticals, the uptake of targeting agents in normal tissues must be minimized for successful diagnosis and/or therapy. Methodological developments include the application of extracorporeal elimination of excess targeting agents in systemic circulation [86] and the reduction of renal uptake by amino acid infusion [87]. Another method is to use antibodies with specificity for the targeting agent to form large molecular complexes [88], which are taken up and degraded by the reticuloendothelial system. Various

methods using pretargeting [89, 90] have also been tried for improved selective tumour uptake.

8.3.1. Single dose and repeated dose toxicity studies

Unlike most other products, medical imaging drugs are typically administered infrequently or in a single dose; they are not administered to achieve a steady state. Therefore, the development programme can omit long term (i.e. three months or longer) repeat dose toxicity studies, and if toxicity studies are performed on the combined components of the test compound and no significant toxicity is found, toxicological studies of individual components are seldom required [91].

Radiation toxicity studies of therapeutic biological radiopharmaceuticals should include a range of levels (at least twice the maximum planned human radiation dose), to identify the 'no observed adverse effect' level (NOAEL) as well as dose related mild to severe radiation toxicity, establishing the maximum tolerated dose to be used to define the starting dose in Phase I clinical trials. The studies should also include the cold formulation as a control group to distinguish its potential effects from specific radiation effects [92, 93].

The studies should identify organs at risk and establish a margin of safety for early and late radiation toxicity. The time period in which radiation injury becomes clinically apparent is determined in part by the turnover time. In organs with rapid cell turnover (e.g. bone marrow, epidermis, small intestine), radiation injury can cause bone marrow failure, desquamation, nausea, and vomiting and diarrhoea within days or weeks of an acute dose radiation (an accepted time is less than 60 d). Radiation injury to these organs is called early or acute radiation toxicity and is often reversible. However, in organs with slow cell turnover rate (e.g. brain, liver, kidneys), symptoms of radiation injury can cause brain radionecrosis and liver or kidney failure within several months to years, with a latency period with relatively normal organ functioning (an accepted time is more than 60 d). Radiation injury to these organs is referred to as late radiation toxicity and is usually progressive and irreversible. Therefore, animal studies designed to elucidate the late radiation toxicity effects of a biological therapeutic radiopharmaceutical should last for at least one year post-dosing, and a study duration of less than one year should be justified. A recovery period should generally be included to determine the possible reversal effect. When possible, these studies should also include a toxicokinetic design.

8.3.2. Immunogenicity

Biological radiopharmaceuticals are frequently immunogenic, and the development of antibodies after intermittent, repeated administration can alter the pharmacokinetic/toxicokinetic, biodistribution, safety, and/or imaging/therapeutic properties, and greatly complicates the study interpretation. The development of such antibodies should be tested and characterized during the study (see Section 9).

8.3.3. Local tolerance studies

Local tolerance should be evaluated. In some cases, the potential adverse local effect of the product can be evaluated in single or repeated dose toxicity studies, thus obviating the need for separate local tolerance studies. The effect of misadministration should be evaluated in a manner that is appropriate for the intended route of administration (e.g. in the case of biological radiopharmaceuticals intended for intravenous administration, extravasations or spill on the skin effects should be evaluated).

8.4. INTERPRETATION OF RESULTS

Severe organ toxicities have been reported with therapeutic biological radiopharmaceuticals. Therefore, dosimetry estimates should be required prior to clinical studies; they should be developed with simulation models using an appropriate diagnostic or therapeutic radioisotope.

The information available on pharmacokinetics/toxicokinetics should be sufficient for radiation dosimetry calculations.

It is recommended that calculations of absorbed dose to organs be carried out in accordance with the MIRD or OLINDA schedules. The model used for calculations of the cumulated activity (time integral of the activity) in source organs should be explained, and the origin of the data used, such as animal studies, should be stated. The absorbed dose to the organ receiving the highest exposure and that to all organs included in the calculation of the effective dose equivalent should be stated. The unit must be milliGrays per unit of activity administered (mGy/MBq).

The estimation of the radiation dose should be summarized in terms of the effective dose equivalent using the weighting factors given by the International Commission on Radiological Protection (ICRP). The unit must be milliSieverts per unit of activity (mSv/MBq) (radiopharmaceuticals based on MAbs).

8.5. AVAILABLE GUIDELINES FOR PRECLINICAL STUDIES

The main guidelines available are those of the European Nuclear Medicine Society (EANM), which has updated the previous guidelines from the Society of Nuclear Medicine (SNM).

9. CLINICAL STUDIES

9.1. INTRODUCTION

The guidance provided here is intended to clarify which scientific and regulatory issues should be considered when planning clinical studies in humans with newly developed (i.e. investigational) or marketed radiopharmaceutical products of either a chemical or a bio(techno)logical origin. Existing regulations allow a great deal of flexibility in the amount of data that must be submitted with a clinical trial application (CTA), depending on the goals of the proposed clinical trial, the specific human testing proposed and the expected risks.

This section aims to provide the necessary guidance on general procedures for compiling and submitting a CTA to the concerned regulatory bodies in order to obtain official approval for initiation of a clinical study.

9.2. DEFINITIONS

In general, the clinical development of new drugs can be divided into four different phases, with each clinical phase having its own specific study objectives. In this context, radiopharmaceuticals can be used as a tool to develop new drugs. In such cases, the radiopharmaceutical is applied as an imaging probe to indirectly evaluate the therapeutic efficacy of the new drug. Alternatively, the radiopharmaceutical itself can be developed as a new drug product for either diagnostic or therapeutic purposes. The different phases of clinical drug development are generally defined as in the following (based on the ICH E8 guideline on General Considerations for Clinical Trials).

9.2.1. Phase 1 clinical studies

Phase 1 includes the initial introduction of an investigational new drug into humans. These studies are closely monitored and may be conducted in patients; however, they are usually conducted in healthy volunteer subjects. They are designed to determine the metabolic and pharmacological actions of the drug in humans and the side effects associated with increasing doses, and, if possible, to gain early evidence of effectiveness. During Phase 1, sufficient information about the drug's pharmacokinetics and pharmacological effects should be obtained to permit the design of well controlled, scientifically valid Phase 2 clinical studies.

Phase 1 clinical studies also evaluate drug metabolism, structure–activity relationships and the mechanism of action in humans. These studies also determine which investigational drugs are used as research tools to explore biological phenomena or disease processes. The total number of subjects included in Phase 1 studies varies with the drug, but is generally in the range of 20–80. Phase 1 studies normally include radiation dosimetry studies of investigational radiopharmaceutical products.

The local authorities or, in the United States of America, the Center for Drug Evaluation and Research (CDER) can prohibit a Phase 1 study from proceeding or can stop a trial that has started, for reasons of safety or because of a sponsor's failure to accurately disclose the study risks to investigators. Although the local authorities or the CDER routinely provide advice in such cases, investigators may choose to ignore any advice regarding the design of Phase 1 clinical studies in areas other than patient safety.

9.2.2. Phase 2 clinical studies

Phase 2 includes early controlled clinical studies conducted to obtain preliminary data on the effectiveness of the drug for a particular indication or indications in patients with a given disease or condition. This phase of testing also helps determine the common short term side effects and risks associated with the drug. Phase 2 clinical studies are typically well controlled, closely monitored and conducted in a relatively small number of patients, usually involving several hundred subjects.

9.2.3. Phase 3 clinical studies

Phase 3 clinical studies are expanded controlled and uncontrolled trials. They are performed after preliminary evidence suggesting effectiveness of the drug has been obtained in Phase 2, and are intended to gather the additional

information about effectiveness and safety that is needed to evaluate the overall benefit–risk relationship of the drug. Phase 3 studies also provide an adequate basis for extrapolating the results to the general population and transmitting that information in the physician labelling. Phase 3 studies usually include several hundred to several thousand subjects.

In both Phase 2 and Phase 3, local authorities or the CDER can impose a clinical hold if a study is unsafe (as in Phase 1), or if the protocol design is clearly deficient in meeting its stated objectives. Great care is taken to ensure that this determination is not made in isolation but reflects current scientific knowledge, experience with the design of clinical trials and experience with the class of drugs under investigation.

9.2.4. Phase 4 clinical studies

Clinical studies started after marketing of the drug has begun are usually defined as Phase 4 studies. Therapeutic use studies go beyond the prior demonstration of the drug's safety, efficacy and dose definition.

Studies in Phase 4 include all studies (other than routine surveillance) that are performed after drug approval and that are related to the approved indication. They are studies that are not considered necessary for approval but that are often important for optimizing the drug's use. They may be of any type, but should have valid scientific objectives. Commonly conducted studies include additional drug–drug interaction, dose response or safety studies.

For example, a variety of definitions related to the use of an investigational medicinal product (IMP) in clinical studies can be found in the ICH E6 guideline on Good Clinical Practice and in several texts of the European pharmaceutical legislation (e.g. Directives 2001/20/EC and 2005/28/EC). The most relevant definitions have also been included in Annex I of this publication.

9.3. REQUIREMENTS FOR ETHICAL COMMITTEE APPROVAL AND COMPETENT AUTHORITY APPROVAL

9.3.1. Liability regarding submission of a clinical trial

Throughout drug development, emerging chemical/pharmaceutical, preclinical (e.g. animal toxicology) and clinical data should be reviewed and evaluated by qualified experts, to assess the quality, safety and efficacy of the investigational medicinal product dossier (IMPD) and the implications for the general safety of trial subjects. In response to such findings, future studies and,

where necessary, studies in progress should be appropriately modified in a timely fashion to maintain the safety of trial subjects.

In this context, the investigator and the sponsor of a clinical study share the responsibility for protecting clinical trial subjects with the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) and the local national health authority (hereinafter called the 'competent authority' (CA)). The principles and practices concerning protection of trial subjects are stated in the ICH guideline on Good Clinical Practice (ICH E6) and define the responsibilities of the different parties involved in a clinical trial. These principles have their origins in the Declaration of Helsinki and should be observed in the conduct of all human drug investigations. National legislation should be complied with in each Member State.

With respect to the ICH GCP regulations and the pharmaceutical legislation existing in the different ICH regions, written approval should normally be obtained from the IRB/IEC and the CA to authorize the initiation of a clinical trial using an IMP in order to fully ensure the quality, safety and efficacy of the IMP under investigation. For multicentre clinical trials, approval should be sought in every country involved in the trial. For this purpose, a CTA should be compiled and submitted (e.g. in parallel) to the IRB/IEC and CA by the applicant.

9.3.2. Legislation and liability

9.3.2.1. General regulations regarding the safety of clinical trial subjects

As previously outlined, clinical trials using IMPs should be conducted in accordance with the ethical principles originating in the Declaration of Helsinki. Furthermore, all clinical studies should be designed, conducted, analysed and reported in compliance with the internationally accepted ICH guidelines (ICH E6, ICH E8) and the applicable national regulatory requirement(s).

The most prominent ICH GCP principles are as follows:

(1) Before a trial is initiated, foreseeable risks and inconveniences should be weighed against the anticipated benefit for the individual trial subject and for society. A trial should be initiated and continued only if the anticipated benefits justify the risks.
(2) The rights, safety and well-being of the trial subjects are the most important considerations and should prevail over the interests of science and society.

(3) The available chemical/pharmaceutical, preclinical and clinical information on an investigational product should be adequate to support the proposed clinical trial. The proposed study should be scientifically justified and based on the latest scientific knowledge.
(4) Clinical trials should be scientifically sound and should be described in a clear, detailed protocol. In addition, the study protocol must have been approved by the IEC.
(5) A trial should be conducted in compliance with a protocol that has received prior IRB/IEC approval/favourable opinion.
(6) The proposed study should also be approved by the CA prior to startup, if so demanded by national regulatory requirements.
(7) The medical care given to, and medical decisions made on behalf of, subjects should always be the responsibility of a qualified physician.
(8) Each staff member involved in conducting a trial should be qualified by education, training and experience to perform his or her task(s).
(9) Freely given informed consent should be obtained from every subject prior to clinical trial participation.
(10) All clinical trial information should be recorded, handled and stored in a way that allows its accurate reporting, interpretation and verification.
(11) The confidentiality of records that could identify subjects should be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).
(12) IMPs should be manufactured, handled and stored in accordance with local GMP regulations. They should be used in accordance with the approved protocol.
(13) Systems with procedures that assure the quality of every aspect of the trial should be implemented.
(14) The liability of the investigator and the sponsor for any harm or injury caused, directly or indirectly, to the patient participating in the trial or the rightful claimant should be covered by insurance. For this purpose, the sponsor is obliged to take out insurance prior to initiation of the study to cover its liability as well as the liability of any person intervening in the study, independently of the relation that exists between the sponsor and the intervening party. Finally, the sponsor or its legal representative should be located in its national (legal) district.

9.3.2.2. Format and content of CTAs

As mentioned previously, prior to the start of the study, an approval/favourable opinion should be obtained from the appropriate IRB/IEC and the CA for any mono- or multicentre clinical study using IMPs.

The legal status, composition, function, operations and regulatory requirements pertaining to IECs may differ among countries, but should allow the IEC to act in agreement with GCP, as described in the guidance provided here.

Although evaluation and approval of the study protocol or investigator's brochure (including previous clinical data obtained with the IMP) are generally performed by the IEC, some exceptions do exist in certain countries. Furthermore, certain data contained in the study protocol or investigator's brochure may also be considered by the CA in the course of its evaluation of the study, even if this evaluation is normally focused on the quality data and, possibly, the preclinical data of the IMP.

When submitting CTAs to the CA, in addition to internationally accepted requirements, country specific regulatory requirements should be taken into account. Moreover, the submission fees and legally determined evaluation deadlines applied by CAs often vary from country to country. In some ICH regions, it may be possible that no written approval is actually sent to the applicant (as with approval of the US Food and Drug Administration (FDA)). In such cases, the applicant is authorized to start with the clinical study provided that it has received no objections from the CA within a given period (e.g. 30 d) after submission of the study. This type of approval is often referred to as a 'silent' or 'tacit' approval. In some countries, the CA still uses a 'notification' system for clinical studies, rather than performing a full evaluation of the CTA.

9.3.2.3. Format and content of CTAs to the IRB/IEC and/or the CA

Applicants should be aware that the regulatory bodies of different countries have specific requirements regarding the format of the CTA — often referred to as the common technical document (CTD) format. Detailed information on the format and content of CTAs to be submitted to the IRB/IEC can be found in several guidance documents applied either in the EU or the United States of America. A representative list of the information required in the EU is given in Table 2.

TABLE 2. INFORMATION REQUIRED FOR ETHICS COMMITTEES

General

Covering letter
Application form
Clinical trial protocol
Investigator's brochure
List of CAs to which the application has been submitted and details of decisions, if available

Subject related

Informed consent form
Subject information leaflet
Arrangements for recruitment of subjects

Protocol related

Summary of the protocol in the national language
Outline of all active trials with the same IMP
Peer review of the scientific value of the trial, if available (not compulsory)
Ethics assessment made by the principal/coordinating investigator, if not given in the application form

IMP related

Investigational medicinal product dossier (IMPD)
Simplified IMPD for known products
Summary of product characteristics (SmPC) (for products with marketing authorization in the European Union (EU))
If the IMP is manufactured in the EU:

- A copy of the manufacturer authorization referred to in Article 13.1 of the Directive stating the scope of this authorization

If the IMP is not manufactured in the EU:

- The declaration by the QP that the manufacturing site works in compliance with GMP at least equivalent to EU GMP
- A copy of the importer authorization referred to in Article 13.1 of the Directive, certificate of analysis for test product in exceptional cases: where impurities are not justified by the specification or when unexpected impurities (not covered by specification) are detected

Viral safety studies
Examples of the label in the national language
Applicable authorizations to cover trials or products with special characteristics (if available; e.g. genetically modified organisms, radiopharmaceuticals)
Transmissible spongiform encephalopathy (TSE) certificate of suitability, where applicable
Declaration of GMP status of active biological substance

TABLE 2. INFORMATION REQUIRED FOR ETHICS COMMITTEES (cont.)

Facilities and staff related
Facilities for the trial
CV of the coordinating investigator in the State concerned (for multicentre clinical trials)
CV of each investigator responsible for the conduct of the trial at a site in the State concerned (principal investigator)
Information on supporting staff at each site
Finance related
Provision for indemnity or compensation in the event of injury or death attributable to the clinical trial
Any insurance or indemnity to cover the liability of the investigator and the sponsor
Compensation to subjects
Compensation to investigators
Agreement between the sponsor and the trial sites
Agreement between the investigators and the trial sites
Certificate of agreement between the sponsor and the investigator, if not in the protocol

A representative list of the information required by CAs in the EU is given in Table 3.

TABLE 3. INFORMATION REQUIRED FOR CAs IN THE EU

General
Receipt of confirmation of trial identification number (e.g. EudraCT number)
Covering letter
Application form
List of CAs within the EU to which the application has been submitted and details of decisions
Copy of ethics committee opinion in the State concerned, if available
Copy/summary of any scientific advice
If the applicant is not the sponsor, a letter of authorization enabling the applicant to act on behalf of the sponsor
Note: Application to CAs in English is acceptable
Subject related
Informed consent form
Subject information leaflet
Arrangements for recruitment of subjects
Protocol related
Protocol with all current amendments
Summary of the protocol in the national language
Peer review of trial, if available (not compulsory)
Ethics assessment made by the principal/coordinating investigator

TABLE 3. INFORMATION REQUIRED FOR CAs IN THE EU (cont.)

IMP related

Investigator's brochure
IMPD
Simplified IMPD for known products (see Table 2)
SmPC (for products with marketing authorization in the EU)
Outline of all active trials with the same IMP
If the IMP is manufactured in the EU and if there is no marketing authorization in the EU:

- Copy of the manufacturing authorization referred to in Article 13.1 of the Directive stating the scope of this authorization

If the IMP is not manufactured in the EU and if there is no marketing authorization in the EU:

- Certification by the QP that the manufacturing site works in compliance with GMP at least equivalent to EU GMP, or that each production batch has undergone all relevant analyses, tests or checks necessary to confirm its quality
- Certification of GMP status of the active biological substance
- Copy of the importer's manufacturing authorization referred to in Article 13.1 of the Directive
- Certificate of analysis for test product in exceptional cases: where impurities are not justified by the specification or when unexpected impurities (not covered by specification) are detected

Viral safety studies, if applicable
Applicable authorizations to cover trials or products with special characteristics (if available; e.g. genetically modified organisms, radiopharmaceuticals)
TSE certificate of suitability, if applicable
Examples of the label in the national language

Facilities and staff related

Facilities for the trial
CV of the coordinating investigator in the State concerned (for multicentre clinical trials)
CV of each investigator responsible for the conduct of a trial at a site in the State concerned (principal investigator)
Information about supporting staff

Finance related

Provision for indemnity or compensation in the event of injury or death attributable to the clinical trial
Any insurance or indemnity to cover the liability of the sponsor or the investigator
Compensation to investigators
Compensation to subjects
Agreement between the sponsor and the trial sites
Agreement between the investigators and the trial sites
Certificate of agreement between the sponsor and the investigator, if not stated in the protocol

9.4. INVESTIGATIONAL MEDICINAL PRODUCT DOSSIER

In general, the IMPD of a CTA comprises three major sections related to (1) chemistry, manufacturing and controls (generally referred to as quality data); (2) preclinical data (i.e. animal pharmacology and toxicology data); and (3) previously obtained clinical information.

Applicants should take into account that, owing to country specific national laws, the quality requirements for medicinal products such as radiopharmaceuticals used in clinical studies often differ from one country to another. The same is true for current legislation regarding preparation, dispensing and use of radiopharmaceuticals, radioactive precursors or radionuclides that are routinely used in hospitals for diagnostic or therapeutic purposes. In addition, the scope of the national legislation that is based on the definition of an IMP may also differ from that of the corresponding international legislation, and therefore may have a substantial impact on the way clinical research is approved and conducted in a specific country.

As stated in the pharmaceutical legislation, the manufacture and importation of IMPs should in general comply with current good manufacturing practice (cGMP) requirements. General guidance regarding cGMP requirements during Phase 1 clinical studies can be found on the web site of the US FDA (see Annex III of this publication). The GMP requirements for the manufacture of IMPs that are applied in the EU can be found in annex 13 of volume 4 of the European pharmaceutical legislation (Eudralex; see Annex I). Specific guidance on the cGMP requirements for PET radiopharmaceuticals has been developed by the FDA.

Nevertheless, many CAs apply country specific standards regarding the level of GMP requirements to be followed for manufacturing, dispensing and using radiopharmaceuticals or radionuclides that are prepared in hospital radiopharmacies or in centralized radiopharmacies for daily clinical practice in hospitals. In most cases, this concerns formulations for intravenous use that, owing to the short half-life of the radioisotope used, are prepared in-house under the responsibility of a QP. Consequently, a variety of procedures are being used across the Member States of different ICH regions, as considerable uncertainty remains regarding the preparation conditions, quality requirements for starting materials and analyses of the finished product to be followed, as well as the technical profile of the QP, who in some countries is a (hospital) pharmacist, chemist or biologist, or, sometimes, a technician. Therefore, CTA applicants are advised to inform themselves of the specific GMP requirements of the country concerned early in the development phase of a new radiopharmaceutical, particularly when the radiopharmaceutical is being manufactured in a hospital pharmacy or

non-commercial research centre. In the IAEA's guidance on hospital radiopharmacy, clinical trials fall under operational level 3 [3]. In this context, the EANM has elaborated draft guidelines on cGRP in the preparation of radiopharmaceuticals.

Before any clinical trial is carried out, results of preclinical or previous studies in humans should be sufficient to indicate that the drug is safe for the proposed investigation in humans. The purpose and timing of animal pharmacology and toxicology studies intended to support clinical trials of a given duration are discussed in ICH guideline M3 on Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals. The role of such studies for biotechnological products is described in ICH guideline S6 on Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals. Protection of the subject of the clinical trial is safeguarded through risk assessment based on the results of toxicological experiments prior to any clinical trial, screening by ethics committees and Member State CAs, and rules on the protection of personal data.

Detailed information on the content and CTD format required for the IMPD sections (i.e. quality, preclinical and clinical data) can be found in the EU's detailed guidance on requests to the CAs for authorization of a clinical trial of a medicinal product for human use, notification of substantial amendments and declaration of the end of the trial (see Annex II).

9.5. INFORMED PATIENT CONSENT FOR PARTICIPATION IN A TRIAL

According to the GCP guidelines and international regulatory requirements, participation of human volunteers or patients in a clinical study is allowed only on the condition that the persons concerned have freely given their written informed consent after being fully informed concerning at least the following items related to the study:

(a) The nature, extent, objectives, consequences, expected benefits and risks of the study, the conditions in which the study will take place, and identification and final opinion of the IRB/IEC concerned.
(b) The right of each participant (or his or her legal representative) to withdraw (or to withdraw the patient) from the study at any moment, without personal consequences.

If written consent cannot be given, the person can provide his or her consent orally in the presence of at least one witness who is of age and is independent of the sponsor and the investigator of the study.

As mentioned previously, each person participating in a clinical study or his or her legal representative has the right to withdraw the participant's informed consent and to terminate his or her participation at any moment during the study, without personal consequences.

9.6. FIRST STUDIES IN HUMANS

The transition from non-clinical to initial clinical investigations is a quantum leap and requires special consideration. Even with non-clinical testing and having evaluated all the risks associated with the radiopharmaceutical, specific steps should be taken to mitigate and manage risk, as the safety of study participants is the paramount consideration. Initial investigations in humans should be conducted as a single protocol at a single site. The ability of animal studies to predict distribution and safety issues in humans may be limited. These first studies in humans must therefore be carefully documented, and all data obtained should be stored for future evaluation.

Before initiating studies in humans, the investigators should review risk factors and the chances of successful application in humans. The latter will require a review of the nature of the target, the relevance of the animal model and the pharmacokinetics. Differences in the affinity for the molecular target and in the tissue distribution of the molecular target in animals and humans should be accounted for. In vitro tissue or cell studies can provide evidence of species specificity. Some radiolabelled biologicals that are highly species specific can give rise to misinterpretation of pharmacokinetics and pharmacodynamics (i.e. molecular target uptake). Finally, when dealing with products having short half-lives, such as PET tracers, the investigative value is poor when the time to effectively reach the target is considerably longer than the physical half-life of the PET tracer.

The choice of subjects and design protocol for first investigations in humans should be carefully considered with respect to the molecular target, potential accelerated metabolism, pharmacological or toxic events, age and sex differences, diet, use of stimulants such as tobacco and coffee, and drug use/abuse.

When dealing with radiolabelled biologicals and PET tracers, which in general are administered in nano- to microgram amounts, attention should be paid to adsorption to syringes, containers and infusion systems. Wherever

possible, concurrent medication, even with registered medications, should be avoided.

If the tracer is planned for disease or treatment monitoring, the influence of consistency and reproducibility of specific radioactivity and radiochemical purity may be important. Therefore, an adequate level of quality characterization is essential.

When dealing with PET radionuclides coupled to biological material, in particular those involving biological cascade, an interval between investigations and well spaced intervals between repeated doses should be maintained. Care should also be taken with cross-reactivity studies using human and animal tissues, especially with MAbs and peptides. Human specific proteins are likely to be immunogenic in animal species; therefore, repeat studies in animals may be of little predictive value.

The radiation dose from radiolabelled biological PET tracers is normally much higher than that from SPECT tracers; therefore, protocols involving repeated doses should be carefully considered. The first evaluation in humans should be conducted in an environment with immediate access to equipment and sufficiently trained staff for acute emergencies. Attention should also be focused on radioactive metabolites, which may interfere and provide erroneous interpretation. All levels of human metabolic systems should be carefully monitored. An adequate period of observation and subsequent follow-up is also essential.

9.7. CLINICAL TRIAL DATABASES

Identification of a clinical trial is generally the first mandatory step in the procedure for submitting a CTA. For this purpose, the applicant should request a unique identification number (in Europe this number is the "EudraCTnr."), to be assigned by the CA in order to identify each clinical trial. Normally, one identification number is issued per study protocol, irrespective of the number of clinical trial sites or Member States involved.

As a result, regulatory bodies make use of a clinical trials database to obtain an overview of clinical trials being conducted in a specific ICH region. Such a database is needed to facilitate communication on these clinical trials between the authorities of different countries, to enable each to maintain an overview of clinical trials and IMP development, and to provide for enhanced protection of clinical trial subjects and patients receiving investigational IMPs. More detailed information on the EudraCT database can be found in the detailed guidance on the European clinical trials database (see Annex III).

9.8. ADVERSE REACTION/EVENT REPORTING

With respect to GCP and general pharmacovigilance regulations, it is imperative that any adverse reactions occurring during clinical trials be reported by the investigator to the sponsor of the study (with the exception of those adverse reactions identified in the protocol or investigator's brochure as not being required to be mentioned).

In the case of serious adverse reactions, the sponsor is generally obliged to inform the concerned regulatory bodies of the country or countries involved within a legally determined time frame. In some countries, a rapid alert system is applied, according to which suspected unexpected serious adverse reactions (SUSARs) have to be reported to the regulatory bodies within, for example, seven days of a sponsor's learning of such a case. Relevant follow-up information can be communicated afterwards, within an additional time frame of, for example, eight days.

If the regulatory bodies decide that patient safety can no longer be guaranteed, the clinical trial can be temporarily suspended or terminated. Alternatively, the sponsor of the study may decide to halt the study on a temporary basis or to end the trial completely. Many CAs and IRBs/ECs demand that, throughout the study, the sponsor provide a list of all SUSARs that have occurred. In certain ICH regions (e.g. Europe), a database has been developed in which all SUSARs related to any type of IMP are to be entered. Such a database is needed to facilitate the review of the safety of using these products in clinical trials. The database also facilitates communication on this review and on the safety of these clinical trials between the regulatory bodies involved. This process enables each country concerned to obtain a better overview of the clinical trials and medicinal product development, and to provide for enhanced protection of clinical trial subjects and patients receiving medicinal products (e.g. detailed guidance on adverse reaction reporting and SUSARs).

In addition, a system for reporting adverse reactions to as well as product defects specifically related to radiopharmaceuticals has recently been developed in Europe. More information on the on-line reporting mechanism can be found on the web site of the British Nuclear Medicine Society (see Annex III).

Adverse reaction report forms from the FDA (FDA-3500 for voluntary reporting and FDA-3500A for mandatory reporting) have been revised and re-authorized. These forms can be downloaded in PDF format from: http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm

9.9. CASE REPORT FORM

In accordance with GCP, the investigator should make use of a printed, optical or electronic document designed to record all information on each trial subject required by the protocol, which needs to be reported to the sponsor of the study.

Good case report forms (CRFs) are crucial to conducting a successful clinical trial. CRFs capture data that will be used to evaluate the research questions asked in the protocol and to collect adverse event data for safety reports and processes. Information on developing a CRF is available from the International Clinical Sciences Support Center, and can also be downloaded from http://www.nihtraining.com/cc/ippcr/current/downloads\Mailhot020706.ppt

Good CRFs should: (i) gather complete and accurate data that answer study questions (e.g. by avoiding duplication of data, easing transcription of data onto the CRF and complying with the study protocol); (ii) promote accurate data entry (e.g. by giving visual cues to the person recording the data, such as boxes that clearly indicate where data should be recorded, clear guidance about skip patterns, and a clean, uncrowded layout); (iii) organize data in a format that facilitates data analysis (e.g. by grouping on the same form those data that will be analysed together, where possible). An example of a CRF is given in Annex V of this publication.

9.10. DECLARATION OF HELSINKI

The accepted basis for the conduct of clinical trials in humans is founded on the protection of human rights and the dignity of the human being with regard to the application of biology and medicine, as, for instance, reflected in the 2008 version of the Declaration of Helsinki.

9.11. FUNDING OF THE CLINICAL TRIAL

The financial aspects of a clinical study should be clearly described in an agreement established between the sponsor and the investigator(s) or investigator site(s). With regard to the information that is required by the EU and CAs in this context, reference is made to Tables 2 and 3, especially the finance related sections.

9.12. SCIENTIFIC ADVICE

It should be noted that investigators seeking scientific advice related to the development of any new radiopharmaceutical product can address their request either to the CA of the country concerned or to international regulatory bodies such as the FDA or the European Medicines Agency (EMEA). Recently, a procedure has been installed by which scientific advice on the same IMP can be sought in parallel from both the FDA and the EMEA.

Guidance for companies or research organizations requesting scientific advice and/or protocol assistance can be found on the web site of the EMEA (see Annex III). The EMEA guidance provides an overview of the procedure for obtaining scientific advice or protocol assistance as well as guidance to applicants on preparing a request. It also explains the scope and nature of scientific advice and protocol assistance.

In general, scientific advice can be provided on quality, safety or efficacy issues related to an IMP. Such advice should ensure that the investigator anticipates the appropriate quality, preclinical or clinical tests conforming to regulatory requirements prior to submitting a CTA request to the CA.

With regard to protocol assistance, advice is often requested on criteria for selection of volunteers or patients. With regard to selection of study participants, the investigator should take into account that, for certain IMPs (e.g. MAbs), healthy volunteers should not be considered because of the potential risk of immunogenicity. In addition, for particular therapeutic indications, restricted patient populations are required (e.g. exclusion of children and women of child-bearing age).

10. STUDIES TO EVALUATE IMMUNOGENICITY OF RADIOPHARMACEUTICALS

10.1. INTRODUCTION

Unwanted immunogenicity is a significant issue affecting many biotechnology derived pharmaceuticals, including hormones, enzymes, cytokines and even fully human MAb products, leading to therapeutic failure, or, worse, severe and possibly fatal side effects. Therefore, unwanted immunogenicity must be considered from the outset, during preclinical development, through clinical trials and into the post-registration period for all

biologicals, including radiolabelled biologicals, to ensure the safety and efficacy in the recipients of these products. This requires a careful strategy for assessment of immunogenicity along with monitoring of antibody formation using appropriate assays integrated into the clinical studies (for information on detailed guidance on immunogenicity assessment, see Section 9.4).

10.2. FACTORS INFLUENCING IMMUNOGENICITY

For many therapeutic strategies employing non-human proteins, immunogenicity remains an absolute barrier to clinical success. This is a testament to the sophistication of an immune system that is able to distinguish 'self' from 'non-self' (something foreign) in our bodies, even if the non-self shares identity with one of our own proteins. Consistent with the premise of 'non-self' as a cause of unwanted immunogenicity of animal proteins (e.g. murine antibodies), the history of therapeutic antibody development has shown that 'engineering by homology' to recast the antibody sequence to be more similar to the sequences of antibody present naturally in the host can reduce the likelihood of an immunogenic response. The clinical success of first chimeric (part mouse, part human), then humanized (part mouse, mostly human) and recently 'fully human' antibodies (obtained from human gene repertoires in transgenic mice or recombinant phage libraries) has reinforced the impression that foreignness is the issue and that humans do not raise antibodies to human proteins. However, from an immunological standpoint, this simplistic view is incorrect. 'More human' is not necessarily 'less immunogenic', as examples of engineered antibodies with significant immunogenicity rates show (see Table 4.).

The immune response to a therapeutic protein is dependent on a myriad of factors which converge to result in an immune response in the first instance, and in the clinical consequences of that response (if any) thereafter. These factors include the type of product (relative to the native protein, e.g. glycosylated/non-glycosylated, native sequence/mutein, structural similarity with the native protein, presence of impurities, aggregates, clipped or deamidated forms, degradation products) and its formulation, but also the dose and duration of treatment, the frequency of dosing, the route of administration and the functional activities of the protein, patient related factors including genetic predisposition, immune status and susceptibility of the recipient, and the type of disease being treated (see Table 5). Owing to the multifactorial dependence, the issue of unwanted immunogenicity is further complicated by product related immunogenicity. Some products based on a particular protein may be immunogenic, whereas others, even of the same protein sequence, may

TABLE 4. IMMUNOGENICITY OF THERAPEUTIC ANTIBODIES

Antibody category	Antibody	Indication	Percentage of patients with induced antibodies
Chimeric	Remicade	Crohn's disease and rheumatoid arthritis	10–60
	Simulect	Transplant rejection	1.5
	Rituxan	Lymphoma	1.1
Chimeric Fab	ReoPro	Cardiac	6
Humanized	Anti-A33	Cancer	72
	Anti-B72.3	Cancer	75
	CAMPATH	Leukaemia	50
	Zenapax	Transplant rejection	8.4
	Synagis	Respiratory syncytial virus infection	1.1
	Herceptin	Cancer	0.1
	Xolair	Allergy	0
Fully human	Humira	Crohn's disease and rheumatoid arthritis	12

TABLE 5. FACTORS INFLUENCING UNWANTED IMMUNOGENICITY OF THERAPEUTIC PROTEINS

Product related (intrinsic)
Molecular structure: variation in amino acid sequence and/or glycosylation pattern relative to native protein, novel epitopes
Product impurities: aggregates, degradation products, oxidized or deamidated forms, host cell proteins
Formulation
Biological properties of the therapeutic/protein
Product related (extrinsic)
Dose, route, frequency of administration and duration of treatment
Cellular or soluble target
Endogenous counterpart
Host related
Genetic profile
Immune status
Disease state

be less immunogenic or in some cases even non-immunogenic, with variable clinical consequences, depending on the product.

10.3. CONSEQUENCES OF UNWANTED IMMUNOGENICITY

The clinical sequelae of the induced antibodies, which range from benign to severe, are largely dependent on the characteristics of the antibodies and the intrinsic properties of the protein. The incidence of antibody formation varies from being extremely rare to occurring in a majority of the treated patients, depending on the protein. In most instances, the effect(s) of antibody development is (are) often benign, causing limited, if any, undesirable effects in the recipients. In other instances, however, elicitation of an immune response can lead to mild or severe adverse 'anaphylactoid' reactions, cause neutralization of the biological alone, or alter the pharmacokinetics/pharmacodynamics of the administered protein and compromise its efficacy. In some situations that are representative of the 'worst case' scenario, neutralizing antibodies that cross-react with endogenously produced protein(s) having an essential biological function (e.g. as seen with megakaryocyte growth and development factor and erythropoietin) may be elicited, causing severe and possibly fatal clinical problems. 'Intrinsic' immunogenicity varies considerably between biologicals; for example, some biologicals such as IFN-γ and G-CSF seem incapable of inducing detectable immune responses, while others such as GM-CSF, IFN-α and IFN-β appear to be inherently relatively immunogenic.

10.4. GUIDANCE ON IMMUNOGENICITY ASSESSMENT

Following extensive discussion of procedures and practices undertaken by the industry, a consortium of members of the American Association of Pharmaceutical Sciences (AAPS) has formulated recommendations on the assessment of antibodies during preclinical and clinical development of a biotherapeutic. These published recommendations provide useful guidance regarding critical issues impacting on design, optimization and qualification of antibody assays. Specific criteria that are important during the design and optimization of screening assays, and the design and optimization of cell based assays for detection of neutralizing antibody responses elicited by biotechnology products are briefly described [94, 95]. A risk based bioanalytical strategy for the assessment of antibody immune responses against biological products has recently been published [96].

On the regulatory front, immunogenicity testing in non-clinical and clinical studies has been advocated in several guidance publications. The ICH S6 guideline on Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals [66] specifies that the "measurement of antibodies associated with administration of these types of products should be performed when conducting repeated dose toxicity studies in order to aid in the interpretation of these studies. Antibody responses should be characterized (e.g., titer, number of responding animals, neutralizing or non-neutralizing), and their responses should be correlated with any pharmacological and/or toxicological changes" [66].

The European Medicines Agency Committee for Medicinal Products for Human Use (EMEA CHMP) Guideline on Similar Biological Medicinal Products Containing Biotechnology-Derived Proteins as Active Substance: Non-clinical and Clinical Issues [97] also addresses aspects relating to immunogenicity and indicates that antibody titres, cross-reactivity and neutralizing capacity should be determined as part of non-clinical toxicity studies. Furthermore, for clinical studies, the immunogenicity of a similar biological medicinal product must always be investigated. Specifics regarding the assays indicate that sensitive screening assays should be used, followed by neutralizing antibody assays for further characterization of antibodies [97].

Recently, the EMEA issued a draft Guideline on the Immunogenicity Assessment of Biotechnology-derived Therapeutic Proteins [98]. This very comprehensive and detailed guideline includes an example of a well defined strategy for immunogenicity evaluation involving antibody assays, characterization of the observed immune response, and an evaluation of the impact of antibody development on efficacy and safety. Although the guideline recommends using a risk based strategy, it does not provide guidance on how to apply such a strategy to antibody testing, but provides general guidance on antibody assays and critical issues (e.g. antibody controls, validation, sampling strategies) that need to be considered and adopted for immunogenicity studies. The EMEA guideline states that the impact of a product's immunogenicity on patient safety in terms of acute consequences (e.g. infusion or anaphylactic reactions and unexpected clinical symptoms) and non-acute consequences (e.g. delayed hypersensitivity, immune complex related effects and cross-reactivity with an endogenous antigen) should be considered. The EMEA guideline strongly emphasizes commitment to assessment of unwanted immunogenicity and its clinical significance post-approval, usually as part of pharmacovigilance surveillance. In some cases, post-approval clinical studies may be needed to establish the risk associated with an unwanted immune response. The guidance has recently been revised [98].

Most concepts stated in the EMEA Guideline on the Immunogenicity Assessment of Biotechnology-derived Therapeutic Proteins are also applicable to radiolabelled biologicals and should be considered for these medicines. From a practical perspective, however, some of the issues may need to be modified and adapted, depending on the radiolabelled biological, its specific usage and target population.

10.5. NON-CLINICAL ASSESSMENT OF UNWANTED IMMUNOGENICITY

Non-clinical studies aimed at predicting immunogenicity, its characteristics and clinical significance in humans are not needed. Currently, there is considerable emphasis on determining T cell epitopes in T cell assays (using cells derived from previously exposed or naive individuals) and on computer algorithms for sequence analysis and prediction of T cell epitopes (on the basis of structural analysis by scanning of protein sequences for major histocompatibility complex binding motifs) for their suitability as 'tools' in predicting potential immunogenicity of therapeutics and for selecting those with minimal risk of immunogenicity. Although consideration may be given to these emerging technologies, these approaches are not a requirement.

Since therapeutic proteins show species differences in most cases, human proteins are often recognized as foreign proteins in animals. Therefore, the utility of animal models for predicting immunogenicity is considered to be low. Measurement of antibodies in non-clinical studies is, however, requested as part of repeated dose toxicity studies in conventional animal models, to aid in the interpretation of these studies. During this testing, antibody responses should be characterized (e.g. titre, number of responding animals, neutralizing or non-neutralizing), and their appearance should be correlated with any pharmacological and/or toxicological changes. Specifically, the effects of antibody formation on pharmacokinetic/pharmacodynamic parameters, incidence and/or severity of adverse effects, complement activation, or the emergence of new toxic effects should be considered when interpreting the data. Attention should also be given to evaluation of possible pathological changes related to immune complex formation and deposition (as discussed in Ref. [66]).

As stated in the EMEA guidance, an immune response to a therapeutic protein representing a counterpart to an endogenous protein may result in cross-reactivity directed at the endogenous protein. Any relevant experience concerning the consequences of induction of an immune response to the

endogenous protein or its absence/dysfunction in animal models should be noted and discussed (as discussed in Ref. [98]).

It should be noted that conventional animal models can be useful for comparison of antibody responses to assess the 'relative immunogenicity' (i.e. for different routes of administration, different dosing schedules, different formulations or even different batches of a particular therapeutic) or for comparison of unlabelled versus radiolabelled product, and for changes in manufacturing processes (see Ref. [99]).

10.6. ASSESSMENT OF IMMUNOGENICITY

Currently, the only realistic option for assessing the immunogenicity of a radiolabelled biological is to include carefully planned immunogenicity studies during clinical trials in humans. The need for these studies should be dictated by the intended use/purpose of the radiolabelled biological (e.g. diagnostic or therapeutic use), the frequency of use (i.e. single or multiple injections), the route of administration, the disease type and other relevant issues. It is critical to consider the risk of generating an immune response, the potential severity of the induced response, and the risk/benefit to the target population. For example, frequent administration of a radiolabelled therapeutic in immunocompetent individuals potentially carries a 'high risk' of generating an immune response, and thus there is a need for immunogenicity testing in such a situation.

Depending on the radiolabelled biological, therefore, a rigorous assessment based on state of the art measurement and characterization of antibodies is required in order to understand the immunogenicity of a product. In addition, a well considered strategy for immunogenicity assessment that includes a risk level based bioanalytical testing strategy and a management plan for related adverse events is essential.

10.7. ASSAYS FOR IMMUNOGENICITY ASSESSMENT

Unwanted immunogenicity induced by biologicals can comprise humoral and cellular immune responses. It is important to select and/or develop assays and assay strategies for assessment of such immune responses. Currently, the most practical approach for testing unwanted immunogenicity is by detection, measurement and characterization of antibodies generated specifically against the product.

10.7.1. Assay strategy

Adopting an appropriate strategy for assessment of unwanted immunogenicity is important [94]. This usually includes a screening assay for identification of antibody positive samples or patients, analytical immunochemical procedures for confirming the presence of antibodies and determining antibody specificity, and functional assays for assessment of the neutralizing capacity of the antibodies. In addition, non-antibody assays, for example, assays for relevant biomarkers or pharmacokinetic measurements that assess and characterize the clinical impact of antibodies (and possibly other components of immune responses) if these are detected or induced, are also required (see Ref. [98]).

10.7.2. Types of antibody assay

10.7.2.1. Screening assays

Screening assays determine the presence (or absence) of antibodies based on the ability of the antibodies to recognize the relevant antigenic determinants in the therapeutic protein. They include immunoassays, radioimmunoprecipitation assays and surface plasmon resonance (SPR) assays [100, 101]. These assays have distinct advantages and disadvantages (see Table 6). Immunoassays constitute a large group of assays and are based on a variety of formats and detection systems. They include direct binding assays, bridging assays, capture (sandwich) assays and competitive immunoassays using radioligand, enzymatic, fluorescent, chemiluminescent or electrochemical luminescence detection systems. Each of these platforms must be judged on its relative merits for each product. It is important to ensure that the assay parameters and platform selected are suitable for the particular product.

Screening assays should identify all positive samples and detect antibodies of different classes and specificities (e.g. IgM, IgG subclasses). They should not provide false negative results or underestimate true positives. It is important to realize that a screening assay that is unable to identify any positives following a preclinical or clinical trial casts doubt on the ability of the assay to detect low positive samples.

10.7.2.2. Assays for confirming the presence of antibodies

It is necessary to include a confirmatory step after the initial screening to eliminate false positive samples or patients and to confirm that any positive result is due to the presence of ‘genuine’ antibodies. For this, any of the

methods that are used for screening purposes (e.g. ELISAs, competitive immunoassays, SPR assays) can be used. Often, assay specificity is shown by assaying the serum sample (identified as positive by the screening assay) in the absence or presence of excess antigen such that the latter sample no longer provides a positive signal in the assay employed. Care should be taken in the interpretation of such results, as some substances may also cross-react and bind non-specifically.

In certain instances, it may be possible to select another assay based on a different scientific principle, taking into account the limitations and characteristics of the screening assay and use. Adoption of an immunoblotting procedure, which provides information concerning the specificity of the antibodies detected, is valuable, as the antibodies may have specificity for other components (e.g. contaminants) in the product [101, 102]. For example, very low levels of expression system derived bacterial proteins in rDNA products have been shown to be highly immunogenic compared with the human sequence major protein (the active principle) present in the product. Other procedures (e.g. analytical radioimmunoprecipitation assays) can also be used for specificity studies.

10.7.2.3. Neutralization assays

For a biological therapeutic, assessing the neutralizing capacity of antibodies usually requires the use of bioassays (e.g. cell based assays). However, if neutralizing cell based assays are not feasible, competitive ligand binding assays or other suitable assays may be used to demonstrate the neutralizing capacity/potential of the induced antibodies.

10.7.3. Interpretation of data

It is important to establish clear criteria for interpretation of results (i.e. for distinguishing antibody 'positive' samples from antibody 'negative' samples) and to confirm positive results. Approaches can differ according to assay, for example, and must be decided on accordingly. A common procedure for establishing a positive cut-off for immunoassays is to establish the assay background (see discussion above). A statistical approach should preferably be used to establish the assay cut-off value. Alternatively, real data (e.g. double the background value) can be used to determine what will be considered the lowest positive result [98].

10.7.4. Considerations for antibody assays

It should be considered that the detection, measurement and characterization of antibodies is a significant task, and that achieving valid, useful results involves more than simply ensuring that appropriate tests are performed. The purpose of the assay, suitability of assay platform(s), availability of positive and negative antibodies, and assay design are some of the important issues that must be considered prior to development and design of antibody assays [100, 101]. In addition, assay validation needs to be addressed, particularly if assays are being used for pivotal clinical trials (see Ref. [98]).

Several issues must be considered, including the following:

(1) A suitable and sensitive screening assay should be available for antibody detection. Recommending a general assay format for screening antibodies against all biologicals is difficult, if not impossible. Assay formats tend to vary in suitability for different proteins and very often are dependent on the class of therapeutic protein (e.g. a therapeutic cytokine or a MAb). For example, when determining antibodies against a non-immunoglobulin recombinant protein, all platforms listed in Table 6 are suitable options and must be judged on their relative merits. If the protein

TABLE 6. METHODS USED FOR DETECTION AND CHARACTERIZATION OF ANTIBODIES

Type of assay	Parameter measured	Advantages	Disadvantages
Binding assay: Direct format (coating with antigen and detecting with labelled anti-Ig)	Identifies antibodies capable of binding to the antigen preparation	Rapid Relatively easy to use High throughput assay — often used as a 'screening assay' for antibody detection Good sensitivity	Prone to spurious binding and 'matrix effects' May fail to detect 'low affinity' antibodies Immobilization of antigen may mask or alter epitopes Species specificity and isotype detection determined by secondary reagent Detection reagent may differ between control and sample

TABLE 6. METHODS USED FOR DETECTION AND CHARACTERIZATION OF ANTIBODIES (cont.)

Type of assay	Parameter measured	Advantages	Disadvantages
Binding assay: Indirect format (coating with a specific MAb or biotin, etc., followed by antigen)	Identifies antibodies capable of binding to the antigen preparation	Coating with a specific MAb keeps antigen in oriented position Coating is consistent and maintains antigen conformation	Extensive studies required to demonstrate that the coating MAb does not mask or alter epitopes May fail to detect low affinity antibodies Species specificity and isotype detection determined by secondary reagent
Binding assay: Bridging format (coating with antigen and detecting with labelled antigen)	Identifies antibodies capable of binding to the antigen preparation	High throughput Dual arm binding ensures high specificity Can detect all isotype responses Not species specific	Immobilization of the antigen may alter the conformation of the protein Requires labelled antigen Labelling may alter/denature antigen, mask epitopes May fail to detect rapidly dissociating antibodies
Electrochemi-luminescence: Bridging format	Measures antigen–antibody binding in liquid phase	High throughput Can detect all isotype responses Allows use of high concentrations of matrix Detection signal consistent during life of TAG conjugate Not species specific	Requires two antigen conjugates (biotin and TAG) Antigen labelling may alter/denature antigen, mask/alter epitopes Equipment and reagents are vendor specific
Radioimmuno-precipitation assay	Measures antigen–antibody binding in liquid phase	Moderate to high throughput Good sensitivity Can be specific	Can be isotype specific; may fail to detect rapidly dissociating antibodies Requires radiolabelled antigen; radiolabel may alter/denature antigen, decay of radiolabel may affect antigen stability

TABLE 6. METHODS USED FOR DETECTION AND CHARACTERIZATION OF ANTIBODIES (cont.)

Type of assay	Parameter measured	Advantages	Disadvantages
Surface plasmon resonance	Shows antigen–antibody interaction in liquid phase in real time	Provides information on the specificity, isotype, relative binding affinity and relative concentration Enables detection of both low affinity and high affinity antibodies Detection reagent not required Not species specific	Low throughput; expensive; requires dedicated equipment; reagents are vendor specific May require immobilization of the antigen, which may alter the conformation of the native protein Regeneration step may degrade antigen Sensitivity often less than with binding assay
Immunoblotting	Assesses the specificity of the antibodies for protein in antigen preparations	Dissects the specificity of the antibodies Provides profile of reactivity against subcomponents of the product Good sensitivity	Non-quantitative Relatively low throughput May fail to detect antibodies that do not recognize antigen after SDS PAGE separation Appropriate detection reagents necessary
Bioassay	Assesses the neutralizing potential of the antibodies	Functional assay which distinguishes antibodies with neutralizing potential Quantitates the neutralization capacity (in vitro) of antibodies capable of neutralizing the biological activity of the antigen May correlate with clinical response	Relatively time-consuming Often variable Can be affected by non-specific serum (matrix) effects and non-antibody neutralizing factors (e.g. inhibitors, soluble receptors) Validation can be difficult because of the reagents (e.g. cell lines)

is a humanized or fully human immunoglobulin therapeutic, the choices for detection of human anti-human antibodies are limited. However, even among these proteins, the suitability of a particular platform and/or assay format is highly dependent on the therapeutic itself. A case by case approach is therefore advocated. Should the initial assay selection not meet desired expectations (e.g. if it gives poor sensitivity or high backgrounds), more than one assay may need to be developed and tested, and suitable assay optimization may need to be undertaken to ensure that a valid comparison can be carried out.

Adopting the simplest assay suitable for all requirements is often the preferred approach to assay selection (particularly when high throughput is important; e.g. for screening assays). However, care must be taken to ensure that it does not compromise other stages of the immunogenicity assessment. For example, one must consider whether the selected assay format allows for appropriate epitope exposure and detection of all antibodies, including low affinity antibodies and those of certain isotypes or subclasses. In the former scenario, it may be necessary to adopt a strategy that avoids specific masking of particular epitope(s); for the latter, adopting a more suitable assay format (e.g. the 'bridging' assay, electrochemiluminescent or SPR method) may assist in overcoming this problem.

(2) A design suitable for the intended purpose of the assay is essential. Consideration should be given to determination of optimal assay procedures and parameters for obtaining a valid assay. All assays should exhibit sensitivity, precision, recovery and robustness suitable for the intended purpose of the assay, and appropriate validation depending on the development phase.

(3) Factors such as comedications, dosing regimen, disease specific issues (e.g. presence of rheumatoid factor) and the product itself can cause interference in assays. A residual therapeutic effect (e.g. MAbs, which often have a long half-life) in patient sera can lead to complexation with induced antibodies, competition with bound or soluble antigen from the assay and a reduction of the amount of antibody detected. This may affect assays differently, depending on the assay, the assay format and the antibody characteristics. This can be resolved by assessing the amount of antigen required to reduce the antibody signal (positive) to background for a range of antibody concentrations and by adopting strategies for dissociating immune complexes, removing excess biological by solid phase adsorption, appropriate sampling for antibody assessment (allowing a time interval for the product to be cleared from circulation) and/or use of an assay that allows sufficient sample dilution. Such

approaches may be adopted on a case by case basis. The acceptable degree of interference should be scientifically justified based on the nature of the samples and the significance of the immune response.

(4) For all types of antibody assay, a careful selection of controls (e.g. negative and positive) is critical [98, 100].

Negative standards or controls are needed to establish assay baselines and to characterize and/or validate the assays. The assay baseline for normal (healthy) individuals is fairly easily determined by measuring the assay response using samples derived from an appropriate number of such individuals and analysing this response to provide a statistically valid background value. However, this value may not represent the baseline response of the assay to samples derived from the patient population, which would therefore need to be established separately, using pre-treatment samples from patients or from some other valid, relevant population. In any case, samples from some individuals or patients may contain pre-existing (pre-treatment) antibodies or possibly other substances that produce significant positive responses in assays; thus, screening patients for this is necessary to ensure that post-treatment data can be interpreted correctly.

An antibody positive standard, reference material or control is needed for all assays, to demonstrate assay response; it can also be used for calibration purposes. If possible, this should be a human preparation with a significant antibody content that is available in sufficient quantities for continued use. It should be stored appropriately (lyophilized or frozen at a suitable temperature) and well characterized. Reference preparations for neutralization bioassays should have significant neutralizing activity, but it is also useful to include a non-neutralizing antibody preparation in assays, at least in validation studies.

In most cases, however, sufficient human serum may not be available in the quantities required, or even at all (e.g. early in product development and/or trials), and in such cases, use of an animal serum as a reference is the only realistic option. In such cases, serum from hyperimmunized animals is usually the best source, as it can be acquired in amounts adequate for inclusion in antibody assays throughout the studies. However, this serum must be selected carefully, and its uses are more limited than those of human reference preparations; for example, immunochemical procedures, which involve the use of an anti-human immunoglobulin reagent, will not reliably respond to non-human antibodies, and the characteristics of the response in all assays may differ from those of responses to human antibodies in human samples. It must be taken into account that not all species will produce antibodies with

comparable affinity, avidity and class/subclass types, thus potentially affecting their performance. Purification and quantification of antibody preparations for use as positive controls is required during assay optimization and validation. In some instances, it may be useful to prepare a panel of reference materials containing different amounts of antibodies and antibodies with different characteristics for characterizing/validating assays and for use as assay performance indicators. If possible, this should include one or more preparations with low antibody content (close to the minimum detection limit) and containing low avidity antibodies.

(5) The criteria used for distinguishing antibody positive samples from antibody negative samples or levels of magnitude must be carefully selected and defined [99–101]. Quantification of antibody levels should also be considered. Different approaches can be used for this, but it is best to use the same approach for interpretation of data to ensure comparability between assays.

(6) Prior to testing samples for studies of unwanted immunogenicity, it is important to ensure that consideration has been given to the timing of sample collection and that the use of the radiolabelled product (i.e. single or multiple doses) has been taken into account. Determination of the timing of sample collection can be made from early pharmacokinetic studies so that levels of circulating product do not interfere with the ability of the assay to detect antibodies. In cases where levels of circulating product and immune complexes may occur, adoption of strategies for dissociation of immune complexes may be necessary. It is important to ensure that a pre-treatment baseline sample from the patient is available, as this is often the best negative control for inclusion in antibody assays. If the pre-treatment sample gives a positive result, the nature of the cause (specific or non-specific) should be established. Post-administration samples should be drawn at regular intervals. It may be necessary in some instances to assess antibody development at baseline and in the sequential samples before readministration of the radiolabelled product.

(7) Monitoring of immunogenicity is largely dependent on the product, patient status, duration of use and desired clinical response. In some instances, long term immunogenicity studies involving analysis of samples from a large number of treated patients may be necessary if a complete assessment of immunogenicity (and its consequences) is to be obtained.

(8) It should be determined whether the results of the antibody testing are correlated with product efficacy and adverse events.

10.8. CONCLUSIONS

An application submitted for a radiolabelled product to be administered in a repeated dosing regimen should include a clinically available, validated test that reliably measures human antibody responses to the product if an anti-product response may affect the safety, efficacy or dosing of the product.

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Annex I

SELECTED DEFINITIONS

The definitions given below may not necessarily conform to definitions adopted elsewhere for international use.

approval. The affirmative decision of the Investigational Review Board (IRB)/ Independent Ethics Committee (IEC) or competent authority (CA) that the clinical trial has been reviewed and may be conducted at the institution site within the constraints set forth by the IRB/IEC or CA, the institution, good clinical practice and the applicable regulatory requirements.

case report form (CRF). A printed, optical or electronic document designed to record all the information required by the protocol to be reported to the sponsor on each trial subject.

clinical investigator's brochure (CIB). A compilation of those clinical and non-clinical data on the investigational product(s) that are relevant to the study of the investigational product(s) in human subjects.

clinical trial(s). Any investigation in human subjects intended to discover or verify pharmacological and other pharmacodynamic effects of (an) investigational product(s), and to identify any adverse reactions to (an) investigational product(s), and to study absorption, distribution, metabolism and excretion of (an) investigational product(s) with the object of ascertaining its safety and/or efficacy. The terms 'clinical trial' and 'clinical study' are often used synonymously.

clinical trial/study report. A written description of a trial/study of any therapeutic, prophylactic or diagnostic agent conducted in human subjects, in which the clinical and statistical description, presentations and analyses are fully integrated into a single report.

comparator product. An investigational or marketed product (i.e. active control), or placebo, used as a reference in a clinical trial.

competent authority (CA). A body having the power to regulate. In this GCP guideline, the expression 'competent authority' includes those authorities that review submitted clinical data and those that conduct inspections or

provide scientific advice. These bodies are sometimes referred to as regulatory bodies.

contract. A written, dated and signed agreement between two or more involved parties that sets out any arrangements on delegation and distribution of tasks and obligations, and, if appropriate, on financial matters. The protocol may serve as the basis of a contract.

contract research organization (CRO). A person or an organization (commercial, academic or other) contracted by the sponsor to perform one or more of a sponsor's trial related duties and functions.

European Medicines Agency (EMEA). Established by the European Commission and located in London. Technical and scientific support for ICH activities is provided by the Committee for Medicinal Products for Human Use (CHMP) of the EMEA.

fast protein liquid chromatography (FPLC). An alternative to HPLC that does not use high pressure liquid.

good clinical practice (GCP). An international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety and well-being of trial subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki, and that the clinical trial data are credible.

The objective of the ICH GCP Guideline is to provide a unified standard for the European Union (EU), Japan and the United States of America to facilitate the mutual acceptance of clinical data by the regulatory bodies in these jurisdictions.

The guideline was developed with consideration of the current good clinical practices of the European Union, Japan and the United States of America, as well as those of Australia, Canada, the Nordic countries and the World Health Organization (WHO).

This guideline should be followed when generating clinical trial data that are intended to be submitted to regulatory bodies.

The principles established in this guideline may also be applied to other clinical investigations that may have an impact on the safety and well-being of human subjects.

informed consent. A process by which a subject voluntarily confirms his or her willingness to participate in a particular trial, after having been informed of all aspects of the trial that are relevant to the subject's decision to participate. Informed consent is documented by means of a written, signed and dated informed consent form.

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). A joint initiative involving both regulators and industry as equal partners in the scientific and technical discussions of the testing procedures required to ensure and assess the safety, quality and efficacy of medicines.

The focus of ICH has been on the technical requirements for medicinal products containing new drugs. The vast majority of those new drugs and medicines are developed in western Europe, Japan and the United States of America; therefore, when ICH was established, it was agreed that its scope would be confined to registration in those three regions.

There are Six Parties directly involved in the decision making process.

The Six Parties are the founder members of ICH which represent the regulatory bodies and the research based industry in the European Union, Japan and the United States of America: EU, EFPIA, MHLW, JPMA, FDA and PhRMA.

independent ethics committee (IEC). An independent body (a review board or a committee, institutional, regional, national or supranational) of medical professionals and non-medical members, whose responsibility it is to ensure the protection of the rights, safety and well-being of human subjects involved in a trial and to provide public assurance of that protection, by, among other things, reviewing and approving/providing favourable opinion on the trial protocol, the suitability of the investigator(s), the facilities and the methods and materials to be used in obtaining and documenting informed consent of the trial subjects.

inspection. The act by a regulatory authority (or authorities) of conducting an official review of the documents, facilities, records and any other resources that are deemed by this authority (or authorities) to be related to the clinical trial and that may be located at the site of the trial, at the facilities of the sponsor and/or contract research organization, or at other establishments deemed appropriate by the regulatory authority (or authorities).

instant thin layer chromatography (ITLC). An easy chromatographic method on paper strips, often used for evaluating the labelling efficiency and chemical purity of radiolabelled peptides.

investigational medicinal product (IMP). A pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical trial, including products already having marketing authorization but being used or assembled (formulated or packaged) in a way different from the authorized form, or being used for an unauthorized indication, or being used to gain further information about the authorized form.

investigational new drug (IND). A synonym for IMP used mainly in the United States of America.

investigator. A person responsible for the conduct of the clinical trial at a trial site. If a trial is conducted by a team of individuals at a trial site, the investigator is the responsible leader of the team and may be called the principal investigator.

Investigational Review Board (IRB). An independent body of medical, scientific and non-scientific members, whose responsibility is to ensure the protection of the rights, safety and well-being of human subjects involved in a trial by, among other things, reviewing, approving and providing continuing review of the trial protocol and amendments, and of the methods and materials to be used in obtaining and documenting informed consent of the trial subjects.

matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS). An analytical method to evaluate the molecular weight of all compounds present in a mixture. It works particularly well with peptides and proteins with a molecular weight below 30 000 kDa.

monocentre clinical trial. A clinical trial conducted according to a single protocol and at only one site.

multicentre clinical trial. A clinical trial conducted according to a single protocol but at more than one site, and therefore carried out by more than one investigator.

non-investigational medicinal product (NIMP). Some clinical trial protocols require the use of non-investigational medicinal products (NIMPs) such

as support or escape medication for preventive, diagnostic or therapeutic reasons and/or needed to ensure that adequate medical care is provided for the subject. Such NIMPs may also be used in accordance with the protocol to induce a physiological response. These products do not fall within the definition of investigational medicinal products in the Directive and may be supplied by the sponsor. The sponsor should provide details of these NIMPs and their proposed use in the trial protocol and should ensure that they are of the necessary quality for human use after seeking advice and/or involvement of a qualified person, where appropriate.

quality assurance (QA). All those planned and systematic actions that are established to ensure that the trial is performed and the data are generated, documented (recorded) and reported in compliance with good clinical practice and the applicable regulatory requirement(s).

quality control (QC). The operational techniques and activities undertaken within the quality assurance system to verify that the requirements for quality of the trial related activities have been fulfilled.

regulatory requirement(s). Any law(s) and regulation(s) addressing the conduct of clinical trials of investigational products.

sponsor. An individual, company, institution or organization that takes responsibility for the initiation, management and/or financing of a clinical trial.

study protocol. A document that describes the objective(s), design, methodology, statistical considerations and organization of a trial. The protocol usually also gives the background and rationale for the trial, but these could be provided in other protocol referenced documents. Throughout the GCP guideline, the term 'protocol' refers to protocol and protocol amendments

Annex II

SELECTED GUIDANCE MATERIALS

US Food and Drug Administration (FDA)

Guidance for Industry, Investigators and Reviewers: Exploratory IND Studies, G:\6384dft.doc (April 2005)

Guidance on PET Drug Products – Current Good Manufacturing Practice (cGMP), G:\4259dft.doc (March 2002)

Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use, Docket No. 94D-0259 (February, 1997)

Guidance for Industry: Content and Format of Investigational New Drug Applications (INDs) for Phase 1 Studies of Drugs, Including Well-Characterized, Therapeutic, Biotechnology-Derived Products (November 1995)

Guidance for Industry: Developing Medical Imaging Drugs and Biological Products, Part 1: Conducting Safety Assessments (June 2004)

European Medicines Agency (EMEA)

Guideline on the Requirements to the Chemical and Pharmaceutical Quality Documentation Concerning Investigational Medicinal Products in Clinical Trials, CPMP/CHMP/QWP185401/2004

Note for Guidance on 'Radiopharmaceuticals', Draft 3.0

Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (ICH S6), CPMP/ICH/302/95

Non-clinical Safety Studies for the Conduct of Human Trials for Pharmaceuticals (ICH M3), CPMP/ICH/286/95

Note for Guidance on Radiopharmaceuticals Based on Monoclonal Antibodies, 3AQ21A

Guideline on Virus Safety Evaluation of Biotechnological Medicinal Products, EMEA/CHMP/BWP/398498/2005-corr

Guideline on Radiopharmaceuticals, EMEA/CHMP/QWP/306970/2007, Draft

Guideline on Similar Biological Medicinal Products Containing Biotechnology Derived Proteins as Active Substance: Non-clinical and Clinical Issues, EMEA/CHMP/BMWP/42832/2005

Guideline on the Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins, EMEA/CHMP/BMWP/14327/2006, Draft

Guideline on Comparability of Biotechnology-Derived Medicinal Products after a Change in the Manufacturing Process: Non-clinical and Clinical Issues, CHMP/BMWP/101695/06

Pharmacopoeias

General monograph on radiopharmaceutical preparations and individual monographs on radiopharmaceuticals (European Pharmacopoeia (PhEur))

Monographs on radiopharmaceuticals (United States Pharmacopeia (USP))

Guidelines for Aseptic Compounding and Dispensing of Radiopharmaceuticals (USP), Chapter <797> [1.11] and Chapter <1075> Good Compounding Practices [1.12]

European Commission – Eudralex

Detailed Guidance on the Application Format and Documentation to be Submitted in an Application for an Ethics Committee Opinion on the Clinical Trial on Medicinal Products for Human Use (April 2004)

Detailed Guidance for the Request for Authorisation of a Clinical Trial on a Medicinal Product for Human Use to the Competent Authorities, Notification of Substantial Amendments and Declaration of the End of the Trial (October 2005)

Detailed Guidance on the European Clinical Trials Database (EUDRACT Database)

Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the Approximation of the Laws, Regulations and Administrative Provisions of the Member States Relating to the Implementation of Good Clinical Practice in the Conduct of Clinical Trials on Medicinal Products for Human Use

Commission Directive 2005/28/EC of 8 April 2005 Laying Down Principles and Detailed Guidelines for Good Clinical Practice as Regards Investigational Medicinal Products for Human Use, As Well As the Requirements for Authorisation of the Manufacturing or Importation of Such Products

Detailed Guidance on the Collection, Verification and Presentation of Adverse Reaction Reports Arising from Clinical Trials on Medicinal Products for Human Use (April 2004)

Detailed Guidance on the European Database of Suspected Unexpected Serious Adverse Reactions (Eudravigilance – Clinical Trial Module) (April 2004)

Eudralex – Volume 4, Medicinal Products for Human and Veterinary Use: Good Manufacturing Practice, Annex 13: Manufacturing of Investigational Medicinal Products

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)

Q5E: Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process (June 2005)

S6: Guidance for Industry: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (July 1997)

E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting. This document gives standard definitions and terminology for key aspects of clinical safety reporting. It also gives guidance on mechanisms for handling expedited (rapid) reporting of adverse drug reactions in the investigational phase of drug development (October 1994).

E3: Structure and Content of Clinical Study Reports. This document describes the format and content of a study report that will be acceptable in all three ICH regions. It consists of a core report suitable for all submissions and appendices

that need to be available but will not be submitted in all cases (November 1995).

E6: Good Clinical Practice. This Good Clinical Practice document describes the responsibilities and expectations of all participants in the conduct of clinical trials, including investigators, monitors, sponsors and IRBs. GCPs cover aspects of monitoring, reporting and archiving of clinical trials and incorporating addenda on the Essential Documents and on the Investigator's Brochure which had been agreed earlier through the ICH process (May 1996).

E7: Studies in Support of Special Populations: Geriatrics. This document provides recommendations on the special considerations which apply in the design and conduct of clinical trials of medicines that are likely to have significant use in the elderly (June 1993).

E8: General Considerations for Clinical Trials. The tripartite harmonized ICH guideline was finalized in July 1997. This document sets out the general scientific principles for the conduct, performance and control of clinical trials. The guideline addresses a wide range of subjects in the design and execution of clinical trials.

E9: Statistical Principles for Clinical Trials. This biostatistical guideline describes essential considerations on the design and analysis of clinical trials, especially the "confirmatory" (hypothesis-testing) trials that are the basis for demonstrating effectiveness (February 1998).

E10: Choice of Control Group and Related Issues in Clinical Trials. This document addresses the choice of control groups in clinical trials, considering the ethical and inferential properties and limitations of different kinds of control group. It points out the assay sensitivity problem in active control equivalence/non-inferiority trials that limits the usefulness of trial design in many circumstances (July 2000).

E11: Clinical Investigation of Medicinal Products in the Paediatric Population. This document addresses the conduct of clinical trials of medicines in paediatric populations. It facilitates the development of safe and effective use of medicinal products in paediatrics (July 2000).

Other guidance documents

EANM Guidelines on Current Good Radiopharmacy Practice (cGRPP) in the Preparation of Radiopharmaceuticals,
http://www.eanm.org/scientific_info/guidelines/gl_radioph_cgrpp.pdf

SNM Clinical Trials Group (CTG): The CTG provides the framework and infrastructure necessary to establish support for innovative trials of both diagnostic and therapeutic radiopharmaceuticals. The infrastructure that has been developed to support CTG includes a steering committee, with involvement by all councils and centres; standard operating procedures; and processes for protocol evaluation and review. Ethics and conflict of interest guidelines are being established as well.

Annex III

SELECTED WEB SITES

BNMS	http://www.bnms.org.uk
CORDIS	http://cordis.europa.eu/
CRF samples	http://www.icssc.org/ICTDIR_DM_Presentation_new.ppt
DELPHIAN	http://www.delphian.com
EANM	http://www.eanm.org
EMEA	http://www.emea.eu.int/
ESPACE	http://ep.espacenet.com
European Commission	http://pharmacos.eudra.org/F2/home.html
EUDRACT	http://eudract.emea.europ
FDA (USA)	http://www.fda.gov
FDA: Safety	http://www.fda.gov/medwatch/SAFETY/3500.pdf http://www.fda.gov/medwatch/SAFETY/3500A.pdf
HSFP	http://www.hfsp.org/
IAEA CRP	http://www-crp.iaea.org/
ICH	http://www.ich.org/cache/compo/276-254-1.html
NIH (USA)	http://grants.nih.gov/grants/oer.htm
Proposal Central	http://proposalcentral.altum.com/
SNM	http://www.snm.org
SNM grants	http://www.uphs.upenn.edu/pet/snmerf/
Weizmann Institute	http://www.weizmann.ac.il/RGP_open/rwebpages.shtml

Annex IV

TABLE OF RADIONUCLIDES

Radionuclide	Half-life	Decay mode	Internal toxicity class	Annual limit on intake (mCi)	Container posting level (mCi)	Γ[a] R/h@1cm per mCi	TVL[b] (mmPb)	Radiation types, keV (% per decay)
H-3	12.35 a	β	Low	80	1	—	—	Betas: 19 (100%)
C-11	20.38 months	β+, EC	Low	400	1	5.97	13.7	Positrons: 960 (99.7%) Gammas: 511 (199.5%)
N-13	9.97 months	β+	Low	—	1	5.97	13.7	Positrons: 1199 (99.8%) Gammas: 511 (199.6%)
C-14	5730 a	β	Moderate	2	1	—	—	Betas: 156 (100%)
O-15	122.24 s	β+	Low	—	—	5.97	13.7	Positrons: 1732 (99.9%) Gammas: 511 (199.8%)
F-18	109.77 months	β+	Low	70	1	5.8	13.7	Positrons: 634 (96.7%) Gammas: 511 (193.4%)
Na-22	2.6 a	β+, EC	High	0.4	0.01	12	26.6	Positrons: 545 (89.8%) Gammas: 511 (180%) 1275 (99.9%)
Na-24	15 h	β	Moderate	4	0.1	18.4	52	Betas: 1390 (99.9%) Gammas: 1386 (100%) 2754 (100%)

For footnotes see p. 132.

Radionuclide	Half-life	Decay mode	Internal toxicity class	Annual limit on intake (mCi)	Container posting level (mCi)	Γ[a] R/h@1cm per mCi	TVL[b] (mmPb)	Radiation types, keV (% per decay)
P-32	14.29 d	β	High	0.4	0.01	—	—	Betas: 1710 (100%)
P-33	25.4 d	β	Moderate	3	0.1	—	—	Betas: 250 (100%)
S-35	87.44 d	β	Moderate	2	0.1	—	—	Betas: 167 (100%)
Cl-36	301 000 a	β	High	0.2	0.010	—	—	Betas: 714 (98%)
K-40	1.3×10^9 a	β, EC	High	0.3	0.1	0.7	38.7	Betas: 1312 (89.3%) Gammas: 1460 (10.7%)
K-42	12.36 h	β	Moderate	5	1	1.4	39.8	Betas: 1996 (17.5%) 3521 (82%) Gammas: 1525 (18%)
Ca-45	163 d	β	Moderate	0.8	0.1	—	—	Betas: 257 (100%)
Sc-46	83.83 d	β	High	0.2	0.01	10.9	29.1	Betas: 357 (100%) Electrons: 140 (38%) Gammas: 889 (100%) 1121 (100%) 143 (62%)
Ca-47	4.53 d	β	Moderate	0.8	0.1	5.7	34.4	Betas: 691 (81.7%) 1988 (18%) Gammas: 489 (7.0%) 808 (6.9%) 1297 (74.9%)
V-48	16.24 d	β+	Moderate	0.6	0.1	15.6	30.1	Positrons: 698 (50%) Gammas: 983 (100%) 1312 (97.5%) 2240 (2.4%) 511 (100%) 944 (7.7%)
Cr-51	27.7 d	EC	Low	20	1	0.2	6.3	Gammas: 320 (9.8%)

For footnotes see p. 132.

Radionuclide	Half-life	Decay mode	Internal toxicity class	Annual limit on intake (mCi)	Container posting level (mCi)	Γ[a] R/h@1cm per mCi	TVL[b] (mmPb)	Radiation types, keV (% per decay)
Mn-54	312.5 d	EC	Moderate	0.8	0.1	4.7	24.6	Gammas: 835 (100%)
Fe-55	2.7 a	EC	Moderate	2	0.1	—	—	X rays: 6 (28%)
Co-57	270.9 d	EC	Moderate	0.7	0.1	0.9	0.7	Gammas: 122 (85.5%) 136 (10.6%)
Fe-59	44.53 d	β	High	0.3	0.01	6.4	33.6	Betas: 273 (45.2%) 465 (53.1%) Gammas: 192 (3.0%) 1099 (56.5%) 1292 (43.2%)
Co-60	5.27 a	β	High	0.030	0.001	13.2	34.8	Betas: 318 (100%) Gammas: 1173 (100%) 1332 (100%)
Ni-63	96 a	β	Moderate	0.8	0.1	—	—	Betas: 66 (100%)
Ga-67	3.26 d	EC	Low	7	1	1.1	4.7	Electrons: 84 (26.8%) Gammas: 93 (36%) 185 (19.7%) 300 (15.9%) 394 (4.5%)
Ge-68	288 d	EC	High	0.1	0.010	5.51	14.4	Positrons: 836 (84%) Gammas: 511 (178%) 1 077 (3.3%) 1883 (0.1%) X rays: 9 (39%) 10 (5.5%)

For footnotes see p. 132.

Radionuclide	Half-life	Decay mode	Internal toxicity class	Annual limit on intake (mCi)	Container posting level (mCi)	Γ[a] R/h@1cm per mCi	TVL[b] (mmPb)	Radiation types, keV (% per decay)
As-74	17.76 d	β+	Moderate	0.8	0.1	4.4	16.8	Betas: 718 (16%) 1353 (19%) Positrons: 944 (27%) 944 (27%) 945 (27%) Gammas: 10 (5.1%) 511 (59%) 596 (60%) 608 (5.5%)
Se-75	119.8 d	EC	Moderate	0.5	0.1	2.1	4.6	Gammas: 121 (16.7%) 136 (59.2%) 265 (59.8%) 280 (25.2%) 401 (11.4%)
Kr-85	10.72 a	β	—	—	1	0.4	2.8	Betas: 687 (99.6%) Gammas: 51.4 (43.4%)
Sr-85	64.84 d	EC	Moderate	2	0.1	3.0	13.9	Gammas: 514 (99.2%) 15 (8.7%)
Rb-86	18.66 d	β	Moderate	0.5	0.1	0.5	31.3	Betas: 698 (8.8%) 1774 (94%) Gammas: 1076 (8.8%)
Sr-89	50.5 d	β	High	0.1	0	—	26.8	Betas: 1491 (100%)
Sr-90/Y-90	29.12 a	β	Very high	0.004	0.0001	—	—	Betas: 546 (100%) 2284 (100%)
Y-90	64.0 h	β	High	0.4	0.01	—	—	Betas: 2284 (100%)
Nb-95	35.15 d	β	Moderate	1	0.1	4.3	22.5	Betas: 160 (100%) Gammas: 766 (100%)

For footnotes see p. 132.

Radionuclide	Half-life	Decay mode	Internal toxicity class	Annual limit on intake (mCi)	Container posting level (mCi)	Γ[a] R/h@1cm per mCi	TVL[b] (mmPb)	Radiation types, keV (% per decay)
Mo-99	2.75 d	β	Moderate	1	0.1	1.8	20.5	Betas: 436 (17.3%) 1214 (82.7%) Gammas: 181 (6.2%) 740 (12.8%)
Tc-99m	6.02 h	IT	Low	80	1	0.6	0.9	Electrons: 119 (8.8%) 137 (1.1%) Gammas: 140 (89%)
Pd-103	16.96 d	EC	Low	6	0.1	1.48	0.02	X rays: 20.1 (28.7%) 20.2 (54.4%) 22.7 (16.9%)
Cd-109	464 d	EC	High	0.04	0.001	1.8	—	Electrons: 63 (42%) 84 (44%) 88 (10%) X rays: 22 (84%) 25 (18%)
Ag-110m	249.9 d	IT, β	High	0.09	0.01	—	—	Betas: 22 (67.3%) 531 (30.5%) Gammas: 658 (94.4%) 678 (10.7%) 687 (6.5%) 707 (16.7%) 764 (22.4%) 818 (7.3%) 885 (72.6%) 938 (34.3%) 1384 (24.3%) 1505 (13.1%)
In-111	2.83 d	EC	Moderate	4	0.1	3.4	2.2	Electrons: 145 (8.4%) 219 (4.9%) Gammas: 171 (90.2%) 245 (94%) X rays: 23 (68%) 26 (15%)

For footnotes see p. 132.

Radionuclide	Half-life	Decay mode	Internal toxicity class	Annual limit on intake (mCi)	Container posting level (mCi)	Γ[a] R/h@1cm per mCi	TVL[b] (mmPb)	Radiation types, keV (% per decay)
Sn-113	115.1 d	IT	Moderate	0.5	0.1	1.7	0.05	Electrons: 20 (13%) X rays: 24 (60%) 27 (13%)
Cd-115m	44.6 d	β	High	0.05	0.01	0.2	30.1	Betas: 616 (98%) 1621 (98%)
I-123	13.2 h	EC	Moderate	3	0.1	1.3	1	Electrons: 127 (13.6%) Gammas: 159 (83%) X rays: 27 (70.6%) 31 (16%)
I-125	60.14 d	EC	High	0.04	0.001	0.7	0.06	Electrons: 23 (19.7%) 31 (12.3%) Gammas: 35 (6.5%) X rays: 27 (112%) 31 (25.4%)
I-129	1.6×10^7 a	β	High	0.005	0.001	0.6	0.08	Betas: 152 (100%) Electrons: 34 (11%) Gammas: 40 (7.5%) X rays: 30 (57%) 34 (13%)
I-131	8.04 d	β	High	0.03	0.001	2.1	9.6	Betas: 334 (7.4%) 606 (89.3%) Gammas: 284 (6.2%) 364 (81.2%) 637 (7.3%)
Ba-133	10.74 a	EC	Moderate	0.7	0.1	2.4	5.8	Electrons: 45 (48%) 75 (7.4%) Gammas: 81 (33%) 276 (6.9%) 303 (17.8%) 356 (60%) 383 (8.7%) X rays: 31 (97%) 35 (22.8%)

For footnotes see p. 132.

Radionuclide	Half-life	Decay mode	Internal toxicity class	Annual limit on intake (mCi)	Container posting level (mCi)	Γ[a] R/h@1cm per mCi	TVL[b] (mmPb)	Radiation types, keV (% per decay)
Xe-133	5.25 d	β	—	—	1	0.1	0.4	Betas: 346 (99.3%) Electrons: 45 (53.3%) Gammas: 81 (36.5%) X-rays: 31 (38.9%)
Cs-137	30.0 a	β	High	0.1	0.01	3.5	18.9	Betas: 512 (94.6%) 1173 (5.4%) Electrons: 624 (8.1%) Gammas: 662 (90%)
Ce-141	32.5 d	β	Moderate	0.7	0.1	0.4	0.9	Betas: 435 (71%) 580 (29.5%) Electrons: 103 (18.8%) Gammas: 145 (48.4%) X rays: 36 (13.8%)
Eu-150	34.2 a	EC	High	0.02	0.001	—	—	Electrons: 5 (45.9%) 5 (45.9%) 6 (27.1%) 1 (150%) Gammas: 334 (94%) 584 (51.5%) 737 (9.4%) 748 (5.1%) 1049 (5.2%) X rays: 40 (65.4%) 45 (8.3%)
Eu-152	13.33 a	β, EC	High	0.02	0.001	—	—	Betas: 696 (13.6%) 1475 (8.4%) Electrons: 5 (73.4%) 33 (5.7%) 75 (19.5%) 114 (10.6%)

For footnotes see p. 132.

Radionuclide	Half-life	Decay mode	Internal toxicity class	Annual limit on intake (mCi)	Container posting level (mCi)	Γ[a] R/h@1cm per mCi	TVL[b] (mmPb)	Radiation types, keV (% per decay)
Gd-153	242 d	EC	High	0.1	0.01	0.8	0.2	Electrons: 55 (32.2%) 49 (8.1%) 95 (5.1%) Gammas: 70 (2.6%) 97 (32%) 103 (22.2%) X rays: 41 (100.5%) 47 (25.3%)
Eu-154	8.8 a	β, EC	High	0.02	0.001	6.3	29.1	Betas: 247 (27.9%) 569 (36.5%) 839 (17.4%) 1844 (11.4%) Gammas: 723 (19.7%) 873 (11.5%) 1005 (17.9%) 127 (35.5%)
Yb-169	32.01 d	EC	Moderate	0.7	0.1	1.8	1.6	Electrons: 50 (34.9%) 100 (5.6%) 118 (10.3%) 120 (51.6%) 139 (12.4%) Gammas: 63 (42%) 110 (17%) 131 (12%) 177 (22%) 197 (36%) 307 (10%) X rays: 50 (147%) 58 (39%)
Re-186	3.78 d	β	Moderate	2	0.1	0.2	0.8	Betas: 1070 (94%) 1076 (71%) Gammas: 137 (9.5%)
Re-188	16.98 h	β	Moderate	2	0.1	0.3	16.8	Betas: 2120 (71.4%) Gammas: 155 (15%)

For footnotes see p. 132.

Radionuclide	Half-life	Decay mode	Internal toxicity class	Annual limit on intake (mCi)	Container posting level (mCi)	Γ[a] R/h@1cm per mCi	TVL[b] (mmPb)	Radiation types, keV (% per decay)
Ir-192	74.02 d	β, EC	High	0.2	0.001	4.8	20	Betas: 536 (41.4%) 672 (48.3%) Gammas: 296 (29%) 308 (29.7%) 317 (82.8%) 468 (48%) 604 (8.2%) 612 (5.3%)
Au-198	2.7 d	β	Moderate	1	0.1	2.4	10.1	Betas: 961 (98.6%) Gammas: 412 (95.5%)
Tl-201	3.04 d	EC	Low	20	1	0.4	0.9	Electrons: 84 (15.4%) Gammas: 167 (10%) X rays: 69 (27.4%) 71 (46.5%) 80 (20.5%)
Hg-203	46.6 d	β	Moderate	0.5	0.1	1.3	4.7	Betas: 212 (100%) Electrons: 194 (16.9%) 264 (4.4%) Gammas: 279 (77.3%) X rays: 71 (4.7%) 73 (8.0%)
Bi-206	6.24 d	EC	Moderate	0.6	0.1	17.2	26	Electrons: 96 (22.2%) 256 (5.6%) Gammas: 516 (40%) 803 (98.9%) 881 (66.2%) 1719 (32%)
Bi-207	38 a	EC	High	0.4	0.01	8.3	25.8	Electrons: 976 (7.0%) Gammas: 570 (97.7%) 1064 (75%) 1770 (6.8%)

For footnotes see p. 132.

Radionuclide	Half-life	Decay mode	Internal toxicity class	Annual limit on intake (mCi)	Container posting level (mCi)	Γ[a] R/h@1cm per mCi	TVL[b] (mmPb)	Radiation types, keV (% per decay)
Po-208	2.93 a	α	High	0.014	0.000001	—	—	Alphas: 5110 (100%)
Pb-210	22.3 a	β	Very high	0.0002	0.00001	0.0	0.2	Betas: 17 (80.2%) 63 (19.8%) Electrons: 8 (33.6%) 30 (57.9%) 43 (18.1%) Gammas: 11 (24%)
Po-210	138.38 d	α	Very high	0.0006	0.0001	—	—	Alphas: 5305 (100%)
Rn-222	3.82 d	α	High	0.1	0.001	—	—	Alphas: 5490 (99.9%)
Ra-226	1600 a	α	Very high	0.0006	0.0001	—	—	Alphas: 4602 (5.6%) 4785 (94.6%)
Th-228	1.91 a	α	Very high	0.00001	0.000001	—	—	Alphas: 5341 (26.7%) 5423 (72.7%) Electrons: 9 (9.6%) 65 (19.1%) 80 (5.2%) X rays: 12 (9.6%)
Pu-238	87.74 a	α, SF	Very high	0.000007	0.000001	—	—	Alphas: 5457 (28.3%) 5499 (71.6%) Electrons: 10 (9.1%) 22 (20.7%) 38 (7.6%) X rays: 14 (11.6%)
U-238	4.5×10^9 a	α, SF	Very high	0.00004	0.1	—	—	Alphas: 4147 (23%) 4196 (77%) Electrons: 10 (8.2%) 29 (16.8%) 44 (6.1%) X rays: 13 (9%)

For footnotes see p. 132.

Radionuclide	Half-life	Decay mode	Internal toxicity class	Annual limit on intake (mCi)	Container posting level (mCi)	Γ[a] R/h@1cm per mCi	TVL[b] (mmPb)	Radiation types, keV (% per decay)
Pu-239	24 065 a	α	Very high	0.000006	0.000001	—	—	Alphas: 5105 (11.5%) 5143 (15.1%) 5155 (73.3%) Electrons: 7 (19%)
Am-241	432.2 a	α	Very high	0.000006	0.000001	0.1	0.4	Alphas: 5443 (12.8%) 5486 (85.2%) Gammas: 60 (35.9%)
Cm-244	18.11 a	α, SF	Very high	0.00001	0.000001	—	—	Alphas: 5763 (23.6%) 5805 (76.4%) Electrons: 10 (6.9%) 20 (17.2%) 37 (6.3%) X rays: 14 (10.3%)
Cf-250	13.08 a	α	Very high	0.000009	0.000001	—	—	Alphas: 5989 (16.2%) 6031 (83.4%) Electrons: 18 (12%) X rays: 15 (7.8%)
Cf-252	2.638 a	α, SF	Very high	0.00002	0.000001	—	—	Alphas: 6 076 (15.2%) 6118 (81.6%) Electrons: 19 (11.2%) X rays: 15 (7.3%)

Note: α = alpha decay; β = beta decay; β+ = positron decay; EC = electron capture; IT = isomeric transition (gamma) decay; SF = spontaneous fission.

[a] Γ = specific gamma ray constant.

[b] TVL = tenth value layer.

Annex V

EXAMPLE OF A CASE REPORT FORM

^{99m}Tc-PEPTIDE FOR IMAGING

Number: _____ Date of recruitment: _____

Name and surname: _____

Date of birth: _____/_____/_____ Tel./Fax/Email : _____

Sex: M / F

SHORT HISTORY OF THE PATIENT

Diagnosis: _____

Date of diagnosis: _____/_____/_____

Treatments:

Medical: _____

Surgical: _____

Other therapies: _____

Course of disease:

Complications:

PRESENT HISTORY

Disease activity: ☐ Active ☐ Non-active

Current therapy: _____

Describe here the sites of lesions, if inflammation is present, other signs and symptoms:

_____

_____

SCINTIGRAPHIC STUDY

Date: _____/_____/_____

Radiopharmaceutical: _____ Amount: ____ (mCi/MBq) _____ (mg/µg/nMoles)

Time: _____:_____

Injection site: _____

Injection method: _____

1st Scan: at time _____:_____

Acquisition procedure:

Site: _____

Modality: _____

Acquisition time: _____

Matrix: _____

2nd Scan: at time _____:_____

Acquisition procedure:

Site: _____

Modality: _____

Acquisition time: _____

Matrix: _____

3rd Scan: at time _____:_____

Acquisition procedure:

Site: _____

Modality: _____

Acquisition time: _____

Matrix: _____

CLINICAL EVALUATION AND FOLLOW-UP DATA

LABORATORY TESTS

Type: _____ Date: _____ Result: _____ Normal Values: _____

Type: _____ Date: _____ Result: _____ Normal Values: _____

Type: _____ Date: _____ Result: _____ Normal Values: _____

Type: _____ Date: _____ Result: _____ Normal Values: _____

Type: _____ Date: _____ Result: _____ Normal Values: _____

Type: _____ Date: _____ Result: _____ Normal Values: _____

Type: _____ Date: _____ Result: _____ Normal Values: _____

Type: _____ Date: _____ Result: _____ Normal Values: _____

Type: _____ Date: _____ Result: _____ Normal Values: _____

OTHER DIAGNOSTIC TESTS

CT scan Date:_____/_____/_____

Result: _____

Ultrasound Date:_____/_____/_____

Result: _____

NMR Date:_____/_____/_____

Result: _____

Biopsy Date:_____/_____/_____

Result: _____

Other Date:_____/_____/_____

Result: _____

EVALUATION OF SCINTIGRAPHIC RESULTS
QUALITATIVE ANALYSIS

EVALUATION OF SCINTIGRAPHIC RESULTS
QUANTITATIVE ANALYSIS

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