Strategies for Clinical Implementation and Quality Management of PET Tracers

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STRATEGIES FOR CLINICAL IMPLEMENTATION AND QUALITY MANAGEMENT OF PET TRACERS

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FOREWORD

Positron emission tomography (PET) methodologies, which visualize in vivo biochemical, physiological and pharmacological processes, together with the availability of hybrid imaging modalities (PET with computer tomography (PET/CT) imaging), have revolutionized patient diagnosis, disease staging, disease management and therapy follow-up. These technologies have opened up fascinating possibilities for new non-invasive medical care and individualized patient management unlike those seen before in a clinical setting. There is immense global interest in PET, with accelerated investment in the clinical setting. The radiotracers used for PET are very different from conventional nuclear medicine radiopharmaceuticals. They have extremely short half-lives, which means that they have to be produced close to the clinical user. The time available for the labelling of PET molecules (including purification and quality control) is very limited, which presents new challenges and a need for more proficient systems to be established before clinical use.

The busy and demanding nature of clinical settings adds to the complexities and creates a need for more robust quality management programmes. This publication focuses on clinical settings and was compiled following several IAEA Consultants Meetings and with the benefit of the contributions of individual experts. It aims to raise awareness of the issues involved and suggests ways of reducing risk. The purpose of these guidelines is to encourage a proactive approach to each aspect of parametric release and to propose practical test methods for each criterion for parametric acceptance, thereby helping end users to ensure the quality of PET products. It is hoped that these criteria will stimulate further cooperation among various countries worldwide in the development of a set of harmonized acceptance test criteria for PET systems and sensible quality assurance (QA) standards for all PET tracers.

Many countries and IAEA Member States are in the process of creating rules governing the production of clinically safe and effective PET radiopharmaceuticals for human use. Where such rules are available, it is encouraged that they be used. The guidelines presented in this book will be useful for those Member States who have yet to develop such rules or who simply wish to adopt them for national usage. They are not meant to replace existing regulatory or local oversight. This publication should be a useful resource for both researchers and practitioners. Continuous reliable development and quality supply of radiopharmaceuticals for patients worldwide is vital for sustained development of nuclear medicine.

The IAEA is grateful to all the contributors and reviewers of these guidelines. The IAEA officer responsible for this publication was K.K. Solanki of the Division of Human Health.

EDITORIAL NOTE

CONTENTS

1. INTRODUCTION

1.1. SCOPE

This section provides a brief background and details of essential physical properties of positron emission tomography (PET) isotopes. It discusses differences between traditional radiopharmaceuticals and PET radiopharmaceuticals, elements in the production to release of PET radiopharmaceuticals and a summary of a new class of PET tracers.

1.2. BACKGROUND

There is immense global interest in PET, with accelerated investment in clinical settings. The PET methodologies, which visualize in vivo biochemical, physiological and pharmacological processes, together with the availability of hybrid imaging modalities (PET with computer tomography (PET/CT) imaging), have revolutionized patient diagnosis, disease staging, management and therapy follow-up. These technologies have opened up fascinating possibilities for new non-invasive medical care and individualized patient management, unlike those seen before in clinical settings. The clinical utility of PET imaging is very wide and includes oncology, cardiac imaging, neuroimaging, infection imaging and numerous other areas.

The radiotracers used for PET are very different from conventional nuclear medicine radiopharmaceuticals. Although numerous PET radioisotopes exist, the most common radioactive isotopes in PET radiopharmaceuticals include ^{11}C, ^{18}F, ^{13}N and ^{15}O. These radioisotopes have very short half-lives, so it is desirable that these isotopes are produced close to the clinical facilities where they are used.

In general, radiopharmaceuticals are unique medicinal formulations containing radioisotopes that are used in major clinical areas. Because of multiple safety issues, the facilities and procedures for the production, use and storage of radiopharmaceuticals are generally subject to licensing by national and/or regional authorities. Additional regulations may apply to matters such as transportation or dispensing of radiopharmaceuticals. Each producer or user must be thoroughly cognizant of the national requirements pertaining to the articles concerned.

As with other nuclear medicine radiopharmaceuticals, PET radiopharmaceuticals create challenges in terms of their handling and production. Because of the inherent physical properties of PET isotopes, these

radiopharmaceuticals pose added radiation safety risks to the personnel who prepare and administer these drugs and to the patients to whom they are administered. Specialized techniques are required to minimize these risks to personnel. All personnel involved in any part of the operation are required to have appropriate training pertinent to their specific area. Maintenance personnel and support staff such as cleaners should also receive specific instruction and appropriate supervision while they are in the facilities. In addition, safeguards need to be implemented to minimize the risk to patients. It is essential to ensure that reproducible and clinically reliable results can be obtained. All operations should be carried out or supervised by personnel who have received expert training in handling radioactive materials.

In addition to radiation licences, facilities may be required to register as a pharmaceutical manufacturer or a pharmacy to meet compliance with national regulations related to preparation of medicines. More specifically, facilities should meet the requirements for preparation of sterile or aseptically produced pharmaceuticals. This would require the facilities to be appropriately registered and have authorized staffing. For the latter, if the facilities are registered as a pharmacy they will require oversight by a nationally registered pharmacist. If the facilities are registered as a pharmaceutical manufacturer, then a nationally registered radiopharmacist or a 'qualified' person will be required. To comply with the radiation licence requirements, appropriate radiation protection measures would be required as well as a responsible medical physicist. In addition, the IAEA has numerous publications on safe radioisotope handling, waste and transportation [1–4].

1.3. PET RADIOPHARMACEUTICALS

The use of 2-^{18}F-2-fluoro-deoxy-D-glucose (^{18}F-FDG (see Annex)), in conjunction with PET imaging, has found wide acceptance in oncology, cardiology and neurology. Called the 'molecule of the millennium', ^{18}F-FDG has evolved from being a research radiopharmaceutical in the early 1970s to the forefront of nuclear medicine imaging in the 1990s, supported by concurrent developments in PET imaging hardware and software. The relevance of ^{18}F-FDG in oncology is reinforced by the large number of requests for PET scans. Since the initial introduction of ^{18}F-FDG in the diagnosis and management of cancer at a limited number of hospitals fifteen years ago, hundreds of cancer units in hospitals have installed, or are in the process of installing, dedicated PET scanners for such use. These facilities procure ^{18}F-FDG from commercial sources or, in some cases, from in-house medical cyclotron facilities.

Advances in PET scanners for fast data acquisition and rapid data/image analysis, have resulted in considerable improvements in the resolution and cost effectiveness of PET imaging. In addition, the anatomical co-registration with CT, available in PET/CT machines, means that PET imaging with ^{18}F-FDG is capable of accurately localizing malignancies and metastases and of identifying unknown primaries. Furthermore, the remarkable resolution of PET has stimulated research in developing other PET radiopharmaceuticals labelled with ^{18}F, and other positron emitting radioisotopes, namely, ^{11}C, ^{13}N and ^{15}O, which are readily produced from compact medical cyclotrons capable of producing 11–16 MeV proton beams.

Fluorine-18 has a longer half-life ($T_{1/2}$ = 109.8 min) than ^{11}C ($T_{1/2}$ = 20.4 min), ^{13}N ($T_{1/2}$ = 9.98 min) and ^{15}O ($T_{1/2}$ = 2.03 min) and is therefore more economical to produce and use as a tracer, because ^{18}F labelled radiopharmaceuticals can be used in more patients and, depending on the infrastructure available, can also be transported to other hospitals within a distance of 200 km. Fluorine-18 can be used to replace the –OH group in many compounds, resulting in production of an effective PET radiopharmaceutical. Carbon-11 has also been used to label a large number of useful compounds, as it can replace the stable ^{12}C isotope in them; however, its short half-life precludes its economic use, but attempts to produce ^{18}F labelled alternatives are under way. Nitrogen-13 has been used for very few compounds, but there have been several reports of its effective use as $^{13}NH_3$ for myocardial blood flow studies. The very short lived ^{15}O radioisotope has to be piped directly from the cyclotron to the PET scanner room and is mainly used as $C^{15}O$ or as $H_2{}^{15}O$ for perfusion studies. The routine positron emitters being investigated are listed in Table 1.

TABLE 1. PROPERTIES OF COMMON PET ISOTOPES AND THEIR METHODS OF PRODUCTION

Nuclide	Physical half-life, $T_{1/2}$ (min)	Percentage abundance, mode of decay	Max. energy (keV)	Maximum range (mm)	Common production method	Proton beam energy (MeV)
C-11	20.4	99.8, β	960	4.1	^{14}N(p, α)^{11}C	13→3
	9.98	0.2, EC				
N-13	9.98	100, β	1190	5.4	^{16}O(p, α)^{13}N	16→7
O-15	2.03	100, β	1700	8.0	^{15}N(p, n)^{15}O	10→0
F-18	109.8	97, β	690	2.4	^{18}O(p, n)^{18}F	16→3
		3, EC				

Production of PET radiopharmaceuticals requires a considerably different infrastructure from that for conventional radiopharmacy. The large difference in the requirements for the facilities and equipment of a ^{99m}Tc radiopharmacy and a ^{18}F radiopharmacy is due to the differences in the physical and chemical properties of the radioisotopes handled, and also the chemistry involved in preparing the tracers. Other positron emitters currently being investigated that may become important in the future are listed in Table 2.

TABLE 2. PROPERTIES OF OTHER PROMISING PET ISOTOPES AND THEIR METHODS OF PRODUCTION

Nuclide	Physical half-life, $T_{1/2}$	Mode of decay (%)	X ray energy (keV)	Percentage abundance	Common production method	Proton beam energy (MeV)
I-124	4.2 d	β^+ (23) EC (77)	511 603 1691	46 61 10.4	^{124}Te(p, n)^{124}I	18
Tc-94m	52 min	β^+ (70) EC (30)	511 871 1521 1868	140 94 4.5 5.7	^{94}Mo(p, n)^{94m}Tc	11–12
Ga-68	68 min (generator)	β^+ (89) EC (11)	511	178	^{68}Ge–^{68}Ga ^{68}Zn(p, n)^{68}Ga	40
Rb-82	75 s (generator)	β^+ (95) EC (5)	511 777	190 13.4	^{98}Mo ↓spallation ^{82}Sr($T_{1/2}$ = 25.6 d) → ^{82}Rb	40
Cu-64	12.7 h	β^+ (19.3) EC (41) β (39.63)	511 1350	38.6 0.6	^{64}Ni(p, n)^{64}Cu	12

1.4. DIFFERENCES BETWEEN ^{99m}Tc AND PET RADIOPHARMACY

Unlike ^{99m}Tc, typical nuclear medicine radionuclides are produced differently and have very different properties, both physical and nuclidic; leading to different handling and monitoring requirements. Table 3 summarizes the major differences between ^{99m}Tc labelled radiopharmaceuticals and PET radiopharmaceuticals.

TABLE 3. IMPORTANT DIFFERENCES BETWEEN Tc-99m AND PET RADIOPHARMACY

Tc-99m radiopharmacy	PET radiopharmacy
$T_{1/2}$ = 6 h	$T_{1/2}$ = 110 min for F-18, 20 min for C-11, 10 min for N-13 and 2 min for O-15
Sterile $^{99m}TcO_4$ produced on-site from a generator	Isotope produced on-site from a cyclotron
Tc-99m radiopharmaceuticals are complexes of Tc-99m and a chelating agent attached to biologically active molecules with the desired properties, and easy to prepare, with no chemical synthesis.	PET radiopharmaceuticals are biologically active molecules that are synthesized with the PET isotope attached covalently at a specific site within the molecule.
'Cold' sterile reagent and Tc-99m available in separate sterile vials — preparation requires addition of sterile $^{99m}TcO_4^-$ with a 'cold kit' and good mixing (no further processing or minimal without opening the vial)	Rapid chemical synthesis of the PET radiopharmaceutical is necessary using special precursors (to reduce synthesis time) for F-18 and C-11 labelled radiopharmaceuticals. Only simple molecules possible with N-13 and O-15
Quality control (QC) of radiopharmaceutical is minimal since the cold kit used for making the Tc-99m radiopharmaceutical is certified to be sterile and free of endotoxins.	Quality control is more elaborate, since the PET radiopharmaceutical is synthesized every day.
'Parametric release' permitted if quality assurance (QA) is validated	'Parametric release' permitted if QA is validated

TABLE 3. IMPORTANT DIFFERENCES BETWEEN Tc-99m AND PET RADIOPHARMACY (cont.)

Tc-99m radiopharmacy	PET radiopharmacy
The radiopharmaceutical prepared can be used throughout the day — for at least eight hours.	Because of the short physical half-life of its isotope, ^{18}F-RP has to be used within a few hours, perhaps requiring multiple syntheses on the same day. In the case with ^{11}C radiopharmaceutical, each synthesis could cover one or two patients.
γ = 140 keV, 20 mm lead shielding is adequate.	β^+ emitters, $\gamma = 2 \times 511$ keV; 70 mm lead shielding required
Multipatient dose in a single vial possible. Up to 12.95 GBq (350 mCi) of Tc-99m	Preferred as maximum of two to three doses in a vial. Doses preferred in syringes, ready to inject to minimize handling
Should be sterile	Should be sterile
Endotoxin levels below 175/V[a] (IU)[b]	Endotoxin levels below 175/V (IU)

[a] V: Maximum recommended dose.
[b] IU: International units.

The time available for the labelling of PET molecules (including purification and quality control (QC)) is limited, which raises new challenges and a need for more efficient systems to be implemented before clinical use. The busy and demanding nature of clinical settings compounds these complexities. Establishing criteria for ensuring better acceptance practices for PET systems and parametric acceptance of PET tracers for clinical applications is therefore essential.

This class of radiopharmaceuticals includes isotopes with relatively short half-lives (2–110 min), and therefore makes it difficult to follow rules prescribed for therapeutic radiopharmaceuticals or even those prescribed for diagnostic radiopharmaceuticals with longer half-lives.

PET radiopharmaceuticals, as with all human parenteral drugs, must be produced in a work environment designed for production of sterile injectable products. In addition, these radiopharmaceuticals should pass the local governing pharmacopoeial tests or equivalent specifications on various properties including: (a) chemical purity, (b) pH, (c) isotonicity, (d) sterility, (e) apyrogenicity and (f) toxicity, as well as on (g) radionuclidic purity and (h) radiochemical purity, prior to administration to humans.

The complexities involved in establishing a radiopharmaceutical preparation facility arise from the cooperation required from multiple regulatory and governing agencies to ensure safety. Unlike typical pharmaceutical production facilities, the risk from radiopharmaceuticals results mainly from their inherent radiations. Therefore, in addition to microbial contamination risks, radiopharmaceutical facilities should be compliant with local radiation safety guidelines.

In addition to pharmacopoeial regulations, PET radiopharmaceuticals, owing to their radioactive nature, also have to comply with local radiation safety regulations. There are separate implementation authorities for these two sets of regulations and, hence, a PET radiopharmaceutical production facility requires clearances from both regulators. Fulfilling both regulations is a prerequisite for a PET radiopharmaceutical production facility, and has been the topic of much debate. A prominent concern is adequate shielding of the cyclotron, because there are very high ambient gamma and neutron radiations during irradiation of the target. Adequate shielding is required for the transport of the radioactivity produced from the cyclotron to the radiochemistry hot cells where the synthesis of the PET radiopharmaceutical takes place, as well as a shielded area for doing the QC tests, packaging and dispatch of the PET radiopharmaceutical. Adequate monitoring for radioactive spillage/contamination and the need for radiation safety personnel to make regular checks are also necessary. Minimum specifications for radiation safety while handling radioactive isotopes is mandatory by law, and permission to handle radioactivity can only be granted after the prescribed specifications have been met.

All radiopharmaceuticals have a limited shelf life, although this can vary from minutes to a few weeks. For PET radiopharmaceuticals, the maximum shelf life is a few hours for ^{18}F products and minutes for ^{13}N and ^{11}C products. Hence, they have typically been prepared in-house or 'compounded' by the nuclear medicine department of a hospital, and QC and use were supervised by the radiopharmacist in charge of compounding. However, different radiopharmacists have adopted different procedures and protocols to compound the same PET radiopharmaceutical. For example, ^{18}F-FDG can be compounded using either nucleophilic or electrophilic substitution reactions, using different precursors and different synthesis routes. As a result, yields and specific activities of the final product vary considerably. The ^{18}F-FDG produced via any of the synthesis routes would fulfil radiochemical and pharmaceutical purity requirements; however, there were various impurities (unreacted precursors, unhydrolysed ^{18}F precursors, residual phase transfer catalysts, solvents, etc.), which were present to varying extents. Production of PET radiopharmaceuticals cannot be controlled in the same way as ^{99m}Tc radiopharmaceuticals,

which are mainly produced by simply mixing the sterile $^{99m}TcO_4^-$ solution with the sterile complexing agent supplied in a vial. There has been no consensus amongst PET radiochemists and radiopharmacists regarding what should constitute a common acceptable good radiopharmacy practice for ^{18}F-FDG, let alone PET radiopharmaceuticals in general.

There is limited time available immediately after production and prior to their administration for tests to be performed. Criteria for 'parametric release' have therefore been established in the form of a carefully devised standard operating procedure (SOP), whereby many production runs of the radiopharmaceutical are devoted exclusively to the QC tests listed above. The SOP is said to be validated, to a high degree of confidence, when a specified number of production runs comply with all the QC tests, without any failures (at least three clear runs). This 'high degree of confidence' protocol allows the PET radiopharmacy laboratory to release a PET radiopharmaceutical product suitable for patients, having carried out essential QC tests and doing the remaining tests 'post-decay', i.e. after several hours or even days (collectively testing samples from several production runs), by which time all the radioactivity has decayed completely. These are sometimes referred to as 'after the fact' tests.

1.5. IAEA OPERATIONAL GUIDANCE ON HOSPITAL RADIOPHARMACY

While there are numerous similarities between traditional nuclear medicine radiopharmaceuticals and PET radiopharmaceuticals, PET radionuclides require additional precautions and care. This section provides assistance for understanding the differences and additional requirements for PET radiopharmaceuticals. The radiopharmaceuticals used in most clinical nuclear medicine imaging sites can be broadly categorized as being in one of three groups [5]:

(1) Group 1 — Ready to use radiopharmaceuticals purchased from a vendor:
    (a) Tc-99m and related radiopharmaceuticals;
    (b) Radiopharmaceuticals containing radioiodine.
(2) Group 2 — Kits and generator produced radiopharmaceuticals:
    (a) Tc-99m kits and generators;
    (b) Red blood cell and white blood cell labelling kits.

(3) Group 3 — In-house prepared radiopharmaceuticals:
   (a) Kits not available for ^{99m}Tc or other nuclear medicine imaging products;
   (b) Labelled monoclonal antibodies;
   (c) PET radiopharmaceuticals.

1.5.1. Staff and training

Staff should be trained in all aspects related to compounding, dispensing and use of positron emitting radionuclides, internal doses and their generators in clinical settings. Competency in PET radiochemistry is essential.

In many nuclear medicine facilities, it may be customary to operate Group 1 and 2 radiopharmaceutical facilities as a component of medical practice and, therefore, under the supervision and control of a nuclear medicine physician. Operation of a Group 3 radiopharmaceutical facility would, however, require special handling before the radiopharmaceuticals can be released for patient use. Radiopharmaceuticals from this group need to be prepared and certified by a qualified individual, nationally registered, pharmacist or 'qualified person' for safety and quality in their intended use. This publication aims to include Group 3 radiopharmaceuticals for PET imaging, although the same principles could be applied for non-PET radiopharmaceuticals.

1.5.2. Facilities

Production of PET radiopharmaceuticals requires an infrastructure considerably different from that needed for conventional radiopharmacy. While large differences in the requirements for the facilities and equipment of a ^{99m}Tc radiopharmacy and a ^{18}F radiopharmacy were mentioned earlier, differences in the physical and chemical properties of the radioisotopes leading to differences in the chemistries involved in preparing the PET tracers remain the primary reason.

Hot cells (Fig. 1) and complete chemistry systems are essential for PET. Ideally, the synthesis boxes and hot cells should be placed in an EU Grade C (ISO 7) environment next to an EU Grade A (ISO 5) laminar airflow (LAF) workbench used for dispensing. The whole system should be located in a room that meets EU Grade D level of cleanliness.

FIG. 1. A typical PET hot cell.

Long lived generators should be housed in a separate Type II LAF/ isolator with an operational EU Grade A (ISO 5) environment or isolator technology, and with a background in the room at a standard higher than EU Grade D (ISO 8).

Suitable shielding, according to the energy and type of emission of the radionuclide, should be taken into account, and the cabinet performance should be validated.

While acquisition of a cyclotron and synthesis modules is the key component in establishing a radiopharmaceutical production facility, other factors such as production facility qualifications and staff training are equally important. Installation of a cyclotron and synthesis modules are outside the scope of this publication.

1.5.3. Operations

A full quality assurance (QA) programme should be in place when preparing PET or therapeutic radiopharmaceuticals for administration to humans. Due care is essential to prevent any cross-contamination.

1.5.4. Record keeping

Control details and records of each batch prepared are paramount. Careful monitoring of yields and full accountability for each operation should be undertaken after each batch run, including in-process checking. Any deviation and changes must be taken into account, and should be an integral part of the official batch release.

1.5.5. Quality control

The very short half-lives of many PET radionuclides have additional implications for quality systems. Rapid quality control checks of radiochemical purities can be made using a high pressure liquid chromatography (HPLC) system whilst in transit, but before release of products for clinical use. Better communication links and follow-up are required. Pre-validation of the system and the whole operation is essential to ensure consistency. Any changes or deviations with respect to starting materials, equipment or processes must be validated before the initial production run for clinical use.

Sterility, bacterial endotoxin and physicochemical purity tests should be routinely performed, and there should be close monitoring for all PET radiopharmaceuticals, as well as for long lived generators. If tests cannot be prepared before release, they should be done retrospectively.

Ongoing microbiological assessment is essential.

1.6. OVERVIEW OF QUALITY PROGRAMME

The QA and quality management requirements (i.e. test procedure, acceptance criteria and testing schedule) for some PET tracers are quite different, mainly due to the very short half-lives of the products. Figure 2 provides an overview of a typical QC programme. Good validation and systems controls are essential to ensure consistent resultant PET tracers and to reduce the risk of product failure. Semi-automation and automation in general are essential not only for safer handling of high energy PET radioisotopes but also for improving final product quality. Despite this, there are no set guidelines for acceptance of automated systems. These are essential starting blocks for PET in a clinical setting.

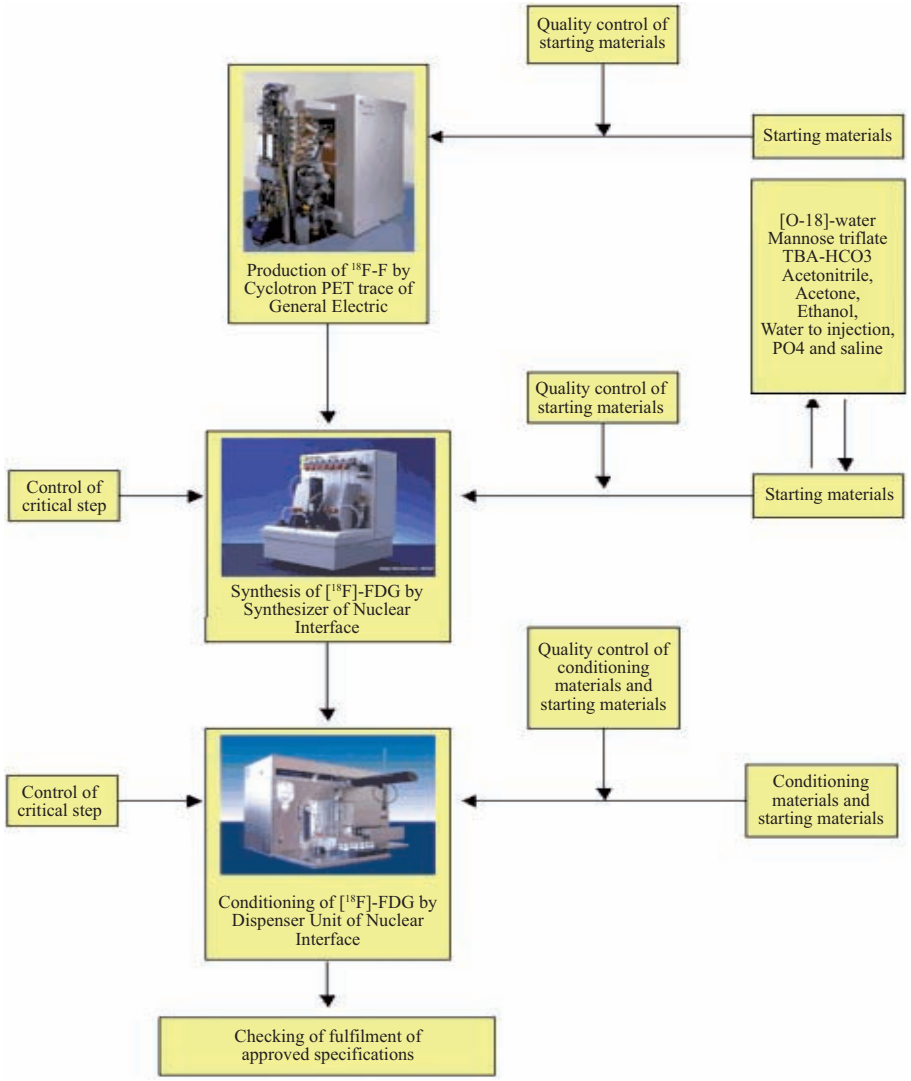


FIG. 2. Overview of a typical QC programme.

The QC requirements for some PET tracers, for example, injection of fludeoxyglucose ^{18}F (^{18}F-FDG: see Annex) are listed in major pharmacopoeias, the WHO International Pharmacopoeia (Ph. Int.), the United States Pharmacopeia (USP) and the European Pharmacopoeia (EP), as well as in the chemistry, manufacturing and controls (CMC) instructions issued by some

regulators. Furthermore, there were key differences between the pharmacopoeial requirements. All PET products administered to humans must, in due course, meet appropriate pharmacopoeial requirements and/or conform to drug approval procedures (including validation and acceptance systems testing, any issued CMC or QA specifications, and continuous performance requirements).

Sterility is important as most PET tracers are administrated intravenously. Pharmacopoeial sterility tests normally require at least two weeks. More robust quality systems are therefore essential to ensure aseptic practices and microbiological risk reduction. The associated validation systems were also deemed to be important.

These guidelines are designed to encourage proactive discussion on the advantages and disadvantages of each aspect of parametric release and to additionally propose practical test methods for each criterion for parametric acceptance, thereby helping end users to ensure the quality of PET products. It is hoped that these criteria will encourage further cooperation among various countries worldwide in the development of a set of harmonized acceptance test criteria for PET systems and sensible QA standards for all PET drug products. The guide should be a useful resource for both researchers and practitioners alike. Continuous and reliable development, together with a good supply of radiopharmaceuticals for patients worldwide, is vital for sustained development of nuclear medicine. Figure 3 provides an overview of a QC programme applied to PET tracers.

1.7. CRITERIA FOR SYNTHESIS OF A NEW PET RADIOPHARMACEUTICAL

Ideally, a new PET radiopharmaceutical for clinical application should fulfil the following criteria for synthesis:

(a) Labelling yield — a high labelling yield including purification is mandatory.
(b) Labelling time — rapid labelling including purification and essential QC is mandatory.
(c) Cost — the precursor and reagents should be available either commercially or via in-house synthesis.
(d) Automation — automatic synthesis is mandatory for routine clinical application.

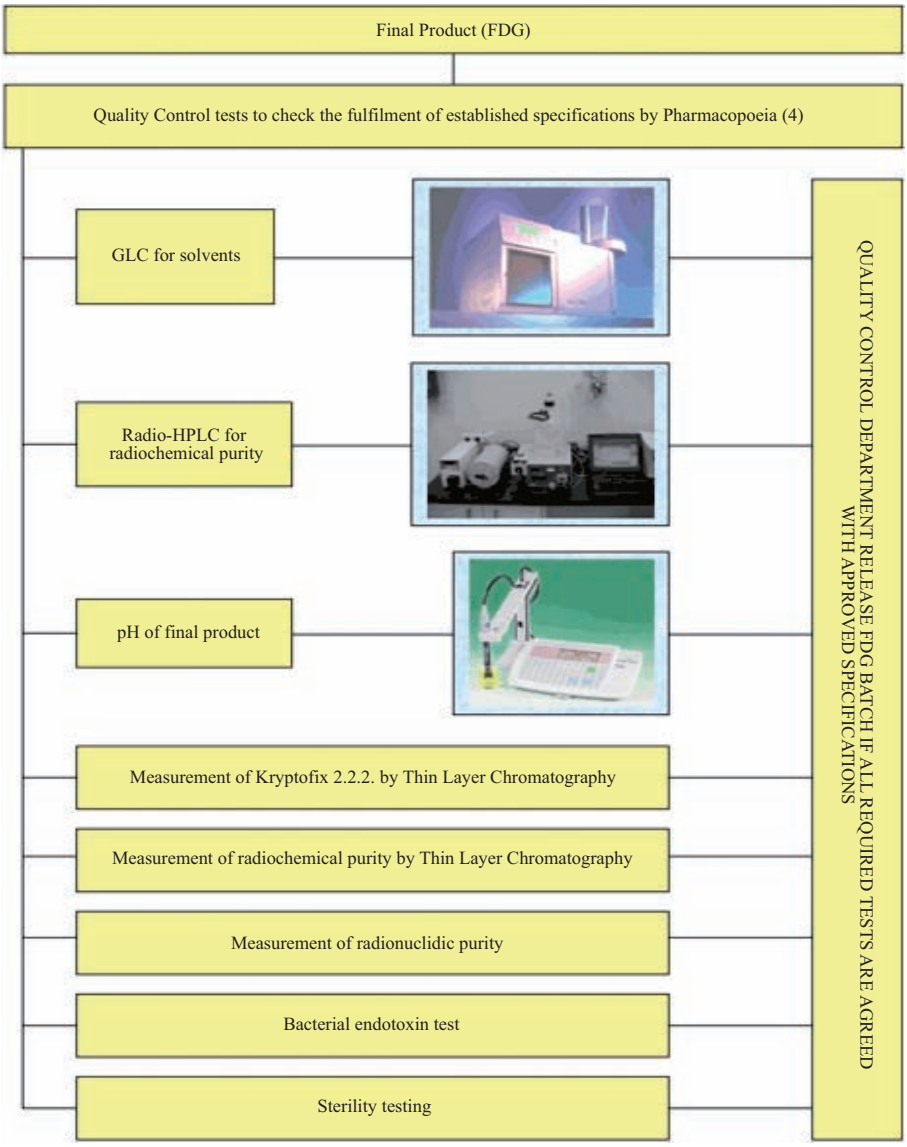


FIG. 3. Overview of a typical QC programme for PET tracers.

(e) Quality control should include the following:
- —A pH measurement;
- —A visual inspection;
- —A radiochemical purity test;
- —A radionuclidic purity test;

— A specific activity test;
— A test of the integrity of the sterile filter membrane;
— A bacterial endotoxin test (after release).

1.8. SUMMARY OF A NEW CLASS OF PET TRACERS

A more detailed description of many new PET tracers is provided in Section 9. Three broad categories of PET tracers are emerging: metabolic agents, receptor agents and peptides. The clinical usefulness of many of these new PET tracers is still under consideration.

1.8.1. Metabolic agents

For metabolic PET agents, the following factors are relevant:

(1) Clinical importance of the target metabolism — the target metabolism should represent a specific cell condition or pathological state.
(2) The agent should accumulate in special cells due to the metabolic procedure.
(3) Rapid clearance from blood and non-target tissues is mandatory.
(4) A sufficiently high specific activity is required, although the importance of this is lower than for other agents.
(5) For whole body PET, excretion through the kidneys is preferable to excretion through the hepatobiliary tract.

Imaging the metabolism of cells is the most common and efficient method currently in the clinical field (Table 4).

Among the metabolic PET agents, ^{18}F-FDG achieved the greatest success, which provided the major momentum for the promotion of PET. Another eminently successful metabolic PET agent in the clinical fields is ^{11}C-methionine.

Metabolic agents are taken up by the cells that use them as a fuel for energy metabolism or building blocks for biosynthesis. Thus, each PET agent should represent the target metabolism accurately.

In general, the capacity of cells to take up the metabolic PET agent is higher relative to other PET agents such as receptor binding agents. This is due to the continuous spend (consumption or utilization) of the metabolic PET agent. Thus, the specific activity of the agent is less important than those for receptor binding agents. However, the specific activity of metabolic agents such as ^{18}F-fluorodopa can be too low because it is labelled by carrier added ^{18}F gas.

TABLE 4. PET AGENTS FOR IMAGING METABOLISM

Metabolism	PET agent
Glucose	^{18}F-FDG
Amino acid	^{11}C-methionine, ^{18}F-fluoroethyltyrosine
Fatty acid	^{11}C-palmitic acid
TCA[a] cycle	^{11}C-acetic acid
Membrane lipid synthesis	^{11}C-choline ^{18}F-fluorocholine
Nucleoside	^{18}F-FLT[b] ^{8}F-FHBG[c]
Ischaemia	^{18}F-fluoromisonidazole
Dopamine	^{18}F-fluorodopa

[a] TCA: Tricarboxylic acid.
[b] ^{18}F-FLT: 3′-deoxy-3′-fluorothymidine.
[c] ^{18}F-FHBG: 9-(4-^{18}F-fluoro-3-hydroxymethylbutyl)-guanine.

1.8.2. Receptor agents

For receptor PET agents, the following factors are relevant:

(a) The clinical importance of the target receptor — the target receptor should represent a specific cell condition or a pathological state.
(b) Brain PET receptor agents require high lipophilicity for easy penetration through the blood–brain barrier. Log *P* should be between 2 and 4, where *P* is the partition coefficient.
(c) Brain PET receptor agents require a low molecular weight, preferably less than 500 daltons, for easy penetration through the blood–brain barrier.
(d) A high affinity to the target receptor is mandatory.
(e) A high specificity to a target receptor is recommended.
(f) No or a low metabolism is required for accurate receptor imaging.
(g) Rapid clearance from blood and non-target tissues is mandatory.
(h) For whole body PET, excretion through the kidneys is preferable to excretion through the hepatobiliary tract.

Although receptor binding PET agents (Table 5) are the most intensively studied of the agents used, their clinical application was less successful than that of metabolic PET agents.

The number of receptors expressed on cell surfaces or inside cells is limited. As a result, only a limited number of radiopharmaceutical molecules can bind to receptors. This greatly restricts the use of receptor binding radiopharmaceuticals for imaging receptors.

Another limitation of receptor binding agents is their binding affinity. In general, dissociation constant (Kd) values of nanomolar affinity are required for good receptor imaging. If the affinity of the agent is low, the ratio of bound to free PET ligand concentration would decrease and will subsequently reduce the target to non-target ratio.

For brain receptor imaging agents, lipophilicity and molecular weight are also important factors for penetration into brain tissue. In general, a log *P* value of 2–4 is recommended for brain receptor binding agents. If the log *P* value is lower than 2, penetration through the blood–brain barrier will be low. On the other hand, if the log *P* value is higher than 4, its protein binding would be too high, which would also reduce the brain uptake of the agent. In general, a

TABLE 5. RECEPTOR BINDING PET AGENTS

Receptor	PET agent
Dopamine D1	^{11}C-Schring23390
Dopamine D2	^{11}C-FLB457 ^{11}C-Raclopride
Dopamine transporter	^{11}C-WIN35428 (β-CFT) ^{18}F-FP-CIT
Serotonin transporter	^{11}C-DAS ^{11}C-WAY-100635 ^{18}F-MPPF
Benzodiazepine receptor	^{11}C-flumazenil ^{18}F-fluoroethylflumazenil ^{18}F-fluoroflumazenil
Opioid receptor	^{11}C-1-carfentanil
β-amyloid plaque	^{11}C-PIB
Oestrogen receptor	^{18}F-FES

molecular weight of less than 500 daltons is optimal for penetration through the blood–brain barrier.

Although β-amyloid plaque is not a brain receptor, the mechanism of its imaging is the same as that of other receptor agents. Carbon-11-PIB (Pittsburgh Compound B) is a promising PET agent, owing to its excellent imaging properties and the clinical importance of Alzheimer's disease imaging.

1.8.3. Peptide agents

For peptide PET agents, the following factors are relevant:

(a) The clinical importance of the target peptide receptor. The target receptor should represent a specific cell condition or pathological state.
(b) Small molecular weight is important for rapid blood and non-target tissue clearance, thus producing a high quality image with high target to non-target ratio.
(c) A high affinity to the target receptor is mandatory.
(d) A high specificity to the target receptor is recommended.
(e) No or a low metabolism is required for accurate receptor imaging.
(f) For whole body PET, excretion through the kidneys is preferable to excretion through the hepatobiliary tract.

Peptide PET agents are regarded as the most promising agents for clinical application in the future. Peptides can target numerous different cells (Table 6). The most successful peptide PET agents are octreotide derivatives. Fluorine-18 and ^{68}Ga are recommended positron emitters for peptide labelling. Carbon-11 is not recommended because of its short half-life and the fact that a period of one or two hours washout is required for peptide imaging.

For clinical introduction of peptide PET agents, the clinical importance of target tissue is the most important factor. The affinity of the peptide to the receptor and the abundance of the receptor are also important for a high target to non-target ratio in the image. The molecular weight of the peptide should be less than 30 kilodaltons for rapid clearance from blood and non-target tissues.

Fluorine-18 is preferred because of its relatively long half-life, and ^{68}Ga is preferred because it is easy to prepare. Gallium-68 is obtained from ^{68}Ge–^{68}Ga generators, which enable PET imaging without operation of a cyclotron and complicated automatic radiopharmaceutical synthesizers.

TABLE 6. PEPTIDES AND RECEPTOR TYPES

Regulatory peptide	Number of amino acid residues	Receptor type (subtypes)	In vivo activity
SST (somatostatin)	14	SST receptors (sst1/sst2/sst3/sst4/sst5)	Inhibition of hormone and exocrine secretion
BN/GRP (bombesin/ gastrin releasing peptide)	14	BN/GRP receptors (GRP, NMB and BRS-3)	Gut hormone release and regulation of exocrine secretion
VIP (vasoactive intestinal peptide)	28	VIP receptors (–)	Vasodilation, water and electrolyte secretion in the gut
RGD-containing peptides/ RGD-peptidomimetics	—	GPIIb/IIIa/platelet and vitronectin/integrin receptors	Inhibition of adhesive and aggregatory functions of platelets
R-MSH (R-melanocyte stimulating hormone)	13	R-MSH receptors (–)	Melanogenesis
NT (neurotensin)	13	NT receptors (NT1, NT2, NT3)	Vasoconstriction, regulation of cardiac activity and a rise in vascular permeability
SP (substance P)	11	SP receptors (NK1)	Hypotension, salivary gland secretion and transmission of pain

1.9. IAEA CONSULTANTS MEETING

In November 2005, the first Consultants Meeting on the topic of parametric PET release met for the following purposes:

(a) To assess current and future considerations about the new classes of PET tracers and associated systems in clinical settings. To develop this guidance publication on criteria essential for a systematic approach to safer and effective translation of PET research into clinical practice.
(b) To establish strategies with a view to minimizing or eliminating them or reducing the risk of their presence in the final product. To establish potential sources of toxicity or pharmacologically active contamination for the most common radiopharmaceuticals.
(c) To classify different types of automated systems and the acceptance criteria with a view to identifying the effects of subtle variations in automated systems from preparation process control and quality specifications.
(d) To establish criteria for an acceptance test for PET tracer synthesis modules and parametric acceptance of PET tracers (Fig. 4) for commonly used clinical applications.

Furthermore, the Consultants Meeting proposed practical testing methods for each criterion for parametric acceptance, thereby helping end users to ensure the quality of their PET products.

1.10. THE ROLE OF THIS PUBLICATION

This guidance publication aims to encourage proactive discussion about the advantages and disadvantages of each aspect of parametric release. It is

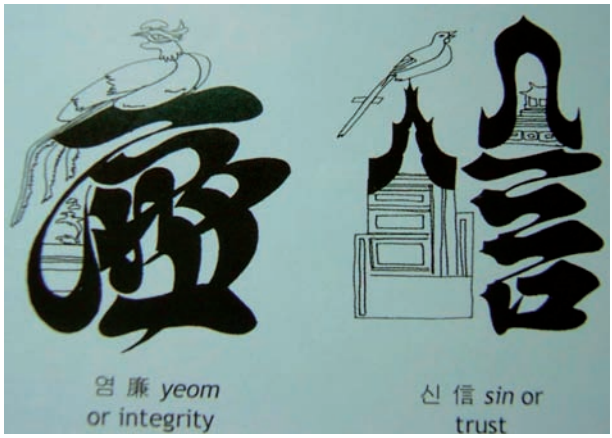


FIG. 4. Essentials of parametric release philosophy: integrity (yeom in Confucius philosophy) and trust (sin in Confucius philosophy).

hoped that these criteria related to parametric release will encourage further cooperation among various countries worldwide in the development of a set of harmonized acceptance test criteria for PET systems and sensible QA standards for all PET drug products. This guide should serve as a useful resource for both researchers and practitioners. Continuous, reliable, development and quality supply of radiopharmaceuticals for patients worldwide is vital for sustained development of nuclear medicine.

This publication should illustrate the importance of qualified staff operating these facilities in order to ensure the uniform quality of radiopharmaceuticals across various facilities.

This publication would also assist Member States in making interim decisions on critical issues while developing their own guidelines to tackle important issues such as:

(a) Development of proper education and training courses to train personnel in safe and effective handling and production of these radiopharmaceuticals;
(b) Development of appropriate legislation to ensure the safety and efficacy of the radiopharmaceuticals produced;
(c) Monitoring of regulatory guidance from various regulatory agencies in developed nations;
(d) Effective translation of PET tracers into clinics.

2. EFFECTIVE TRANSLATION OF PET RESEARCH INTO CLINICAL PRACTICE

2.1. SCOPE

Translation research refers to the conversion of data obtained from basic research into a base for clinical practice. It serves as a bridge between basic research and clinical research. The primary objective of translation research is to ensure that radiopharmaceuticals are safe for use on patients. The purpose of this section is to discuss certain criteria necessary in translating basic research data into clinical practice. The scope of the discussion will focus primarily on the stage between laboratory syntheses and phase I clinical trials. There are separate requirements for other phase (II and III) clinical trials.

The criteria for effective and safe translation of basic PET research into clinical practice may be roughly separated into chemical aspects and biological aspects, which will be described Sections 2.2 and 2.3, respectively.

2.2. CHEMICAL ASPECTS

2.2.1. Established synthesis procedures and formulation

This section deals with the thorough investigation and validation of the synthesis procedures and formulation of radiopharmaceuticals. The validation refers to a number of consecutive productions with satisfactory results in terms of factors such as yield, purity and stability. The number of consecutive productions considered to comprise a satisfactory validation varies among different regulatory bodies and institutional committees, and is commonly of the order of 3–10.

2.2.2. Established procedures for analysis and quality assurance

The establishment of a satisfactory quality of the radiopharmaceutical should cover the following areas:

— Radiochemical purity;
— Radiochemical identity;
— Chemical purity;
— Radionuclidic purity;
— Radionuclidic identity;
— Residual solvents;
— Sterility;
— Pyrogenicity;
— Isotonicity;
— pH.

In general, a radiochemical purity of higher than 95%, a chemical purity of higher than 95%, a radionuclidic purity of at least 99% and acceptable levels of residual solvents are required.

Analysis methods should have been validated. If available, pharmacopoeial analysis methods should also be viewed as methods transferred from other laboratories and tested for suitability in an analytical laboratory.

In most cases, there are no pharmacopoeial analysis methods, and the validation of the new analysis method may include:

— Precision;
— Accuracy;
— Limit of detection;
— Limit of quantitation;
— Specificity;
— Linearity;
— Robustness.

Detailed validation requirements can be found in Section 7.

2.2.3. Established batch stability data and storage conditions

The storage conditions and stability of radiopharmaceuticals should have been established for at least three consecutive batches. The stability testing requirements, however, vary among different regulatory bodies and institutional committees.

2.2.4. Established quality assurance programme

A distinct feature of quality assurance (QA) is that various checks and controls have already been built into the production process, and good laboratory practices (GLPs) are guidelines for setting up good QA programmes. More details of good radiopharmacy practice can be found in Sections 6–8.

2.2.5. Established drug master files/SOPs

Each radiopharmaceutical should have a drug master file/SOP that should include at least:

— The chemical name and structure of the radiopharmaceutical;
— A detailed description of production;
— The identity, grade, quantity, function and supplier of each starting material;
— The specification, quantity, and supplier of containers and closures;
— A list of devices used in preparation;
— Labelling;
— Impurities and their maximum allowed concentrations;
— The QC measures performed;
— The shelf life and storage conditions.

In addition, the drug master file for a new radiopharmaceutical should contain information on validation of methods for preparation and QC, stability tests and dosimetry calculations.

2.3. BIOLOGICAL ASPECTS

2.3.1. Biodistribution data

Biodistribution studies in animals must be conducted. The animal model used should be mammalian (preferably a large animal or primate). Approval from an institutional committee on animal studies must be obtained before conducting any biodistribution studies. The study should cover at least four time points after injection of the radiopharmaceutical. The number of animals utilized at each time point should be statistically significant, usually at least four to six. The method of killing the animals in the experiment should be carefully chosen in order not to alter the biodistribution of the drug. This is of particular importance for neuro-receptor imaging agents. Images of the biodistribution should also be obtained using in vivo and/or ex vivo techniques.

2.3.2. Target to non-target ratio

The target to non-target ratio refers to the ratio of the amount of radioactivity localized in the target organ to that localized in other organs or tissues. Two ratios are of particular importance. The target to blood ratio is important because most organs and tissues are rich in blood supply. A high target to blood ratio predicts that the target organ can be clearly visualized. A target to blood ratio higher than two is preferred. The target to surrounding tissue ratio is also of importance as it predicts that the target organ can be clearly visualized, because the amount of radioactivity inside the target organ is higher than that inside the adjacent normal tissues and organs. A target to surrounding tissue ratio of more than two is also preferred.

2.3.3. Dosimetry data and critical organ

Dosimetry data refer to the radiation dose adsorbed by various organs. From the biodistribution it is possible to determine the dose to each organ as well as the residence time of the radioactivity in various organs. The radiation adsorbed dose to each organ can then be calculated using the MIRDOSE 3 (Medical Internal Radiation Dosimetry) computer program. In general, all

solid organs should have an adsorbed dose of less than 20 mSv. The radiation dose to blood and bone marrow should be less than 3 mSv.

Critical organ/tissue refers to the organ or tissue that receives the highest radiation dose. It is important to identify the critical organ and ensure that the dose to this organ is within allowable limits.

2.3.4. Acute toxicity test and side effect profiles

Acute toxicity tests should be conducted in animal models. The animal models should be mammalian (preferably a large animal). Toxicity can range from necrosis of the injection site to death. A parameter that describes the toxicity of a drug is the Lethal Dose 50 (LD50), which refers to the quantity of the drug that kills 50% of the animals in an experiment. The higher the LD50 value, the less toxic is the drug. It is essential to determine the LD50 value of the drug and ensure that the quantity of radiopharmaceutical injected into humans is far below the LD50 value. Eventual side effects of the drug at the determined dosage should also be evaluated.

The minute amounts of mass, in the nanogram to low microgram scale, which is administered to humans when performing PET studies, has given rise to the microdosing concept [6]. Many regulatory bodies such as the US Food and Drug Administration (FDA) and the European Medicines Evaluation Agency (EMEA) have included this concept in their view of PET radiopharmaceuticals, which has resulted in them reducing the minimum amount of toxicity data to be gathered prior to the first human studies.

Recently, the risk of genotoxic hazard during PET examinations using new radiopharmaceuticals has been evaluated with respect to risk from ionizing radiation and risk from chemical molecules [7]. It was found that the use of high specific radioactivity PET radiopharmaceuticals administrated in less than 10 mg amounts resulted in the genotoxic risk from radiation being higher than the chemical risk, so that no genotoxicity testing was required. As radiation doses at this level are accepted in clinical trials, it was recommended that the regulatory demands on genotoxicity should be reconsidered.

2.3.5. Excretion and metabolism

It is important to determine the route of excretion and the rate of excretion of the drug. Data on excretion can be extracted from biodistribution studies.

2.3.6. Imaging studies

The objective of animal imaging studies is to confirm the data obtained from the biodistribution studies. If the biodistribution data are favourable, imaging studies should be carried out. Images should ideally be obtained in a large animal, preferably a primate. Images should be obtained at various times, and data on the metabolism of the radiopharmaceutical should be collected.

2.4. FIRST STUDIES ON HUMANS

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

Before initiating human studies, investigators should review the factors of risk and the chances of successful human application. The latter will require a review of the nature of the target, the relevance of the animal model and the pharmacokinetics. The differences in affinity for molecular targets and the tissue distribution of molecular targets in animals and humans should be accounted for. In vitro tissue or cell studies can provide evidence of species specificity. Some PET tracers that are highly species specific can give rise to misinterpretation of pharmacokinetics and pharmacodynamics, i.e. molecular target uptake. Finally, when dealing with short half-life products, 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 the first investigations in humans should be carefully considered due to molecular targets, the potential accelerated metabolism, pharmacological or toxicological events, age and gender differences and diet, use of stimulants, such as tobacco and coffee, and drug use and abuse.

When dealing with PET tracers, which are in general administered in nanogram to microgram amounts, attention should be paid to adsorption to syringes, containers and infusion systems. Wherever possible, concurrent medication, even using registered medication, should be avoided.

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

When dealing with PET radionuclides coupled to biological materials, in particular those involved with biological cascades, an interval between each investigation 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 monoclonal antibodies and peptides. Proteins specific to humans are likely to be immunogenic in animal species, so that repeat studies in animals may be of little predictive value.

Radiation doses from PET tracers are normally much higher than those from single photon emission computed tomography (SPECT) tracers, so that 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 focus on radioactive metabolites that may interfere and provide an erroneous interpretation. All levels of human metabolic systems should be carefully monitored. An adequate period of observation and subsequent follow-up is also essential.

2.5. CONCLUSIONS

The criteria described above are not complete. They constitute the minimum amount of data that should be obtained. However, the studies necessary for translation from basic PET research into clinical practice also have to be approved by individual institutional oversight committees and government regulatory bodies. A more detailed description of such oversight committees and regulatory bodies can be found in Section 3.

3. OVERSIGHT COMMITTEES

3.1. SCOPE

Only a few countries have either already established or are in the process of creating governing rules for the production of safe and effective PET

radiopharmaceuticals for human use. Since this class of radiopharmaceuticals incorporates isotopes with relatively short half-lives (2–110 min), it is difficult to follow rules that are prescribed for therapeutic radiopharmaceuticals incorporating isotopes with relatively longer half-lives. Therefore, production and QC of these PET radiopharmaceuticals require somewhat different rules. This section will describe the salient features of different existing governing bodies functioning in various countries, and highlights certain similarities and differences.

3.2. INTRODUCTION

With the ever increasing need for and utility of PET imaging in clinical diagnosis, as well as in drug discovery and development processes, the need for regulatory rules and compliance strategies to ensure safe and effective preparation of emerging radiopharmaceuticals is evident. In the context of preparing PET radiopharmaceuticals for injection, the establishment of specific supervisory controls for adequate QA would enable timely release of short lived radiopharmaceuticals. Once these release criteria have been established and adopted, it is obligatory that local regulatory and governance bodies enforce compliance with those protocols and procedures.

As mentioned in the Introduction to this publication, the references to various oversight committees, their role and composition are intended solely as examples to describe their function in regulating the QA of PET radiopharmaceuticals and are not described as an enforcing tool for Member States. The intent is to establish appropriate international guidelines and standards to create a guidance publication to streamline regulations across Member States. However, the publication is not intended to replace the existing regulatory or oversight committees in Member States.

Initially, Member States may not have a consolidated list of specifications for PET radiopharmaceuticals, but they may wish to install facilities to produce PET radiopharmaceuticals or procure them from contracted manufacturers. In such cases, the production and use of PET radiopharmaceuticals may be regulated by forming local oversight committees, which draw on the information from published experiences on the subject and from the up-to-date regulations and good radiopharmacy practices for PET radiopharmaceuticals contained in universally acknowledged pharmacopoeias, for example, from Ph. Int., USP or EP.

3.3. ROLE OF OVERSIGHT COMMITTEES

The main role of oversight committees operating in various countries can be categorized as:

(1) To establish appropriate guidelines for the production, manufacturing, and QA of PET radiopharmaceuticals:
   (a) Establish controls relating to production methods;
   (b) Establish controls relating to production facilities;
   (c) Establish controls for the 'parametric release' (QC) criteria for radiopharmaceuticals.
(2) To set up safety guidelines for new PET radiopharmaceuticals intended for research use or for the first studies in humans (phase I trials).
(3) To ensure that the regulations laid down for the clinical evaluation of new PET radiopharmaceuticals are realistic, and not impediments to the efforts of researchers to develop them.
(4) To enforce these guidelines to ensure proper implementation and adherence.

Considering the fact that oversight committees are largely formed to formulate QC criteria and to regulate manufacturing practice for PET radiopharmaceuticals, item (3) above would not appear to be relevant. Nonetheless, it is common to find that regulations can become old, unrealistic and burdensome. In those situations, they need to be either revoked or modified. For this reason in 1997, the FDA Modernization Act (FDAMA) was enacted by the US Congress [8] to modify the FDA regulations [9, 10]. More recently, the FDA in the USA developed a 'critical path' initiative to help facilitate the drug discovery process. This process led to the creation of the exploratory investigational new drug (xIND) mechanism. This new path is already effective and has been beneficial in expediting the development of newer drugs and in improving the efficacy evaluation of newer PET radiopharmaceuticals.

3.4. OVERVIEW OF EXISTING REGULATIONS

The following is an overview of existing regulations covering the production of PET radiopharmaceuticals for commercial use and research investigations. The USP and the EP include QA standards in the form of monographs for several PET drugs. It would be helpful for putative oversight committees to know how the regulations overseeing QA have evolved in the USP and EP for PET radiopharmaceuticals.

3.4.1. Regulations in the USA for PET radiopharmaceuticals

3.4.1.1. History

First prepared in 1976, ^{18}F-FDG was found to have immense value in imaging the heart, brain and various cancers. Over the years, most ^{18}F-FDG has been produced under the practice of pharmacy, regulated by pharmacy boards in individual countries.

Historically, small amounts of PET drug products were synthesized by a limited number of producers at academic and not-for-profit institutions for on-site patient use, clinical investigation and academic research. With reimbursements being available for many PET studies, manufacturers with commercial interests (for-profit) are operating an increasing number of PET drug production facilities situated in medical institutions. In addition, an increasing number of stand-alone PET drug production facilities are being established that primarily focus on producing and distributing PET radiopharmaceuticals to a large number of imaging centres. In order to meet increasing demands for ^{18}F-FDG, the FDA recognized that production and good radiopharmacy practice requirements for ^{18}F-FDG production differ between facilities, depending on their size, need and staffing for production operations. A uniform good radiopharmacy practice is therefore required for these facilities.

Largely because of the need for parametric release of ^{18}F-FDG, the FDA published draft guidelines for the manufacture of PET radiopharmaceutical drug products and good radiopharmacy practice for finished PET radiopharmaceuticals [9–11] and published them in a federal register. This guidance document [11] included procedures to comply with good radiopharmacy practice to produce PET radiopharmaceuticals. The recent version of FDA's guidance document for PET drug products can be obtained on the following web site: www.fda.org

3.4.2. The FDA Modernization Act

3.4.2.1. History

Since 1975, the FDA has considered all imaging probes as drugs and regulated them as drugs. Despite the well known fact that these radiopharmaceuticals were used in tracer amounts and exhibited no pharmacological or physiological effects, extensive safety evaluations and testing were mandated by the FDA for these probes. Under older regulations, all human studies involving radioactive entities must be performed under either the Radioactive Drug Research Committee (RDRC) or the Investigational New Drug (IND)

approval mechanism. This requirement added considerable delays in developing and marketing radioactive probes. In 1997, the FDA Modernization Act (FDAMA) was promulgated by the US Congress to expedite the drug discovery process. This Act retracted previous regulations [9–11] and introduced new provisions.

The 1997 FDAMA required FDA to establish approval procedures and good radiopharmacy practice requirements for PET radiopharmaceuticals [8]. Although draft versions of the rules and guidance for PET radiopharmaceuticals were released by the FDA in March 2002 and in September 2005 [12–14], the FDA has not yet issued the final rules for good radiopharmacy practice requirements. The 1997 FDAMA explicitly stated that the FDA may not require a new drug application (NDA) or abbreviated new drug application (ANDA) for a PET drug until two years after publication of a final rule on the good radiopharmacy practice requirements for PET drug products.

Until the proposed good radiopharmacy practice for PET radiopharmaceuticals has been finalized, the FDA will ensure that PET drug products meet safety and identity requirements, as well as strength, quality and purity requirements, as set forth by the regulations under the Federal Food, Drug and Cosmetic Act (FDCA), which are revised from time to time [15–17]. This has been ensured by the amendment to the FDCA instituted by the 1997 FDAMA in which a compounded PET drug is deemed to be adulterated if it is compounded, processed, packed or held other than in accordance with the PET compounding standards and official monographs of the USP (i.e. in compliance with the standards published in USP monographs and general chapters relating to this class of drug) [8]. The FDA's proposal [17] regarding good radiopharmacy practice for PET drugs is the result of almost ten years of consideration of the issue. The FDA stated in the introduction to the proposed rule that the timing of the proposal reflects the increasing number of PET drug production facilities, and facilities where PET scans are performed in the USA.

The USP, through the specifications and monographs it publishes for various drugs from time to time, guides the FDA in its law making. The USP monographs are based on the deliberations by its scientific decision making bodies, namely a council of experts and expert committees drawn from the scientific and health care professional communities. Of the 62 expert committees advising the USP, two deal directly with radiopharmaceuticals. The committee entrusted with setting standards for radiopharmaceuticals is the Expert Committee on Radiopharmaceutical and Medical Imaging Agents, in the Noncomplex Actives and Excipients Division. Drug information related to radiopharmaceuticals is the responsibility of the Expert Committee on Radiopharmaceuticals in the Information Division.

The USP specifications for the various FDA good radiopharmacy practice requirements for PET radiopharmaceuticals are listed in the various general chapters of the USP, as shown in Table 7.

Owing to the inherent nature of PET isotopes, the proposed good radiopharmacy practice requirements for PET drug products differ from the rule for good radiopharmacy practice with general drugs (Regulatory Code 21 CFR 211) in several ways. The key differences in the proposed PET good radiopharmacy practice requirements are as follows:

(a) There are fewer organizational restrictions and fewer personnel are required.
(b) Multiple operations or storage in the same area are permitted as long as the manufacturer employs adequate organization and other controls.
(c) Aseptic processing requirements are streamlined to be consistent with the nature of the production process.
(d) There are streamlined QC requirements for components.
(e) Self-verification of significant steps in PET drug production is permitted.
(f) Same-person oversight of production, review of batch records and authorization of product release are allowed.
(g) There are specialized QC requirements for PET drugs produced in multiple sub-batches.
(h) There are simplified labelling requirements.

3.4.3. Chapter <823> of the USP PET drug products for human use

Investigational and research PET drug products have been excluded and will be discussed later.

Following the 1997 FDAMA, Chapter <823> of the USP (currently 28th edition 2005) 'Radiopharmaceuticals for Positron Emission Tomography—Compounding' has been recognized as the standard for compounding and preparing PET radiopharmaceuticals as per Section 121 of the FDAMA [8] and the FDA draft rule and guidance of current good manufacturing practice (GMP) for PET radiopharmaceuticals [14, 15].

The proposed FDA guidance document (as described under the rule for good manufacturing (Regulatory Code 21 CFR 212) for PET drug products) describes the minimum expected standards for PET drug production at all types of production facilities to ensure that every PET drug product meets the requirements of the FDA. As proposed, the rule contains most of the basic components of the general good radiopharmacy practice, covering:

(a) Personnel and resources;
(b) QA;
(c) Facilities and equipment;
(d) Control of components, containers and closures;
(e) Production and process controls;
(f) Laboratory controls (including stability);
(g) Finished product controls and acceptance criteria (as well as actions to be taken if a product does not conform to specifications);
(h) Labelling and packaging, distribution and complaint handling.

TABLE 7. 2004 USP NATIONAL FORMULARY GENERAL CHAPTERS RELATED TO THE FDA GOOD RADIOPHARMACY PRACTICE REQUIREMENTS FOR PET RADIOPHARMACEUTICALS [16]

USP chapter No.	Title	Citation of draft good radiopharmacy practice rule or guidance
71	Sterility tests	Draft good radiopharmacy practice guidance: X. Finished Drug Product Controls and Acceptance Criteria
85	Bacterial endotoxin test	Draft good radiopharmacy practice guidance: X. Finished Drug Product Controls and Acceptance Criteria
621	Chromatography	Draft good radiopharmacy practice guidance: V. Facilities and Equipment
821	Radioactivity	Draft good radiopharmacy practice guidance: V. Facilities and Equipment
823	Radiopharmaceuticals for positron emission tomography — Compounding	Main principles and concepts for draft cGMP[a] rule and guidance
1015	Automated radiochemical synthesis apparatus	Draft good radiopharmacy practice guidance: V. Facilities and Equipment
1225	Validation of pharmacopoeial methods	Draft good radiopharmacy practice guidance: VIII. Laboratory Controls

[a] cGMP: current good manufacturing practice.

Although the FDA regulates the production and usage of radiopharmaceuticals, there are multiple options to receive FDA approval, depending on the intended use and application of particular radiopharmaceuticals. Major options or programmes (IND and RDRC) available for these approvals are described in Sections 3.4.4 and 3.4.5, respectively.

3.4.4. The Investigational New Drug programme

The IND programme covered by Regulatory Code 21 CFR 312 was established to allow for the clinical investigation of radioactive drugs in human subjects.

The IND programme is intended to conduct clinical investigations and can include:

(a) Research involving therapeutic, diagnostic or preventive benefits to human subjects;
(b) Research to study safety and efficacy (i.e. clinical trials);
(c) Basic research that does not meet the requirements of Regulatory Code 21 CFR 361.1;
(d) Basic research that meets the requirements of Regulatory Code CFR 361.1; however, the investigator chooses the IND pathway.

The IND process is applicable to protocols investigating the safety and efficacy of new radiopharmaceuticals in humans. During the early preclinical development of a new drug, the sponsor's primary goal is to determine if the product is reasonably safe for its use in humans and if the compound exhibits any pharmacological activity. After the product has been identified as a viable candidate for further development, the sponsor then focuses on collecting the data and information necessary to establish that the product will not expose humans to unreasonable risks when used in limited, early stage, clinical studies.

The FDA's role in the development of a new drug begins when the drug's sponsor (usually the manufacturer or potential marketer), having screened the new molecule for pharmacological activity and acute toxicity potential in animals, wants to test its diagnostic or therapeutic potential in humans. At that point, the molecule changes in legal status under the FDCA and becomes a new drug subject to the specific requirements of the drug regulatory system.

3.4.4.1. Types of INDs

There are three types of IND:

(1) An *investigator initiated IND* is submitted by a researcher, who initiates and conducts an investigation, and under whose immediate direction the investigational drug is administered or dispensed. A physician might submit a research IND to propose studying an unapproved drug, or an approved product for a new indication or in a new patient population.
(2) An *emergency use IND* allows the FDA to authorize use of an experimental drug in an emergency situation that does not allow time for submission of an IND. It is also used for patients who do not meet the criteria of an existing study protocol, or in the case of an approved study, when a protocol does not exist.
(3) A *treatment IND* is submitted for experimental drugs showing promise in a clinic for serious or immediately life threatening conditions, while the final clinical work is conducted and the FDA review takes place.

The IND application must contain information in three broad areas:

(1) *Animal pharmacology and toxicology studies.* These include preclinical data to permit an assessment of whether the product is safe for initial testing in humans. Any previous experience with the drug in humans (often used in other countries) is also pertinent for this submission.
(2) *Manufacturing information.* This includes information pertaining to the composition, manufacture, stability and controls used for manufacturing the drug substance and the drug product. This information is assessed to ensure that the company or researcher can adequately produce and supply consistent batches of the drug.
(3) *Clinical protocols and investigator information.* These include detailed protocols for proposed clinical studies with sufficient details to help assess whether the initial phase trials will expose subjects to unnecessary risks. They also include information on the qualifications of clinical investigators — professionals (generally physicians) who oversee the administration of the experimental compound, to assess if they are qualified to fulfil their clinical trial duties. Finally, documentation should be included to show a commitment to obtain informed consent from the research subjects, to obtain a review of the study by an institutional review board (IRB), and to adhere to the investigational new drug regulations.

Once the IND has been submitted, the sponsor must wait 30 calendar days before initiating any clinical trials. During this time, the FDA has an opportunity to review the IND for safety to ensure that research subjects will not be subjected to unreasonable risk.

Exploratory INDs are a relatively new formulation to perform certain preliminary human studies using novel radiopharmaceuticals. In the past, investigators refrained from the IND process because of the time and resources needed to comply with the requirements. This either forced certain studies to remain unexplored or to be performed using an inefficient venue. In order to help investigate certain drugs for a 'go/no-go' decision, the FDA now offers an exploratory IND mechanism. Preliminary studies for go/no-go decisions are better suited under this mechanism and require only limited toxicity data (in one mammalian species, two time points to acquire histopathology information), along with limited regulatory constraints. Once the preliminary results have been acquired, an IND would be necessary to conduct further detailed studies.

3.4.5. The Radioactive Drug Research Committee programme

The RDRC provides oversight of the use of radiopharmaceuticals for research in humans. This committee reports to the FDA but consists of members from academic institutions who perform the research. One of the major functions of this committee is to ensure radiopharmaceutical quality and the relevance of its use for the advancement of science.

The RDRC programme is covered under Regulatory Code 21 CFR 361.1:

(a) The RDRC was established in July 1975 to regulate all the radioactive drugs under the FDA that are generally recognized as safe and effective (GRAS/E) and allows for basic research of radioactive drugs in human subjects without an IND.
(b) The research study should be approved by the Institutional Review Board (see below).

However, if the investigator chooses the IND pathway, simultaneous reporting to the RDRC is not required.

The RDRC programme is intended only for basic research for the purpose of advancing scientific knowledge:

(a) This research is intended to obtain preliminary data in humans to understand the in vivo behaviour of radiopharmaceuticals, such as their kinetics, distribution, dosimetry and localization.

(b) This research is intended to obtain basic information regarding human physiology, pathophysiology and biochemistry of radioactive drugs.
(c) This research is not intended to determine the safety and effectiveness of a radioactive drug in human subjects as a therapy, diagnostic or preventive medical product.
(d) This research is not intended to be of immediate therapeutic, diagnostic or preventive benefit to human study subjects.

3.4.6. Institutional review board

The IRB is covered by Regulatory Code 21 CFR 56.

In the USA, this review board is a federally mandated governing body that monitors research projects and protocols performed using human subjects. Its responsibilities include reviewing the protocols, providing oversight and ensuring protection of human rights.

The two main questions the IRB faces are whether the protocol involves research, and second, whether it involves human subjects. Research is defined by the regulations as "a systematic investigation, including research and development, testing and evaluation, designed to develop or contribute to generalised knowledge". The IRB must include one member whose primary concerns are in scientific areas, one member whose primary concerns are in non-scientific areas, and at least one member who is not otherwise affiliated with the institution and who is not part of the immediate family of a person who is affiliated with the institution. The rest of the committee will be comprised of experts from various fields to determine the research merit of submitted protocols.

Under Federal regulations, the IRB has the authority to approve, to request modifications to or disapprove all research activities that fall within its jurisdiction. Research that has been reviewed and approved by an IRB may be subject to review and disapproval by officials of the institution. However, those officials may not approve research if it has been disapproved by the IRB. The IRB also functions independently of, but in coordination with, other committees. The IRB, however, makes its independent determination of whether to approve or disapprove the protocol based upon whether or not human subjects are adequately protected.

The primary function of the IRB is to review and monitor biomedical research involving human subjects to ensure protection of their rights and welfare. Its responsibilities include:

(a) A review of initial research and subsequent changes;
(b) Authority to approve, to request modification to, or to disapprove research activities;
(c) Authority to suspend or terminate approval of research;
(d) Approval must be obtained prior to implementation;
(e) A continuing review of ongoing research.

The IRB approves research study protocols if the following criteria are demonstrated:

(a) Minimization of risks to subjects and reasonable risks in relation to anticipated benefits;
(b) Equitable selection of subjects;
(c) Compliance with the informed consent requirements of Regulatory Code 21 CFR 50, including subpart D if some subjects are children;
(d) Adequate provision for monitoring data to ensure the safety of subjects;
(e) Protection of rights and welfare of vulnerable subjects;
(f) Adequate provisions to protect privacy and confidentiality.

While the above describes the role and responsibilities of the IND, RDRC and IRB in the context of research studies or evaluations of a new PET radiopharmaceutical, the study PET radiopharmaceutical should be prepared and tested for suitability according to USP 28, Chapter <823>. This is likely to change when the FDA finalizes the good radiopharmacy practice for PET radiopharmaceuticals, and the proposed rules are:

(a) PET drug products for human use, except for investigational and research PET drug products, for which Regulatory Code 21 CFR 212 applies;
(b) For investigational and research PET drugs or drug products, USP 28, Chapter <823>;
(c) For investigational PET drug products being developed for a future marketing application (NDA), it is recommended that Regulatory Code 21 CFR 212 be followed.

3.5. ALTERNATIVE TO EXISTING OVERSIGHT COMMITTEES

In many countries where the production and use of radiopharmaceuticals is limited exclusively to just one or a few major hospitals, local legislative rules and regulations specifically for radiopharmaceuticals may be non-existent. This is particularly true if the numbers of radiopharmaceuticals produced are

limited and are based on 'kit' processes. The methods and procedures used by the radiopharmacist would be according to the knowledge based training received by the personnel in their facility or elsewhere, their experience and the current compounding practice of the hospital where the radiopharmacy is located.

In such situations, a local Oversight Committee for Radiopharmaceuticals (OCRP) (e.g., the Radio-Pharmaceutical Committee in India) may perform the role of the RDRC, IND and, to some extent, that of the NDA to scrutinize the SOPs, QC protocols, sterility/BET records, dispensing and various other responsibilities, as appropriate, and construct a system that will ensure that the manufacturing practice for radiopharmaceuticals are safe for their intended use. The SOPs can be constructed, taking into account the requirements established under good radiopharmacy practice, and the feasibility of adopting the prescribed guidance within Member States.

The PET radiopharmacy investment is major due to the cyclotron and radiochemistry synthesis modules, auxiliary equipment, skilled staff and space. The PET radiopharmacy is complex to operate, as is apparent from the above discussion, even if only ^{18}F-FDG is produced. Operational costs are high, because of the basic cost of ^{18}O-water, certified precursors and reagents, and spares. The 110 min half-life of ^{18}F requires that time schedules of production and supply have to be strictly maintained, the latter by 'parametric release'. Hence, the stakes are high: failures in production and QC add to the operational costs and to a loss of reputation.

All of this implies that the PET radiopharmacy operations are well planned and executed expeditiously. The SOP is the critical document that will ensure that the procedures adopted are such that a PET radiopharmaceutical is produced and delivered on time, and that the QC criteria will be consistently met. It is expected that hospital based PET radiopharmacy will use precursors and other critical reagents procured from established and reliable sources for PET radiopharmaceutical compounding. The staff will have requisite training and hands-on experience suitable for the purpose. With the ready availability of much of the equipment and consumables required for satisfactory radiopharmacy practices, there is no need to look for local alternative equivalents for most of them.

The OCRP can oversee radiopharmaceutical manufacturing compliance standards through MS self-inspection in Member State establishments that lack their own oversight committees. The relevance of self-inspection is to monitor the implementation and compliance with good principles and practices of radiopharmacy, and to propose necessary corrective measures. These may include at least the following items:

(a) Personnel;
(b) Premises and utilities;
(c) Equipment;
(d) Production and in-process control;
(e) QC;
(f) Storage of raw materials and finished products;
(g) Documentation;
(h) Printed packaging materials control;
(i) Distribution of the products;
(j) Calibration of instruments or 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 at which self-inspections are carried out would depend on the size and scope of the facility and the number of products made by it. Self-inspection can be conducted partially (on production lines, facility, SOPs, etc.). However, a complete self-inspection should be carried out once a year.

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

(a) Self-inspection results;
(b) Evaluation and conclusions;
(c) Recommended corrective action;
(d) Implementation and assessment of corrective action.

Finally, it may be stated that oversight committees for radiopharmaceuticals should periodically carry out a quality audit. Quality audits would consist 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.

3.5.1. European Pharmacopoeia guidelines for PET radiopharmaceuticals

Effective from 1 October 2005, the EP has provided guidelines on current Good Radiopharmacy Practice [18], emphasizing the need to adhere to regulations on radiation protection, as well as to appropriate rules of working under aseptic conditions while preparing radiopharmaceuticals for injection.

The good radiopharmacy practice guidelines for PET radiopharmaceutical cover:

(a) Personnel and resources;
(b) QA;
(c) Equipment and facilities;
(d) Documentation;
(e) Production and process controls;
(f) Laboratory controls;
(g) Finished radiopharmaceutical controls and acceptance criteria;
(h) Labelling and packaging;
(i) Distribution;
(j) Complaint handling;
(k) Self-inspection;
(l) Records.

As expected, the good radiopharmacy practice guidelines cover all aspects of PET radiopharmaceutical production, similar to the good radiopharmacy practice of the FDA mentioned earlier. The criteria list of the good radiopharmacy practice appears to have four more points than the good radiopharmacy practice, perhaps owing to the fact that good radiopharmacy practice combined some criteria under one heading. For example, the EP good radiopharmacy practice considers labelling and packaging, distribution and complaint handling as separate criteria, while the FDA good radiopharmacy practice combines them under one document.

3.5.2. Regulations in Europe for PET radiopharmaceuticals

The role of the EMEA cannot be compared with that of the FDA, which is the equivalent authority in the USA. European Union (EU) directives need to be adopted (implemented) by single Member States, which usually takes place with the possibility of introducing changes and on different timeframes. Although this situation can be avoided for marketing authorization (MA) applications, the situation is quite complex for clinical trials where researchers have to refer and apply to their national competent authority. The evolution of the regulations has introduced tighter rules; the latest directives have extended good radiopharmacy practice to R&D medicinal products, and to non-profit and academic research. As in the USA, several organizations, such as the Association of Imaging Producers and Equipment Suppliers (AIPES) and the European Association of Nuclear Medicine (EANM), are lobbying regulators

for a simplification of registration procedures for radiopharmaceuticals or, even better, for adoption of a separate regulation.

Some of the core legislation in Europe can be found in:

(a) Directive 2001/20/EC of the European Parliament and of the EU Council 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;
(b) Directive 2001/83/EC on the European Community code relating to medicinal products for human use.

Directive 2001/83/EC has been amended by other directives, such as 2002/98/EC for regulating medicines based on human blood and blood components, and 2004/24/EC on special issues as regards traditional herbal medicinal products. Directive 2003/63/EC directly dealt with radiopharmaceuticals and, in particular, introduced a revised version of analytical, pharmacotoxicological and clinical, together with protocols in respect of testing of medicinal products aiming at giving more precise indications and instructions on the marketing applications. In particular, Part III on particular medicinal products contains a chapter dedicated to radiopharmaceuticals and precursors.

Directive 2003/94/EC explicitly provides guidelines for good radiopharmacy practice of investigational medicinal products (IMPs). More recently, Directive 2005/28/EC lays down the principles and detailed guidelines for good clinical practice as regards investigational medicinal products for human use, as well as the requirements for authorization of the manufacturing or importation of such products. In Europe, as the Members States have authority on clinical trial approval, the methods for implementing this directive and translating it into national regulations will be very important. Although any clinical trial of an IMP has to receive approval by the ethical committee and the national competent agency, they might operate with different references and will thus translate this directive differently.

The EMEA has recently published a concept paper on the use of the microdosing concept in drug development. The relevant EMEA documents on radiopharmaceuticals and on diagnostic agents are to be revised soon, and some simplification due to the microdosing concept might be expected in these documents.

3.6. EANM GUIDELINES FOR PET RADIOPHARMACY

The radiopharmacy committee of the EANM has published guidelines on good radiopharmacy practice (GRPP) [18], emphasizing the need to adhere to regulations on radiation protection as well as to appropriate rules of working under aseptic conditions while preparing radiopharmaceuticals for injection. The handling of radiopharmaceuticals is potentially hazardous. The level of risk depends upon the types of radiation emitted and the half-lives of the radioactive isotopes. Particular attention must be paid to the prevention of cross-contamination and to waste disposal. The EANM radiopharmacy also states that a continuous assessment of the effectiveness of the QA system is essential to prove that the procedures applied in the radiopharmacy department lead to the expected quality.

Like the USP, the EANM radiopharmacy also recognizes the complexities in preparing PET radiopharmaceuticals vis-à-vis those of ^{99m}Tc radiopharmaceuticals. The latter are referred to as classical radiopharmaceuticals using kit procedures, and the EANM radiopharmacy provides separate guidelines for both. Part A covers the classical ^{99m}Tc radiopharmaceuticals and Part B covers PET radiopharmaceuticals.

Good radiopharmacy practice for PET radiopharmaceuticals covers:

(a) Personnel and resources;
(b) QA;
(c) Equipment and facilities;
(d) Documentation;
(e) Production and process controls;
(f) Laboratory controls;
(g) Finished radiopharmaceutical controls and acceptance criteria;
(h) Labelling and packaging;
(i) Distribution;
(j) Complaint handling;
(k) Self-inspection;
(l) Records.

As expected the good radiopharmacy practice guidelines cover all aspects of PET radiopharmaceutical production, such as the good radiopharmacy practice of the FDCA. The EANM criteria list for good radiopharmacy practice appears to have four more points than the FDA good radiopharmacy practice, but this is not so as the latter has combined some criteria under one heading. For example, the EANM good radiopharmacy practice considers

labelling and packaging, distribution, and complaint handling as separate criteria, while the FDA good radiopharmacy practice takes them as one.

It is good radiopharmacy practice to have rigorous guidelines for the preparation and manufacture of PET radiopharmaceuticals for injection. The good radiopharmacy practice guidelines are intended for in-house PET radiopharmaceuticals, as well as for other radiopharmaceuticals that do not follow kit procedures. There is no mention of whether the guidelines are adequate for commercial manufacturers of PET radiopharmaceuticals, or whether additional controls and regulations are required.

4. TERMINOLOGY

4.1. RADIOPHARMACEUTICALS

Positron emission tomography tracers are radiopharmaceuticals and are therefore defined accordingly. Radiopharmaceuticals are medicinal products suitable for human use that contain a radionuclide. In terms of PET, these are mainly ^{11}C, ^{18}F, ^{13}N and ^{15}O. The radionuclide is integral to the medicinal application of the radiopharmaceutical, making it appropriate for one or more diagnostic application. This can be divided into four sections, however, the latter three ((2)–(4) below) are deemed as starting material or precursor/s:

(1) *Radiopharmaceutical.* A radiopharmaceutical is a PET tracer in a ready to use form that contains one or more radionuclides.
(2) *Radionuclide generator*. Such a generator is a system in which a daughter radionuclide (short half-life) is eluted (separated) from parent radionuclide/s (long half-life) and later used for preparation of a radiopharmaceutical for a PET tracer.
(3) *Radiopharmaceutical starting material.* Such a material is any substance or a radionuclide produced for the synthesis or radiolabelling process with a resultant product becoming an aimed PET tracer.

(4) *Kit for radiopharmaceutical preparation*. This is in general a vial or synthesis set containing essential pre-dispensed precursor/s, in general pre-sterilized, pre-validated products to which the appropriate radionuclide is added and diluted before medical use. In most cases, this is a multidose vial, which may require additional steps, including boiling or filtration. The kits or synthesis sets are designed for immediate use after preparation.

4.2. CHARGED PARTICLE BOMBARDMENT OF TARGET MATERIALS

Charged particle bombardment of target materials is increasingly common in cyclotrons and in accelerators. The composition and purity of the target material will determine the relative percentages of the principal radionuclide and ultimately the radionuclidic purity. For very short lived radionuclides, including PET tracers, determination of the chemical state and purity of the radionuclide before patient use is difficult. Therefore, before use of these radionuclides in clinics, extensive validations and strict operational conditions are essential. Strict control of range of specified quantity and quality is also essential. Any subsequent change in operational conditions should be revalidated.

4.2.1. Neutron bombardment

Neutron bombardment of target materials occurs in nuclear reactors, and the desired nuclear reaction will be influenced by the energy of the incident particle together with the isotopic composition and purity of the target material.

4.2.2. Target materials

Normally, isotopically enriched target material in which the abundance of the required target nuclide has been artificially increased is held in the gaseous, liquid or solid state in order to be irradiated by a beam of particles. The chemical purity, form and chemical additives will determine the relative yield and percentages of the principal radionuclide and radionuclide impurities.

4.3. RADIOACTIVE DECAY

Radioactivity decays at an exponential rate with a decay constant characteristic of each radionuclide.

4.3.1. Half-life period measurement

Testing of preparations should take place after appropriate dilution, to avoid dead time losses, using an ionization chamber, a Geiger–Müller counter, a scintillation counter or a semiconductor detector. The activity must be sufficiently high to allow detection during several estimated half-lives. The measured half-life should not deviate by more than 5% from the half-life stated in Ph. Int.

4.4. STARTING MATERIAL

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

4.4.1. Precursors for synthesis

These are not produced on a large scale and are accepted for use in a batch using validation data and a certificate of analysis (COA) demonstrating consistent reliability.

4.4.2. 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 less than 2 g/d, the identification threshold is ≥0.1%, the reporting threshold is ≥0.05% and the qualification threshold is ≥0.15%.

4.4.3. Certificates of analysis/conformity

Since most PET tracers are formulated as injections, the starting materials should ideally be sterile products. Where this is not the case, the responsible radiopharmacist or qualified person should ensure that the product

is of appropriate quality by means of specifications, Certificates of Analysis/ Conformity, QC tests, bioburden or a combination of these.

4.4.4. Components

Ideally, components (including reconstitution devices, syringes and needles, the product contact parts of filling systems, transfer tubing and final containers purchased in pre-sterilized form from manufacturers) should be either marked 'Conforms to evaluation' (CE) or have a documented form of approval.

Synthesis sets and filling systems should not be modified, but, if this is the case, the modification should be validated and demonstrated so that such modifications do not jeopardize patient products.

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 PET synthesis 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 in the bioburden on the surface of the primary packaging.

Any filters used should be pre-assembled by the manufacturer and guaranteed to be sterile. In addition, these should be pre-validated using volumes, pH and pressure as per the actual patient batch. Standard bubble point testing following filtration of PET tracers should be carried out with great care, due to high radiation risk because of the destructive nature of the test.

4.4.5. Local sterilization

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

4.5. PRODUCTION

Materials for medicinal uses are processed and manufactured by procedures that are designed to ensure a consistent quality and that comply with the requirements of pharmacopoeia monographs or approved specifications.

4.5.1. Clean rooms

A clean room is a room in which the number and concentration of viable and non-viable airborne particles are controlled. The room is constructed and used in such a manner as to minimize the introduction, generation and retention of particles inside the room. Other relevant parameters such as temperature and humidity should be controlled as necessary.

4.5.2. Support rooms

A support room is a dedicated room that is used for activities ancillary to the aseptic preparation process. Such activities may include component assembly, generation of documentation, labelling, checking and packaging.

4.5.3. Controlled work spaces

A controlled work space is an enclosed area constructed and operated in a controlled manner and equipped with appropriate air handling and filtration systems to reduce the introduction, generation and retention of contaminants to a pre-defined level.

4.5.4. Aseptic work zones

The more viable organisms there are in the immediate environment of the process at the point that the container is breached, the greater the risk that some will enter the sterile product. Environmental standards, and the monitoring of those standards, are therefore a fundamental part of every QA system.

4.5.5. Open or closed procedures

Closed transfer procedures between two sealed containers using an integrated transfer device have a far lower risk of contamination when compared with an open procedure in which the sterile container is open to the atmosphere, albeit only for a few seconds. For open procedures, the standard of the environment at the point of fill or transfer is particularly critical.

4.5.6. Critical zones

The critical zone is that part of the controlled workspace where containers are opened and the product is exposed. Particulate and microbiological contamination should be reduced to levels appropriate for the intended use.

4.6. STERILIZATION AND RADIOPHARMACEUTICALS

The general Ph. Int. principles apply to radiopharmaceuticals. However, for radiopharmaceuticals containing a radionuclide of very short life (especially PET tracers), these principles would impose considerable constraints if the general requirements stated in Ph. Int. were truly applied. When possible, terminal sterilization is recommended for radiopharmaceuticals. The main concern would be the practical safety of handling high levels of radiation during the terminal sterilization process.

Further considerations are fundamental when dealing with thermolabile, biological or autologous radiolabelled products. In most of these cases, strict aseptic processes and an appropriate level of validation would be essential to ensure the safety of final radiopharmaceuticals for patient use.

Filtration methods are widely used in radiopharmaceutical practices; however, caution is advised. The use of the bubble point technique to establish integrity testing of final filtration units is fundamental. However, the test process is destructive, and can cause spillage and spread of radioactive materials, which is also unacceptable. The levels of all risks must be carefully considered, and expert advice and due care are of paramount importance for these topics.

4.7. QUALITY ASSURANCE

Quality assurance is the sum of measures taken to ensure that quality is built into the product, especially since most PET radiopharmaceuticals are used within minutes of preparation and without a complete set of normal QCs. Only well documented and validated processes that are essential before the first run for patient use can be used for parametric release. Therefore, any change needs to be validated before use on patients.

4.7.1. Qualified release

A formal recorded approval should be given by a radiopharmacist or qualified person who was not involved in preparation of the PET tracer before a preparation can be released for patient use. Before qualified release, the responsible person should:

(a) Ensure that the product has been produced in accordance with the approved and validated operating procedures, and be aware of all the quality checks undertaken;
(b) Carry out a reconciliation of associated documentation, empty and partially used component containers and starting materials;
(c) Carry out a visual inspection of the product;
(d) Ensure that the product complies with the appropriate specification, including labelling;
(e) Be aware of recent retrospective test results for the products concerned;
(f) Ensure that the daily monitoring records for the unit are satisfactory, for example, those with regard to pressure differentials and cleaning;
(g) Review any quality exception reports (Section 4.7.3);
(h) Be aware of recent microbiological and environmental results for the facilities.

4.7.2. Parametric release

Parametric release is a system of release that provides assurance of quality based on information collected during the process of manufacture and on compliance with the total quality system. Parametric release is useful if based on existing batch and validation data, derived by internationally agreed analytical principles.

4.7.3. Quality exception report

A quality exception report describes any deviation that occurred during the preparation process. It should also indicate any remedial action taken.

4.7.4. Validation

Validation usually refers to the process and can be based on evidence obtained through testing and/or on the analysis of accumulated (historical) data. For more information, see Section 7.

4.7.5. Qualification

Qualification is a component of validation and is a term used for equipment, utilities and systems.

4.8. RESIDUAL SOLVENTS

The International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use (Geneva) has impurity guidelines for the amount of residual solvents that may remain in active substances, excipients and medicinal products after processing. The World Health Organization (WHO) uses the 'acceptable daily intake' (ADI) as a method for classification of residual solvent by risk assessment:

— Class 1 solvents: solvents to be avoided, for example, benzene;
— Class 2 solvents: solvents to be limited, for eample, acetonitrile;
— Class 3 solvents: solvents with low toxic potential, for example, ethanol.

Always refer to individual pharmacopoeia monographs or updated ICH guidelines.

A PET radiopharmaceutical can be released for use before completion of the test on the basis of validation runs and the parametric criteria established.

Some of the common solvents used in PET radiopharmaceuticals are listed in Table 8.

4.9. ESSENTIAL TERMINOLOGY ASSOCIATED WITH VALIDATION (IN ALPHABETICAL ORDER)

4.9.1. Calibration

Calibration is the set of operations that establish, under specified conditions, the relationship between values indicated by an instrument or system for measuring (especially weighing), recording and controlling, or the

TABLE 8. RESIDUAL SOLVENTS USED IN PET RADIOPHARMACEUTICALS AND OTHER MEDICINES

Residual solvent	Maximum mg/V (V is maximum recommended dose in millilitres)
Acetone	50
Acetonitrile	4.1
Chloroform	0.6
1, 2-dichloroethane	18.7
Methanol	30
Methylbutylketone	0.5

values represented by a material measure, and the corresponding known values of a reference standard. Limits for acceptance of the results of measuring should be established.

4.9.2. Change revalidation

Change revalidation involves the repeat of the initial validation process to provide assurance that changes in the process and/or in the process environment, whether intentional or unintentional, do not adversely affect process characteristics and product quality.

4.9.3. Computer validation

Computer validation is documented evidence that provides a high degree of assurance that a computerized system records data correctly and that data processing complies with predetermined specifications.

4.9.4. Cleaning validation

Cleaning validation is documented evidence to ensure that cleaning procedures are removing residues to predetermined levels of acceptability, taking into consideration factors such as batch size, dose, toxicology and equipment size.

4.9.5. Design qualification

Design qualification is documented evidence stating that the premises, supporting utilities, equipment and processes have been designed in accordance with the requirements of good radiopharmacy practice.

4.9.6. Installation qualification

Installation qualification consists of the performance of tests to ensure that the installations (such as machines, measuring devices, utilities and manufacturing areas) used in a manufacturing process are appropriately selected and correctly installed and operate in accordance with established specifications.

4.9.7. Operational qualification

Operational qualification is documented verification that the system, or subsystem, performs as intended over all anticipated operating ranges.

4.9.8. Performance qualification

Performance qualification is the documentary evidence which verifies that the equipment or system operates consistently and gives reproducibility within defined specifications and parameters for prolonged periods (sometimes termed process validation).

Process validation is documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality characteristics.

4.9.9. Qualification

Qualification consists of proof and documentation that premises, systems and equipment are properly installed and/or work correctly, and lead to the expected results. Qualification is often a part (initial stage) of validation, but the individual qualification steps alone do not constitute the validation process.

4.9.10. Retrospective validation

Retrospective validation consists of an examination of past production experience on the assumption that composition, procedures and equipment remain unchanged.

4.9.11. Revalidation

Revalidation is repeated validation of an approved process (or a part thereof) to ensure continued compliance with established requirements.

4.9.12. Prospective validation of risk analysis

Prospective validation of risk analysis is validation carried out during the development stage by means of a risk analysis of the production process, which is broken down into individual steps; these are then evaluated on the basis of past experience to determine whether they might lead to critical situations.

4.9.13. Standard operating procedures

A SOP is an authorized written procedure that provides instructions for performing operations not necessarily specific to a given product or material, but rather of a more general nature (e.g., equipment operation, maintenance and cleaning; validation; cleaning of premises and environmental control; sampling and inspection). Certain SOPs may be used to supplement product specific master and batch production documentation.

4.9.14. Validation

Validation is an action of proving and documenting that any process, procedure or method actually leads to the expected results (see also Qualification (Section 4.9.9)).

The test approach, which is applicable to both prospective and concurrent validation, may include:

(a) Extensive product testing, which may involve extensive sample testing, with the estimation of confidence limits for individual results and batch homogeneity;
(b) Simulation process trials;
(c) Challenge/worst case tests, which determine the robustness of the process;
(d) Control of process parameters being monitored during normal production runs to obtain additional information on the reliability of the process.

A validation protocol is a document describing the activities to be performed in a validation, including the acceptance criteria for the approval of a manufacturing process, or a part thereof, for routine use.

A validation report is a document in which the records, results and evaluation of a completed validation programme are assembled. It may also contain proposals for the improvement of processes and/or equipment.

4.9.15. Validation master plan

A validation master plan (VMP) is a high level document that establishes an umbrella validation plan for the entire project and summarizes the manufacturer's overall philosophy and approach, to be used for establishing the adequacy of performance. It provides information on the manufacturer's validation work programme and defines details of and timescales for the validation work to be performed, including stating the responsibilities relating to the plan.

The VMP should reflect the key elements of the validation programme. It should be concise and clear and contain at least:

(a) A validation policy;
(b) An organizational structure of validation activities;
(c) A summary of facilities, systems, equipment and processes validated and to be validated;
(d) A documentation format (e.g. a protocol and report format);
(e) Planning and scheduling;
(f) Change control;
(g) References to existing documents.

4.9.16. Verification

The application of methods, procedures, tests, and other evaluation and monitoring, in order to determine compliance with good practices.

4.9.17. Worst case

A condition or set of conditions encompassing upper and lower processing limits and circumstances, within SOPs, which pose the greatest chance of product or process failure when compared with ideal conditions. Such conditions do not necessarily include product or process failure.

5. TYPES OF AUTOMATED SYSTEM

5.1. SCOPE

The scope is to classify different types of automated system and the acceptance criteria with a view to identifying the effects of subtle variations in automated systems from preparation process control and quality specification.

5.2. INTRODUCTION

A radiopharmaceutical is defined as a pharmaceutical that contains one or more radioisotope/s. For PET imaging, these radiopharmaceuticals contain a PET radioisotope. As mentioned in Section 1, the most commonly employed PET isotopes are ^{18}F ($t_{1/2}$ = 110 min), ^{11}C ($t_{1/2}$ = 20 min), ^{13}N ($t_{1/2}$ = 10 min) and ^{15}O ($t_{1/2}$ = 2 min). Besides these isotopes, ^{68}Ga, ^{82}Rb, ^{64}Cu, ^{124}I and ^{75}Br are among a long list of PET isotopes with certain clinical relevance. Advances in medical imaging devices, as well as an increased understanding of the physiological processes involved in human disease, have increased the need for newer and novel imaging agents. Therefore, it is increasingly important to develop synthesis of new facile radioligands. While ^{18}F labelled molecular imaging probes have been developed at a fast pace, isotope handling and usage in radiochemical synthesis require particular care. While the need for new radiopharmaceuticals is emerging, it is important to consider four major properties of these PET isotopes that are critical in developing radiopharmaceuticals incorporating these isotopes.

(1) Short physical half-life;
(2) Higher radiation dose due to inherent positron energies;
(3) Necessity of larger quantities of starting activity;
(4) Complexity of radiochemical synthesis.

Because of the short half-life, it is imperative that synthetic strategies and schemes should be rapid, facile, reliable and reproducible, and have the highest possible yield. In addition, the QA criteria and analysis should be tailored to ensure timely completion.

When using radioisotopes with short half-lives, synthesis of imaging agents requires large quantities of starting activity in order to yield meaningful quantities of finished radiopharmaceutical. For safe and effective handling of large quantities of these isotopes, it is important to implement remote handling

techniques. Positron emitting isotopes are high energy particles that impart 511 keV positron energies. After annihilation, these nuclides deposit considerably higher radiation doses than do routinely used nuclear medicine isotopes. For example, a 37 MBq (1 mCi) unshielded gamma emitting nuclear medicine isotope exerts an exposure of 0.2–2.2 rad/h (0.002–0.022 Gy/h) at 1 cm from the surface (depending on gamma energies), while exposure from a 1 mCi PET isotope at 1 cm would be about 5.8 rad/h (0.058 Gy/h), a 2–10 fold higher dose; signifying a need for more careful and cautious handling. These two properties of PET isotopes lead to added complexity. In addition, it is difficult to adapt complex chemical synthesis procedures that require multiple purifications and handling while handling large quantities of these rapidly decaying isotopes.

An automated and remote handling procedure for the delivery of radioisotopes and for synthesis of PET radiopharmaceuticals is essential for safe handling of these radioisotopes and to minimize exposure of personnel. To accomplish this, several research groups and commercial companies have devoted their time and resources to develop automated synthesis modules. An additional benefit from synthesizing radiopharmaceuticals using an automated module is the batch-to-batch reproducibility of the synthesized product in terms of quality and quantity. Radiopharmaceuticals produced manually require added steps to ensure batch-to-batch consistency in quality. Added QCs may be necessary for manual production. In essence, PET radiopharmaceutical production laboratories require a safe and effectively shielded workplace as well as remotely controlled and/or automated synthesis modules to prepare radiopharmaceuticals. Although automation appears straightforward, it encompasses the entire process of manufacturing a radiopharmaceutical starting from radioisotope production, to certifying it as a safe and effective radiopharmaceutical. For simplicity and ease of understanding, the safe handling of radiopharmaceuticals during production and QC can be divided into two major categories:

(1) A safe workplace with effective shielding and remote handling;
(2) Automated and remotely controlled synthesis modules.

5.3. SAFE WORKPLACE WITH EFFECTIVE SHIELDING AND REMOTE HANDLING

It is imperative that radiopharmaceutical laboratory design should be focused on local regulatory agency requirements and personnel safety. The level of shielding and automation depends on the workload, resources and

available expertise. There are several options to minimize personnel exposure in a PET radiochemistry laboratory. To achieve effective shielding, the practical and most effective means to handle PET radioisotopes safely and to protect laboratory personnel from radiation is to use 60–75 mm thick lead lining. Therefore, the simplest way to provide this added protection is to enclose all work areas with lead walls. Unlike a decade ago, there are multiple choices for the safe and effective handling of PET isotopes.

5.3.1. Lead lined hoods

A primitive but economical and practical option for remote handling of radioisotopes is the lead lined fume hood or lead cave (Fig. 5). The reagents are added remotely to the reaction vessel from behind the lead wall using syringes and valves controlled by push buttons (on the right hand side of the sash in Fig. 5).

FIG. 5. A lead lined hood: a fume hood converted to a lead lined cave for remote handling of radioisotopes.

The work area is lined with lead bricks and the radiochemistry synthesis apparatus is placed inside this lead lined area. The reagents can be added from outside using syringes and valves that are located on the outside of the lead lined area without entering the radiation field.

The lead glass windows are an aid to monitoring the reaction without having to lean over the lead wall. These lead caves are suitable for housing computer controlled synthesis boxes or for using a remotely controlled procedure where minimal or no operator interventions are necessary inside the work area. The use of these lead lined hoods is well suited for developmental work using small amounts of radioactivity, because manual access to the inside is feasible. In addition, maximum quantities of radioactivity in one of these lead caves should be restricted, as it is not completely shielded on all sides with lead.

5.3.2. Hot cells

The next option to create a safe workplace is the use of hot cells (Fig. 6). The hot cell concept is similar to that described for the lead cave. The hot cells are primarily lead caves but provide lead shielding in all six directions. The access to the inside work area is limited to the front and sides of the cells through panels or doors that are closed during operation. As these cells are shielded at the top and bottom, radiation exposure within and around the radiochemistry laboratories is significantly reduced. The hot cells provide access to the inside space for preparation work before synthesis takes place and for cleanup after use. During the synthesis time, all doors and side panels are closed, to minimize and/or eliminate laboratory radiation exposure and to ensure that the inside environment can be controlled. The operator uses robotic arms (manipulators) from outside the cell for safe and effective handling of radiopharmaceuticals. These hot cells are well suited for installing synthesis boxes that can be operated by automated procedures.

As can be seen in Figs 6 and 7, operators can reach the reaction vials and other items that are located inside the cell remotely using the arms located outside the cell and without encountering any radiation exposure.

Figure 8 shows a set of two hot cells, with robotic arms providing support for manual interaction without jeopardizing safety. Once the finished radiopharmaceutical is ready, the final product can be either delivered to a side drawer lined with lead or accessed from a side panel, depending on the model purchased, without opening the hot cell doors. It is advisable to wait for the radioactivity to decay before opening the hot cell doors for cleanup or for setting it up for reuse. There are several manufacturers that sell hot cells internationally. Hot cells require a large investment, and it is therefore important to

FIG. 6. An operator working on a hot cell with robotic arms.

FIG. 7. Inside view of a hot cell through the hot cell window in the door. The inside part of the robotic arm is reaching for the reaction vessel, while the operator is working with the manipulators outside.

match the options that a particular vendor offers and the requirements for safe and effective operation of the radiopharmaceutical manufacturing site before choosing a hot cell vendor. It should not be assumed that all hot cell vendors would provide the same options, designs and accessories.

FIG. 8. A set of two hot cells with robotic arms.

A large number of laboratories acquire remote controlled arms (manipulators) to help remote handling of radioactivity within the hot cell (Figs 6–8). If semi-automated synthesis or manual interventions are anticipated during the radiosynthesis, installation of these manipulators will add safety as well as the option to perform manual operations in the process. This option allows safe handling of large quantities of radioactivity while maintaining safety. As shown in Figs 6 and 7, the operator works with manipulator arms from outside the hot cell to simulate manual operations.

The arms aid in moving and transferring radioactive materials, as well as activating the next process as performed during manual processing. Several vendors manufacture manipulators that can be used in hot cells, and most of them export these items internationally. Most hot cell vendors could retrofit manipulators in existing hot cells. If deferring the manipulator purchase at the time of hot cell purchase, it is advisable to opt for manipulator hole cut-out plugs. The manufacturer will make the housing insert for manipulators and will cover these with lead plugs to retain their integrity while providing the flexibility to install manipulators by removing these plugs during future refits.

Hot cell manufacturers have responded to ever increasing regulatory requirements for the production of safe and effective radiopharmaceuticals. A stringent pharmaceutical QC criterion can be met with the high energy particulate airfilter (HEPA) option available with some hot cells. Once the radiopharmaceutical has been prepared and collected in a sterile vial under a

sterile environment, further doses can be drawn using the dose drawing options available from certain hot cell manufacturers.

5.4. AUTOMATED AND REMOTE CONTROLLED SYNTHESIS MODULES

It is important to develop novel radiotracers to advance the field of radiopharmaceutical chemistry. The descriptive path to develop radiotracers of clinical relevance is to explore the potential of various radiotracers. To achieve that goal, one needs to combine expertise from medicinal chemistry, organic chemistry, radiochemistry, radiation safety and regulatory compliance with a good understanding of clinical issues. It is important to adapt oneself to bridge the gap between preclinical testing of the recently developed radiotracers and careful and cautious testing and use of these new imaging agents in humans. This requires reliable, safe and effective production of new radiopharmaceuticals.

5.4.1. Fluorination synthesis modules

Figure 9 shows the automated synthesis modules available from different manufacturers to produce ^{18}F-FDGs. Each module has its own intricacies and advantages. There are several types of laboratory automation available at this time.

Although several manufacturers are developing automated synthesis boxes, their application is mainly limited to the synthesis of radiopharmaceuticals that are fully developed and close to routine use. It is important to mention that a large number of PET tracers are at present synthesized using remote control facilities with limited or no automation.

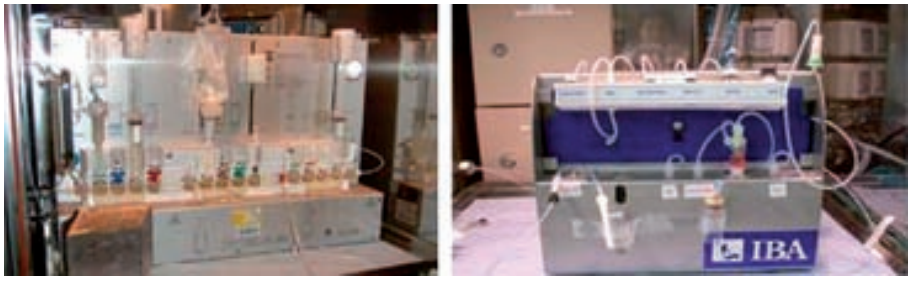


FIG. 9. Different types of module for producing ^{18}F-FDGs.

For ^{18}F-FDG production, fully automated boxes using sterile single use components with all the sterile reagents in separate vials can be obtained. This enables multiple batches of ^{18}F-FDG to be produced with one set-up routine. Two of the commercially available modules for synthesis of ^{18}F-FDG are shown in Fig. 9. There are many vendors that sell synthesis modules for ^{18}F-FDG production. While some ^{18}F-FDG boxes can be used for preparation of multiple ^{18}F radiotracers utilizing similar types of radiochemistry, adaptation of synthesis of other ligands to these modules, without experienced radiochemistry personnel, can be difficult. Therefore, there is a growing need for more flexible automation systems.

In addition to the ^{18}F-FDG modules, several manufacturers offer commercial modules that can perform various types of radiochemical synthesis varying from simple to complex. Most of these modules are computer controlled and are well adapted to hot cells, caves or even minicells.

The three major categories of chemistry based module, other than the ^{18}F-FDG synthesis box, available on the market are: ^{18}F nucleophilic fluorination modules, ^{18}F electrophilic fluorination modules, and ^{11}C-methyl iodide/triflate production and methylation modules. Although not all syntheses can be accomplished using one of the available categories of module, these modules can be customized relatively easily or optimized for the production of some other radiopharmaceuticals of interest. It is important to remember that some of the fully automated modules are quite expensive and could cost well above US $100 000. Although the cost is quite high, these modules provide reliable production of radiopharmaceuticals that are compliant with regulations.

Use of an automated synthesis module is by far the most effective, safe and practical approach to manufacturing radiopharmaceuticals for human use. Amongst the nucleophilic synthesis modules, an example of one of the low cost automated synthesis boxes commercially available for a specific application is shown in Fig. 10. This module can trap ^{18}F-fluoride to reclaim ^{18}O-water and then release ^{18}F-fluoride to perform a single step radiochemical reaction, followed by transferral of the reactants to a chromatography system for separation and purification.

Since each ligand synthesis uses slightly different procedures, it becomes cumbersome to automate each individual synthesis. Therefore, a large number of research groups assemble semi-automated synthesis modules by using components such as electric valves that are operated either by remote control, computer (PC) control or programmable logic controllers (PLCs). The complicating aspect of this option is that these modules are custom-made for individual needs and are prone to fail if not monitored carefully. Since these

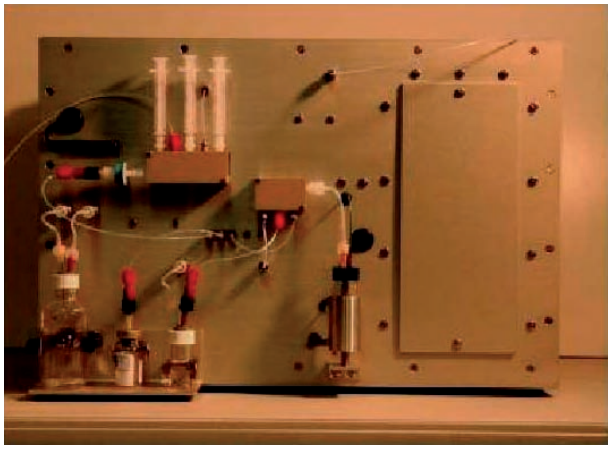


FIG. 10. An automated module to produce ^{18}F labelled fluorobromomethane from ^{18}F-fluoride.

modules are highly individualized, it is difficult for others to adapt and use them with any reproducibility and reliability. Acquiring a fully automated module remains a better choice, unless well qualified staff are available to assemble and maintain these semi-automated modules.

More robust and fully automated modules for nucleophilic fluorination reactions for use in research and production are shown in Fig. 11. These modules can be optimized for one of the several synthesis schemes available. While this module can be optimized for back-to-back synthesis of more than one type of tracer, cleaning and refilling the vessels with reagents for the next synthesis does pose a risk of exposure. Therefore, great care is necessary to utilize such modules for back-to-back multi-tracer synthesis. An effective option for electrophilic fluorination modules is also similar to that described above, except that it is fitted with trapping systems to trap carrier gases, as well as electrophilic fluorination, effectively and efficiently.

5.4.2. Carbon-11 synthesis modules

There are two major synthesis routes for the production of ^{11}C radiolabelled radiopharmaceuticals. The first route is a wet chemistry method employing lithium aluminium hydride (LAH) as the reducing agent for the ^{11}C-CO_2, followed by reaction with HI to produce ^{11}C-methyl iodide. The second route is the gas phase method, employing nickel catalysed reduction of ^{11}C-CO_2 to CH_4, followed by subsequent reaction with HI to yield ^{11}C-methyl iodide. While both methods provide excellent radiochemical yields of the reacting species ^{11}C-methyl iodide, the gas phase method is simpler and seems

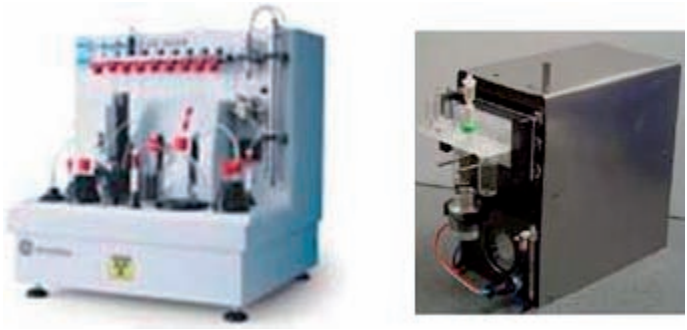


FIG. 11. Automated modules from two different vendors to produce ^{18}F labelled radiopharmaceuticals using the nucleophilic fluorination reaction.

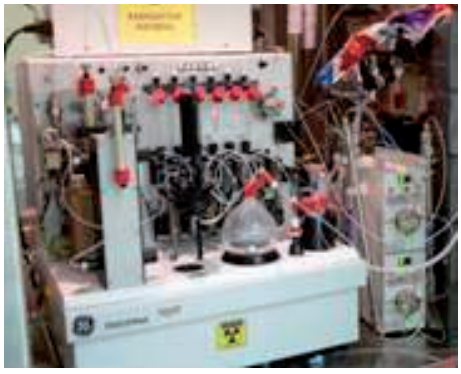


FIG. 12. An automated synthesis module that uses gas phase chemistry to produce ^{11}C labelled radiopharmaceuticals.

to provide a product with a better specific activity. Figure 12 shows a photograph of a gas phase synthesis unit. This module is designed to synthesize ^{11}C labelled molecules. Since a large number of neuro-receptor ligands and other ^{11}C radiopharmaceuticals use a common synthon (^{11}C-methyl iodide or triflate), this module can be used to synthesize a large number of different radiopharmaceuticals. The reproducibility of the results from these modules is quite consistent, and they provide the option of safe and effective multiple back-to-back synthesis. In addition, use of this module does not require the user to learn all of the mechanics behind the module.

5.4.3. Installation site requirements

Prior to installation of any module, it is essential that the site be prepared systematically. The checklist provided in Table 9 should ensure a more standardized approach.

In conclusion, production of novel radiopharmaceuticals has become more facile and reproducible, despite increased demand requiring manufacturing of larger batches of these radiopharmaceuticals.

TABLE 9. CHECKLIST FOR MODULE INSTALLATION SITE REQUIREMENTS

Consideration	Yes/No	Comments
Adequate shielding/hot cell		
Appropriate hot cell environment		
Adequate space requirement		
Clarity of arrangement of the module in the hot cell		
Possibility of thorough cleaning of the synthesis module		
Adequate space requirements outside of the hot cell for any of the following: —HPLC pump and eluent bottles —Dewar for cooling —Regulator bar —Printer, computer and control unit —Other items		
Installation qualification		
Delivery checklist		
Complete items list		
Missing items list		
Damaged parts list		

TABLE 9. CHECKLIST FOR MODULE INSTALLATION SITE REQUIREMENTS (cont.)

Consideration	Yes/No	Comments
Set-up checklist: —Synthesizer —Suitable shield —Control unit —HPLC pump —Ultraviolet (UV) detector —Laptop computer —Printer		
Installation checklist including: —Electrical connections —Compressed air connections —Inert gas connections and type —Hydrogen connections —Target line connections		
Operational qualification		
Operational tests including: —Software —Controls —Values —Pressure transducers —Heater —Radiation detector —Reactor needles —Movement check —Fluid detector —HPLC operations		
Test syntheses including: —Cold run —Clean synthesis —Line clearance —Hot synthesis and yields —Radiochemical purities		
Training consideration in the following areas: —Preparation —Operations —Maintenance —Calibration		

6. QMS — A: STRATEGIES AND PRACTICES TO REDUCE RISKS OF ERROR IN THE FINAL PRODUCT

A well documented quality management system (QMS) is a prerequisite for the QA of PET radiopharmaceuticals. The QMS shall cover, as a minimum, the following key quality elements:

— A site master file;
— Personnel files;
— Documentation and an SOP;
— A validation policy;
— Well controlled equipment and facilities;
— A materials control procedure;
— Well controlled synthesis procedures;
— Quality control procedures;
— A release procedure;
— An out of specification (OoS) procedure;
— Recall, complaint and change control procedures;
— Internal audit plans.

6.1. THE SITE MASTER FILE

The site master file is the core document in the QMS of a facility producing PET radiopharmaceuticals. The structure of this document may vary depending on the size and complexity of the facility. As a minimum, it should always include the following parts:

6.1.1. The main objectives of the facility

The objectives may be commercial, scientific, clinical or a mixture of these.

6.1.2. A functional (operational) description

This part explains the operational flow during PET radiopharmaceutical production, and includes a list of the various departments or units involved in the production. It also explains the responsibility relationship among the personnel taking part in the production of PET radiopharmaceuticals.

6.1.3. A quality system

This part provides a general description of the quality system of the facility, and includes functions such as QA and quality management, as well as a list of personnel responsible for quality. Documents concerning quality, such as SOPs and standard management procedures, are also included.

6.1.4. Management and administration

Depending on the objectives of the facility, the means for management and administration of the facility are varied, and these means should be explained in this part. The system for storage of raw data and printed reports that are generated in the production of radiopharmaceuticals, patient studies and other activities should also be explained in this part.

6.1.5. Cooperation agreements

This part includes a list, together with general descriptions, of agreements with commercial enterprises, research facilities and hospitals.

6.1.6. Personnel

A general description of the various levels of personnel and of their training and qualifications should be given.

6.1.7. Premises

This part includes a list and description (with drawings) of the laboratories and other rooms where PET radiopharmaceuticals are produced.

6.1.8. Equipment

This part includes a list of the major equipment used in the production of PET radiopharmaceuticals. Service schedules and service agreements for this equipment should also be included. Quality control equipment should be validated and recalibrated at least every 12 months.

6.1.9. Guidelines

The guidelines (good radiopharmacy practice, GLP, and international and domestic regulations) that are to be respected in the production of PET radiopharmaceuticals at the facility should be given in this part.

6.1.10. Radiopharmaceutical production

This part provides a list of radiopharmaceuticals produced with reference to SOPs for their production.

6.1.11. Storage rooms

This part provides a description of the rooms where products are stored before release and where returned products are stored after incurring complaints.

6.1.12. Audits

The frequency of internal audits and a list of personnel responsible for their scheduling and planning should be given in this part.

Most PET radiopharmaceuticals are produced in-house with a limited available time due to the short half-lives of most PET radioisotopes. Hence, QA is a priority in producing PET radiopharmaceuticals. The objective of QA is to minimize errors in the final products. The following sections describe the strategies or concepts commonly employed to reduce risks in a QA system.

6.2. TRAINING OF STAFF

The highest priority in establishing a good QA system is to have good staff training. It is essential that all staff have a sound knowledge of the quality requirements of PET radiopharmaceuticals and aseptic techniques, as well as an understanding of the importance of reducing errors in the final products. The requirements for training are as follows:

(a) All personnel should be properly trained to carry out their assigned duties.
(b) There is proper training and records, including objectives, training details and assessments.

(c) There are job descriptions that are specific and with no extensive overlap.
(d) There are leave procedures to ensure coverage of duties.
(e) There are health screens to ensure that production staff are not colour blind and do not have any infectious diseases.

6.2.1. Minimum requirements

The application of standardized practices and professionally accepted norms should be at the core of any service. The IAEA recently published some of the basic practices in its Nuclear Medicine Resources Manual [19]. Table 10 provides details of the minimum requirements of professional profiles.

TABLE 10. MINIMUM REQUIREMENTS FOR PROFESSIONAL STAFF

Professional staff	Requirements
Nuclear medicine technologists	BSc plus one year practice qualification or minimum three years practice qualification plus IAEA distance assisted training (DAT) graduate
Radiographers	BSc in radiography with key module in nuclear medicine
Medical physicists	Qualified, with postgraduate to MSc in medical physics with major in nuclear medicine If involved with hot laboratory, to have adequate radiopharmaceutical training
Hot laboratory assistants with no support from a qualified radiopharmacist	Nationally/professionally approved training or minimum IAEA competency based programme with three month internship
Radiopharmaceutical scientists and radiopharmacists for major centres, including PET facilities	Qualified and registered pharmacists, ideally with a postgraduate qualification and experience with radiopharmaceuticals, or MSc radiopharmaceutical scientists recognized as authorized or qualified persons
Nurses	Qualified and registered nurses with internship in nuclear medicine If involved with dispensing, adequate training in radiation protection and essential hot laboratory practices

There should be an adequate number of qualified staff (with a minimum of three) with clearly defined responsibilities, i.e. cyclotron operation production and QC. There should be adequate training for staff on radiochemistry, aseptic techniques, radiopharmacy, etc. A clear and updated rotation schedule is essential with no grey areas in terms of responsibility. In a QMS, the focus is on a systems approach, and there is therefore no scope for systems that are dependent on any one person.

Supervisors and staff should have discussions regarding training needs, so that adequate and timely training can be provided. It is the responsibility of supervisors to arrange training for staff, but it is the responsibility of individual staff to attend all the training provided. There should be proper assessment of staff after training. Training records should be properly maintained for future reference.

Staff should be encouraged and allowed time to attend continuing education. The training needs of staff should also be reviewed periodically.

6.3. DOCUMENTATION

All procedures must be properly documented. It is assumed that any event did not occur if it is not documented. The different types of documentation include:

(a) Standard operation procedures — These are instructions for staff to carry out their tasks.
(b) Validation plans and records — These should describe the basics of validation work, how to perform the validation work, including detailed work instructions, staff responsibilities and timetables, as well as the results of each validation test.
(c) Batch records — These are records of the manufacture of each batch of products. The records should at least include the name of the product, lot number of the product, date of manufacture, staff carrying out the manufacturing, raw materials used, equipment used, facility where the manufacture took place, manufacturing method, monitoring of the manufacturing reaction, as well as monitoring of the manufacturing environment.
(d) Quality control records — These are records of the QC tests performed on each batch of product. The record should include at least the name of the product, lot number of the product, date of tests, staff carrying out QC tests, reagent used, equipment used, test method, results, acceptance limits and interpretation of the results. These records should be signed by

the person in charge of QC and include a statement confirming whether the product has passed the QC tests.

(e) Approved forms — There should be different forms for different purposes.

(f) Specifications — These refer to raw material requirements, final products, test reagents, manufacture and QC equipment, manufacturing facility, etc.

(g) Data sheets.

(h) Log books, etc.

6.3.1. Records

Proper records are helpful for reducing errors. Records must be completed in a timely and accurate manner. The objective of having records is not to find fault with staff, but to identify problems so that they can be avoided in future.

Supervisors and staff should periodically and routinely review records. By reviewing records, staff can monitor equipment performance and changes in environment, so that necessary actions such as maintenance, calibration or repairs can be carried out.

The following records should usually be kept:

(a) Production batch records — These are detailed records of how the product is manufactured. The records should include descriptions of the starting materials, manufacturing procedures, manufacturing environments, manufacturing staff and labelling.

(b) Checklist records of release for supply — These are records of the final check of a product before it is released from the facility. A PET product, because of its ultra-short half-life, can be released without a completed release for the supply checklist records. These records provide assurance that the product and the manufacturing process have been cross-checked before it is released.

(c) Dispensing records — These are records of the dispensing of the product. They should provide details of the time of release of a certain dose, the identity of the patient receiving the dose, dosage, time of dispensing, lot number of the product and the staff dispensing the dose.

(d) Quality control records — These are records of the QC tests of the product. The QC should be signed by the staff performing the QC, then reviewed and signed by the person in charge of QC.

(e) Equipment use records — These records track how equipment is used, calibrated and maintained. They also describe the functional status of the equipment and ensure that all equipment is functioning properly.
(f) Environment monitoring records — These records track the manufacturing environment to ensure that it is suitable for the manufacture of PET radiopharmaceuticals.
(g) Cleaning records of equipment and facility — These records monitor the cleanliness of the manufacturing equipment and facility according to SOPs. It is used to check that the facility and equipment are functioning within acceptable limits.
(h) Validation records of the equipment and facility — These records ensure that the manufacturing facility and equipment have been validated.
(i) Maintenance records of the equipment and facility.
(j) Calibration records of equipment.
(k) Staff training records — These records ensure that staff have been properly trained in certain procedures.
(l) Check-in records of raw materials — These records ensure that the raw materials received are acceptable according to the specification set. Each batch of raw materials received should be checked.
(m) Out of specification investigation records — These records ensure that a proper investigation has been carried out if a product has failed a QC test.
(n) Corrective and preventive action records — These records ensure that appropriate action has been taken in response to any problem outside normal specifications.
(o) Recall and complaints records — These records ensure that proper action has been taken in the case of a complaint or product failure.

6.3.2. Standard operating procedures

All procedures must be clearly written and reviewed, and all staff must be familiar with all SOPs. Standard operating procedures, which are properly written and adhered to, can prevent human error and individual variations, as well as maintain uniformity among batches.

Standard operating procedures must be constantly reviewed. Before an SOP is put into use, it should be tried or 'field tested'. A well written SOP should include:

(a) Version number — To keep track of the revision history of the SOP. The SOP in use should be the one with the latest version number.
(b) Author — Usually the author who wrote the SOP is the one who will rewrite or update it.

(c) Signature, date of adoption of the SOP — All SOPs should be reviewed and approved by the person in charge. This means that the SOP has been tested and found to be suitable for use. An SOP is not official without the signature of the person responsible for approval.
(d) Objective of the SOP — This is a description of what the SOP is used for. For every procedure, there should be a specific SOP. Staff should use the correct SOP.
(e) Persons responsible for carrying out the SOP — This must clearly identify the responsibility of the staff when performing a certain procedure, in order to avoid confusion and, subsequently, mistakes.
(f) An overview or flow diagram of the procedures — This is a summary of the procedures to be performed.
(g) Procedures in detail — Instructions should be as detailed as possible. The instructions should also be written clearly so that any new staff can follow them.
(h) Interpretation of results (if any).
(i) Actions to be taken if failures occur (if any).
(j) Affiliated documents.

6.4. VALIDATION POLICY

Validation refers to the process of establishing documentary evidence that provides a high degree of assurance that a specific process or piece of equipment will consistently produce results meeting its predetermined specifications and quality attributes (Section 4). Good radiopharmacy practice requires that:

— There is a VMP.
— There is a validation timetable.
— Synthesis equipment has been validated.
— Quality control equipment has been validated.
— Analysis methods have been validated.
— Cleaning procedures have been validated.
— Media filling procedures have been validated.

6.5. WELL CONTROLLED EQUIPMENT AND FACILITIES

Entry to manufacturing facilities should be restricted to authorized personnel only. The facility should be cleaned using a validated cleaning

method. The facility should also be routinely validated. The environment parameters of the facility, such as temperature, pressure, humidity and bacterial counts, should be monitored and recorded. Figure 13 shows a typical PET facility.

Manufacturing equipment should be fully validated and routinely maintained. The equipment should be properly cleaned and tested before each synthesis operation. The use of synthesis equipment should be restricted to properly trained staff, and this use should be properly recorded.

6.5.1. Premises and equipment validation

It is essential to have validation processes for premises and equipment. These should include:

(a) Installation qualification — All components/modules are installed properly, while technical manuals are present and documented.
(b) Operation qualification/commissioning — Equipment functions satisfactorily to the specifications of the manufacturer.
(c) Performance qualification/validation — Equipment meets requirements for performing the specific tasks required by users.

FIG. 13. A typical PET facility.

In addition, equipment must be maintained, calibrated and revalidated routinely, and these actions should be properly recorded. In daily use, equipment should be used properly, and this daily use should also be recorded.

6.5.2. Environment

The quality of the environment is defined for the different pharmaceuticals produced. The WHO, the Pharmaceutical Inspection Co-operation Scheme (PICS), the International Organization for Standardization (ISO) and the FDA have different classifications of clean rooms for manufacturing environments. Staff must be familiar with these classifications. Since most PET radiopharmaceuticals are for intravenous use, they must be manufactured in the proper environment (minimum Class C in Europe (PICS and WHO classifications), and Class 10,000 in the FDA classification).

PET radiopharmaceuticals must also be dispensed in the environment specified for aseptic dispensing (Class A in the PICS and WHO classifications, and Class 100 in the FDA classification).

The environment must be validated routinely according to established SOPs to confirm that it complies with the specifications. These include tests in:

(a) Particle counts and viable counts (in terms of microbial growth);
(b) Airflow velocity and direction;
(c) Pressure differential.

The environment must be properly monitored in order to verify that it fulfils the required specifications during each session or at least daily. A media fill simulation should be carried out to ensure that the synthesis system is enclosed and that there is no entry of bacteria from the environment. This is a simulation of the manufacturing procedures using bacterial culture media instead of raw material. The simulation can also test the aseptic technique of the staff carrying out the operations.

6.5.3. Cleaning and maintenance

The environment must be properly maintained to ensure that it is stable and functioning. Proper maintenance procedures include:

— Approved cleaning procedures;
— Properly trained cleaning personnel;
— Validation of cleaning.

Cleaning and maintenance should include the following:

(a) The synthesis modules, production hot cell and workbench should be cleaned with sterile isopropyl or ethyl alcohol before each operation.
(b) The clean room should be cleaned with bactericidal detergent weekly, with a change of detergent every six months.
(c) The synthesis module and production hot cell should be serviced according to the schedule recommended by the manufacturer.
(d) The dispensing hot cell should be validated once a year with changes to HEPAs.
(e) The clean room should be validated once a year.
(f) Quality control equipment (HPLC, gas chromatography (GC) and the thin layer chromatography (TLC) scanners) should be serviced once a year.

6.6. MATERIAL CONTROLS 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 increased emphasis on ensuring that all starting materials, also referred to as active pharmaceutical ingredients (APIs), are prepared under an appropriate quality management system. Active pharmaceutical ingredients can be raw materials, and intermediate, isolated or purified substances used in the preparation of PET tracers intended for administration to humans. They should conform to API guidelines. Regular quality reviews of API should be conducted with the objective of verifying their consistency (see Table 11 on guidance on the COA).

Materials should be carefully checked using an SOP before being used in synthesis. 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 COA should be checked to confirm the quality and identity; for example, the melting point of mannose triflate. Approved raw materials should be stored in a clearly identified secured location away from material not related to production. Only approved raw materials can be used in the production process. Rejected raw materials should be separated, destroyed or returned to the vendor as soon as possible.

A quality assessment of the components (including ingredients, reagents, target solutions and gases), containers and caps on bottles of medicine, and other materials (e.g. transfer lines, purification devices and membrane filters) that come into contact with the final PET agents, should be conducted.

TABLE 11. GUIDANCE ON THE CERTIFICATE OF ANALYSIS

Number	Key point
1	A COA from a third party can be used to ensure the quality of materials (APIs).
2	The radiopharmacist or qualified person (QP) must ensure that the COA is from a competent organization.
3	The COA must: —State the organization issuing it —Clearly state the name, product code and specification (e.g., Ph.Int. or EP) —State batch number of the API, method of testing, date, result, identified impurities, limitations of test and conformance —State who is the authorizing person (QP).
4	Even with a COA, the identity may need to be confirmed.
5	The purchaser is responsible for the correctness of the material used in the PET tracer.
6	It is useful to have knowledge of the COAs of any intermediates.
7	Conformance of processing and packaging.
8	If no expiry date is designated by the manufacturer, an expiry date is to be assigned to the component, material or supply on the basis of a knowledge of its physical and chemical properties, and prior experience with its use.

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 components, containers and caps on bottles of medicine, materials and supplies used for the compounding of PET radiopharmaceuticals.

6.7. WELL CONTROLLED SYNTHESIS PROCEDURES

The synthesis procedures should be pharmacopoeial or properly validated. The equipment used should be fully validated, tested and cleaned before each synthesis. The transfer lines should be cleaned. The staff performing the synthesis should be well trained in synthesis and aseptic technique. There should be proper recording of the synthesis procedures, and ongoing reviews should be carried out. All details related to manufacture, including raw materials, staff and environment, should be recorded in the batch

production records. The PET radiopharmaceutical should be synthesized according to SOPs, to ensure consistency and reproducibility. A minimum of three consecutive successful trials is essential. Environmental monitoring and a stability test of the products should be carried out simultaneously. All in-process QC processes and equipment should be validated in addition to the preparation process. Any change should be written and verified. When changes in compounding procedures are implemented or reviewed, written records should be updated and verified. Records should be routinely verified at a minimum interval of two years, to ensure that they are current. All outdated copies should be retrieved, cross-checked and ideally destroyed. A single copy of the written compounding procedures should be retained, separate from the master file, for review purposes.

6.7.1. Controls over computers

Computer and related automated equipment should be appropriately controlled to ensure that only authorized personnel can institute changes in compounding software. Such changes should be documented and verified, and only current versions of the software should be available for use in PET agent compounding procedures. A magnetic, optical or memory chip copy and printout of the current computer software programs used in the compounding of each PET agent is to be maintained within a master file located in the PET facility.

Whenever there is a change in the compounding procedures, computer software program or component specifications, verification procedures and studies must be conducted. A minimum of three consecutive studies verifying minimum acceptance criteria are to be performed prior to approval for human use of new or revised compounding procedures for a given PET agent. Routine and verified processes being used with consistent success should undergo a minimum of one verification study that meets acceptance criteria annually. Outdated copies of computer software programs shall also be retained, separate from the master file, for review purposes.

6.7.2. Final label

The final PET radiopharmaceutical container or dispensing–administration assembly should be labelled prior to initiation of the compounding procedure. This label should conform to the requirements of Ph. Int. The information given in Table 12 should appear on the label of the immediate container, for example, a vial or syringe (p: primary packaging; s: secondary packaging).

TABLE 12. FINAL LABEL REQUIREMENTS

'Caution — Radioactive Material'	p and s
The name of the radiopharmaceutical preparation	p and s
The route of administration	p and s
A statement that the product is radioactive	p and s
The total radioactivity present at a stated time	p and s
The expiry date or expiry period	p and s
A batch or lot number	p and s
In the case of solutions, the total volume together with a dosage form	p and s
Special storage requirements with respect to temperature and light	s
In some cases, the name and concentration of any added microbial preservatives	s
Any additional national or regional legal labelling requirements	p and s

Note: In the case of a solution, a statement of the radioactive concentration (e.g., in MBq/mL of the solution) may be given, instead of a statement of the total radioactivity.

6.8. QUALITY CONTROL

Quality control procedures should use pharmacopoeial methods and be validated with proper reference standards. Staff should be well trained in the QC procedures. Quality control of incoming raw materials, packaging materials, intermediate materials and finished products should also be conducted. The results should be properly recorded and constantly reviewed. Most pharmacopoeial standards require a dedicated HPLC system for PET QC such as the one shown in Fig. 14.

The QC instruments should be fully validated, and routinely maintained and calibrated. The use of each QC instrument should be restricted to properly trained staff and should be properly recorded. The staff performing the QC procedure should be different from the staff performing the production. The person in charge of QC has the final authority to decide if a certain batch has passed or failed the QC checks.

There should be quality assessment of analytical supplies (e.g. solvents and chromatography columns), sterility test media, endotoxin test reagents and other supplies intended for use in PET agent QC procedures.

FIG. 14. A typical HPLC set-up for PET QC.

The following principles apply to well controlled QC:

(a) Staff involved in preparation cannot perform QC on the same batch.
(b) The decision of the person performing the QC test is final.
(c) The radiochemical purity of every batch should be tested by TLC or HPLC (and should ideally conform to pharmacopoeial methods).
(d) Sterility and endotoxin tests (which should ideally conform to pharmacopoeial methods) should be performed.
(e) A bubble point test should be performed on each incoming batch (with a minimum of three items per batch).
(f) A bubble point test on every batch of product should be performed within 24 h.
(g) The following data should be included in QC records:
    (i) Batch number;
    (ii) Date (and time) of performing the QC test;
    (iii) Name of staff;
    (iv) Manufacturer, product number and lot number of TLC plate and/or HPLC column;

(v) Relative fraction (R_f) or retention time;
(vi) Percentage radiochemical purity (optional) if already indicated on the report from the TLC scanner or HPLC;
(vii) The acceptance limit;
(viii) Conformity of acceptance limit/action list if the batch has been failed.

6.9. RELEASE PROCEDURES

Before being released for clinical use, the drug product should be guaranteed until a release for a supply check has been done. The objective of the release for supply checks is to confirm that the synthesis has been carried out in a well controlled manner.

The release for supply checks should include at least confirmation that:

(a) The starting materials have been checked and approved for synthesis;
(b) The synthesis of the drug product has been carried out according to SOPs;
(c) The synthesis equipment has been cleaned and tested;
(d) The synthesis environment has been monitored;
(e) The labels are correct.

6.9.1. Line clearance

The compounding and dispensing area, as well as all equipment related to cleanliness, should be inspected immediately before use. Before initiation of compounding and dispensing activities, extraneous materials and labels must be removed from involved areas and equipment.

6.10. OUT OF SPECIFICATION PROCEDURES

Results of QC tests showing the quality of the radiopharmaceutical to be less than that required by the accepted product specifications are a clear indication that one or several of the production steps have not been carried out properly. The reasons for the malfunctioning of the production process must be established, and it is essential that there be an SOP on the procedure to perform an OoS investigation. The results of this investigation have to be properly recorded and the conclusions of the investigation should be clearly formulated to recommend corrective actions, in order to restore high quality radiopharmaceutical production. The monitoring of the malfunctioning

process step should also often be improved to prevent future failure in radiopharmaceutical production. Revalidation may be necessary.

6.11. RECALL, COMPLAINT AND CHANGE CONTROL PROCEDURES

Good radiopharmacy practice requires that there should be a procedure to handle customer complaints, recall of products that have been released and change control. The SOP for recall, complaint and change control should include information on:

— The personnel responsible for making the recall decision;
— The person operationally responsible for the recall;
— The timeframe within which the recall procedure must be completed;
— Records of the recall;
— The follow-up measures taken after the recall;
— Routine recall drills;
— Change control procedures.

6.12. INTERNAL AUDIT PLANS

The objective of internal audits is to examine the QA system objectively, resulting in a continuous improvement of the QA system (details of self-audits are given in Tables 13 and 14). Good radiopharmacy practice requires that:

(a) There should be an overall plan for the performance of internal audits, to ensure that all departments of the production facility are audited at regular intervals.
(b) Internal audits should be carried out on a regular basis. These audits should be conducted objectively and be properly recorded.
(c) If the results of an audit are not satisfactory, corrective actions and changes should be proposed, carried out and documented.

In Table 13, standards are set at three levels, A, B and C:

A Class A standards are those required by legislation, IAEA technical documents or external standard setting bodies. Any failure to reach the class A standard is therefore regarded as serious, and urgent corrective actions should be instituted in such cases.

B Class B standards are those that, although not compulsory, are expected to be reached by all departments. In cases of failure to achieve the class B standard, corrective actions are recommended.

C Achievement of class C standards is desirable but not essential. Corrective actions to achieve the class C standard may improve overall function.

In conclusion, a detailed QA programme is necessary to reduce the risks of errors, misunderstandings and contamination in the manufacture of radiopharmaceuticals. Successful application of good practice in radiopharmaceutical preparation requires understanding and sincere commitment at the management level. It also requires commitment and cooperation among all groups of staff. The resultant product should have quality built in to it, and be safe and efficacious.

7. QMS — B: VALIDATION

7.1. INTRODUCTION

These guidelines focus mainly on the overall concept of validation and serve as general guidelines only. They are not intended to be prescriptive in specific validation requirements. Validation of specific processes and products, such as sterile or aseptic product manufacture or preparation, requires further consideration.

Not all of the many factors affecting the different types of validation are defined and addressed in this publication. Manufacturers should plan validation appropriately in a manner that will ensure regulatory compliance, and ensure that product quality, safety and consistency are not compromised.

TABLE 13. PET RADIOPHARMACY AUDIT

Item No.	Component	Class	Verifiable: Manual, reference documentation, SOP, QC data, patient file, etc.	Yes/No	Comments/ planned action	Date achieved
	Quality management					
3c.1	Are standards set for the nuclear medicine service, preferably in the form of a quality manual and/or flow charts and/or SOPs?	B				
3c.2	Are there systems for monitoring compliance with standards, with defined criteria of acceptability?	B				
3c.3	Is there an SOP for handling non-compliance, including recording and correction/prevention?	B				
3c.4	Is this specification used for acceptance testing and validation of all equipment?	B				
3c.5	Are there documented policies and protocols for the operation, QC and assurance for equipment in use, and do these policies conform to the manufacturer's instruction manual?	A				

TABLE 13. PET RADIOPHARMACY AUDIT (cont.)

Item No.	Component	Class	Verifiable: Manual, reference documentation, SOP, QC data, patient file, etc.	Yes/No	Comments/ planned action	Date achieved
3c.6	Are SOPs written and independently approved for each procedure or activity associated with the operation of the unit?	B				
3c.7	Are SOPs reviewed at specified intervals?	B				
3c.8	Are records of validation available for all of the following areas: —Operation design? —Installation validation? —Process operational qualification? —Microbiological validation of operators, processes and facilities? —Validation of the cleaning process? —Validation of training? —Validation of computer systems and software?	B				
3c.9	Is there a procedure to ensure that any equipment or material that fails a quality test is not used unless specifically authorized by a designated member of staff?	A				

TABLE 13. PET RADIOPHARMACY AUDIT (cont.)

Item No.	Component	Class	Verifiable: Manual, reference documentation, SOP, QC data, patient file, etc.	Yes/No	Comments/ planned action	Date achieved
3c.10	Is there an SOP for checking and validating both hardware and software?	A				
3c.11	Are details of the responsible professional provided?	A				
3c.12	In practice, who approves all systems of work and documentation associated with radiopharmaceutical preparation and processes?	B				
3c.13	In practice who and how are the requirements met for verifying that all prescriptions or protocols have been adhered to before preparation commences?	A				
3c.14	In practice by whom and how are the requirements met for performing final checking of all products prepared, and released for issue?	A				
	Staffing					
3c.15	Is the radiopharmacy unit operated under the direction of a person with appropriate training as defined by local or national regulations?	A				

TABLE 13. PET RADIOPHARMACY AUDIT (cont.)

Item No.	Component	Class	Verifiable: Manual, reference documentation, SOP, QC data, patient file, etc.	Yes/No	Comments/ planned action	Date achieved
3c.16	Is there a staff training manual in writing for all grades of staff?	B				
3c.17	Are there training records for training appropriate to the tasks performed and FDG, including aseptic practice?	A				
3c.18	Who approves formal release of radiopharmaceuticals for patient use (ideally this must be a nominated radiopharmacist or qualified/authorized person (QP))?	A				
3c.19	Is staff training provided for performing final checking of all products prepared before release for patient use?	A				
3c.20	Are there annual performance reviews to check the competence of staff to perform adequately and are additional training needs identified to rectify deficiencies?	B				
	Facilities					
3c.21	Does the unit have adequate lighting, ventilation and environmental conditions (including appropriate finishes to walls, floors and ceilings)?	B				

TABLE 13. PET RADIOPHARMACY AUDIT (cont.)

Item No.	Component	Class	Verifiable: Manual, reference documentation, SOP, QC data, patient file, etc.	Yes/No	Comments/ planned action	Date achieved
3c.22	Where clean rooms are used, are overpressures recorded daily, and are gauges on LAF cabinets and isolators also monitored daily where appropriate?	B				
3c.23	Are the air velocities determined at inlet ducts or terminal inlet filters supplying clean rooms, and is the rate of air change calculated for each room?	B				
3c.24	Are pressure differentials across all HEPA filters recorded daily?	B				
3c.25	Are there records of the determination of air velocities carried out for LAF cabinets or hot cells?	B				
3c.26	What grade of clean environment is operationally achieved within the changing facilities and support area?	B				
3c.27	Is challenge testing of the HEPA filters in hot cells, laminar airflow cabinets and isolators carried out annually?	B				

TABLE 13. PET RADIOPHARMACY AUDIT (cont.)

Item No.	Component	Class	Verifiable: Manual, reference documentation, SOP, QC data, patient file, etc.	Yes/No	Comments/ planned action	Date achieved
3c.28	Is an operator protection test carried out annually on microbiological safety cabinets using the potassium iodide disc method?	A				
3c.29	For negative pressure hot cells, is a routine leak test performed?	B				
3c.30	If radiopharmacy isolators are used, are there visual inspection records of the gloves or gauntlets used daily? Is there a record of integrity testing by means of a glove (manometer) tester before preparation takes place, and after any change of gloves or gauntlets?	A				
3c.31	Are there records and logs kept for all equipment, irrespective of whether maintenance and calibration is performed in-house or by external contractors?	B				
3c.32	Is there a system of planned preventive maintenance?	B				

TABLE 13. PET RADIOPHARMACY AUDIT (cont.)

Item No.	Component	Class	Verifiable: Manual, reference documentation, SOP, QC data, patient file, etc.	Yes/No	Comments/ planned action	Date achieved
	Purchase of materials					
3c.33	Is there a specification prepared for each starting material, component and reagent?	B				
3c.34	Are all goods that are received checked against the order for correctness of delivery?	B				
3c.35	Are records of batch numbers and quantities received kept?	B				
3c.36	Is there a visual inspection prior to acceptance?	B				
3c.37	Do products or components have approval, CE marks or COA, etc?	A				
3c.38	How many unauthorized or unapproved products or radio-chemicals are used each year?	A				
3c.39	Who takes responsibility for purchasing and quality?	B				
	Preparation protocols					
3c.40	Are there written procedures for the entire operation?	B				

TABLE 13. PET RADIOPHARMACY AUDIT (cont.)

Item No.	Component	Class	Verifiable: Manual, reference documentation, SOP, QC data, patient file, etc.	Yes/No	Comments/ planned action	Date achieved
3c.41	Are there written procedures for aseptic preparation and for dispensing of unit doses?	B				
3c.42	Are there written procedures for calibration assay of each PET isotope and calibrator check before preparation and dispensing?	A				
3c.43	Is each step checked and cross-checked on the working document at the time of completion of that step?	B				
3c.44	Are there written procedures for the operation of the PET radionuclide generator? (if applicable)	B				
3c.45	Are there set criteria before release of a preparation for patient use?	B				
3c.46	Is there a system of documentation in operation such that the history of each preparation can be adequately traced from the starting material, equipment used, operator, QC and final usage?	B				

TABLE 13. PET RADIOPHARMACY AUDIT (cont.)

Item No.	Component	Class	Verifiable: Manual, reference documentation, SOP, QC data, patient file, etc.	Yes/No	Comments/ planned action	Date achieved
	Quality assurance/quality control					
3c.47	Are daily QA checks performed on radionuclide calibrators, specifically for functionality of PET settings?	A				
3c.48	Are checks of constancy using a long lived radioactive source with radionuclides traceable to national standards performed?	B				
3c.49	Is regular calibration undertaken for radionuclides, containers, and/or the sample volume or configuration used?	A				
3c.50	Is there a linearity check of the dose calibrator response over the complete range of activities for all measured PET radionuclides at least annually?	A				
3c.51	Are there a set of programmes for checking the QC of radiopharmaceuticals against standards in EP, USP or Ph. Int?	B				
3c.52	What QA checks are performed before purchase of a radiopharmaceutical?	B				

TABLE 13. PET RADIOPHARMACY AUDIT (cont.)

Item No.	Component	Class	Verifiable: Manual, reference documentation, SOP, QC data, patient file, etc.	Yes/No	Comments/ planned action	Date achieved
3c.53	How are changes that affect the radiopharmaceutical quality checked and managed? For example, the following factors: source of any starting material, diluents or vehicle used, needles, syringes, swabs and sterile containers?	A				
3c.54	Is breakthrough checked on the first eluate from a PET generator? Are periodic checks of breakthrough on a PET generator conducted?	A				
3c.55	Is the radiochemical purity of generator eluates checked?	B				
3c.56	Are tests of radiochemical purity conducted regularly? For example, on first use of a new batch or first delivery of a starting material or a critical component for PET tracer preparation?	B				
3c.57	Are tests and trend analysis of radiochemical purity regularly carried out?	B				
3c.58	Are there routine checks of residual solvent/s in each preparation?	B				

TABLE 13. PET RADIOPHARMACY AUDIT (cont.)

Item No.	Component	Class	Verifiable: Manual, reference documentation, SOP, QC data, patient file, etc.	Yes/No	Comments/ planned action	Date achieved
3c.59	Is pH testing regularly carried out?	B				
3c.60	Are there routine checks of half-life and radionuclide purity of radioactivity content?	A				
3c.61	If rapid alternative methods are employed, how often are these cross-checked with standard techniques?	A				
3c.62	Is there an analysis of the results of radiochemical purity tests subject to both intra- and inter-operator variability even when carried out according to a strict protocol?	B				
3c.63	How often are bubble point checks on any batch of sterilizing filters undertaken?	A				
3c.64	Are checks on chromatography and/or HPLC reproducibility undertaken?	B				
3c.65	Is there a written procedure for dealing with products that fail to meet the required standard?	B				
3c.66	Are there written procedures for the recall of defective products?	B				

TABLE 13. PET RADIOPHARMACY AUDIT (cont.)

Item No.	Component	Class	Verifiable: Manual, reference documentation, SOP, QC data, patient file, etc.	Yes/No	Comments/ planned action	Date achieved
3c.67	Is there a record of complaints and any associated follow-up and investigation?	B				
3c.68	Is there a written procedure for regular contamination surveys of the unit?	A				
3c.69	Is there a written procedure for the microbiological testing of the unit?	B				
3c.70	Are there records of microbiological monitoring of the environment carried out using a combination of settle plating, surface sampling, active air sampling and finger dabs, at appropriate intervals?	B				
3c.71	Is there a written procedure for the verification of operator technique (e.g., broth transfer)?	B				
3c.72	Is sterility of remnants or residue of PET preparation tested each week?	B				
3c.73	Are there records of bacterial endotoxin testing?	B				

TABLE 13. PET RADIOPHARMACY AUDIT (cont.)

Item No.	Component	Class	Verifiable: Manual, reference documentation, SOP, QC data, patient file, etc.	Yes/No	Comments/ planned action	Date achieved
3c.74	Is there a system of recorded self-inspection and evaluation of reports?	B				
3c.75	Is there a system for an external audit or peer review process?	B				
	Waste					
3c.76	Are there written procedures for the disposal of radioactive and non-active waste?	A				
3c.77	Is there an audit of arrival and use of radioactive materials, including an audit of disposal of all radioactive waste?	A				
3c.78	Are there usage logs of solid sources and logs of transfers for disposal?	A				

TABLE 14. AUDIT SUMMARY PRIORITIES: A RADIOPHARMACY MAJOR CORRECTIVE SUMMARY

Item No.	Comment	Action	Timeframe	Date achieved

Validation is an essential part of good radiopharmaceutical practices and GMPs. It is, therefore, an element of the quality management and QA programme associated with a particular product or process. The basic principles of QA have, as their goal, the production of products that are fit for their intended use. These principles state that:

(a) Quality, safety and efficacy must be designed and built into products.
(b) Quality cannot be added to products through inspections or tests.
(c) Each critical step of the manufacturing process must be validated. Other steps in the process must be under control, to maximize the probability that the finished product meets all the quality and design specifications.

Validation of processes and systems is fundamental to achieving these goals. Design and validation can help to establish confidence that medicinal products will consistently meet their product specifications. The validation process is crucial for PET tracers due to their very short half-lives. Ongoing validation is fundamental and cannot be emphasized enough in the preparation and use of PET tracers in human applications.

The documentation associated with validation includes:

— SOPs;
— Specifications;
— VMPs;
— Qualification protocols and reports;
— Validation protocols and reports.

The implementation of validation work requires considerable resources, such as:

(a) Time — In general, validation work is subject to rigorous time schedules.
(b) Financial — Validation often requires specialized personnel and expensive technology.
(c) Human — Collaboration of experts of various disciplines (e.g. a multi-disciplinary team, comprising QA, engineering, manufacturing and other disciplines, depending on the product and process to be validated).
(d) Robust operating systems.

It is important to take note of the essential terminology (which is included in Section 2) used for validation.

7.2. RELATIONSHIP BETWEEN VALIDATION AND QUALIFICATION

Validation and qualification are essentially components of the same concept. The term qualification is normally used for equipment, utilities and systems, and validation for processes. In this sense, qualification is part of validation. Validation also refers to the overall concept of validation.

7.3. QUALIFICATION OF SYSTEMS AND EQUIPMENT

With PET, much of the focus is on cyclotrons, hot cells and other pieces of equipment. It is therefore essential to start with appropriate qualification of the system, such as that described in Fig. 15.

Qualification of systems should take place before that of equipment. New systems and equipment should undergo all the stages of qualification, including design qualification, installation qualification, operational qualification and performance qualification, as appropriate. The manufacturer should have a qualification policy; however, end users should also have a robust policy for qualification of systems and equipment. There should be independent assessment and comparison before final acceptance of the system or equipment. The extent of the qualification should be based on the criticality of the system or equipment. A single test is inadequate. Ideally, assessments should be undertaken over a number of days, if not over a period of time. The continued suitable performance of equipment is important to ensure batch-to-batch consistency. Critical equipment should therefore be qualified.

Qualification should be carried out in accordance with predetermined and approved qualification protocols. The results of the qualification should be recorded and reflected in qualification reports. The relevant documentation associated with qualification, including SOPs, specifications and acceptance criteria, certificates and manuals, should be maintained.

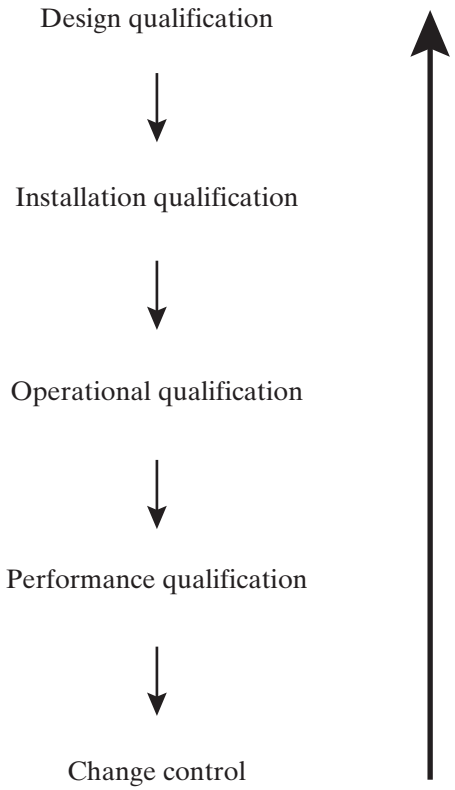


FIG. 15. The stages of qualification.

7.4. INSTALLATION QUALIFICATION

Systems and equipment should be correctly installed in accordance with an installation plan and installation qualification protocol. Measurement, control and indicating devices should be calibrated against appropriate national or international standards that are traceable. There should be documented records for the installation (an installation qualification report) to indicate the satisfactory state of the installation, and these records should include the details of the supplier and manufacturer, the name, model and serial number of the system or piece of equipment, the date of installation, the spare parts held in storage, relevant procedures and certificates.

7.5. OPERATIONAL QUALIFICATION

Systems and equipment should operate correctly, and the operation should be verified in accordance with an operational qualification protocol. Standard operating procedures for operation should be finalized and approved. Operational qualification should include verification of operation of all system elements, parts, services, controls, gauges and other components. There should be documented records for the verification of operation (an operational qualification report) to indicate satisfactory operation. Systems and equipment should be released for routine use after completion of operational qualification, provided that all calibration, cleaning, maintenance, training and related tests and results were found to be acceptable. Figure 16 shows a typical schematic diagram of FDG operations and tubing from target vial up to synthesis vial and valves V7, V8 and V9. Replacing these tubes with PEEK coated tubes, and flushing the tubes and valves reduces damage considerably. The whole process is a major undertaking; however, it is essential.

7.6. PERFORMANCE QUALIFICATION

Systems and equipment should perform consistently in accordance with design specifications. The performance should be verified in accordance with a performance qualification protocol. There should be documented records for the verification of performance (performance qualification report) to indicate satisfactory performance over a period of time. Manufacturers should define the selected period over which performance qualification is carried out.

7.7. REQUALIFICATION

Periodic requalification should also be conducted. Requalification of systems and equipment should be carried out in accordance with a defined schedule. The frequency of requalification may be determined on the basis of factors such as analysis of the results relating to calibration, verification and maintenance. Requalification should also take place after changes have been made. The extent of requalification after a change should be determined on the basis of a risk assessment of the change. Requalification after a change should be considered as part of the change control procedure.

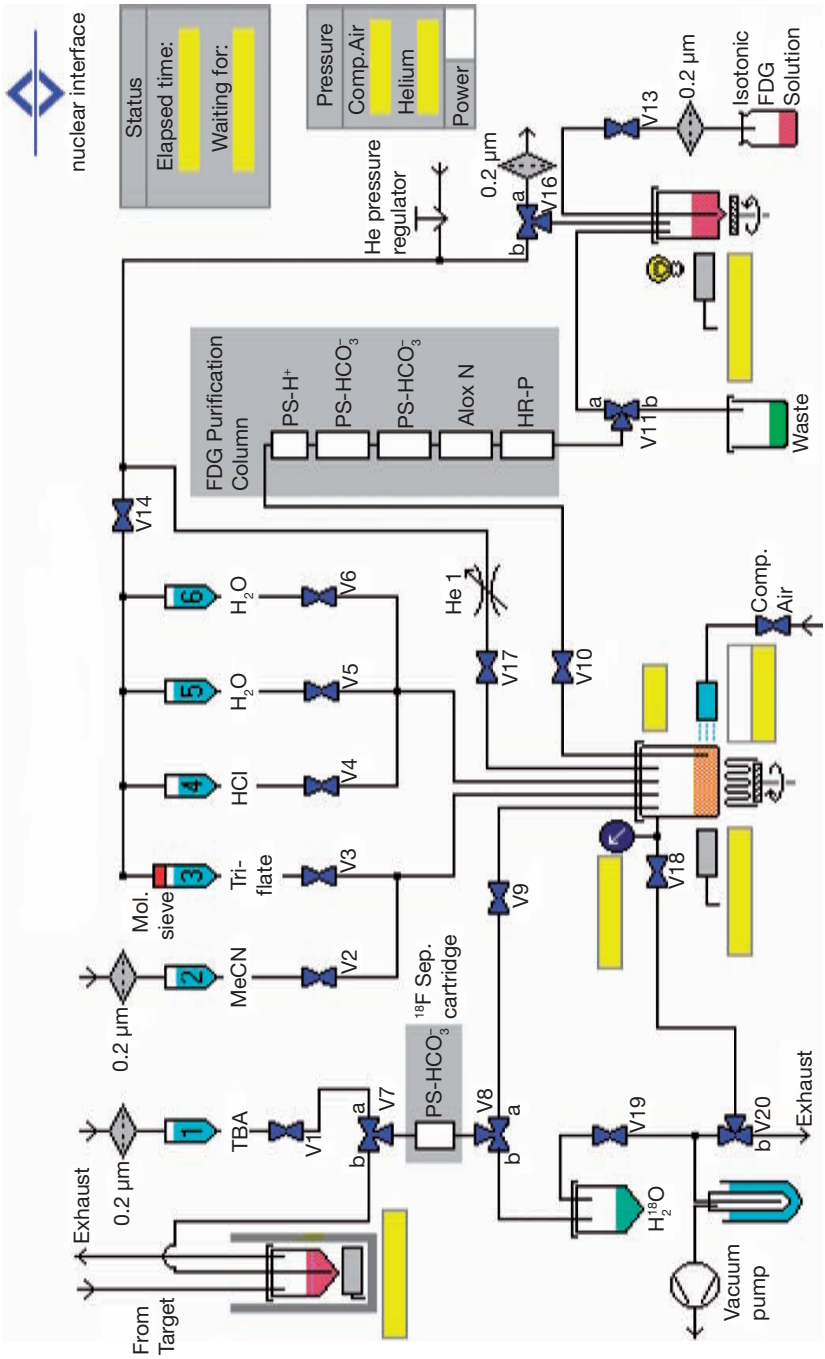


FIG. 16. A typical schematic diagram of 2-^{18}F-FDG synthesis.

7.8. ESSENTIALS OF VALIDATION

There should be an appropriate and sufficient system, including an organizational structure and documentation infrastructure, sufficient personnel (management and persons responsible for QA) and financial resources, to perform validation tasks in a timely manner.

Validation should be performed:

(a) For new premises, equipment, utilities and systems, and for processes and procedures;
(b) At periodic intervals;
(c) After major changes have been made.

Qualification should be completed before process validation is performed. The process of qualification should be logical and systematic, and it should start from the design phase of the premises, equipment, utilities and equipment.

A multidisciplinary team of personnel with appropriate qualifications and experience should be responsible for performing validation.

There should be proper preparation and planning before validation is carried out. Validation should be carried out in a structured way according to documented procedures and protocols. The outcome of the validation should be reflected in written reports.

When a new manufacturing formula or method is adopted, steps should be taken to demonstrate its suitability for routine processing. The defined process, using the materials and equipment specified, should be shown to consistently yield a product of the required quality.

With PET tracers, the objective is to monitor the process continuously. There should be a clear distinction between in-process controls and validation. In-process tests are performed during the manufacture of each batch using specifications and methods devised during the development phase.

Validation should be carried out over a period of time; for example, at least three consecutive batches (scale appropriately — the size of the batches should be appropriate) should be validated, to show consistency. Worst case situations should be considered. A risk assessment approach should be used to determine the scope and extent of validation.

More rigorous validation work is required to prove control of the critical aspects of operations.

7.9. CALIBRATION AND VERIFICATION

Calibration should normally be performed by officially recognized bodies. Personnel who are responsible for calibration and preventive maintenance should have appropriate qualifications and training. A calibration programme should be available and indicate information such as calibration standards and limits, responsible persons, calibration intervals, records and actions to be taken when problems are identified.

There should be traceability to the calibration standards (e.g. national, regional or international standards) used. Calibrated equipment, instruments and other devices should be labelled, coded or otherwise identified to indicate the status of calibration and the date when recalibration is due.

Calibration and verification of the equipment, instruments and other devices as applicable, used in production and QC, should be carried out at regular intervals. When the equipment, instruments and other devices have not been used for a certain period of time, their function and calibration status should be verified and shown to be satisfactory before use. Figure 17 illustrates erosion of miniature solenoid valves due to radiation damage incurred with use.

7.10. PERIODIC REVALIDATION

Periodic revalidations should be carried out, as process changes may occur gradually over a period of time, or because of wear of equipment.

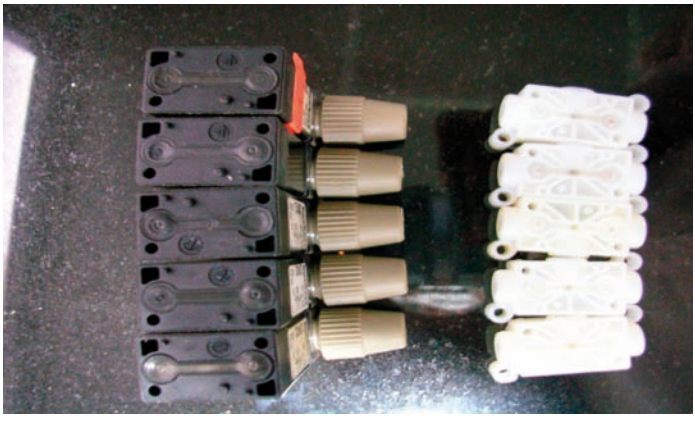


FIG. 17. Miniature solenoid valves eroded with use due to radiation damage.

The following matters should be considered when periodic revalidations are carried out:

— Master formulas and specifications;
— SOPs;
— Records (e.g. calibration, maintenance and cleaning records);
— Analytical methods;
— Wear and tear or critical service schedules.

The frequency and extent of revalidation should be determined using a risk based approach and a review of historical data. Revalidation should be carried out in accordance with a defined schedule. Processes and procedures should undergo revalidation to ensure that they remain capable of achieving the intended results. There should be periodic revalidation, as well as revalidation after changes.

Changes should be controlled in accordance with an SOP, as changes may have an impact on a qualified utility, system or piece of equipment, as well as with validated processes and or procedures. Changes should be formally requested, documented and approved before implementation. Records should be maintained. The procedure should describe the actions to be taken, including the need and extent of qualification or validation to be carried out. Changes requiring revalidation include:

(a) A change of starting materials (physical properties, such as density, viscosity or particle size distribution, may affect the process or product).
(b) A change of the manufacturer of the starting material.
(c) A transfer of processes to another site (including a change of facilities and installations that influence the process).
(d) Changes of primary packaging material (e.g. substitution of plastic for glass).
(e) Changes in the manufacturing process (e.g. different mixing times and drying temperatures).
(f) Changes in the equipment (e.g. addition of automatic detection systems, installation of new equipment, and major revisions to machinery or apparatus).
(g) Changes of equipment that involve the replacement of equipment on a like for like basis would not normally require a revalidation. For example, a new centrifugal pump replacing an older model would not necessarily require revalidation.

(h) Production area and support system changes (e.g. rearrangement of areas or a new water treatment method).
(i) The appearance of negative quality trends.
(j) The appearance of new findings based on current knowledge, for example, new technology.
(k) Changes in support systems.

7.11. ANALYTICAL METHOD VALIDATION

In general, analytical results should be reliable, accurate and reproducible. Specifications and standard test methods in pharmacopoeia (pharmacopoeial methods), or suitably developed specifications or test methods (non-pharmacopoeial methods), as approved by the national drug regulatory authority, should be used. Well characterized reference materials, with documented purity, should be used in the validation study. Pharmacopoeial methods used for determination of content or impurities in pharmaceutical products should also demonstrate that the methods are specific with respect to the substance (no placebo interference).

Verification or revalidation should be carried out when relevant (Table 15). This may be necessary when there are:

(a) Changes in the synthesis of the drug substance;
(b) Changes in the composition of the finished product;
(c) Changes in the analytical procedure;
(d) On transferral of analytical methods from one laboratory to another laboratory;
(e) Changes of major pieces of equipment/instruments.

Analytical methods, whether stability indicating or not, should be validated.

Non-pharmacopoeial methods should be appropriately validated. A justification should be provided if non-pharmacopoeial methods are used when pharmacopoeial methods are available. The justification should include data such as comparative data with the pharmacopoeia or other methods. The validated analytical method should be transferred from research and development to the QC unit when appropriate, with due care taken and appropriate consideration given.

TABLE 15. CHARACTERISTICS TO CONSIDER DURING ANALYTICAL VALIDATION

Analytical procedure characteristics	Identification	Testing for impurities		Assay–dissolution (measurement only) –content/potency
		Quantitative tests	Limit tests	
Accuracy	–[a]	+[b]	–	+
Precision repeatability	–	+	–	+
Intermediate precision[c]	–	+	–	+
Specificity	+	+	+	+
Detection limit	–	–[d]	+	–
Quantitation limit	–	+	–	–
Linearity	–	+	–	+
Range	–	+	–	+

[a] –: Characteristic is normally not evaluated.
[b] +: Characteristic should normally be evaluated.
[c] In cases where reproducibility has been tested, intermediate precision is not needed.
[d] The detection limit may be needed in some cases.

The characteristics that should be considered during validation of analytical methods include:

— Specificity;
— Linearity;
— Range;
— Accuracy;
— Precision;
— Detection limit;
— Quantitation limit;
— Robustness;
— System suitability testing (e.g. for chromatographic determination).

7.11.1. Accuracy

Accuracy is the degree of agreement of test results with the true value, or the closeness of the results obtained by the procedure to the true value. It is normally established on samples of the material to be examined that have been prepared to quantitative accuracy. Accuracy should be established across the specified range of the analytical procedure. (Note: It is acceptable that a 'spiked' placebo be used, where a known quantity or concentration of a reference material is used.)

7.11.2. Precision

Precision is the degree of agreement among individual results. The complete procedure should be applied repeatedly to separate, identical, samples drawn from the same homogeneous batch of material. Precision should be measured by the scatter of individual results from the mean (good grouping) and expressed as the standard deviation.

7.11.2.1. Repeatability

Repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure, for example, three concentrations/three replicates each, or a minimum of six determinations at 100% of the test concentration.

7.11.2.2. Intermediate precision

Intermediate precision expresses variations within a laboratory (usually different days, different analysts and different pieces of equipment). If the reproducibility is confirmed, intermediate precision is not required.

7.11.2.3. Reproducibility

Reproducibility expresses precision between laboratories.

7.11.3. Robustness

Robustness (or ruggedness) is the ability of a procedure to provide analytical results of acceptable accuracy and precision under a variety of conditions. The results from separate samples are influenced by changes in the operational or environmental conditions. Robustness should be considered

during the development phase, and should show the reliability of an analysis with respect to deliberate variations in method parameters.

Factors that can have an effect on chromatographic analysis include:

— Stability of test and of standard samples and solutions;
— Reagents (e.g. different suppliers);
— Different columns (e.g. different lots and/or suppliers);
— Extraction time;
— Variations of pH of a mobile phase;
— Variations in mobile phase composition;
— Temperature;
— Flow rate.

7.11.4. Linearity

Linearity indicates the ability to produce results that are directly proportional to the concentration of the analyte in samples. A series of samples should be prepared having analyte concentrations spanning the claimed range of the procedure. If there is a linear relationship, test results should be evaluated by appropriate statistical methods. A minimum of five (5) concentrations should be used.

7.11.5. Range

Range is an expression of the lowest and highest levels of analyte that have been demonstrated to be determinable for the product. The specified range is normally derived from linearity studies.

7.11.6. Specificity

Specificity (selectivity) is the ability to measure unequivocally the analyte in the presence of components such as excipients and impurities that may be expected to be present. An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities and assays.

7.11.7. Detection limit

The detection limit (limit of detection) is the lowest level of an analyte that can be detected, but not necessarily determined, in a quantitative fashion.

Approaches may include procedures that are instrumental or non-instrumental and could include those based on:

— A visual evaluation;
— The signal to noise ratio;
— The standard deviation of the response to the slope;
— The standard deviation of a blank;
— The calibration curve.

7.11.8. The limit of quantitation

The quantitation limit (limit of quantitation) is the lowest level of an analyte in a sample that can be determined with acceptable accuracy and precision. Approaches include procedures that are instrumental or non-instrumental and can include those stated above for the detection limit.

7.12. ASEPTIC VALIDATION

Most PET tracers are parental pharmaceuticals and therefore must be sterile, free of any pyrogenic substance and fit for administration to humans. Microbiological contamination remains a key risk. Taking into consideration the fact that normal pharmacopoeial sterility tests require 14 days incubation, these ultra-short-lived isotopes are released on validation data referred to as parametric release. Most PET tracers are therefore for 'immediate' use. Ideally, these products, prepared under controlled conditions for immediate use and administration, should be released as soon as practicable after preparation has been completed. As for all aseptically prepared products, there are six major sources of contamination:

(1) Airborne contamination;
(2) Contamination by touch;
(3) Surface contamination of components;
(4) Contamination during storage;
(5) Contamination during manufacture;
(6) Contamination during administration.

Ideally, most starting materials should be sterile, have sterile disposal components, and, where possible, closed procedures should be used to minimize microbial risks. Most PET tracers are prepared using multiple steps that necessitate strict control of the environment. The more viable organisms

there are in the immediate environment of the process at the point the container is breached, the greater the risk that some will enter the sterile product. Therefore, environmental standards, and the monitoring of those standards, are a fundamental part of every QA system. Wherever possible, semi-automated closed systems should be employed during preparation of PET tracers. In addition, wherever possible, the final product should be terminally sterilized.

If the product is stored or transported, it should be recognized that the optimum incubation temperatures for the majority of microorganisms are 25–35°C. At fridge temperatures (2–8°C), growth will usually be retarded or stopped. However, when organisms are moved from a fridge to room or body temperature, they will begin to grow after a recovery lag period of a few hours.

7.12.1. Operator

The training of operators is a critical aspect of aseptic processing and must be supported by regular validation of technique. Members of staff are a major source of contamination during production of PET tracers. All members of staff should receive training that will provide them with:

(a) An appropriate knowledge of GMP;
(b) A knowledge of radiation safety and local rules;
(c) Competence in the necessary aseptic skills;
(d) A knowledge of pharmaceutical microbiology;
(e) A working knowledge of all the elements essential to ensure that a product is fit for administration to humans.

Before undertaking aseptic work, all staff should be trained to an agreed level, which should be assessed. In particular, radiopharmacy staff must have achieved ‘adequate training’ as defined in rules related to the Ionizing Radiation (Medical Exposure) Regulations. Regular reassessment of the competency of each member of staff should be undertaken, and revision or retraining provided where necessary. The health status of staff should also be monitored regularly. Ideally, the day to day supervision of the service provided can be delegated to an authorized (radio)pharmacist or ‘qualified person’, provided that this pharmacist is given clear and precise training in both their duties and their limits of authority and responsibility.

7.12.2. Facilities

The performance criteria for the facility should be established prior to construction. The construction of the clean room should comply with good radiopharmacy practices. Access to the clean room should be restricted to authorized personnel. During use, only those persons who are actively involved in the process should be in the room. Good radiopharmacy practice requirements (Ph.Int.) recommend that walls, floor and ceiling should be smooth and impervious, to allow cleaning; bare wood and other unsealed surfaces must be avoided. To reduce dust accumulation and facilitate cleaning, there should be as little in the way of shelves and other projecting fittings as possible. The junctions of walls, floor and ceiling should be covered. The clean room must not contain a sink and must be entered through a changing room.

Changing rooms should be designed as airlocks and be used to provide separation of the different stages of change, and thus minimize microbial and particulate contamination of protective clothing. A social hand wash prior to entry to the room, followed by a disinfectant hand rub at the point of using gloves, is recommended. Hand washing facilities and water supplied to them should be regularly monitored for compliance with appropriate limits (e.g. the limits for potable water are 100 cfu/mL at 25°C and 10 cfu/mL at 35°C). Here, cfu/mL stands for colony forming units per millilitre.

There should be a support room from which materials can be passed into and out of the clean room through a hatch(es). The doors of the hatch(es) should be interlocked. A validated transfer process should be used to transfer materials into the clean room. Hot cells should be situated inside the clean room and away from the exit. Hot cells should be situated in a dedicated room used only for the hot cell and its ancillary equipment, as well as for related activities. The interior surfaces of the room (walls, floor and ceilings) should be smooth and free from cracks and open joints. They should not shed particulate matter and should allow easy and effective cleaning and sanitation measures.

Adherence to design specifications should be demonstrated by a clear programme of qualification and validation monitoring of the facility from commissioning onwards and during operational use. Ideally, clean air devices should run continuously. Should it be necessary to switch off such a device, for example, for cleaning or maintenance purposes, aseptic manipulation should not be carried out until a satisfactory environment has been achieved, as demonstrated by appropriate validation studies. Pressure differentials across inlet HEPA filters in cabinets, isolators and clean rooms, as well as between rooms with different classifications, should be constantly monitored. There should be alarms to indicate malfunctions. Pressure differentials should be recorded at defined intervals. All rooms and equipment used for preparation

activities should be cleaned and disinfected regularly and frequently in accordance with an agreed written procedure. The procedure should require written confirmation that cleaning has been carried out.

All areas associated with the aseptic preparation process should be assessed on the following occasions by the QA officer, for compliance with the appropriate standards:

— On commissioning;
— After maintenance procedures;
— Routinely at an agreed frequency.

A written report about the test data, indicating the significance of the results and recommended action, must be brought to the attention of all relevant staff, and full records kept on file for future reference.

The ability to sterilize hot cells and equipment with a gaseous agent should be considered at the time of purchase.

7.12.3. Cleaning

The correct level of cleanliness is such as to ensure that the properly designed and maintained area is clean and dry. Dry dusting alone is not recommended. Wet or damp cleaning with effective detergents should be the method of choice. Dedicated equipment should be used, and stored to minimize microbiological contamination. Mop-heads must be disposed of or resterilized after each cleaning session. Depending on the monitoring results, the use of a disinfectant may need to be considered. However, disinfection is difficult to achieve in an area with even small amounts of dirt. Cleaning and disinfecting agents should be free from viable microorganisms. Dilutions should be freshly prepared for each cleaning session and should be periodically monitored for microbiological contamination before and after use. Clean areas must be regularly cleaned and, where necessary, disinfected according to a written approval procedure. A log should be kept, indicating the areas cleaned and the agents used. This should be checked for completeness before filing. The use of 70% isopropyl alcohol is not adequate for disinfection, as it cannot kill bacterial spores. It is necessary to use sterile 70% isopropyl alcohol or, better, quaternary ammonium disinfectants. The disinfectant should be changed at regular intervals to avoid bacterial resistance.

Cleaning effectiveness should be routinely demonstrated, at least weekly, by microbiological surface sampling, for example, with contact plates or swabs. If results show an increase in microbiological contamination, the use of disinfection and/or an alternative disinfectant should be considered.

Surface disinfection prior to the introduction of items into hatches, both for conventional clean rooms and for isolators, is a vital step in preventing ingress of contamination into critical areas. The process must have a written and validated SOP. The contact time should be clearly stated, validated and accurately measured in practice.

7.12.4. Programme of monitoring

Each unit should have a programme of daily, weekly, monthly, quarterly and annual testing sessions, with all results documented and retained for inspection. Guidelines for recommended monitoring frequency are given in Tables 16–18, and these should be considered as a minimum requirement. The optimum frequency of testing will be a function of the individual unit and the activity within the unit. The programme should confirm that environmental control is maintained within the required standards. It is not a substitute for the continuous vigilance of operators in ensuring the correct functioning of all equipment.

7.12.5. Viable particle growth

Use should be made of a growth medium with low selectivity, i.e. one that is capable of supporting a broad spectrum of microorganisms, including bacteria, fungi, yeast and moulds:

— TSA (Tryptone soya agar) supports general microbial colonies.
— SDA (Sabouraud dextrose agar) supports yeast and fungal colonies.

TSA plates are incubated at 30–35°C for three days, whereas SDA plates are incubated at 20–25°C for five days.

When necessary to detect or search for a particular type of micro-organism, a selective culture medium should be used.

When undertaking microbiological testing, data require careful analysis because of imprecise testing methods compared with chemical and physical analyses, and the low expected levels of contamination. Warning levels should be established well within the guideline limits provided. Exceeding the warning levels on isolated occasions may not require any action other than examination of control systems; however, the frequency of exceeding the limit should be examined and it should be low. If the frequency is high or shows an upward trend, then action should be taken.

TABLE 16. MONITORING INTERVALS FOR A TYPICAL MICROBIOLOGICAL MONITORING PROGRAMME

Test	Critical zone	Clean room suite
Finger dabs	S[a]	n.a.[b]
Settle plates	S	W[c]
Surface sample	W	W
Active air sampler	3M[d]	3M

[a] Every session.
[b] n.a.: not applicable.
[c] W: weekly.
[d] 3M: every three months.

TABLE 17. MONITORING INTERVALS FOR A TYPICAL PHYSICAL MONITORING PROGRAMME

Test	Critical zone	Clean room suite
Pressure differential between rooms	n.a.[a]	D[b]
Pressure differential across HEPAs	W[c]	3M[d]
Particle counts	3M	3M
Air changes/hour, rooms	n.a.	3M
Air velocity, devices	3M	n.a.
HEPA filter integrity and leaks	A[e]	A
Operator protection test EN 12469:2000	A	n.a.
Hot cell/isolator: glove integrity	S[f]	n.a.
Hot cell/isolator: leak test	W	n.a.
Hot cell/isolator: alarm function	W	n.a.

[a] n.a.: not applicable.
[b] D: daily.
[c] W: weekly.
[d] 3M: every three months.
[e] A: annually.
[f] S: every session.

TABLE 18. ENVIRONMENTAL MONITORING OF CONTROLLED AREAS AND DEVICES IN OPERATION: LIMITS FOR MICRO-BIOLOGICAL TEST METHODS

Grade	Finger dabs (cfu/hand)	Settle plates (90 mm diameter) (cfu/4 h)	Surface sample (cfu/16 cm^2)	Active air sample (cfu/m^3)
ISO5 EU A (device)	<1	<1	<1	<1
ISO6 EU B	5	5	5	10
ISO7 EU C	n.a.	50	25	100
ISO 8 EU D	n.a.	100	50	200

Note: If settle plates are exposed for less than four hours, then the warning levels should be adjusted accordingly.

The microbiological media used must be proved to be capable of supporting a broad spectrum of bacterial and fungal growth. Settle plates should not be contaminated prior to their introduction into the aseptic processing environment. The COA for irradiated plates should be checked. When incubating the plates, it is necessary to perform a growth promotion test simultaneously. The objective is to ensure that the bacteria can grow in these culture plates, so that the chance of a false negative will be minimized. The details of growth promotion tests can be found in any major pharmacopoeia.

Process validation using broth to simulate the aseptic procedure should be performed initially, and subsequently on a regular basis. Careful monitoring of the critical zone of the hot cell is required. Figure 18 shows the result for a set of settle plates. This may be achieved by the exposure of settle plates, finger dabs of the gloved hand at the end of the work session and, if required, active air sampling.

7.13. STARTING MATERIALS, COMPONENTS AND OTHER CONSUMABLES

Starting materials should ideally be sterile products with a product approval. Where unlicensed products are used, it is incumbent upon the responsible pharmacist or qualified person to ensure that the product is of

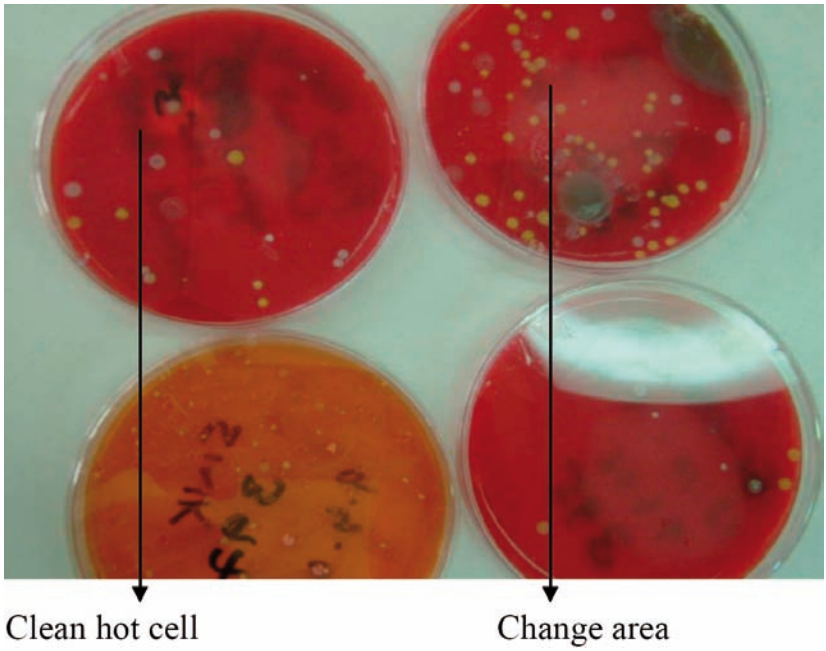


FIG. 18. Result for a set of settle plates: the plates show relative levels of microbiological growth between a relatively 'clean' hot cell and that encountered in the change area.

appropriate quality by means of specifications, COAs and certificates of conformity, QC tests or a combination of these. Figure 19 shows typical starting materials and a typical COA. Unlicensed materials should always be obtained from a supplier with an appropriate manufacturer's approval. Non-sterile starting materials should not be used. Figure 20 illustrates an example of a sterile component of synthesis assembly. All starting materials should be inspected according to approved written procedures for them being used in production. Those starting materials that have been inspected and approved for production should be stored separately.

Components, including reconstitution devices, syringes and needles, the product contact parts of filling systems, transfer tubing and final containers, should ideally be purchased pre-sterilized from the manufacturer. A typical disposable synthesis assembly is shown in Fig. 21. The product should be either CE marked or have a documented form of approval. It should be packaged in such a way that they can be passed into the aseptic environment without increasing the risk of product or environmental contamination. Sterile components should be stored so as to minimize any increase in the bioburden on the surface of the primary packaging. Any filters used should be

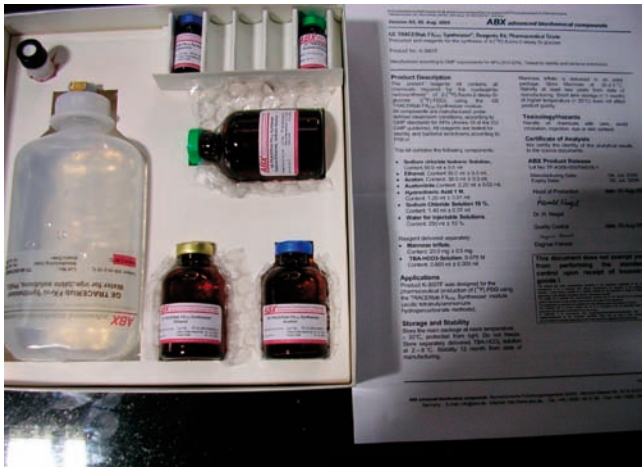


FIG. 19. Typical starting materials and a typical COA.

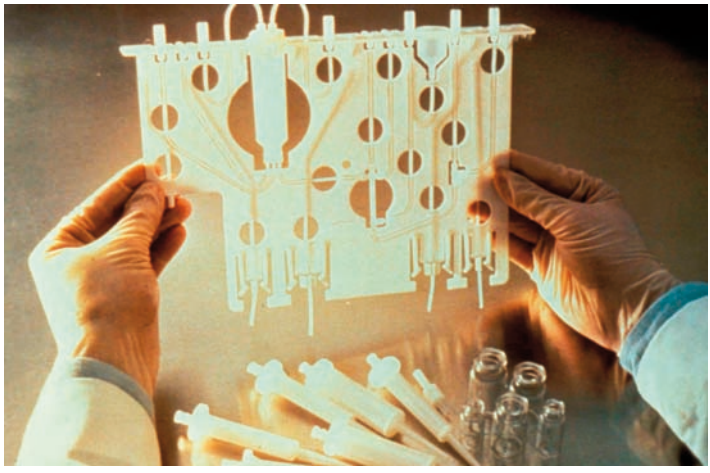


FIG. 20. A typical starting synthesis assembly.

pre-assembled by the manufacturer and guaranteed to be sterile. Sterile single use components should not be used beyond one work session.

Local sterilization of non-sterile components and equipment is acceptable provided that sterility is assured. Such sterilization processes should be validated, appropriately monitored and meet all current standards. An audit trail should be available.

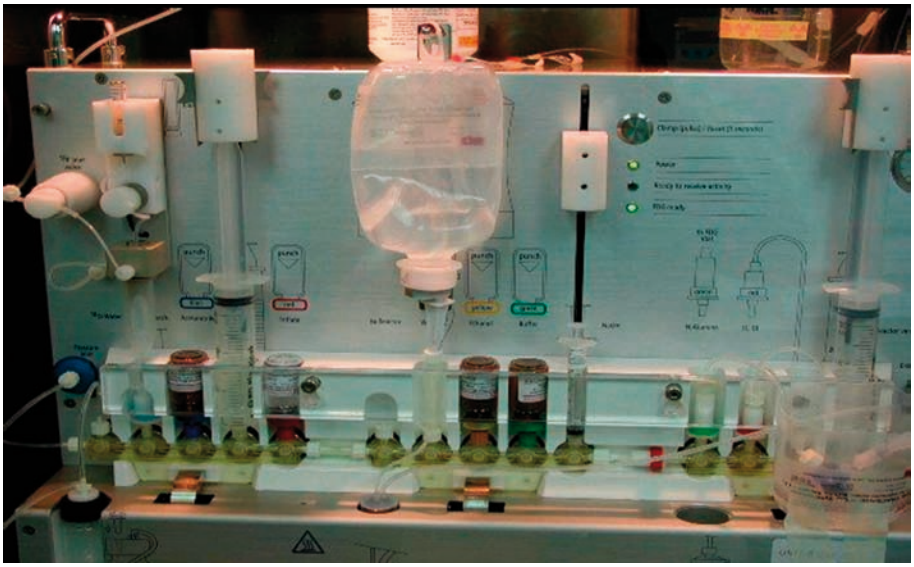


FIG. 21. A typical disposable synthesis assembly.

7.14. MONITORING OF FINISHED PRODUCTS

In addition to normal pharmacopoeial sterility and bacterial endotoxin testing, there should be a planned programme of physical, chemical and microbiological analysis of the finished product as appropriate. Samples may be obtained from unused products, extra samples specially prepared, or from an in-process sample taken at the end of the compounding procedure before the final seals are in place and before removal from the critical zone. Sampling of the final container after completion of preparation and prior to issue may be a threat to product integrity and is therefore not recommended.

The personnel in the testing laboratory must be fully conversant with the technical background and requirements in aseptic preparation, together with the validated methodology for analysing the products and samples. The responsible pharmacist or qualified person should ensure that the testing laboratory has a comprehensive knowledge of all tests, including pharmaceutical microbiology ones.

7.15. STABILITY AND BATCH DATA

7.15.1. Stability under the proposed storage conditions beyond expiry

For each radiopharmaceutical synthesized, a stability test should be performed under the proposed storage conditions beyond the expiry date. There should be stability data for at least three consecutive batches.

7.15.2. Stability at the upper limit of the radioactivity concentration

For each radiopharmaceutical synthesized, there should be stability data and release data for at least three consecutive batches at the upper limit of the proposed radioactivity concentration under the proposed storage conditions.

7.15.3. Stability at the upper limit of the proposed storage conditions

For each radiopharmaceutical synthesized, there should be stability and release data for at least three consecutive batches at the upper limit of the proposed storage conditions.

7.15.4. Post-approval stability tests

For each radiopharmaceutical approved, there should be a stability test for at least one batch per year.

7.16. CLEANING VALIDATION

Pharmaceutical products can be contaminated by a variety of substances, such as contaminants associated with microbes, previous products (both APIs and excipient residues), residues of cleaning agents, airborne matter, such as dust and particulate matter, lubricants and ancillary material, such as disinfectants and decomposition residues, which include product residue breakdown occasioned by, for example, use of strong acids and alkalis during the process and breakdown products of the detergents, acids and alkalis that may be part of the cleaning process.

The objective of cleaning validation is to prove that the equipment is consistently cleaned of product, detergent and microbial residues to an acceptable level, to prevent possible contamination and cross-contamination. There should be written SOPs providing details of the cleaning process for equipment and apparatus. Cleaning procedures should be validated. Cleaning

validation should be described in cleaning validation protocols, which should be formally approved, for example, by the QC or QA unit. In addition, clear swabbing (Fig. 22) and all validation studies should include a worst case scenario.

In preparing the cleaning validation protocol, the following questions should be considered:

— Disassembly of the system;
— Pre-cleaning;
— Cleaning agent, concentration, solution volume and water quality;
— Time and temperature;
— Cleaning interval;
— Flow rate, pressure and rinsing;
— Complexity and design of the equipment;
— Training of operators;
— Size of the system.

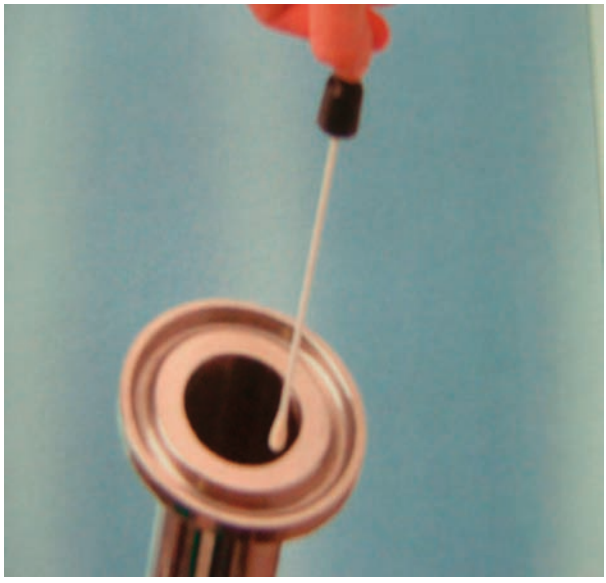


FIG. 22. The swabbing technique employed.

The cleaning validation protocol should include:

(a) The objectives of the validation process;
(b) The responsibilities for performing and approving the validation study;
(c) The description of the equipment to be used, including a list of the equipment, make, model, serial number or other unique code;
(d) The interval between the end of production and cleaning, and the commencement of the cleaning procedure (the interval may be part of the validation challenge study itself): the maximum period that equipment may be left dirty before being cleaned, as well as the establishment of the time after cleaning and before use;
(e) The microbiological levels (bioburden);
(f) The cleaning procedures (documented in an existing SOP, including definitions of any automated processes) to be used for each product, each manufacturing system or each piece of equipment;
(g) All the routine monitoring equipment used, for example, conductivity meters, pH meters and total organic carbon (TOC) analysers;
(h) The number of cleaning cycles to be performed consecutively;
(i) The sampling procedures used (direct sampling, rinse sampling, in-process monitoring and sampling locations) and the rationale;
(j) The data on recovery studies (the efficiency of the recovery of the sampling technique should be established);
(k) The analytical methods (specificity and sensitivity) used, including the limit of detection and the limit of quantification;
(l) The acceptance criteria (with the rationale for setting specific limits), including a margin for error and for sampling efficiency;
(m) The choice of the cleaning agent should be documented and approved by the quality unit, and should be scientifically justified on the basis of, for example:
    — The solubility of the materials to be removed;
    — The design and construction of the equipment and surface materials to be cleaned;
    — The safety of the cleaning agent;
    — Ease of removal and detection;
    — The product attributes;
    — The minimum temperatures and volumes of the cleaning agent and rinse solution;
    — The manufacturer's recommendations;
    — Revalidation requirements.

Personnel and operators who routinely perform cleaning should be trained and should have effective supervision. A programme for training revalidation is essential.

Normally only cleaning procedures for product contact surfaces of the equipment need to be validated. Consideration should be given to non-contact parts into which product or any process materials may migrate. Critical areas should be identified (independently from the method of cleaning), particularly in large systems employing semi-automatic or fully automatic clean-in-place systems. Microbiological aspects of equipment cleaning should be considered. Control of the bioburden through adequate cleaning and storage of equipment is important, to ensure that subsequent sterilization or sanitization procedures achieve the necessary assurance of sterility and the control of any pyrogenic substances in sterile processing. Equipment sterilization processes may not be adequate to achieve significant inactivation or removal of pyrogenic substances.

7.16.1. Analytical validation for cleaning validation

The analytical methods should be validated before cleaning validation is carried out. Samples should be taken throughout manufacture. Traces of the preceding products should be sought in these samples. (Note that the sensitivity of an assay may be greatly reduced by dilution of the contaminant.) Suitable methods that are sensitive and specific should be used where possible and may include chromatographic methods (e.g. HPLC, GC and high pressure, thin layer chromatography (HPTLC)). Other methods may include (alone or in combination) TOC, pH, conductivity, ultraviolet (UV) spectroscopy and enzyme linked immuno-sorbent assay (ELISA). The establishment of acceptance criteria for contaminant levels in the sample should be practical, achievable and verifiable. The rationale for the residue limits established should be logical and based on a knowledge of the materials involved. Limits may be expressed as a concentration in a subsequent product (ppm), in limit per surface area ($\mu g/cm^2$) or in rinse water (ppm). The three most common criteria are:

(1) Equipment should be visually clean (no quantity of residue should be visible on equipment after cleaning). Spiking studies should determine the concentration at which most active ingredients are visible. This criterion may not be suitable for high potency, low dosage drugs. Reports of consistent results of 4 $\mu g/cm^2$ are available.
(2) No more than 10 ppm of one product will appear in another product (the basis for heavy metals in starting materials).

(3) No more than 0.1% of the normal therapeutic dose of one product will appear in the maximum daily dose of a subsequent product.

7.17. VALIDATION OF COMPUTERIZED SYSTEMS

The purpose of validation of computerized systems is to ensure a degree of evidence (documented raw data), confidence (dependability and thorough, rigorous achievement of predetermined specifications), fitness for intended use, accuracy, consistency and reliability. Aspects to be validated include both the system specifications and the functional specifications. Periodic (or ongoing) evaluation should be performed after the initial validation (Table 19). There should be written procedures for performance monitoring, change control, program and data security, calibration and maintenance, personnel training, emergency recovery and periodic re-evaluation. Regular backups of all files and data should be made, and stored in a secure location to prevent intentional or accidental damage.

The system should be qualified after installation. The extent of the qualification should depend on the complexity of the system. The system should be evaluated and performance qualification, change control, maintenance and calibration, security, contingency planning, SOPs, training, performance monitoring and periodic re-evaluation should be addressed.

The following general good radiopharmacy practice requirements are applicable to computer systems:

(a) Verification and revalidation (after a suitable period of running a new system, it should be independently reviewed and compared with the system specification and functional specification);
(b) Change control (alterations should only be made in accordance with a defined procedure, which should include provision for checking, approving and implementing the change);
(c) Checks (data should be checked periodically to confirm that they have been accurately and reliably transferred).

Aspects of computerized operations that should be considered include:

— Networks;
— Manual backups;
— Input/output checks;
— Process documentation;

— Monitoring;
— Alarms;
— Recovery after shutdown.

TABLE 19. SUMMARY OF VALIDATION REQUIREMENTS FOR COMPUTER SYSTEMS

Hardware	Software
1. Types 1.1. Input device 1.2. Output device 1.3. Signal converter 1.4. Central processing unit (CPU) 1.5. Distribution system 1.6. Peripheral devices	1. Level 1.1. Machine language 1.2. Assembly language 1.3. High level language 1.4. Application language
2. Key aspects 2.1. Location, environment, distance and input devices 2.2. Signal conversion 2.3. Input/output operation 2.4. Command overrides 2.5. Maintenance	2. Software identification 2.1. Language 2.2. Name 2.3. Function 2.4. Input 2.5. Output 2.6. Fixed set point 2.7. Variable set point 2.8. Edits 2.9. Input manipulation 2.10. Program overrides
3. Validation 3.1. Function 3.2. Limits 3.3. Worst case 3.4. Reproducibility/consistency 3.5. Documentation 3.6. Revalidation	3. Key aspects 3.1. Software development 3.2. Software security
	4. Validation 4.1. Function 4.2. Worst case 4.3. Repeats 4.4. Documentation 4.5. Revalidation

7.17.1. Hardware

The validation and qualification of the hardware should prove that:

(a) The capacity of the hardware matches its assigned function (e.g. language compatibility).
(b) The hardware operates within its operational limits (e.g. for memory, connector ports and input ports).
(c) The hardware performs under the worst case conditions (e.g. long hours).
(d) Reproducibility/consistency (e.g. at least three runs covering different conditions) requirements are met.

Validation should be carried out in accordance with written qualification protocols, and the results should be recorded in the qualification reports.

7.17.2. Software

Software validation should provide assurance that computer programs (especially those that control manufacturing and processing) will consistently perform as they are intended to, within pre-established limits. When planning the validation, the following points should be considered:

(a) Function: Does the program match the assigned operational function (e.g. generate batch documentation and list different batches of material used in a batch)?
(b) Worst case: Has a validation been carried out under different conditions (e.g. speed, data volume and frequency)?
(c) Repeats: Is there an adequate number of repetitions (replicate data entries)?
(d) Documentation: Are protocols and reports adequate?
(e) Revalidation: Has there been a revalidation when significant changes have been made?

8. QMS — C: CRITERIA FOR ACCEPTANCE FOR PET SYNTHESIS

8.1. ROUTINE QUALITY CONTROL

Radiopharmaceuticals based on PET are short lived, leaving little time for tests to be performed after production and prior to their administration. Hence, the practice is to establish criteria for 'parametric release', wherein many production runs of the radiopharmaceutical are produced exclusively for all the QC tests listed above according to carefully devised SOPs. The SOP is said to be validated, to a high degree of confidence, when a repeated number of production runs are made and all of them comply with all the QC tests, without any failures. The high degree of confidence in the production method is justified, because all the QC parameters are met continuously in sequential production runs, i.e. the chance of a product failing the QC tests is negligible. This high degree of confidence permits the PET radiopharmacy laboratory to release a PET radiopharmaceutical product for patient use by carrying out the essential QC tests and doing the remaining tests 'post-decay', i.e. after several hours or even days (collectively testing samples from several production runs), by which time all the radioactivity has completely decayed. This is sometimes referred to as 'after the fact' testing.

This section describes the tests and procedures necessary for the release of a radiopharmaceutical for use. There are certain tests that need to be conducted, and stated criteria met, at the time of release, while other tests can be performed after the release of a radiopharmaceutical. Post-release tests are subject to initial validation tests. One should be aware that there are certain exceptions to this rule. Some tests are dependent on the radiopharmaceutical and may not apply to all radiopharmaceuticals. For example, a specific activity test that may apply to ligands for receptor studies may be irrelevant to tracers for metabolism studies. In addition, there are situations in which different sets of parametric release criteria have to be applied to certain radiopharmaceuticals due to the extremely short half-life of the isotope involved, for example, ^{15}O-water and bacterial endotoxin testing.

There are certain tests that are essential to ensure product safety and efficacy. These tests are listed below. These tests should be performed prior to the release of PET radiopharmaceuticals. A designated person (or team) shall be responsible for ensuring that these activities are carried out and completed properly.

There are three areas within QA for parametric release, comprising assurance of:

(1) Production;
(2) Product quality;
(3) Usage.

8.1.1. Assurance of production

It is important to ensure the integrity and consistency of radiopharmaceutical preparations.

8.1.1.1. Starting materials

The acquisition, acceptance and use of starting (raw) materials of high quality should be documented and verified.

8.1.1.2. Synthesis precursor

The acceptance of the pharmaceutical precursor (main component, the API) should be documented, and it should be ensured that the acceptance criteria are followed rigorously.

8.1.1.3. Facilities

It is important to ensure that the workplace is clean and free of contamination.

To ensure assurance of production, the following requirements should be adhered to:

(a) It is important to adhere to the expiry dates of the chemicals and components used.
(b) A laminar flow hood should be used to assemble the final product collection vials.
(c) A validated cleaning procedure for the production module should be employed to avoid cross-contamination.
(d) Only written procedures should be used following approval.
(e) There should be a training programme for the production and QC staff.
(f) Drug components that are used in production should be appropriately labelled, stored and separated from chemicals used for other applications.

8.1.2. Assurance of product quality

Despite such a wide array of QC guidance from different governing agencies, one goal remains common, that of ensuring patient safety. For the countries or groups that are just entering into such programmes and lack such regulatory agencies, it is advisable to create an oversight group from outside the production and a user group that includes experts from related fields with no apparent conflict of interest. Irrespective of the governing agency, the following criteria should be proposed and adhered to. Where pharmacopoeial specifications are available, these should be met. Those countries that lack specific radiopharmaceutical pharmacopoeial standards in their national pharmacopoeias can refer to the WHO Ph. Int.

The WHO pharmcopoeial monograph contains qualitative and quantitative assessments and quality control tests to be performed on products (see Annex). The earlier assessment includes conforming to the general requirements stated in the monograph, which include basic description, radionuclide production, radiochemical synthesis, identification tests, clarity and appearance. In addition, a product must meet QC monograph limits on each batch for radionuclide purity, radiochemical purity, chemical purity, residual solvent/s, biodistribution studies, sterility and bacterial endotoxin tests. However, not all tests are required to be undertaken prior to release of the product for administration to patients. The exceptions are stated in the monograph. Only when the product meets the standards of the monograph can the label on the product name state, for example, 'FDG Ph. Int.', which provides clear assurance as to which pharmacopoeial standards apply to the product.

8.1.2.1. Essential QC measures for individual batches of radiopharmaceuticals

The following QC measures should be performed on individual batches of PET agents:

— A pH measurement;
— A visual inspection;
— A test of radiochemical purity;
— Establishment of the radiochemical identity;
— A radionuclidic purity test;
— A specific activity test (if applicable);
— A test of the integrity of the sterile filter membrane;

— A sterility test[1] (completed after release);
— A bacterial endotoxin test[1] (completed after release).

It is also important to set the acceptable test procedures and the minimum acceptance limit criteria for these tests.

8.1.2.2. Test procedures and limits

If there are existing national or local rules, those rules and regulations should be adhered to. Sections 8.1.2.3–8.1.2.13 describe the various key QA tests that aid in the safety and QA of radiopharmaceuticals.

For countries or groups that are just starting such programmes and that lack such regulatory agencies, it is advisable to create an oversight group from outside the production facility and a user group that includes experts from related fields, with no apparent conflicts of interest.

8.1.2.3. Radionuclidic purity

With regard to radionuclidic purity:

(a) No less than 95% of the content should be the desired isotope, demonstrated by a gamma ray spectrum (measured by a gamma ray counter), or by GeLi detector, multichannel analyser or half-life measurements. Use of any one of these methods is sufficient.
(b) This test should be performed annually.

8.1.2.4. Radionuclidic identity

With regard to radionuclidic identity:

(a) An isotope half-life measurement method can be used for this test.

[1] Before initiation of use of a radiopharmaceutical on humans, three consecutive batches of radiopharmaceutical should be prepared and tested using the bacterial endotoxin test and sterility testing. If any of the three batches fails, the set of three production runs for QC testing needs to be repeated. Once the qualifying runs pass all QC tests, subsequent production batches of the radiopharmaceutical can be released while results from sterility and endotoxin tests are pending.

(b) A small sample of isotope is measured in the dose calibrator at time t_0 and then measured again at t_1 (after a few minutes). These two readings can be used to derive the physical half-life decay of the isotope.
(c) The measured half-life under this test should be within 5% of the half-life of the isotope given in the literature.

8.1.2.5. Visual assessment

This test should be performed on each radiopharmaceutical batch. To ensure that the radiopharmaceutical is free of turbidity, check the colour and make an assessment for any particulates. A visual check should be performed on each batch (lot) of radiopharmaceutical by looking at the product vial through a lead glass window in front of a light black and white background (Ph. Int.). This test is performed to ensure that the product is free of particles, turbidity and any colour.

8.1.2.6. pH

With regard to pH:

(a) This test can be performed on each batch (lot) produced, using a pH test strip, pH paper or micro-pH meter.
(b) The pH of the radiopharmaceutical solution should be within the range 4.5–8.0.
(c) If the pH is outside the range given in (b), it can be adjusted using a sterile pyrogen-free solution of acid or base (as needed).
(d) If adjustment of the pH is needed, the radiopharmaceutical batch should be given a different (new) lot number, and should be retested for radiochemical purity, sterility and bacterial endotoxins.

8.1.2.7. Radiochemical purity

With regard to radiochemical purity:

(a) A test for radiochemical purity can be performed using HPLC or a TLC scanner. The radiopharmaceutical should have a radiochemical purity of at least 90%.
(b) This test should be performed on each lot produced and should be completed before the release of the radiopharmaceutical.

(c) If, for any reason, a sample was reprocessed (filtered, reformulated, had its pH adjusted or concentrated), the finished product should be given a different (new) lot number and this should be retested for radiochemical purity.

8.1.2.8. Radiochemical identity

With regard to radiochemical identity:

(a) A test for radiochemical identity can be performed using HPLC or TLC. The identity of the radiopharmaceutical should be between 0.95 and 1.05 of the retention time (or Rf) of the reference drug standard.
(b) This test should be performed on each lot produced, and the test should be completed before the radiopharmaceutical is released.
(c) If, for any reason, a sample has been reprocessed (filtered, reformulated, pH adjusted or concentrated), the finished product should be given a different (new) lot number and this should be retested for radiochemical purity.

8.1.2.9. Chemical checks

The product should be assessed chemically, and intermediate chemical checks carried out as described in various pharmacopoeias.

8.1.2.10. Specific activity

For certain radiopharmaceuticals, especially those for receptor imaging and neuro-imaging applications, specific activity measurements are important. The presence of high amounts of non-radioactive mass in those radiopharmaceuticals may render them useless:

(a) Establish HPLC procedures to measure the non-radioactive mass associated with that radiopharmaceutical.
(b) Establish a procedure to calculate the mass per unit radioactivity.
(c) Establish limits on the quantity of mass per unit of radioactivity that are acceptable. In general, a specific activity of ≈15 GBq/μmol (400 mCi/μmol) is currently suitable for the radioactive sources used for imaging applications. Nonetheless, toxicity and other factors govern the limit of mass allowed for a particular radiopharmaceutical.

(d) If applicable, this test needs to be performed on each batch before release of the radiopharmaceutical.

8.1.2.11. Solvent residues in radiopharmaceuticals

With regard to solvent residues in radiopharmaceuticals:

(a) The residual solvents in the radiopharmaceutical should be below the limits set in the pharmacopoeia.
(b) A test for solvent residues should be performed either by the chemical analysis method or by the GC method.
(c) This test can be performed at the time a facility starts operations, and at the time of validating the synthesis of a particular radiopharmaceutical. In some institutions, this test is subsequently performed quarterly on the assumption there is no change to the fully validated preparation procedure.
(d) If the manufacturing device is altered in a major way or replaced, the procedure should be validated, and a test for solvent residues should be performed.

8.1.2.12. Sterility testing

With regard to sterility testing:

(a) Every production batch should be sterilized using the active culture method for both aerobic and anaerobic bacteria (using soy broth and tripticase broth (or any alternative suitable method) (see the pharmacopoeial requirements in the Annex).
(b) To validate the procedure initially, three consecutive batches should be passed as lacking bacterial contamination.
(c) The sterility test is not a release criteria test. Once validated on three consecutive batches, this test can be performed after release of the radiopharmaceutical (this test must be initiated within 24 h of production).
(d) All production batches should be tested for their sterility.

8.1.2.13. Bacterial endotoxin or pyrogen testing

The pyrogen test is also known as the limulus amebocyte lysate (LAL) test. It can be performed using an LAL (or equivalent) method:

(a) This test should be performed for three consecutive batches of a particular radiopharmaceutical for validation.
(b) Thereafter, this test should be performed on every batch produced.
(c) This test is not a release criteria test. Once validated on three consecutive batches, this test can be performed after release of the radiopharmaceutical (test initiated within 48 h or other acceptable time period after production).
(d) If selected as a release criteria, a 60 min test method (LAL) is recommended for the release of most radiopharmaceuticals, but for radiopharmaceuticals with short lived isotopes (^{11}C) a costly 20 min rapid LAL test method is acceptable.
(e) For radiopharmaceuticals with ultra-short half-life isotopes (^{15}O and ^{13}N), an LAL test can be performed on the first production batch of the day, and subsequent batches can be used without this testing unless the synthesis procedure or main equipment is altered or changed in-between.

8.1.3. Assurance of usage

It is equally important to ensure the safe and effective use of these radiopharmaceuticals. This requires the following general good clinical and laboratory practices:

(a) Before drawing a dose into the syringe, verify the label;
(b) Assay the radiopharmaceutical before injection to ensure that the correct quantity is dispensed;
(c) Ensure that the correct clinical protocols are in place and that they are used for these studies;
(d) Ensure that the required approvals have been obtained and are available for use and review;
(e) Survey the facility for surface contamination.

8.1.4. Microbiological validation

Each batch of radiopharmaceuticals produced must be tested for sterility and apyrogenicity according to USP, EP or Ph. Int. The test should also include a bacterial growth test as a 'positive control'.

A media fill validation should be performed routinely at fixed time intervals. A bacterial culture medium (soybean casein digest medium (SCDM)) is used instead of a raw material in the synthesis procedure. The medium is then cultured for any bacteria captured. The objective of such a validation is to

ensure that the aseptic part of the synthesis system is tight enough to prevent entry of bacteria into the radiopharmaceutical.

8.1.5. Environment assessment

The environment of the production hot cell, dispensing hot cell and radiochemistry clean room should be monitored using settle culture plates (SCDM for bacteria and sheep blood agar (SBA) for fungi) and contact culture plates (SCDM). The plates are then cultured for any bacteria captured.

The pressure differences between inside the radiochemistry clean room and outside, between the production hot cell and the radiochemistry clean room, and between the dispensing hot cell and the radiochemistry clean room, should be monitored during the synthesis.

The performance of the radiochemistry clean room and the dispensing hot cell should be validated at least once a year.

8.2. QUALITY CONTROL FOR A NEW CLASS OF PET RADIOPHARMACEUTICAL

Most of the new classes of PET radiopharmaceuticals are prepared in-house due to their ultra-short half-lives. There are large variations in equipment, staff, facilities and management among different PET radiopharmaceutical production centres. Hence, there should be general criteria in place to ensure the quality of the radiopharmaceuticals produced. Section 8.1 above provides a description of the general criteria for QC for routine production of PET radiopharmaceuticals for clinical use, and these also apply to new tracers; however, there is an additional requirement that is described below. Individual institutes often have additional local requirements in addition to these general criteria.

8.2.1. Radiopharmaceutical substance

Animal toxicity studies should be conducted to ensure that the radiopharmaceutical substance is non-toxic or of low toxicity. Biodistribution studies should be conducted to confirm that the radiopharmaceutical substance has an acceptable target to non-target ratio. In addition, the radiopharmaceutical substance should be compatible with other added substances in the radiopharmaceutical formulation and stable for the period of use. For many PET radiopharmaceuticals it is important that the product be of high specific radioactivity.

8.2.2. Excipients

Excipients (other ingredients) in the radiopharmaceutical product include preservatives, stabilizers and buffers. These other ingredients must have low toxicity and should not generate toxic substances. In addition, they should not affect the stability and biodistribution of the radiopharmaceutical substance.

8.2.3. Composition — Radioactivity concentration

There should be a high radioactivity concentration at the end of synthesis (EOS).

8.2.4. Composition — Batch

A high radioactivity concentration per batch at the end of preparation is desired. This would reduce the volume to be injected into patients. However, sensitivity of the product to radiolysis can be a limiting factor at high radioactivity concentrations.

8.2.5. Reference standards

Reference substances are used for qualitative and quantitative assay of radiopharmaceutical components and ingredients. Many reference substances can be obtained from companies specialized in producing precursors and reference substances for PET radiopharmaceutical production. A COA should accompany each reference substance purchased and should also be established for reference substances prepared in-house or obtained as research samples.

8.2.6. Setting up of a new tracer test facility

There should be sufficient space for preparation and QC of new radiopharmaceuticals. The facility should be designed to control the flow of raw materials and personnel in the facility, in order to ensure traceability and prevent accidental mixing up and contamination of raw materials with routine batches. Each activity should be carried out in a designated area (Table 20).

TABLE 20. DESIGNATED AREA AND ACTIVITY

Designated area	Activity
Quarantine area	Area for short term holding of raw materials awaiting acceptance
Stock area	Area for storage of approved raw materials for preparation of radiopharmaceuticals
Radiopharmaceutical preparation	Designated area(s) for synthesis and aseptic procedures
Quality control room	Area for QC of radiopharmaceuticals
Rejected material area	Area, such as a separated refrigerator, for short term storage of rejected raw materials and rejected products awaiting further investigation and disposal

The quarantine area and stock area must be clearly designated, and access to these areas must be restricted. Raw materials being held in the quarantine area and the stock area must be properly recorded. Line clearance is paramount and should be checked and recorded.

Good QC practices are essential, and equipment should be cleared for routine batches. The arrangement of instruments should be clear and not create any confusion. Access to the QC room must be restricted. The QC room must be cleaned daily. All leftover samples or reagents must be cleared or removed after each QC routine.

8.2.7. Microbiological validation

Each new batch of drug products produced must be tested for sterility and pyrogenicity according to the Int. Ph., USP, BP or EP. The test should also include a bacterial growth test as a positive control. However, validation of the test procedure is necessary.

A media fill validation for a new tracer should be performed routinely at fixed time intervals. A bacterial culture medium (SCDM) is used instead of raw material in the synthesis procedure. The medium is then cultured for any bacteria captured. The objective of such a validation is to ensure that the synthesis system is tight enough to prevent entry of bacteria into the drug product.

8.2.8. Environment assessment

A new tracer procedure for environment testing and validation is necessary. This includes monitoring the environment of the production hot cell, the dispensing hot cell and the radiochemistry clean rooms using settle culture plates (SCDM for bacteria and SBA for fungi) and contact culture plates (SCDM). The plates are then cultured for any bacteria captured.

9. NEW CLASS OF PET TRACERS

9.1. SCOPE

For the last two decades, ^{18}F-FDG has been in use in a clinical setting, and it has proven to be a successful radiotracer for oncology, cardiology and neuropsychiatry PET examinations. The expanded role of PET imaging still depends largely on the availability of multiple radiopharmaceuticals. A wide variety of radiotracers exist that incorporate a number of positron emitting radionuclides that are appropriate for translation into medical practice. These radiotracers have all been documented in the scientific literature. Some of these compounds that appear potentially appropriate, but are not as yet widely translated into routine clinical practice, are summarized in Section 9.2.

9.2. POTENTIAL TRACERS FOR ONCOLOGY

Examination of cancer patients is still the area in which PET is clinically most extensively used. Positron emission tomography has found widespread use in both the initial diagnosis and the evaluation of the efficacy of treatment by surgery, chemotherapy, radiotherapy or a combination of these treatments. Metabolic tracers are the most commonly used radiopharmaceuticals for PET oncology studies. Metabolic tracers are taken up by cells that use them as fuel for energy metabolism or as building blocks for biosynthesis. Thus, each metabolic PET tracer should represent the target metabolism accurately. In general, the capacity of cells to take up a metabolic PET tracer is relatively higher than other PET tracers, such as receptor binding tracers. This is in most cases due to the continuous use of the metabolic PET tracer. Thus, the specific radioactivity of the tracer is less important than those for receptor binding

tracers. However, for some metabolic tracers such as ^{18}F-6-F-fluorodopa, the specific radioactivity can be too low when they are labelled by carrier added ^{18}F-fluorine gas.

The recent introduction of dual imaging devices such as PET/CT scanners has enabled a precise morphological localization of the lesions, which has improved both the diagnostic accuracy and the precision of radiotherapy and surgery. The metabolic index of malignancies is measured in ^{18}F-FDG PET by ^{18}F-FDG being trapped in tissue with a high glucose consumption. This new class of PET tracers gives measures of other physiological processes, such as amino acid transport, hypoxia, cell proliferation, receptors and enzymes. Some of these tracers are summarized in Table 21 on pp. 171–173.

9.2.1. Tracers of amino acid transport

9.2.1.1. [Methyl-^{11}C]L-methionine

Methionine is one of the PET amino acid tracers; the chemical formulas of others are shown in Fig. 23. Methionine is transported across cell membranes mainly by the amino acid transport system L, with minor contributions from systems A and ASC. During the 40 min time span of a ^{11}C-methionine PET study, protein synthesis and trans-methylation reactions will only have a minor influence on the distribution of ^{11}C. The utilization of ^{11}C-methionine has mainly been in the study of patients with brain or head and neck malignancies. It is particularly useful for delineation of, and monitoring treatment of, brain

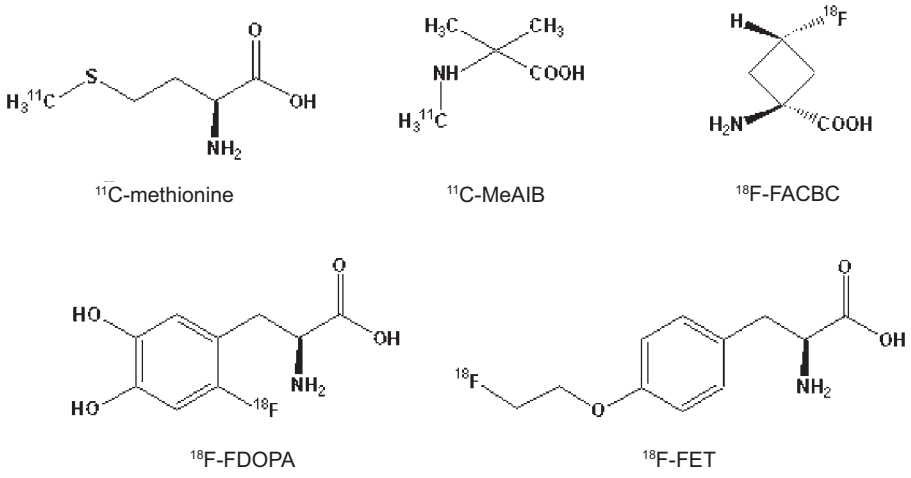


FIG. 23. PET tracers for detection of amino acid transport.

tumours (e.g. astrocytoma, anaplastic oligoastrocytoma and glioblastoma progression).

Figure 24 illustrates monitoring the treatment response of oligoastrocytoma grade III. The upper row of images shows magnetic resonance imaging (MRI) results. The middle row of images shows a high uptake of ^{11}C-methionine before chemotherapy, and the last row of images shows a reduction of ^{11}C-methionine uptake after three cycles of chemotherapy with temozolomide.

Preparation of ^{11}C-methionine from ^{11}C-methyl iodide or ^{11}C-methyl triflate gives a high radiochemical yield [20–23]. On-line and Al_2O_3/KF catalysed reactions have also been employed [24, 25]. In the original methods, HLPC was used for purification of ^{11}C-methionine, and several alternative

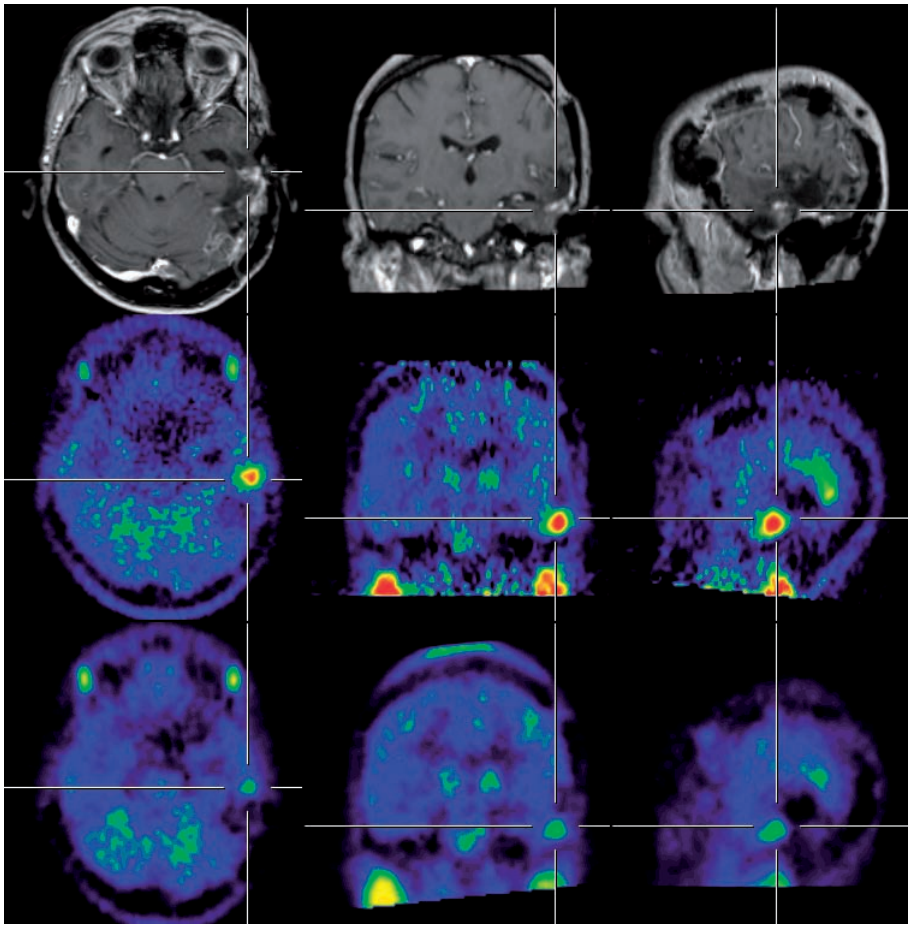


FIG. 24. Monitoring the treatment response of oligoastrocytoma grade III.

purification methods have recently used a combination of short columns, such as Sep-Paks, as described in Refs [26–28].

There are several aspects about the radiochemical purity of ^{11}C-methionine produced by these methods [29–31]. The optical purity has been demonstrated to be dependent on the amount of base used in the in situ generation of the reactive species, the thiolate anion. In order to achieve an acceptable optical purity, i.e. a ^{11}C-D-methionine content of less than 5%, the amount of base should be kept at a minimum [30]. The radioactive by-products ^{11}C-methionine sulphoxide and ^{11}C-methionine sulphone are formed in various amounts, dependent on the reaction conditions [31], and determination of these labelled species should be included in the QC of the final product. Use of ^{11}C-methionine PET gives a relatively low dose of radiation to patients [32].

9.2.1.2. [Methyl-^{11}C]L-MeAIB

MeAIB (^{11}C-methylaminoisobutyric acid) is a metabolically stable amino acid analogue, which is transported from the plasma into the cell exclusively by system A and does not participate in protein synthesis. High yield preparation of ^{11}C labelled MeAIB is by a two step reaction from ^{11}C-methyl iodide or ^{11}C-methyl triflate and AIB methyl ester [33]. Purification by HPLC yields a high purity of the final product. It has been found that ^{11}C-MeAIB is useful in the diagnosis of cancers of the head and neck [34], and human dosimetry data show that ^{11}C-MeAIB PET studies can be performed with a relatively low dose to patients [35].

9.2.1.3. ^{18}F-FACBC

^{18}F-FACBC (^{18}F-*anti*-1-amino-3-fluorocyclobutyl-1-carboxylic acid) is prepared by nucleophilic reaction of ^{18}F-fluoride with a Boc and methyl protected triflate precursor, followed by acidic deprotection of the protection groups and purification on an ion exchange column [36]. This synthetic sequence has recently been fully automated, and the major impurity in the final product has been identified as *anti*-1-amino-3-hydroxycyclobutane-1-carboxylic acid [37].

9.2.1.4. ^{18}F-FET

^{18}F-FET (O-(2′-^{18}F-fluoroethyl)-L-tyrosine) is transported by the specific amino acid uptake system L [38]. It has been proposed that FET might even be specifically transported by a subtype of system L, named LAT2 [39]. There is

only a minor degradation of ^{18}F-FET in the human body and no relevant participation in protein synthesis.

Two different synthesis methods have been used in the production of ^{18}F-FET. The first method involves the reaction of ^{18}F-fluoroethyltosylate with the disodium salt of L-tyrosine [40]. This method is difficult to automate, as two separate HPLC purifications of the ^{18}F-fluoroethyltosylate and the final product are used. The second method uses a nucleophilic ^{18}F-fluorination of a protected precursor followed by subsequent deprotection [41]. A high yield of the product is obtained, and after sterile filtration the ^{18}F-FET containing HPLC eluate can be used directly for human application. Human dosimetry data have shown that 370 MBq of ^{18}F-FET will lead to an effective dose of 6.1 mSv [42]. It has been found that ^{18}F-FET is effective in the detection of brain tumours and of squamous cell carcinomas of the head and neck [39].

9.2.1.5. ^{18}F-FDOPA

^{18}F-FDOPA (^{18}F-6-fluoro-L-DOPA) has for many years been used in studies of the presynaptic dopaminergic system with PET. After discovering that ^{11}C-DOPA was a suitable PET tracer for diagnosis of pancreatic tumours [43], ^{18}F-FDOPA was also demonstrated to be able to localize gastrointestinal tumours [44]. This has led to a greatly increased demand for ^{18}F-FDOPA as a clinical PET tracer.

A full list of all the numerous proposed methods for the production of ^{18}F-FDOPA is beyond the scope of this book. The majority of the procedures for production of ^{18}F-FDOPA use electrophilic fluorination. In the original procedure [45], ^{18}F-F_2 was used in a direct fluorination of L-DOPA in anhydrous hydrogen fluoride. This procedure gave a mixture of 2-, 5- and 6-^{18}F-FDOPA, which was separated using HPLC to give 6-^{18}F-FDOPA with a 2–3% radiochemical yield. The use of ^{18}F-acetylhypofluorite as the labelled precursor in the reaction with 3-methyl-L-DOPA, followed by hydrolysis using HBr, gave a 4% radiochemical yield of the HPLC purified product [46]. By use of a fully protected L-DOPA derivative, 3-methyl-4,*N*-diacetyl-L-DOPA-methyl ester and ^{18}F-acetylhypofluorite, a radiochemical yield of 8% was obtained [47].

By reacting L-DOPA with phosgene, the 5-(benzyl-3′, 4′-carbonate)oxazolidine-2,5-dione was obtained. This precursor gave, upon reaction with ^{18}F-acetylhypofluorite, ^{18}F-FDOPA, with a total radiochemical yield of 21% [48].

A regioselective electrophilic substitution of a 6-trifluoroacetylmercury derivative of L-DOPA has been introduced by Adam and Jivan [49]. They used *N*-acetyl-3′,4′-dimethyl-6-trifluoroacetylmercury-L-DOPA methyl ester and

^{18}F-acetylhypofluorite to give a 12% radiochemical yield of ^{18}F-FDOPA. Another regioselective synthesis used *N*-acetyl-3′,4′-dimethyl-6-trimethylstannyl-L-DOPA ethyl ester and ^{18}F-F_2 or ^{18}F-acetylhypofluorite to give a 25% radiochemical yield of the HPLC purified product [50]. Several other protected trimethylstannyl derivatives of L-DOPA have since been proposed as precursors for ^{18}F-FDOPA. The *N*-3,4-triBoc-L-DOPA-ethyl ester gave a 26% radiochemical yield of pure ^{18}F-FDOPA [51]. Details of an improved synthesis of this labelling precursor have recently been published [52].

There are also nucleophilic approaches to the production of ^{18}F-FDOPA that use either chromatography to separate a racemic product mixture or either asymmetric synthesis or enzymatic synthesis to produce an enantiomerically enriched product. Although these methods are not used in many PET centres, very active research in asymmetric methods during the last decade indicates that they might be the methods of choice in the future for the production of ^{18}F labelled aromatic amino acids. One obvious reason is that nucleophilic ^{18}F-fluoride can be produced in a much higher specific radioactivity than electrophilic ^{18}F-fluorine.

The ^{18}F labelled precursors used in the racemic and asymmetric methods are differently protected ^{18}F-3,4-dihydroxy-6-fluoro-benzaldehydes. ^{18}F-3,4-dimethoxy-6-fluoro-benzaldehyde (^{18}F-6-fluoro-veratralaldehyde) and ^{18}F-3,4-methylene-dioxy-6-fluoro-benzaldehyde (^{18}F-6-fluoro-piperonal) are the most commonly used ^{18}F labelled precursors.

Lemaire et al. prepared racemic ^{18}F-FDOPA from the reaction of ^{18}F-6-fluoro-veratralaldehyde or ^{18}F-6-fluoro-piperonal with phenyloxazolone followed by deprotection using red phosphorous in hydriodic acid. Pure ^{18}F-6-fluoro-L-DOPA was obtained after chiral chromatography with a 10% radiochemical yield [53]. Reddy et al. have published a remote controlled version of this method [54].

Lemaire et al. have also actively explored asymmetric synthesis methods using different chiral glycine synthons. Boc-BMI, which is sold by Merck, Darmstadt, was found to give a satisfactory optical purity (>98%) and a high radiochemical yield (15–20%) of final ^{18}F-6-fluoro-L-DOPA [55]. Recently, by using a chiral quaternary ammonium salt derived from a Cinchona alkaloid as a phase transfer catalyst in the alkylation of a racemic glycine synthon, a 25–30% radiochemical yield of ^{18}F-fluoro-L-DOPA with an optical purity higher than 97.5% was obtained [56].

4-^{18}F-fluorocatechol was used as the labelled precursor in the enzymatic synthesis of ^{18}F-6-fluoro-L-DOPA, which used ^{18}F-tyrosinase to couple the precursor with pyruvic acid and ammonia to give an optically pure product at a 2% yield with a high specific radioactivity of more than 200 MBq/nmol [57].

9.2.2. Tracers of hypoxia

Tumour cells at low oxygen tension are relatively chemo- and radio-resistant. Figure 25 shows the chemical formulas of PET tracers for detection of hypoxia. The hypoxic fractions of individual tumours before, during and after therapy are likely to have prognostic value, but their diagnosis still awaits an accurate and acceptable assay. Many ^{18}F labelled compounds have been evaluated for this purpose, a few of which are entering clinical practice.

9.2.2.1. ^{18}F-FMISO

^{18}F-FMISO (1H-1-(3-^{18}F-fluoro-2-hydroxypropyl)-2-nitroimidazole) is the most extensively used ^{18}F labelled hypoxia tracer. Preparation of ^{18}F-FMISO was independently reported by two groups in 1989 [58, 59]. The original synthesis method used the reaction of ^{18}F-epifluorhydrin with 2-nitroimidazole. This synthesis gave a moderate radiochemical yield, 20–40%, in a synthesis time of 1.5–2 hours, and was difficult to automate. An improved synthesis was later developed that used the direct nucleophilic reaction of ^{18}F-fluoride with a tetrahydropyranyl protected tosylate precursor, followed by acidic deprotection of the tetrahydropyranyl group [60]. This method gives a 55–80% radiochemical yield in 50 min and is easy to automate. As reported in

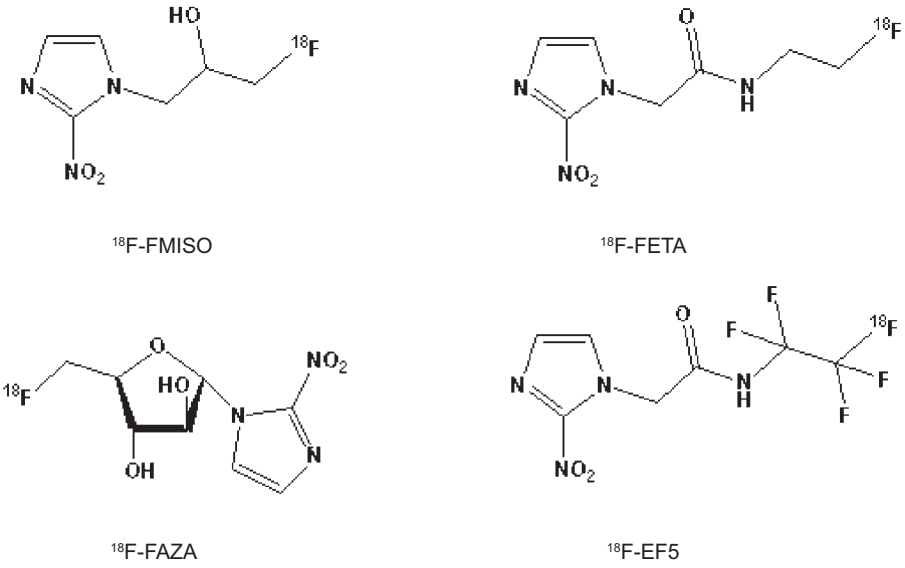


FIG. 25. PET tracers for detection of hypoxia.

a recent publication [61], this method has been adopted in a fully automated synthesis of ^{18}F-FMISO, including HPLC purification using a commercial ^{18}F-FDG module. ^{18}F-FMISO PET is associated with a moderate radiation dose to the patient [62].

9.2.2.2. ^{18}F-etanidazole

^{18}F-etanidazole (^{18}F-N-(2-fluoroethyl)-2-(2-nitroimidazol-1-yl)-acetamide), also known as ^{18}F-FETA, is prepared by reaction of ^{18}F-fluoroethylamine and 2,3,5,6-tetrafluorophenyl-2-(2-nitro-imidazol-1-yl)-acetate. Production of the alkylation reagent ^{18}F-fluoroethylamine is cumbersome, and the reagent is purified by distillation into the reaction mixture [63]. The product is purified with HPLC, giving the pure product in an aqueous solution that needs only to be made isotonic before sterile filtration. So far, only rodent studies have been reported with this hypoxia tracer [64, 65].

9.2.2.3. ^{18}F-FAZA

^{18}F-FAZA (^{18}F-1-(5-fluoro-5-deoxy-α-D-arabinofuranosyl)-2-nitroimidazole) is prepared in high yields by a one step nucleophilic substitution reaction of ^{18}F-fluoride with the tosylate precursor [66]. The product is purified with HPLC, giving the pure product in a 5% ethanol aqueous solution. A fully automated synthesis has been performed on a modified commercial unit [66]. Up to the present time, only rodent studies have been reported with this hypoxia tracer [67, 68].

9.2.2.4. ^{18}F-EF5

^{18}F-EF5 (^{18}F-2-(2-nitro-1[H]-imidazol-1-yl)-*N*-(2,2,3,3-pentafluoropropyl)-acetamide) is prepared in modest yields by direct fluorination of the corresponding 2,3,3-trifluoroallyl precursor using ^{18}F-F_2 in trifluoroacetic acid [69]. After extraction with organic solvents to remove ^{18}F-fluoride, the crude product is purified with HPLC. Micro-PET imaging of ^{18}F-EF5 distributions in tumour bearing rats has shown the potential of this new hypoxia PET tracer [70]. A high specific radioactivity of ^{18}F-EF5 is obviously not a prerequisite, as these micro-PET studies were performed using 30 mg/kg of carrier non-radioactive EF5.

9.2.3. Tracers of proliferation and metabolism

9.2.3.1. ^{18}F-FLT

^{18}F-FLT (^{18}F-3′-deoxy-3′-fluorothymidine) was suggested already in 1991 as a tracer for PET evaluation of drugs against AIDS [71]. Since then, its potential as a PET diagnostic agent for oncology has been realized, with numerous applications, such as assessment of primary tumours [72] and therapeutic response [73] in breast cancer. FLT is one of the tracers for detection of proliferation of cancers and the related metabolism. The chemical formulas of such tracers are shown in Fig. 26.

Various precursors have been used for preparation of ^{18}F-FLT. In the original procedure [71], the use of the 3′-mesyl-5′-trityl precursor gave a 7% radiochemical yield. An *N*-2,4-dimethoxybenzyl-3′-nosyl-5′-(4,4′-dimethoxytrityl) precursor gave a 13% radiochemical yield [74]. An evaluation of various precursors showed that an *N*-Boc-3′-nosyl-5′-(4,4′-dimethoxytrityl) precursor gave the highest radiochemical yield, 20%, within 85 min [75]. A subsequent optimization of the reaction conditions using this precursor enabled a radiochemical yield of 40% within 60 min [76]. Recently, the introduction of an ionic liquid, [bmim][OTf], as a reaction solvent enabled a fourfold reduction of precursor amount, with only a slight reduction in radiochemical yield [77]. A commercial ^{18}F-FDG synthesis unit has been used for a fully automated synthesis of ^{18}F-FLT, including HPLC purification, giving a 50% radiochemical yield within 60 min [78], while another commercial ^{18}F-FDG synthesis unit has been used for semi-automated synthesis of ^{18}F-FLT using Sep-Pak purification, giving a 38% radiochemical yield within 50 min [79].

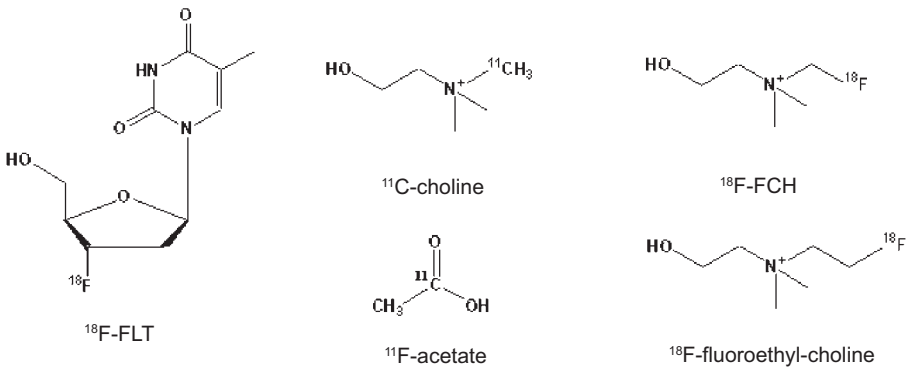


FIG. 26. PET tracers for detection of proliferation of cancers and the related metabolism.

^{18}F-FLT is now being evaluated at a number of PET centres worldwide as the radiotracer for imaging tumour proliferative activity. FLT is an analogue of thymidine and is phosphorylated, by cellular thymidine kinase during the S phase of the cell cycle, to its monophosphate form and metabolically entrapped inside the proliferating tumour cell. ^{18}F-FLT can be used to readily image a number of different tumour types, for example, as lung, breast, brain and oesophageal tumours. Initial PET imaging studies with ^{18}F-FLT demonstrated that it can produce high contrast images of tumours, such as lung cancer. Uptake of ^{18}F-FLT has been found to generally correlate with proliferation of a tumour when compared with measurement of levels of the endogenous proliferation marker Ki-67 made on biopsy specimens, but this has not been found in all situations. Overall, ^{18}F-FLT appears to produce images with less contrast than ^{18}F-FDG. Clinically, the images from FLT are different to those of traditional FDG investigations, as illustrated by Fig. 27 for non-small-cell lung carcinoma (NSCLC).

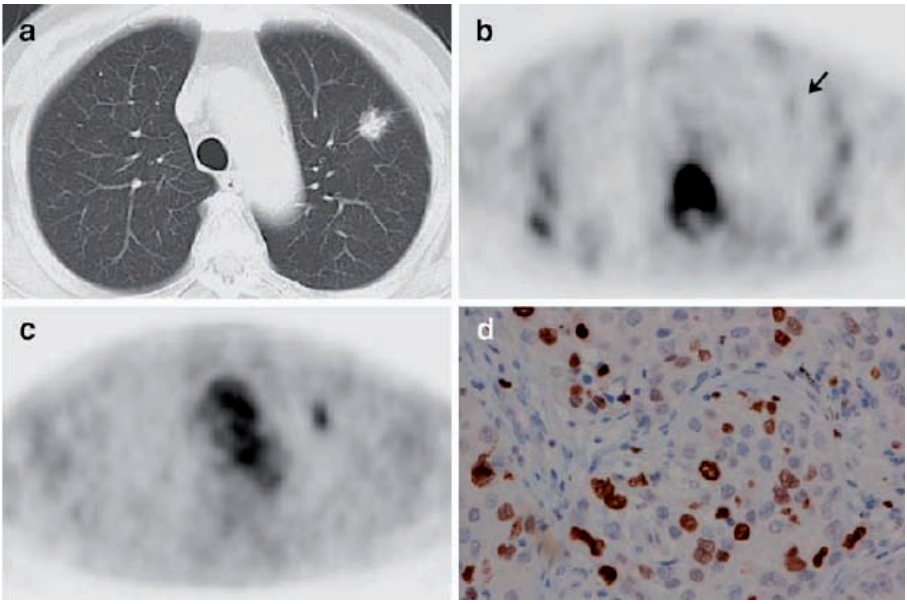


FIG. 27. Imaging by FLT of proliferation activity in a case of NSCLC, showing proliferation of disease, indicated by the arrow in part (b). Part (a) is the associated CT image, (b) is an FLT image, (c) is an FDG image and (d) is an image of tissue stained with proliferation stain.

9.2.3.2. ^{11}C-choline

^{11}C-choline was originally developed as a tracer for the brain central cholinergic system [80]. In the original synthesis, ^{11}C-methyl iodide was used to methylate diethylaminoethanol (DMAE). The reaction solvent acetone was evaporated before HPLC purification on a reversed phase cyano column, using 100% water as solvent. The same article [80] also notes that uptake by the brain is inhibited by even small amounts of the precursor DMAE. This precursor must thus be evaporated together with the reaction solvent before HPLC purification.

The applications of ^{11}C-choline in oncology were discovered one decade ago [81]. This initiated an intensified use of ^{11}C-choline as a PET tracer, now for tumour detection. Can ^{11}C-choline PET play a role in diagnostic imaging of prostate cancer (Fig. 28) and other malignancies, including recurrent glioblastomas, lung carcinomas and metastases? Owing to the mostly low FDG uptake in prostate cancer, other radiopharmaceuticals have been studied: ^{11}C-choline and ^{18}F labelled choline derivatives, including ^{18}F-fluoroethylcholine and ^{18}F-fluoromethylcholine (FCH).

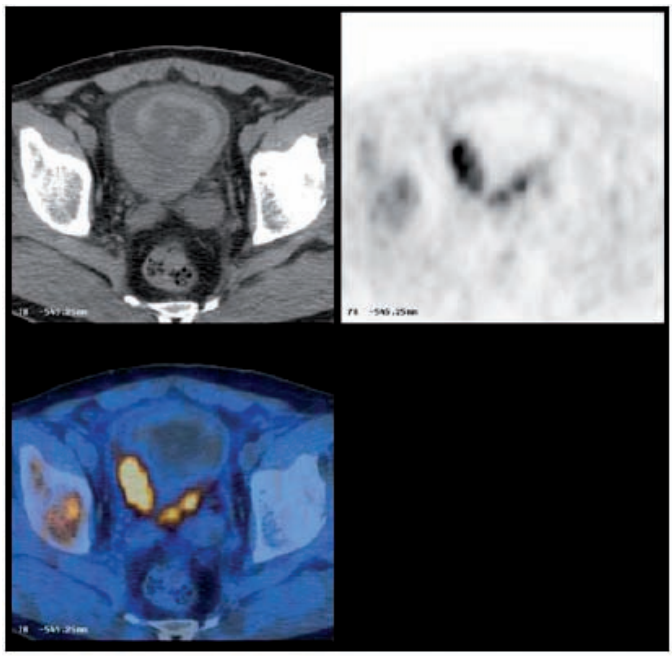


FIG. 28. A case of recurrent urothelial carcinoma: upper right part is a CT image, upper left part is an image using ^{11}C-choline and the lower part is a combined CT/PET image.

Recently, a number of ^{11}C-choline synthesis descriptions have been reported, mainly using simpler purification conditions. Hara and Yuasa have described an automated synthesis using a Sep-Pak cartridge for purification [82]. This system gives an 85% total radiochemical yield in 20 min. Pascali et al. performed both the ^{11}C-methylation and the subsequent purification on a Sep-Pak cartridge [83]. This system substantially simplifies automation. Reischl et al. used Tefzel tubing instead of a conventional reaction vessel and the Sep-Pak purification method in a fully automated system [84]. Recently, an improved radiochemical yield of 96.5% was achieved using substantially reduced amounts (2 mg instead of 60 mg) of DMAE [85]. In order to reliably quantify choline and DMAE concentrations in ^{11}C-choline preparations, an improved HPLC method using a conductivity detector has been developed by Mishani et al. [86].

9.2.3.3. ^{18}F-fluorocholines

^{18}F-fluorocholine ([^{18}F]*N,N*-dimethyl-*N*-fluoromethyl-ethanolamine), also known as ^{18}F-FCH, is prepared in a similar manner to ^{11}C-choline using ^{18}F-fluoromethylbromide as the alkylating reagent [87]. ^{18}F-fluoromethylbromide is purified with preparative GC before reaction with dimethylaminoethanol, and the product is purified on a Sep-Pak cartridge giving ^{18}F-FCH with a 20–40% radiochemical yield in 40 min. ^{18}F-FCH is the only ^{18}F labelled choline analogue that has been evaluated with PET in cancer patients. Human radiation dosimetry measurements have given a maximum dose of 4 MBq/kg when following FDA limits for human research studies [88].

^{18}F-fluoroethylcholine (^{18}F-*N,N*-dimethyl-*N*-fluoroethyl-ethanolamine) has been prepared in an elaborate automated synthesis [89]. ^{18}F-fluoroethyl tosylate was used as the alkylating reagent. After HPLC purification of the product, ions in the HPLC effluent were removed using ion exchange columns from which the product was finally eluted with saline. Comparative PET studies using ^{18}F-fluoroethylcholine and ^{11}C-choline in prostate cancer patients showed that they gave comparable results [89].

Several other ^{18}F-fluoroalkylated choline analogues have been prepared using ^{18}F-fluoroalkyl (ethyl, propyl or butyl) bromides or tosylates as precursors [90, 91]. None of these analogues have, however, so far been evaluated in cancer patients.

9.2.3.4. ^{11}C-acetate

^{11}C-acetate was originally used for studies of myocardial metabolism. About ten years ago, ^{11}C-acetate was also found to be useful in PET

examinations of various tumours, and this accelerated the use of ^{11}C-acetate also for oncological PET studies. ^{11}C-acetate seems to be highly promising in a variety of tumour types. It was first reported to have a high uptake in renal cell carcinoma, and the tracer has the potential to clearly differentiate between malignant and benign tissues. A marked uptake of ^{11}C-acetate has been shown to occur in prostate cancer, and ^{11}C-acetate PET is clearly more sensitive than ^{18}F-FDG PET in detecting prostate cancer (Fig. 29).

The synthesis of ^{11}C-acetate takes place by a straightforward reaction of ^{11}C-carbon dioxide with methyl magnesium bromide. During the last 20 years, a multitude of publications have described various aspects of ^{11}C-acetate synthesis, purification, automation and QC. The first method used freshly prepared methyl magnesium bromide in the synthesis, and stepwise acidic and

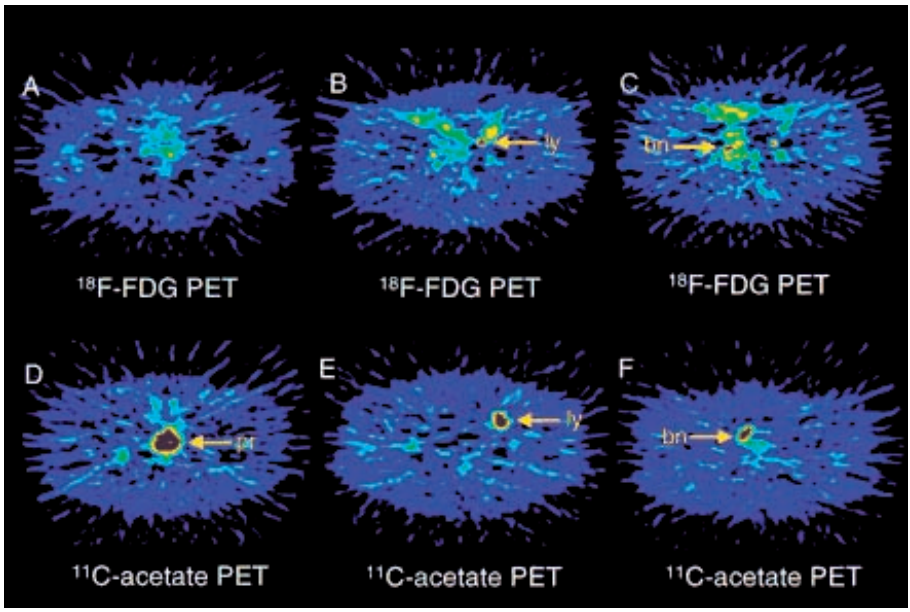


FIG. 29. Comparative PET imaging of prostate carcinomas with ^{18}F-FDG and ^{11}C-acetate: PET images of prostate, lymph node and bone metastases obtained using ^{18}F-FDG and ^{11}C-acetate from a 73 year old man with poorly differentiated (Gleason sum = 7) adenocarcinoma of the prostate. ^{18}F-FDG PET shows low uptake in the prostate (A), with a standardized uptake value (SUV) of 2.87: ^{18}F-FDG uptake in (B) a left-hand-side iliac lymph node metastatic lesion, and in (C) a right-hand-side pubic bone metastatic lesion. ^{11}C-acetate PET shows a high uptake in (D) the prostate, with an SUV of 5.45, in (E) a left-hand-side iliac lymph node metastatic lesion, and in (F) a right-hand-side pubic bone metastatic lesion. In these images, bn denotes bone, ly denotes lymph node and pr denotes prostate.

basic extractions for purification [92]. A radiochemical yield of 72% was obtained in 20 min. Remotely controlled and automated versions of this ^{11}C-acetate preparation were subsequently developed, with a total synthesis time of either 20 min [93] or 12 min [94].

One alternative method for purification of ^{11}C-acetate is distillation. The first distillation method trapped the distilled ^{11}C-acetate in water, and filtered the product solution through a C_{18} Sep-Pak, giving a 43% radiochemical yield in 15 min [95]. Another distillation method trapped the distilled ^{11}C-acetate in sodium bicarbonate solution, which was evaporated and redissolved in saline, giving a 45% radiochemical yield in 20 min [96]. A recent distillation method trapped the distilled ^{11}C-acetate in a physiological phosphate solution, and filtered the product solution through a C_{18} Sep-Pak, giving a 36% radiochemical yield in 14 min [97]. An advantage with this system is that it uses inert, sterile and, whenever possible, disposable, components.

Purification of ^{11}C-acetate with HPLC has been reported to give a 40% radiochemical yield of high purity (>99%) ^{11}C-acetate in 25 min [98]. Several publications have described purification of ^{11}C-acetate in short columns. The first method used two commercially available kieselguhr columns, one for synthesis and one for purification, with a radiochemical yield of more than 90% in 15 min [99]. The second method used a combination of an Ag^+ column and an anion exchange column for purification of ^{11}C-acetate, and gave a 60–65% radiochemical yield in 15 min [100]. In the third method, a robotic system with a combination of an Ag^+ column and a C_{18} Sep-Pak has been reported to give a 60–69% radiochemical yield in 23 min [101].

Two separate methods have used polypropylene loops for the reaction of ^{11}C-carbon dioxide with methyl magnesium bromide. The first method used HPLC purification of the product, giving a 72% radiochemical yield in 16 min [102]. The second method used a combination of an Ag^+ column and an anion exchange column for purification of ^{11}C-acetate [103]. As the eluate of the anion exchange column was found to contain 5–10% of ^{11}C-carbonate, the acidic solution was flushed with nitrogen to remove ^{11}C-carbonate before neutralization with a bicarbonate solution. This method gave a 60–70% radiochemical yield in 12 min [89].

Grignard reactions are not selective for carbon dioxide. Nitrogen oxides are formed in various amounts during proton bombardment of nitrogen gas targets as in the production of ^{11}C-carbon dioxide. These nitrogen oxides can react with organometallic reagents in competition with ^{11}C-carbon dioxide, and reduce the radiochemical yield and purity of radiopharmaceuticals produced from ^{11}C-carbon dioxide. To overcome these problems, a trap for removal of nitrogen oxides from ^{11}C-carbon dioxide was developed by Tewson et al. [104]. This trap was found to improve the yields of reactions with ^{11}C-carbon dioxide

and to enable use of smaller amounts of organometallic reagent. The use of this trap is especially recommended when the content of oxygen in the target gas is relatively high, such as 1%. Recently, this trap has also been recommended for production of ^{11}C-acetate from methyl magnesium chloride [105].

The reaction of ^{11}C-carbon dioxide with methyl magnesium halides also produces other labelled species in addition to ^{11}C-acetate. When ^{11}C-carbon dioxide reacts with two or three molecules of methyl magnesium halide, ^{11}C-labelled isopropanol, acetone and tert-butanol are formed in various amounts, depending on the choice of Grignard reagent and the reaction conditions [106]. The analytical HPLC procedures used in the QC of ^{11}C-acetate batches have thus to separate and identify these ^{11}C labelled species in addition to ^{11}C-carbonate and ^{11}C-acetate. The by-products bromide and magnesium in ^{11}C-acetate batches are typically in amounts (2–20 μg) that are non-toxic (the USFDA daily allowance of magnesium being 300 mg) [107].

9.2.3.5. ^{18}F-sodium fluoride

^{18}F-fluoride is the common product from cyclotron production of ^{18}F. In the original preparation of ^{18}F-sodium fluoride, the target water was simply diluted with saline and sterile filtered [108]. This method is still used by several laboratories. Methods have since been developed for purification of ^{18}F-sodium fluoride using sorption and desorption on anion exchange columns [109]. Quality control of the product is performed by HPLC on ion exchange columns [110].

9.2.4. Neuronal tracers

9.2.4.1. ^{11}C-HED

^{11}C-HED (^{11}C-meta-hydroxy-ephedrine) was originally developed as a tracer for heart neuronal imaging [111]. It has since proved useful in the detection of pheochromocytoma [112]. Figure 30 illustrates a number of neuronal and enzyme tracers for oncology. Preparation is straightforward, using ^{11}C-methyl iodide [111] or ^{11}C-methyl triflate [113] as the labelled precursor, followed by HPLC purification of the crude product. Propylene glycol has been reported to react with ^{11}C-HED during evaporation of HPLC solvent, and should thus not be used as a radiolysis scavenger for ^{11}C-HED [114].

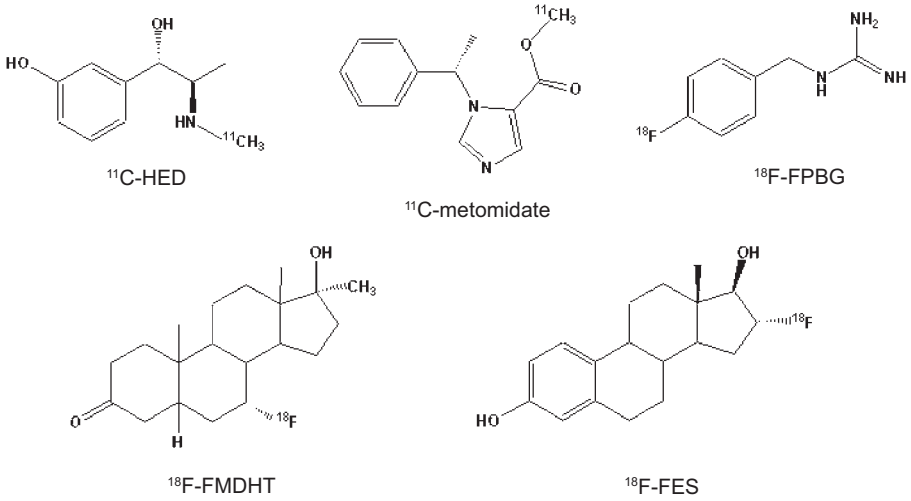


FIG. 30. Neuronal and enzyme PET tracers for oncological applications.

9.2.4.2. ^{18}F-PFBG

^{18}F-PFBG (para-^{18}F-fluorobenzyl guanidine) has been prepared in three steps, starting from a fluoro molecule for nitro molecule exchange on 4-nitrobenzonitrile; however, the product of this reaction route was found to be contaminated with impurities that interfered with the binding to the target [115]. Exchange of the 4-nitrobenzonitrile precursor to 4-nitrilophenyl trimethylammonium trifluoromethanesulphonate eliminated problems with impurities in the final product and also shortened the synthesis time by 10 min [115]. It has been found that ^{18}F-PFBG is specifically accumulated by sympathetic nerve terminals in rats [116].

9.2.4.3. ^{18}F-FMDHT

^{18}F-FMDHT (7α-fluoro-17α-methyl-5α-dihydrotestosterone), which binds to androgen receptors (ARs) with higher selectivity and specificity, has been prepared by nucleophilic reaction of ^{18}F-fluoride with the triflate precursor for 45 min at 60–70°C, followed by HPLC purification on a C_{18} reversed phase column to give a 6–9% radiochemical yield [117]. ^{18}F-FMDHT possesses a methyl group in the 17α position, which provides the added in vivo stability to the molecule towards metabolic degradation. In vivo biodistribution studies show a preferential uptake of ^{18}F-FMDHT in the target tissues, with uptake in the prostate and with a prostate to muscle ratio ranging from

8.06 ± 2.46 at 1 h to 18.81 ± 4.90 at 3 h [118]. The in vivo studies show a high selectivity and specificity of ^{18}F-FMDHT towards AR-rich tissues, and suggest that ^{18}F-FMDHT may be a useful in vivo PET imaging ligand.

9.2.4.4. ^{18}F-FES

^{18}F-FES (^{18}F-16α-fluoro-17β-estradiol) was originally prepared by nucleophilic reaction of ^{18}F-fluoride with the triflate precursor, followed by HPLC purification on a silica column to give a 46% radiochemical yield in 75–90 min [119]. The use of a 3-MOM-protected cyclic sulphone as precursor in nucleophilic ^{18}F-fluorination, followed by hydrolysis of the methoxymethyl (MOM) group, gave similar yields [120]. Optimization of the latter method using optimized deprotection conditions gave ^{18}F-FES in 70% radiochemical yield after 60 min [121]. By use of two different types of ^{18}F-FDG modules, a 50% radiochemical yield in 50 min [122] and a 42% radiochemical yield in 88 min [123] have been reported.

9.2.5. Tracers of enzymes

9.2.5.1. ^{11}C-metomidate

^{11}C-metomidate ([O-methyl-^{11}C]-(R)-1-(1-phenethyl)-1H-imidazole-5-carboxylic acid methyl ester) is a potent inhibitor of 11β-hydroxylase, a key enzyme in the synthesis of cortisol and aldosterone within the adrenal cortex, and is used to image adrenal cortical tumours with PET [124]. In the original preparation, ^{11}C-methyl iodide was used as the precursor, and the product was purified on HPLC using an ethanol–water mixture. The eluate containing the product was diluted with a phosphate buffer before sterile filtration, as evaporation of the eluate caused radiolysis of the product [124]. In a modification of this method, ^{11}C-methyl triflate was used as the precursor and propylene glycol was added to the HPLC eluate, which gave minimal (<2%) radiolysis during evaporation of HPLC solvent [125]. Recently, three different purification methods have been evaluated for the purification of ^{11}C-metomidate [126].

9.2.6. Other tracers

9.2.6.1. ^{18}F or ^{68}Ga labelled monoclonal antibodies, fragments and peptides

Peptide PET tracers are regarded as the most promising agents for clinical applications in the future. Peptides can target numerous different cells

(Table 21 on p. 171). The most successful peptide PET tracers have octreotide derivatives. Fluorine-18 and ^{68}Ga are the recommended positron emitters for peptide labelling. Labelling of monoclonal antibodies, antibody fragments and peptides with ^{18}F represents a potentially very useful strategy for PET examination of cancer patients. Recently, progress has been made with new strategies for easy introduction of ^{18}F into peptide analogues, and also with the design of new analogues with improved physiological distribution patterns. The increased commercial availability of ^{68}Ga generators implies that ^{68}Ga labelled monoclonal antibodies, fragments and peptides will also have a great clinical impact in the future. A description of the numerous methods for labelling of peptides and monoclonal antibodies is outside the scope of this book.

9.3. POTENTIAL TRACERS FOR NEUROLOGY

9.3.1. Tracers of the dopaminergic system

9.3.1.1. ^{11}C-raclopride

^{11}C-raclopride, otherwise known as ^{11}C-FLA 870 ([methoxy-^{11}C]-(s)-(-)-3,5-dichloro-*N*-((1-ethyl-2-pyrrolidinyl)methyl)-2-hydroxy-6-methoxybenzamide), is the most commonly used PET radiopharmaceutical for the study of striatal and thalamic dopamine D_2 receptors. Figure 31 shows the chemical forms of the other PET tracers for the dopaminergic system. In the original preparation, ^{11}C-methyl iodide was used in the reaction of desmethyl-raclopride hydrobromide in NaOH/DMSO (dimethyl sulphoxide) for 15 min, and the product was purified by use of HPLC [127]. A product was obtained having a 40–50% radiochemical yield in 50 min, with a specific radioactivity of 7400–18 500 MBq/μmol (200–500 mCi/μmol). A faster preparation method having a reaction time of 3 min and a similar yield was subsequently developed [128]. The early radiochemistry and QA aspects of ^{11}C-raclopride preparation are summarized in two articles from an EU concerted action on PET [129, 130].

A somewhat lower radiochemical yield (30–45%) of ^{11}C-raclopride was reported using ^{11}C-methyl iodide and the loop method, with heating lasting for 5 min [131].

The preparation of ^{11}C-raclopride from the more reactive precursor ^{11}C-methyl triflate has been studied in detail [132]. It was found that the use of desmethyl-raclopride hydrobromide as precursor resulted in ^{11}C-methyl bromide as the main labelled product. This was overcome by the use of a desmethyl-raclopride-free base as precursor. A high radiochemical yield of

55–65% was obtained after trapping of ^{11}C-methyl triflate in the reaction solution containing 50–100 μg of precursor.

The loop method and the on-column method have been compared with the standard method in solution for preparation of ^{11}C-raclopride from ^{11}C-methyl triflate [133]. While the on-column method gave a low radiochemical yield, the loop method was comparable with the in-solution method. Desmethyl-raclopride triflate was used as the precursor, to prevent formation of ^{11}C-methyl bromide.

A moderate radiochemical yield (20–34%) was reported when ^{11}C-methyl triflate, prepared from ^{11}C-methyl bromide, was used in the preparation of ^{11}C-raclopride [134].

^{11}C-raclopride is one of the PET tracers that are listed in the USP [135]. This USP entry recommends a minimum specific radioactivity of 4662 MBq/μmol (126 mCi/μmol) for ^{11}C-raclopride, based on a 185 MBq (5 mCi) dosage.

9.3.1.2. ^{18}F-fluoroethyl spiperone

^{18}F-N-(2-fluoroethyl)spiroperidol, also known as 3-(2′-^{18}F-fluoroethyl)spiperone or ^{18}F-FESP (8-[4-(4-fluorophenyl)-4-oxobutyl]-3-(2-^{18}F-fluoroethyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one), is used to study

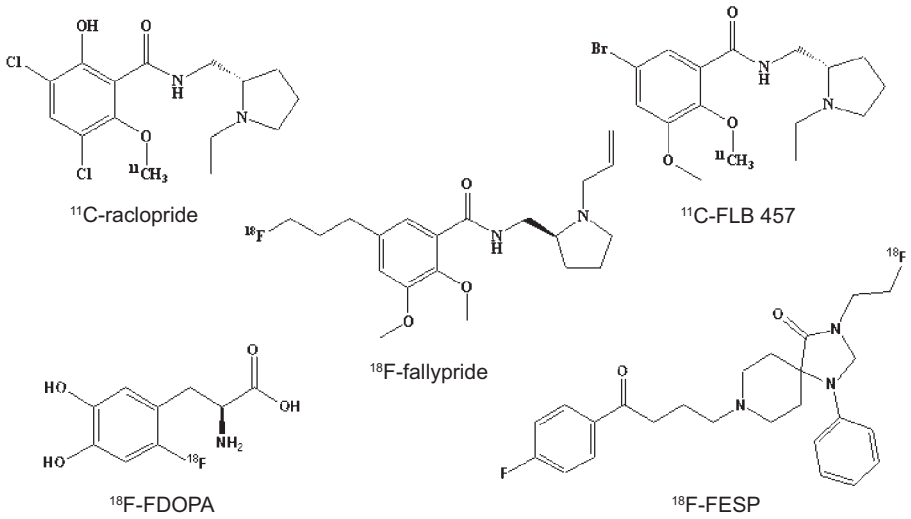


FIG. 31. PET tracers of the dopaminergic system.

striatal D_2 receptors and cortical $5HT_2$ receptors with PET [136]. The first syntheses of this radioligand were independently reported by the University of California, Los Angeles, St. Louis University, Brookhaven National Laboratory and Jülich PET groups [137–140]. All four groups investigated synthesis via ^{18}F-fluoroalkylation of spiperone. ^{18}F-fluoroethylbromide, prepared from bromoethylmesylate [137], bromoethyltriflate [138] or dibromoethane [139], gave the product in 5–8, 30–40 and 10–20% radiochemical yields, respectively. ^{18}F-fluoroethyltosylate gave a 10–20% radiochemical yield of the product [140]. Direct fluorination of the ethylbromide or ethyltosylate of spiperone gave the product with a 30–40% radiochemical yield within one hour [137]. The product was found to be sensitive to radiolysis, which was suppressed by addition of ethanol [137]. A subsequent thorough study of a number of precursors indicated that ketalized tosyl or trimethylphenylsulphonyl precursors were even better for direct fluorination, giving 60% of the final product [141].

9.3.1.3. ^{11}C-FLB 457

^{11}C-FLB 457 (^{11}C-(S)-(-)5-bromo-*N*-((1-ethyl-2-pyrrolidinyl)methyl)-2,3-dimethoxybenzamide) was the first and is the most used radioligand for PET studies of extrastriatal D_2 receptors. The first synthesis of this radioligand used ^{11}C-methyl iodide in the reaction of FLB 604 in NaOH/DMSO for 3 min, and the product was purified on reversed phase HPLC [142]. The radiochemical yield of the product was 25–35%, with a specific radioactivity of about 48 100 MBq/μmol (1300 mCi/μmol). By changing to ^{11}C-methyl triflate as precursor and normal phase HPLC for purification, the radiochemical yield and the specific radioactivity were increased to 40–50% and 74 000–92 500 MBq/μmol (2000–2500 mCi/μmol) [143]. The use of the gas phase production of ^{11}C-methyl iodide in a commercial system and subsequent conversion to ^{11}C-methyl trifoliate resulted in an increased specific radioactivity of 125 800 MBq/μmol (3400 mCi/μmol) of final ^{11}C-FLB 457 product [144]. By using target produced ^{11}C-methane, the specific radioactivity of the final ^{11}C-FLB 457 product has been increased to 6700–11000 mCi/μmol [145]. Recently, even higher values (370 000–3 700 000 MBq/μmol (10 000–100 000 mCi/μmol) for final ^{11}C-FLB 457 product) have been reported by several research groups by using target produced ^{11}C-methane. As the affinity of ^{11}C-FLB 457 for D_2 receptors is very high (20pM) and the concentration of extrastriatal receptors is very low, a high specific radioactivity is crucial to avoid high extrastriatal D_2 occupancy of the injected ^{11}C-FLB 457. A minimum value of 185 000 MBq/μmol (5000 mCi/μmol) at the time of administration has been calculated for a 185 MBq (5 mCi) injection in a human subject [146]. This

implies that target produced ^{11}C-methane is necessary for the production of ^{11}C-FLB 457 for human PET studies.

9.3.1.4. ^{18}F-fallypride

^{18}F-fallypride ((S)-*N*-[(1-allyl-2-pyrrolidinyl)methyl]-5-(3-^{18}F-fluoropropyl)-2,3-dimethoxybenzamide) is useful for the study of both striatal and extra-striatal dopamine D_2 receptors. ^{18}F-fallypride is prepared by nucleophilic substitution with ^{18}F-F^- and a tosylate precursor for 30 min at 85–90°C, and the product was purified using reversed phase HPLC [147]. The final product was obtained with a 20–40% radiochemical yield within 60 min. This synthesis has been successfully performed on a custom manufactured fluorination module with retained radiochemical yield (15%) and specific radioactivity [148].

9.3.1.5. ^{18}F-FDOPA

See Section 9.2.1.5.

9.3.2. Tracers of the serotonergic system

9.3.2.1. ^{11}C-WAY-100635

^{11}C-WAY-100635 ([carbonyl-^{11}C]*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)-ethyl)-*N*-(2-pyridyl)-cyclohexane-carboxamide) is the most commonly used radiopharmaceutical for human PET studies of 5-HT_{1A} receptors. Figure 32 shows the chemical forms of the other PET tracers of the serotonergic system. ^{11}C-WAY-100635 was originally prepared using a one-pot three-step procedure [149]. The reaction of ^{11}C-carbon dioxide with cyclohexylmagnesium chloride and the subsequent reaction with thionyl chloride for 2 min at 70°C generated ^{11}C-cyclohexylcarbonyl chloride. Addition of the amine WAY-100634 (60 mg) and triethylamine, followed by another 15 min reaction at 70°C, gave a crude product that was purified with an ^{18}C Sep-Pak cartridge to give a product with a 60% radiochemical yield within a total synthesis time of 20 min [149].

The original procedure was subsequently modified for preparation of ^{11}C-WAY-100635 for use in human studies [150]. The reaction of ^{11}C-cyclohexylcarbonyl chloride with the amine WAY-100634 (13 mg) for 3–5 min was performed in a separate vessel, and HPLC purification of the crude product that enabled separation from the amine WAY-100634 was performed with an overall radiochemical yield of 15–20% within 45 min.

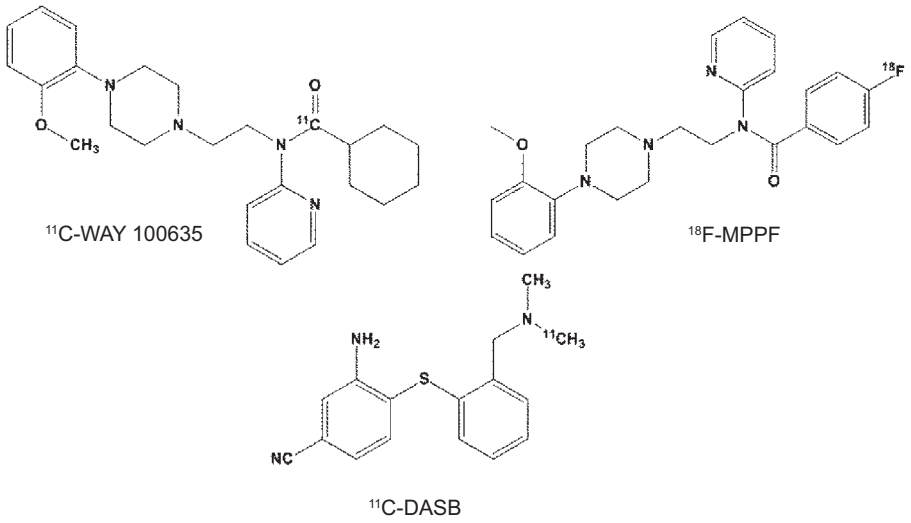


FIG. 32. PET tracers of the serotonergic system.

In another modification of the original one pot procedure, excess cyclohexylmagnesium chloride was quenched by reaction with anhydrous HCl before addition of thionyl chloride. The final product was obtained after HPLC purification with an 11% radiochemical yield within 45 min [151]. This modification was reported to increase the reliability of ^{11}C-WAY-100635 production.

The reaction of ^{11}C-carbon dioxide with cyclohexylmagnesium chloride has also been performed by the loop method, i.e. cyclohexylmagnesium chloride was coated on to the inner wall of a narrow polypropylene tube and ^{11}C-carbon dioxide passed into the tube in a nitrogen stream [152]. Passing a solution of thionylchloride through the tubing converted the trapped radioactive adduct into ^{11}C-cyclohexylcarbonyl chloride, which was released into a vial with the amine WAY-100634 (13 mg) and triethylamine. After heating for 5 min at 70°C, the crude product was purified using HPLC, giving ^{11}C-WAY-100635 with a 50–70% radiochemical yield, counted on ^{11}C-cyclohexylcarbonyl chloride within 45 min [152].

Automation of the loop synthesis of ^{11}C-WAY-100635 has been reported by two research groups. Matarrese and colleagues used a modified nuclear interface PET tracer synthesizer for a fully automated synthesis that gave ^{11}C-WAY-100635 with a 15–20% total radiochemical yield [153]. Truong et al. used an in-house synthesis module and a small amount of the amine

WAY-100634 (3 mg), which gave a comparative yield in a short time, 28–30 min [154].

The one pot synthesis of ^{11}C-WAY-100635 has also been automated using 2–3 mg of the amine WAY-100634, giving similar radiochemical yields within 45 min [155]. ^{11}C-WAY-100635 is one of the more demanding PET radiopharmaceuticals to produce, and failures in production are relatively frequent due to the complex procedure and the corrosive reagents.

A low level of contamination with the amine WAY-100634 in the final ^{11}C-WAY-100635 product has been reported to be acceptable. In rats, co-administration of less than 0.2 μg WAY-100634 per kilogram body weight did not affect the uptake of ^{11}C-WAY-100635 [152].

9.3.2.2. ^{18}F-MPPF

^{18}F-MPPF (^{18}F-4-(2′-methoxyphenyl)-1-[2′-[*N*-(2″-pyridinyl)-p-fluorobenzamido]ethyl]-piperazine) is also used for human PET studies of 5-HT_{1A} receptors. The original procedure for preparation of ^{18}F-MPPF used nucleophilic aromatic substitution with ^{18}F-fluoride on a nitro precursor with heating for 20 min at 140°C [156]. After HPLC purification, the product was obtained with a 10% radiochemical yield within 90 min. A modification of this procedure used microwave heating for 3 min and a Sep-Pak formulation (instead of evaporation) of the HPLC purified product [157]. These modifications gave a 25% radiochemical yield within 70 min. ^{18}F-MPPF has also been prepared by an automated procedure using a coincidence FDG synthesizer [158]. Heating for 10 min at 190°C, followed by HPLC purification and Sep-Pak formulation, gave the product with a 20–25% radiochemical yield within 75 min.

MPPF has also been shown to have low toxicity, as 0.23 mg/kg in mice did not induce any clinical signs or adverse affects. Furthermore, no mutagenic activity was observed using a bacterial reverse mutation test with doses of 78–5000 μg/plate [159].

9.3.2.3. ^{11}C-DAS

^{11}C-DASB ([*N*-methyl-^{11}C])*N*,*N*-dimethyl-2-(2′-amino-4′methylphenyl-thio) benzonitrile) is the most commonly used radioligand for PET examinations of serotonin reuptake sites in the human brain. The original procedure for the preparation of ^{11}C-DASB used ^{11}C-methyl iodide with heating for 4 min at 90°C [160]. After HPLC purification, the product was obtained with a 25–55% radiochemical yield, counted on ^{11}C-methyl iodide, within 25–30 min. The synthesis has also been performed in a 2 mL HPLC loop with 5 min heating at room temperature with similar yields. By using ^{11}C-methyl triflate as the

precursor, heating of the reaction mixture can be omitted [161]. A fully automated production of ^{11}C-DASB using commercial synthesis devices, ^{11}C-methyl iodide as the precursor and Sep-Pak formulation of the HPLC purified product has recently been reported [162]. This procedure provided ^{11}C-DASB with a 43% radiochemical yield within 45 min.

9.3.3. Tracers of benzodiazepine receptors

Benzodiazepines bind to two distinct types of binding sites in the human brain: central benzodiazepine receptors and peripheral benzodiazepine receptor binding (PBR) binding sites. Figure 33 shows the chemical forms of the PET tracers for benzodiazepine receptors and brain amyloid. The early development of PET radioligands for these binding sites has been summarized in a report from the EEC Concerted Action on PET [163].

9.3.3.1. ^{11}C-flumazenil

^{11}C-flumazenil, also known as ^{11}C-Ro 15-1788 ([*N*-methyl-^{11}C]ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate), is the most commonly used radioligand for PET examinations of central benzodiazepine reuptake sites in the human brain, and has been

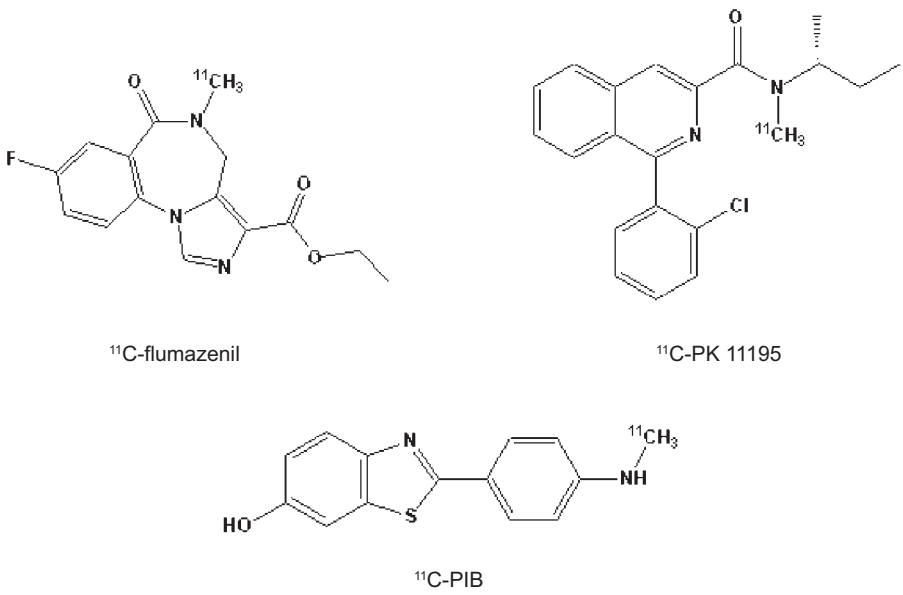


FIG. 33. PET tracers for benzodiazepine receptors and brain amyloid.

especially useful in the diagnosis of epilepsy [164]. The most convenient route for preparation of ^{11}C labelled flumazenil uses either ^{11}C-methyl iodide [165–167] or ^{11}C-methyl triflate. [*O*-ethyl-^{11}C]flumazenil has been prepared and used in a comparison with [*N*-methyl-^{11}C]flumazenil in order to elucidate the metabolic pattern of these differently labelled flumazenil compounds [167]. This study demonstrated that [*N*-methyl-^{11}C]flumazenil does not generate metabolites that disturb the study of central benzodiazepine receptors in humans.

High purity ^{11}C-flumazenil is obtained by reversed phase HPLC purification of the crude product. After evaporation of the purified product, alcohols such as propylene glycol and ethanol are used as co-solvents in the formulation of ^{11}C-flumazenil. Production of ^{11}C-flumazenil has also been achieved by the use of computer control [168] and robotics [169].

9.3.3.2. ^{11}C-PK 11195

^{11}C-PK 11195 is prepared by reaction of ^{11}C-methyl iodide with the anion of the amide precursor. Various bases have been used to extract the proton of the precursor, and different solvents have also been used. In the original procedure [170], dry potassium hydroxide in DMSO was used to trap ^{11}C-methyl iodide. The reaction was reported to be instantaneous. After HPLC purification and evaporation, the product was dissolved in saline and sterile filtered. Fifty per cent of the product was lost in the sterile filter.

A modified procedure [171] used NaH as the base in DMF/DMSO with 1 min heating at room temperature. Oil (Tween-80) and ethanol were used as co-solvents in the final formulation. A more recent procedure [172] used tetrabutylammonium hydroxide as base in DMSO, with 3 min heating at 80°C. Ethanol was used as co-solvent in the final formulation. Base promoted dechlorination of ^{11}C-PK 11195 using the original procedure (dry potassium hydroxide in DMSO) has recently been reported [173]. This dechlorination leads to a labelled by-product that is difficult to separate from ^{11}C-PK 11195. The use of a mild base such as tetrabutylammonium hydroxide is thus recommended to avoid dechlorination.

The first procedures for ^{11}C-PK 11195 production gave the racemic product. The Hammersmith PET group has evaluated the two enantiomers of ^{11}C-PK 11195 and found that the *R*-enantiomer has a twofold higher affinity for PBRs than the *S*-enantiomer [174]. The *R*-enantiomer of the precursor is now commercially available, and subsequently *R*-^{11}C-PK 11195 is now the preferred PET tracer.

9.3.4. Tracers of brain amyloid

A multitude of tracers have recently been developed for imaging of β-amyloid plaques in Alzheimer's disease and mild cognitive impairment [175]. Of these, ^{11}C-6-OH-BTA-1, also known as ^{11}C-PIB ([*N*-methyl-^{11}C]-2-(4′-methylaminophenyl)-6-hydroxybenzothiazole) has become a standard PET tracer that is used in many PET centres.

9.3.4.1. ^{11}C-PIB

The original procedure for the production of ^{11}C-PIB used the reaction of ^{11}C-methyl iodide with the 6-MOM-protected precursor for 5 min, followed by removal of the MOM protection group using HCl in methanol for another 5 min [176]. The radiochemical yield from ^{11}C-methyl iodide was 12%. Three studies have dealt with the production of ^{11}C-PIB using the reaction of ^{11}C-methyl triflate with the non-protected precursor, 6-OH-BTA-0 [177–179]. Wilson et al. [177] used the reaction on the inner walls of an HPLC sample loop or in solution, and reported a 50–60% radiochemical yield from ^{11}C-methyl triflate with either method using 0.4 mg of 6-OH-BTA-0. Solbach et al. [162] used the reaction in solution and reported a 68% radiochemical yield using 4–8 mg of 6-OH-BTA-0. Kemppainen et al. [179] used 0.8 mg of 6-OH-BTA-0 and obtained a 40–60% radiochemical yield. These studies demonstrate that ^{11}C-methyl triflate is the labelled precursor of choice in the production of ^{11}C-PIB and that a high radiochemical yield is obtained using less than 1 mg of 6-OH-BTA-0. When using such low amounts of precursor, HPLC on a reversed phase column is an efficient method for purification of ^{11}C-PIB. Co-solvents such as ethanol and propylene glycol are needed for an efficient solubilization of the final product.

9.3.5. Tracers of blood flow and blood volume

A wide variety of tracers is used to measure blood flow and blood volume in the human brain in vivo with SPECT and PET. The isotopes used for labelling of this kind of PET tracer are mainly ^{11}C, ^{13}N and ^{15}O. Of these three, ^{15}O has the shortest half-life (2 min), which has enabled many repeated studies in a short timeframe. Oxygen-15 can be produced by conventional medical cyclotrons used in ^{11}C and ^{18}F production, and it can also be produced by smaller cyclotrons, so-called oxygen generators. Owing to the short half-life of ^{15}O, the production of ^{15}O tracers is often fully automated, and devices for this production can be obtained from the companies supplying the cyclotrons.

9.3.5.1. ^{15}O-water

^{15}O-water can be produced directly in the target. However, the most commonly used method is to react the cyclotron produced ^{15}O-oxygen gas with hydrogen gas on-line using palladium or platinum as catalyst [180, 181]. Quality control is most conveniently carried out using GC equipped with a radioactivity detector [180, 182].

9.3.5.2. ^{15}O-carbon monoxide

^{15}O-carbon monoxide is most commonly made by reaction of cyclotron produced ^{15}O-oxygen gas with carbon on-line, using high temperatures of 900–950°C [181]. Quality control is most conveniently carried out using GC equipped with a radioactivity detector [180, 182].

9.4. POTENTIAL TRACERS FOR CARDIOLOGY

9.4.1. Metabolic tracers

Figure 34 decribes PET tracers for cardiology.

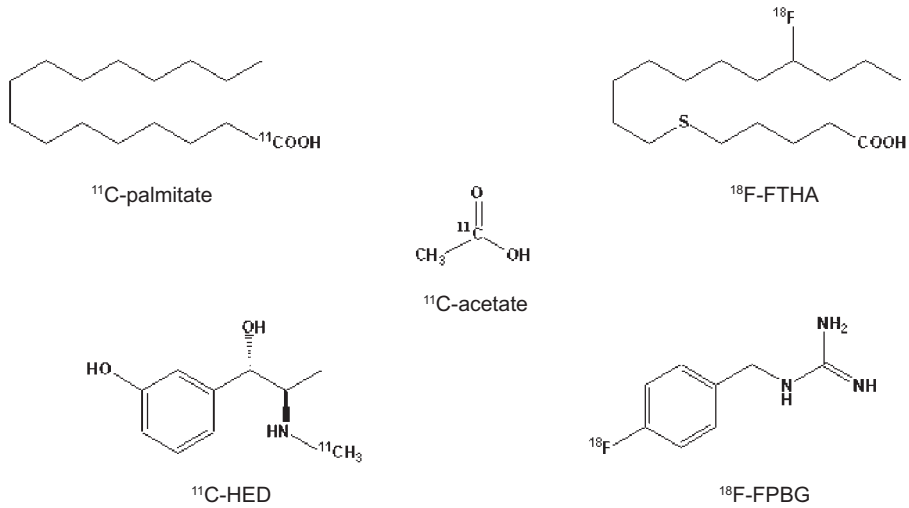


FIG. 34. PET tracers for application in cardiology.

9.4.1.1. ^{11}C-acetate

See Section 9.2.3.4.

9.4.1.2. ^{11}C-palmitate

^{11}C-palmitate is prepared by a Grignard reaction using ^{11}C-CO_2 and *n*-pentadecylmagnesium bromide in diethyl ether or tetrahydrofuran (THF). The reaction is usually carried out in solution, although a reaction on solid supports such as microporous polypropylene powder has been reported [183]. After quenching the reaction mixture with an acid, such as HCl or H_2SO_4, the purification can proceed in various ways. One method, which is still used in many laboratories, is purification on an alumina oxide column from which the product is eluted with acetic acid in diethyl ether [184]. Purification with HPLC has also been reported [185, 186]. Formulation of the purified product is commonly accomplished using as co-solvents sodium bicarbonate and human serum albumin. ^{11}C-palmitate is one of the oldest PET radiopharmaceuticals, and many devices for its remotely controlled or automated production have been described [186–189].

9.4.1.3. ^{18}F-fluoro-fatty acids and fluoropalmitate

Several ^{18}F labelled fatty acid analogues have been proposed for cardiac PET studies. The ^{18}F-6-F- and ^{18}F-7-fluoropalmitic acids were prepared from displacement of the corresponding 6- and 7-mesyl-palmitic acid benzyl esters with ^{18}F-fluoride, followed by hydrolysis of the benzyl ester function. After HPLC purification, the products were obtained with a 6% yield. Preliminary studies in rats indicated a myocardial uptake similar to that of ^{11}C-palmitate [187]. ^{18}F-17-fluoroheptadecanoic acid was prepared using an aminopolyether supported displacement of the corresponding 17-bromo-heptadecanoic acid methyl ester with ^{18}F-fluoride, followed by hydrolysis of the methyl ester function. After HPLC purification, the product was obtained with an 82% yield [190].

The most commonly used ^{18}F labelled fatty acid has been ^{18}F-FTHA (14(*R*, *S*)-^{18}F-fluoro-6-thia-heptadecanoic acid). It is prepared by an amino-polyether supported displacement of the corresponding 14-tosyloxy-6-thia-heptadecanoic acid benzyl ester with ^{18}F-fluoride, followed by hydrolysis of the benzyl ester function. After purification using HPLC, the product was obtained with a 35–65% yield [191]. A microcomputer controlled production of ^{18}F-FTHA giving a 10–15% radiochemical yield has been reported [192].

9.4.2. Neuronal tracers

9.4.2.1. ^{11}C-HED

See Section 9.2.4.1.

9.4.2.2. ^{18}F-PFBG

See Section 9.2.4.2.

9.4.3. Tracers of blood flow and oxygen consumption

Two tracers are commonly used for measurement of cardiac blood flow using PET: ^{13}N-ammonia and ^{15}O-water. Cardiac oxygen consumption is either measured directly using ^{15}O-oxygen gas or indirectly using ^{11}C-acetate. Owing to the short half-life of ^{15}O and ^{13}N, the production of ^{15}O and ^{13}N tracers is often fully automated, and devices for this production can be obtained from the companies supplying the cyclotrons.

9.4.3.1. ^{13}N-ammonia

In most laboratories, ^{13}N-ammonia is produced by reducing the in-target formed ^{13}N nitrates and nitrites by using either DeVarda's alloy in aqueous sodium hydroxide [193] or titanium salts [194]. ^{13}N-ammonia is formed as the primary product in the target and converted by radiolytic oxidation to ^{13}N nitrates and nitrites. By addition of radiolysis scavengers such as alcohols, hydrogen or methane to the target, an in-target production of ^{13}N-ammonia can be achieved [195–197]. The radiochemical purity of ^{13}N-ammonia is determined using HPLC on cation exchange columns [198] or by TLC [199]. Owing to the general importance of ^{13}N-ammonia as a diagnostic PET tracer, there are guidelines for its QC in both the European and US pharmacopoeias.

9.4.3.2. ^{15}O-water

See Section 9.3.5.1.

9.4.3.3. ^{15}O-oxygen gas

^{15}O-oxygen gas is the primary product obtained from cyclotron production of ^{15}O. Quality control is most conveniently carried out using GC equipped with a radioactivity detector [180, 182].

9.5. OTHER TRACERS

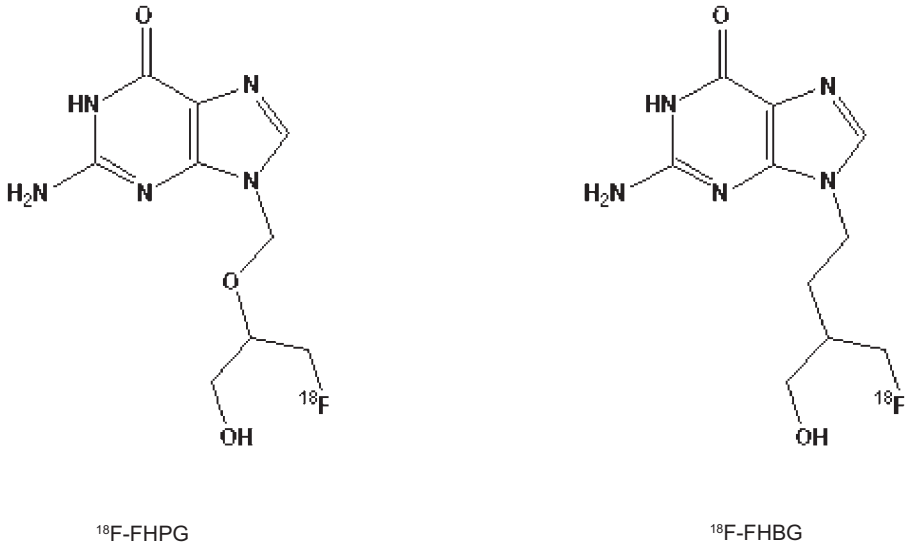
9.5.1. Gene reporter imaging tracers

A multitude of imaging tracers have been prepared and used in imaging of gene therapy in humans [200]. Of these, the tracers of choice for PET imaging (Fig. 35 shows the chemical forms of PET gene receptor imaging tracers) have been ^{18}F-FHPG and recently especially ^{18}F-FHBG [201].

9.5.1.1. ^{18}F-FHPG

^{18}F-FHPG (9-[(3-^{18}F-fluoro-1-hydroxy-2-propoxy)methyl]guanine) was originally prepared by a kryptofix supported reaction of ^{18}F-KF with 9-[N^2, O-bis(methoxytrityl)-3-tosyl-2-propoxy)methyl]guanine, followed by acid hydrolysis of the methoxytrityl protecting groups [202]. After HPLC purification, the product was obtained with a 7–17% radiochemical yield in a total synthesis time of 70–80 min.

The use of 9-[(1, 3-di-tosyl-2-propoxy)methyl]guanine as precursor, followed by basic hydrolysis and HPLC purification gave a 3% radiochemical yield [203]. The use of *N*^{2}-(*p*-anisyldiphenylmethyl)-9-{[1-(*p*-anisyldiphenylmethoxy)-3-tosyl-2-propoxy]methyl}guanine as precursor, followed by acidic hydrolysis and HPLC purification, gave similar results as the original



^{18}F-FHPG ^{18}F-FHBG

FIG. 35. PET gene receptor imaging tracers.

procedure, and by using Sep-Pak purification, the total synthesis time was reduced to 60 min [204]. By using the latter precursor and Sep-Pak purifications both before and after the hydrolysis, the radiochemical yield was increased to 15–30% [205].

A robotic synthesis of ^{18}F-FHPG using the originally proposed precursor and Sep-Pak purifications both before and after the hydrolysis has recently been developed, giving a 16% radiochemical yield in a total synthesis time of 80 min [206].

9.5.1.2. ^{18}F-FHBG

^{18}F-FHBG (9-(4-^{18}F-fluoro-3-hydroxymethylbutyl)guanine) was originally prepared by a kryptofix supported reaction of ^{18}F-KF with 9-[*N*]^2,*O*-bis(methoxytrityl)-3-(tosylmethylbutyl)-guanine, followed by acid hydrolysis of the methoxytrityl protecting groups [207]. After HPLC purification, the product was obtained with a 8–22% radiochemical yield in a total synthesis time of 90–100 min.

The use of N^2-monomethoxytrityl-9-[(4-(tosyl)-3-monomethoxytrityl-methylbutyl]guanine as precursor, followed by acidic hydrolysis and HPLC purification, gave similar results as the original procedure, and by using Sep-Pak purification the total synthesis time was decreased to 60 min [204]. By using the latter precursor and Sep-Pak purifications both before and after hydrolysis the radiochemical yield was increased to 15–30% [205]. The same precursor was used in a microwave and kryptofix mediated nucleophilic fluorination with ^{18}F-KF [208]. Following acidic hydrolysis, Sep-Pak and HPLC purifications, the product was obtained with a 12% radiochemical yield in 55–60 min. The same precursor has also been used in a robotic synthesis with Sep-Pak purifications both before and after the hydrolysis, giving a 19% radiochemical yield in a total synthesis time of 80 min [206].

A simplified procedure using N^2-(*p*-anisyldiphenylmethyl)-9-[(4-tosyl)-3-*p*-anisyldiphenylmethoxymethylbutyl]guanine as precursor and Sep-Pak purifications before and after acidic hydrolysis gave a 20–25% radiochemical yield in 70 min [209]. A fully automated production of ^{18}F-FHBG used the latter precursor and Sep-Pak purification before acidic hydrolysis. After HPLC purification, the product was obtained with a 10–15% radiochemical yield in a total synthesis time of 65 min [210].

^{18}F-FHBG has been found to have an acceptable radiation dosimetry in humans [211]. An acute toxicity evaluation of FHBG in rats of 100 times the expected human dose did not indicate harm to organ function or tissues, and accordingly the FDA has accepted FHBG as an investigational new drug [212].

To summarize, there are many exciting developments that could have the potential to make a clinical difference to patients. These are summarized in Table 21.

TABLE 21. POTENTIAL NEW PET TRACERS

Radiopharmaceutical	Potential application
1. Oncological applications	
^{11}C-methionine	Amino acid transport (brain and non-Hodgkin's lymphoma)
^{11}C-MeAIB	Amino acid transport (uptake system A)
^{18}F-FACBC	Amino acid transport (uptake system A)
^{18}F-FDOPA (6-^{18}F-fluoro-L-DOPA)	Amino acid transport (uptake system L?)
^{18}F-FET (*O*-(2′-^{18}F-fluoroethyl)-L-tyrosine)	Amino acid transport (uptake system L)
^{18}F-F-misonidazole	Hypoxia
^{18}F-fluoro-etanidazole	Hypoxia
^{18}F-F-FAZA	Hypoxia
^{18}F-EF5	Hypoxia
^{18}F-FLT (3′-deoxy-3-^{18}F-fluorothymidine)	Cell proliferation
^{11}C-choline	Brain, lung, prostate and oesophagus cancers
^{18}F-fluorocholine	Prostate cancer
^{11}C-acetate	Prostate cancer
^{18}F-NaF	Bone lesions
^{11}C-HED	Phaeochromocytoma
^{18}F-PFBG (para-^{18}F-fluorobenzylguanidine)	Neuroendocrine tumours

TABLE 21. POTENTIAL NEW PET TRACERS (cont.)

Radiopharmaceutical	Potential application
^{18}F-FMDHT (7α-^{18}F-fluoro-17α-methyl-5α-dihydrotestosterone)	Prostate imaging via androgen receptors
^{18}F-FES (^{18}F-fluoro-17β-estradiol)	Breast cancer
^{11}C-metomidate	Adrenal and cortical cancers
^{18}F labelled monoclonal antibodies, fragments and peptides	Tumour imaging
2. Neuropsychiatric applications	
^{11}C-raclopride	Striatal D_2 receptors
^{18}F-fluoroethyl spiperone	Dopaminergic system and D_2 receptors
^{11}C-FLB 457	Extrastriatal D_2 receptors
^{18}F-fallypride	Dopaminergic system and D_2 receptors
^{18}F-FDOPA (6-^{18}F-fluoro-L-DOPA)	Dopaminergic terminal density
^{11}C-WAY-100635	Serotonergic system, 5-HT_{1a} receptors and epilepsy
^{18}F-MPPF	Serotonergic system and 5-HT_{1a} receptors
^{11}C-DASB	Serotonin reuptake
^{11}C-flumazenil	Epilepsy and strokes
^{11}C-PK 11195	Dementia
^{11}C-PIB	Alzheimer's disease
^{15}O-water	Blood flow
^{15}O-carbon monoxide	Blood volume

TABLE 21. POTENTIAL NEW PET TRACERS (cont.)

Radiopharmaceutical	Potential application
3. Cardiac tracers	
^{11}C-acetate	Cardiomyopathy, heart failure and myocardial efficiency
^{11}C-palmitate	Energy substrate metabolism
^{18}F-fluoro-fatty acids and fluoropalmitate	Energy substrate metabolism
^{11}C-HED	Cardiomyopathy, chronic heart failure and heart transplantation
^{18}F-PFBG (para-^{18}F-fluorobenzylguanidine)	Detection of neuronal damage
^{13}N-ammonia	Blood flow
^{15}O-water	Blood flow
^{15}O-oxygen gas	Oxygen consumption
4. Other applications	
^{18}F-FHPG (9-[(3-[^{18}F]-fluoro-1-hydroxy-2-propoxy)methyl]guanine)	Reporter gene imaging
^{18}F-FHBG (9-[(4-[^{18}F]-fluoro-3-hydroxymethylbutyl)]guanine)	Reporter gene imaging

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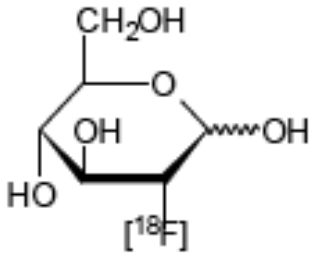
Annex

FLUDEOXYGLUCOSI (^{18}F) INJECTIO
FLUDEOXYGLUCOSE (^{18}F) INJECTION

A monograph on FDG from the WHO International Pharmacopoeia (Ph. Int.) is reproduced in this annex. The other sections of Ph. Int. referred to in this annex are not reproduced here. The contents of this annex have not been edited by the IAEA.



Other names. ^{18}F-FDG injection.

Description. Fludeoxyglucose (^{18}F) injection is a colourless or slightly yellow solution.

Fluorine-18 has a half-life of 109.8 minutes.

Category. Diagnostic.

Additional information. Wherever V is used within the tests of this monograph, V is the maximum recommended dose.

Labelling. State the synthesis pathway (nucleophilic or electrophilic) to prepare 2-[^{18}F]fluoro-2-deoxy-D-glucose.

Requirements

Complies with the monograph for "Parenteral Preparations" and with that for "Radiopharmaceuticals".

Definition. Fludeoxyglucose (^{18}F) injection is a sterile solution of fluorine-18 in the form of 2-[^{18}F]fluoro-2-deoxy-D-glucopyranose (2-[^{18}F]fluoro-2-deoxy-D-glucose), suitable for intravenous administration and that contains sufficient sodium chloride to make the solution isotonic with blood. It contains not less than 90% and not more than 110% of the content of fluorine-18 stated on the label at the reference date and time stated on the label. Not less than 99% of the total radioactivity is due to fluorine-18. Not less than 95% of the total fluorine-18 radioactivity is present as 2-[^{18}F]fluoro-2-deoxy-D-glucose and 2-[^{18}F]fluoro-2-deoxy-D-mannose with the latter not exceeding 10% of the total. The content of 2-fluoro-2-deoxy-D-glucose is not more than 10 mg per V.

Manufacture

Radionuclide production. Fluorine-18 may be prepared by proton irradiation of oxygen-18, deuteron irradiation of neon-20 or alpha irradiation of oxygen-16 and processed in a manner that fluorine-18 obtained is carrier free.

Radiochemical synthesis. 2-[^{18}F]Fluoro-2-deoxy-D-glucose may be synthesized by a nucleophilic or electrophilic pathway, which lead to different products in terms of specific radioactivity, by-products and possible impurities.

STARTING MATERIAL

Precursors for organic synthesis

— *1,3,4,6-Tetra-O-acetyl-2-O-trifluoromethanesulfonyl-β-D-mannopyranose.* Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the reference spectrum of 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-*β*-D-mannopyranose.
Melting range: 119-122°C.

— *3,4,6-Tri-O-acetyl-D-glucal.* Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the *reference spectrum* of 3,4,6-tri-*O*-acetyl-D-glucal.
Melting range: 53–55°C.

A. Nucleophilic pathway
This is the most widely used method by a phase transfer catalysed nucleophilic substitution of 1,3,4,6-tetra-*O*-trifluoromethanesulfonyl-β-D-mannopyranose with [^{18}F]fluoride. Generally [^{18}F]fluoride is absorbed on an anion-exchange resin and eluted with a solution of potassium carbonate which is then evaporated to dryness. Addition of a phase transfer catalyst such as an amino-polyether in dry acetonitrile may be used to enhance the nucleophilicity of the [^{18}F]fluoride. Hydrolysis using hydrochloric acid may lead to the formation of 2-chloro-2-deoxy-D-glucose. Hydrolysis under alkaline conditions may lead to the formation of 2-[^{18}F]fluoro-2-deoxy-D-mannose as a by-product. Variations of the method substitute the aminopolyether by a tetra-alkyl ammonium salt, or use solid phase catalysed nucleophilic substitution on derivatized anion-exchange resin, e.g. derivatized with 4-(4-methylpiperidin-1-yl)pyridine.

B. Electrophilic pathway
This method for production of 2-[^{18}F]fluoro-2-deoxy-D-glucose proceeds by the reaction of molecular fluorine-18 or [^{18}F]acetylhypofluorite with 3,4,6-tri-*O*-acetyl-D-glucal. [^{18}F]Acetylhypofluorite is obtained by conversion of molecular fluorine-18 on a solid complex of acetic acid and potassium acetate. The production of molecular fluorine-18 requires the addition of small amounts of fluorine to the neon target gas, usually from 0.1 per cent to 1 per cent, resulting in the reduction of the specific radioactivity of the end product. Hydrolysis of the *O*-acetyl protected [^{18}F]fluorinated sugar yields 2-[^{18}F]fluoro-2-deoxy-D-glucose and usually small amounts of 2-[^{18}F]fluoro-2-deoxy-D-mannose.

The preparation can be purified by serial chromatography on combinations of ion-retardation resin, ion-exchange resin, alumina and octadecyl derivatized silica gel. Removal of the phase transfer catalyst can be achieved by different methods, all using combinations of separation cartridges.

Aminopolyether. This test is performed only on the bulk solution before addition of sodium chloride by the producer and it is not intended for the final preparation to be injected. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel for chromatography R as the coating substance and a mixture of 1 volume of ammonia R and 9 volumes of methanol R as the mobile phase.

Apply separately to the plate 2 μL of the following 2 solutions. For solution A use the injection to be examined. For solution B dissolve 0.110 g of aminopolyether R in water R and dilute to 10.0 mL with the same solvent. Dilute 0.2 ml of this solution to V with the same solvent.

Develop the plate for a distance of about 8 cm. After removing the plate from the chromatographic chamber, allow it to dry in air for 15 minutes. Expose the plate to iodine vapour for at least 10 minutes. Examine the chromatogram in daylight.

Any spot due to aminopolyether in the chromatogram obtained with solution A is not more intense than the corresponding spot obtained with solution B (2.2 mg per V).

Tetra-alkyl ammonium salts. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (12.5 cm × 4.0 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 μm). As the mobile phase, use a mixture of 25 volumes of a 0.95 g/L solution of toluenesulphonic acid R and 75 volumes of acetonitrile R.

For solution A use the injection to be examined. For solution B dilute 2.1 mL of tetrabutylammonium hydroxide (0.1 mol/L) VS to 20 mL with water R. Dilute 1 mL of this solution to V with the same solvent. Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 254 nm. Inject separately 10 μL each of solutions A and B.

In the chromatogram obtained with solution A, the area of the peak corresponding to tetrabutylammonium ions is not greater than that of the corresponding peak obtained with solution B (2.75 mg per V).

Solid phase derivatization agent 4-(4-methylpiperidin-1-yl)pyridine. Measure the absorbance of the following solutions at the maximum of 263 nm. For solution A, use the preparation to be examined. For solution B dissolve 20 mg of 4-(4-methylpiperidin-1-yl)pyridine R in water R and dilute to 100.0 mL with the same solvent. Dilute 0.1 mL of this solution to V with the same solvent.

The absorbance obtained from solution A is not greater than the absorbance obtained from solution B (0.02 mg per V).

Production of radiopharmaceutical preparation. Fludeoxyglucose (^{18}F) injection may contain antimicrobial preservatives and/or stabilizing agents. The injection may be sterilized by "Heating in an autoclave" (see 5.8 Methods of sterilization).

Identity tests

- Either tests A and C or tests B and C may be applied.

A. Record the gamma-ray spectrum using a suitable instrument with a sample of fluorine-18, suitably diluted if needed. The spectrum is concordant with the *reference spectrum* of a specimen of fluorine-18 in that it exhibits a major peak of 511 keV, maximum of 633 keV. A sum peak of 1020 keV may also be seen depending on geometry and detector efficiency.
Standardized fluorine-18 solutions are available from laboratories recognized by the relevant national or regional authority.

B. The half-life determined using a suitable detector system is between 105 and 115 minutes.

C. Examine the radiochromatogram obtained in the test for radiochemical purity. The distribution of the radioactivity contributes to the identification of the preparation.

pH value. Carry out the test as described under 1.13 Determination of pH, modified as described in the monograph for "Radiopharmaceuticals". pH of the injection, 4.5 to 8.5.

Sterility. The injection complies with the test described under 3.2 Test for sterility, modified as described in the monograph for "Radiopharmaceuticals". Test for sterility will be initiated on the day of manufacture. The injection may be released for use before completion of the test.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins, modified as described in the monograph for "Radiopharmaceuticals". The injection contains not more than 175/V IU of endotoxins per millilitre. The injection may be released for use before completion of the test.

Radionuclidic purity. Record the gamma ray spectrum using a suitable instrument and measure the half-life using a suitable method. Determine the relative amounts of fluorine-18 and other radionuclidic impurities that may be present. Not less than 99% of the total radioactivity is due to fluorine-18.

Radiochemical purity. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel for chromatography R as the coating substance and a mixture of 95 volumes of acetonitrile R and 5 volumes of water R as the mobile phase. Apply to the plate about 5 μL of the injection, suitably diluted with water R to give an optimum count rate. Allow the plate to dry in air and determine the radioactivity distribution by a suitable method. In this system, fludeoxyglucose has an Rf value of about 0.4. Not less than 90% of the total radioactivity is in the spot corresponding to fludeoxyglucose.

Chemical purity

Toxic substances or by-products including aminopolyether*, tetra-alkyl ammonium salts and 2-chloro-3-deoxy-D-glucose are to be controlled at appropriate limits.

2-Fluoro-2-deoxy-D-glucose and 2-chloro-2-deoxy-D-glucose. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (25 cm × 4.0 mm) packed with strongly basic anion-exchange resin for chromatography R (10 μm). As the mobile phase, use carbonate-free sodium hydroxide (0.1 mol/L) VS.

Operate with a flow rate of 1 mL per minute. As a detector, use suitable detectors for radioactivity and for carbohydrates in the required concentration range.

A. If the product is prepared by electrophilic pathway, prepare the following solutions.

For solution (1) use the injection to be examined. For solution (2) dissolve 10 mg of glucose R in water R and dilute to 100 mL with the same solvent. For solution (3) dissolve 10 mg of 2-fluoro-2-deoxy-D-glucose R in water R and dilute to V with the same solvent.

Inject alternately 10 μL of solutions (1), (2) and (3).

* kryptofix.

In the chromatogram obtained with the detector for carbohydrates and solution (1) the principal peak corresponds to the principal peak in the chromatogram obtained with solution (3) (2-fluoro-2-deoxy-D-glucose).

In the chromatogram obtained with solution (1), the area of the peak corresponding to 2-fluoro-2-deoxy-D-glucose is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (10 mg per V).

B. If the product is prepared by the nucleophilic pathway, prepare the following solutions.

For solutions (1), (2) and (3) use the solutions described under A for electrophilic pathway. For solution (4) dissolve 1.0 mg of 2-chloro-2-deoxy-D-glucose R in water R and dilute to 2 mL with the same solvent then dilute 1 mL of this solution to V with the same solvent. For solution (5) dissolve 1.0 mg of 2-fluoro-2-deoxy-D-mannose R in water R and dilute to 2 mL with the same solvent. Mix 0.5 mL of this solution with 0.5 mL of solution (3).

Inject alternately 10 μL of solutions (1), (2), (3), (4) and (5).

In the chromatogram obtained with the detector for carbohydrates and solution (1) the principal peak corresponds to the principal peak in the chromatogram obtained with solution (2) (D-glucose). The peaks, if present, are eluted at the following retention times with reference to 2-fluoro-2-deoxy-D-glucose (retention time 12 minutes): 2-fluoro-2-deoxy-D-mannose about 0.9; 2-chloro-2-deoxy-D-glucose about 1.1.

In the chromatogram obtained with the detector for carbohydrates and solution (5), the test is not valid unless the resolution between the peaks due to 2-fluoro-2-deoxy-D-mannose and 2-fluoro-2-deoxy-D-glucose is at least 1.5 and the peak due to 2-fluoro-2-deoxy-D-glucose is detected with a signal-to-noise ratio of at least 10.

In the chromatogram obtained with solution (1):

— the area of the peak corresponding to 2-fluoro-2-deoxy-D-glucose is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (10 mg per V);

— the area of any peak corresponding to 2-chloro-2-deoxy-D-glucose is not greater than the area of the corresponding peak in the chromatogram obtained with solution (4) (0.5 mg per V).

Radioactivity. Measure the radioactivity in a suitable counting equipment by comparison with a standardized fluorine-18 solution or by measurement in an instrument calibrated with the aid of such a solution.

Standardized fluorine-18 solutions are available from laboratories recognized by the relevant national or regional authority.

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