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IAEA-TECDOC-1856

Quality Control in the Production of Radiopharmaceuticals

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QUALITY CONTROL IN THE PRODUCTION OF RADIOPHARMACEUTICALS

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QUALITY CONTROL IN THE PRODUCTION OF RADIOPHARMACEUTICALS

INTERNATIONAL ATOMIC ENERGY AGENCY
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FOREWORD

Radiopharmaceuticals are central to nuclear medicine applications for the diagnosis and therapy of human diseases, especially cancers and cardiovascular diseases. In the past few decades, advances in the production and quality control of emerging radiopharmaceuticals have led to the production of new radiopharmaceuticals and the availability of new production routes and methods for existing agents. Various new diagnostic agents in the field (such as ^{68}Ga radiopharmaceuticals and generators) as well as therapeutic agents (such as alpha emitters) have already been added to the clinician toolkit. However, the lack of generic and peer-reviewed quality control guidelines and recommendations for their application in human patients is a major concern.

To address this need, and in response to requests from Member States and professional societies, the IAEA developed this publication on quality control in the production of radiopharmaceuticals. It is expected to be of use for all professionals involved in the production and quality control of radiopharmaceuticals worldwide.

This publication is an outcome of the work of an international team of experts in the field between 2016 and 2018. The IAEA wishes to thank the experts for their contributions. Special thanks are due to J. Ballinger (United Kingdom) and F. Dollé (France) for compiling the material, and J.S. Vera Araujo (Bolivarian Republic of Venezuela) for her editorial support. The IAEA officer responsible for this publication was A.R. Jalilian of the Division of Physical and Chemical Sciences.

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1. INTRODUCTION

1.1.BACKGROUND

Quality control and quality assurance are important for all classes of pharmaceuticals, but there are several specific issues for radiopharmaceuticals. A poor quality diagnostic radiopharmaceutical, while not in itself unsafe, could give incorrect information about the patient's condition leading to an inappropriate choice of therapy. A poor quality therapeutic radiopharmaceutical could lead to excess radiation exposure to non-target tissues along with reduced efficacy. The short physical half-lives of radiopharmaceuticals create logistical challenges to perform quality control before the product's release for use. Therefore, it is essential that radiopharmaceuticals are prepared within a quality system in which there is outstanding control of materials and personnel, adequate documentation, and continuous review of ongoing results.

Radiopharmaceuticals are considered a safe class of agents, in part due to the small chemical quantities administered in most cases [1]. However, if a study had to be repeated because of a poor quality radiopharmaceutical, the patient would receive an unnecessary radiation dose with useless consequences. The preparation of radiopharmaceuticals is generally reliable, but a vast range of major and minor problems have been encountered over the years [2, 3]. The reliability of radiopharmaceuticals depends on both, the design of preparation procedures (e.g. generators and kits, automated synthesis units) and quality control measures before release.

The $^{99}Mo/^{99m}Tc$ generator and kits have been used worldwide for more than 40 years. Over the last 20 years, PET ^{18}F radiopharmaceuticals have become more widely available. In the last 5 years, ^{68}Ga has entered the scene and is expected to expand within the availability of kit preparations. Therapeutic radiopharmaceuticals have been important for more than 60 years but are taking new directions, in particular the recent introduction of the alpha particle emitters ^{223}Ra and ^{213}Bi.

This report draws on the experience of a group of experts who collectively have worked with all these classes of radiopharmaceuticals. The goal is to assemble practical information which can help users, who are unfamiliar with certain classes of radiopharmaceuticals, to establish adequate quality control procedures to ensure the safe production of radiopharmaceuticals. The report also gives an overview of radiopharmaceutical legislation in various parts of the world.

One class of agents which will not be addressed is radiolabelled autologous cells, the topic of a recent IAEA publication [4]. Furthermore, this report will focus only on quality control procedures and equipment and will not address quality assurance and documentation.

1.2.OBJECTIVE

These guidelines are intended to help laboratories producing radiopharmaceuticals to set up QC systems, for permitting sufficient testing of the produced radiopharmaceuticals. They aim to be used by hospital radiopharmacies (mainly SPECT), PET production units, and commercial manufacturers. These guidelines do not represent legally enforceable regulations but rather best practices for quality control in the production of radiopharmaceuticals. They address the

minimum requirements for appropriate equipment, processes, and controls and resources needed for QCs.

1.3. SCOPE

This publication was designed to serve as specific guide for quality control of radiopharmaceuticals for all Member States with emphasis on the agents developed in the last few decades. An overview of the quality control processes has been presented in a generic format regardless of the radiopharmaceuticals being prepared in house or receiving them from another radiopharmaceutical producer for routine control and validation purposes. The document describes the equipment together with qualification of these instruments, the techniques and protocols of quality control of radiopharmaceuticals. Annex-1 describes the radionuclides used in the production of radiopharmaceuticals and Annex-II describes the quality control of these precursors. Annex-III demonstrates the quality control methods in detail for each of the selected representative radiopharmaceuticals. Last but not the least, Annex IV describes the status of existing legislation related to radiopharmaceuticals in various global regions. The most important radioactive isotopes covered in this document are ^{99m}Tc, ^{68}Ga, ^{90}Y, ^{213}Bi derived from related generators, as well as directly produced ^{68}Ga and ^{99m}Tc using cyclotrons. On the other hand, the appropriate quality control procedures for the most widely used radiopharmaceuticals produced from mentioned radioisotopes have been described in detail.

1.4 STRUCTURE

This TECDOC describes the essential protocols, techniques and equipment used in the area of Quality Control of Radiopharmaceuticals. It intends to help the radiopharmacy professionals to safely produce medical radioisotopes and radiopharmaceuticals for diagnosis and therapeutic applications.

Section 1 introduces the topic of the publication. Section 2 describes concepts of the quality control procedures for medical radioisotopes and radiopharmaceuticals. Section 3 explains how to handle out-of-specification results. Section 4 fully describes methods and equipment used in routine quality control of medical radioisotopes and radiopharmaceuticals, including validation of methods and qualification of equipment. Section 5 deals with safety issues related to quality control of radiopharmaceuticals. Section 6 describes how to train personnel to perform the quality control assays.

This document also includes 4 Annexes describing the medical radioisotopes describes in the document, examples of quality control of selected medical radioisotopes and radiopharmaceuticals and finally giving examples of related legislation in different countries and regions.

2. QUALITY CONTROLS OF STARTING MATERIALS

2.1. INCOMING NON-RADIOACTIVE STARTING MATERIALS

The minimum quality controls to be performed for incoming material acceptance are: identification and purity (Certificate of Analysis (CoA)). Additional acceptance criteria may need to be established if required.

2.2. INCOMING RADIOACTIVE STARTING MATERIALS

Regarding radionuclides, the minimum quality controls should be performed for incoming material acceptance: radionuclide identity confirmation and activity confirmation via inspection of CoA, and verified by activity measurement if possible. Additional acceptance criteria may need to be established if required. In addition to these requirements, conformance to the parent radionuclide breakthrough acceptance criteria should be established for the generator.

2.3. IN-HOUSE-PRODUCED RADIOACTIVE STARTING MATERIALS

There are three types of in-house produced radioactive starting materials: generator, reactor and cyclotron produced radioisotopes. First, for a generator-produced radioisotope, there should be at least a parent radionuclide breakthrough, radionuclide purity, chemical purity (e.g. metal contamination), and radiochemical purity tests. These tests may be performed either on the eluate or on the final product. Second, for a reactor-produced radioisotope there should be radionuclide purity, specific activity and chemical purity (isotope-dependent). Third, for a cyclotron-produced radioisotope, there should be done at least radionuclide purity and identity. These tests may be performed either on the radionuclide itself or on QC testing on the final product. Other tests such as chemical purity, radiochemical purity, and specific activity may also apply for example when the radionuclide is produced via irradiation of a solid target.

2.4. IN-HOUSE NON-RADIOACTIVE PRODUCED STARTING MATERIALS

2.4.1. Identity and purity

In a situation when the ‘key intermediate’ materials are synthesized from raw materials by using complex chemical reactions (e.g. radiopharmaceutical precursor material), confirmation of the chemical identity and purity of the prepared material should have the minimum QC required, in order to qualify the material for subsequent clinical radiolabelling. Additional testing (e.g. residual solvents, trace-metal analysis, water content, endotoxin, sterility, or bioburden) may apply if necessary for the specific process. For example, testing of trace metals content may not be necessary when the material will be subsequently radiolabelled with halogens, but is absolutely critical when the material is intended for labelling with radiometals.

In-house preparations that involve simple mixing of compatible materials (e.g. preparing solvents or buffers), generally does not require additional QC testing as long as all of the information related to the preparation process is recorded and traceable and the material is appropriately labelled. There may be situations, however, when additional QC testing may need to be performed because a specific parameter may be critical in subsequent radiopharmaceutical

preparations (e.g. pH). Therefore, the requirement for a specific QC test should be established on a case-by-case basis. In all cases, the quality of the reagents used in preparation should be verified via examination of the CoA for the specific lot of the material.

2.4.1.1. Bioburden

Bioburden is the calculated estimation of the number of microorganisms (also known as colony forming units or CFU's) that is present in the production process prior to terminal sterilization. Bioburden value provides useful information only when the process relies on terminal sterilization to assure sterility, because it demonstrates that the microbial load in a particular closed system (i.e. a cassette or a fluid path) is below the specification that the sterilization method is capable of handling. If the process relies on aseptic processing (i.e. mixing sterile components under aseptic conditions without terminal sterilization), then the bioburden determination does not apply as it should be zero (i.e. sterile). Bioburden testing is normally performed at a contract laboratory, specializing in microbiology testing.

2.5. QUALITY CONTROL OF RADIONUCLIDES

A radiopharmaceutical may be a radionuclide itself or a radionuclide attached to a vector. The purpose of QC is to ensure that the quality of the produced radiopharmaceutical meets predefined acceptance criteria. These criteria should be based on the radionuclide and the nature of the vector used (if any, chemical/biological structure), the process of preparation, formulation and the intended route of administration.

3. QUALITY CONTROL OF RADIONUCLINES AND RADIOPHARMACEUTICALS

3.1. RADIONUCLIDIC IDENTITY DETERMINATION

3.1.1 Half-life determination

The aim is to confirm the radionuclidic identity for relatively short-lived radionuclides. The method is as follows:

— Place a sample of the radiopharmaceutical in a certified dose-calibrator (or any other suitable device);
— Record the activity and the exact time for a pre-selected period;
— Apply the formula $T½ = -0.693\times(\Delta t) / \ln (A_f / A_0)$, where Δt is the measuring period duration, A_0 is the starting activity and A_f is the activity measured at the end of the measuring period.

The European Pharmacopoeia (Ph recommends at least three types of measurement, and a measurement period of one quarter the expected half-life. The standard practices in North America include a 10-minute measurement period.

The specification requires the calculated value be within a predefined range of the accepted half-life value (generally 10%).

3.1.2. Gamma spectrometry

The aim is to confirm the radionuclidic identity for longer lived radionuclides when half-life measurement is not practical. The method is as follows:

— To place a sample of the radiopharmaceutical into a gamma-spectrometer and record the signal per a minimum of 10 000 counts;
— Note that the sample activity loaded should result in less than 5% dead time;
— Confirm that the main gamma peak(s) on the spectrum corresponds to the known gamma peak(s) of the radionuclide.

The specification requires that the main gamma peak(s) on the spectrum corresponds to the known gamma peak(s) of the radionuclide.

3.1.3. Radionuclidic purity

The aim is to determine the radionuclidic purity in validation batches only, not in every batch. The method is as follow:

— To place a decayed sample (of at least 10 half-lives the radionuclide of interest) of the radiopharmaceutical into a gamma-spectrometer;
— Analyse the sample for an extended period of time until trace radionuclide impurity gamma spectrum becomes available.

Note, this analysis is subject to great variability due to the dependence on sample and calibration source geometry. Additionally, the quantification analysis is complex and requires significant physics expertise which may not be available on-site.

The specification requires an isotope-dependent. For example, for ^{18}F, values of 99.9% and 99.5% are recommended by Ph. Eur. and USP, respectively. For ^{99m}Tc, values of 99.88 % and 99.935% are recommended by Ph. Eur. and USP, respectively.

3.1.4. Dose-calibrator measurements

3.1.4.1. Case 1: ^{13}N determination in [^{18}F] NaF.

The aim in some cases are to expect impurities that may have the same emission pathway of the interested radionuclide. For instance, this is what happens with positron emitting radionuclides, which all display the same gamma ray emissions at 511 KeV and 1022 KeV (sum peak) and may thus not be distinguished using gamma spectrometry. A practical example is represented by the contamination of ^{13}N in ^{18}F solutions, which is usually not a concern, unless the ^{18}F labelled radiopharmaceuticals is prepared in a very short time, such as [^{18}F] NaF.

The method is as follows: place a sample of the radiopharmaceutical in a certified dose calibrator [ensure that the correct radionuclide is selected] and record the activity and the exact time for two time points. Note that, due to the very short half-life of ^{13}N, the selected time points

have to be very close in time (e.g. two minutes). Apply the following formula, using a suitable calculation software (e.g. Microsoft Excel™):

- $A_{t2} – [A_{t1}\ e^{-(L}R1^{t)}] / -e^{-(L_{R1}t)} + e^{-(L_{R2}t)} = A_{C1}$ (activity of the main radionuclide, corrected at first determination with dose calibrator);
- $A_{C1}\ e^{-(L_{R2}t)} = A_{C2}$ (activity of the main radionuclide, corrected at second determination with dose calibrator);
- $(A_{C2} / A_{C1}) \times 100 = \%\ A_{R2}$ (percentage of the main radionuclide).

Where:

- t = time elapsed between the two activity measurements;
- R1 = contaminant radionuclide (e.g. ^{13}N);
- R2 = main radionuclide (e.g. ^{18}F);
- A_{t1} = activity determined at first time point;
- A_{t2} = activity determined at second time point;
- λ_{R1}, λ_{R2} = decay constant for contaminant and main radionuclides, respectively.

The specification requires an Isotope-dependent. For example, for [^{18}F] NaF, a value of 5% for ^{13}N may be acceptable.

3.1.4.2. Case 2: Determining the radiochemical purity of cyclotron-produced ^{99m}Tc

The isotopic composition of the target material, the irradiation conditions, and time of injection after the end of the bombardment significantly affect the quality of the cyclotron produced ^{99m}Tc. The presence of Mo isotopes ($^{92\text{-}98}Mo$) in the enriched ^{100}Mo target material is leading to the formation of technetium impurities that are increasing the effective dose to the patient. Considering the emission properties of the potential contaminants and the overwhelming presence of ^{99m}Tc, the determination of the radionuclidic purity by γ-spectrometry is very tedious. In these circumstances, a fast and simple dose-calibrator based on quality control tests for the radionuclidic purity has been established. The method is the following:

— A sample of at least 0.5 GBq of the sodium [^{99m}Tc] pertechnetate is placed in a dose calibrator to ensure that the daily tests have been performed and that the dose calibrator is set for ^{99m}Tc;
— A first measurement is performed and recorded as R_{air};
— The sample is placed in the molybdenum assay shield and a second measurement is saved as R_{Lead}. Using the following formula, the emission rate from impurities per MBq of ^{99m}Tc can be calculated from the readings R_{air} and R_{Lead}: $r = k \times (R_{Lead} / R_{air})$

Where:

— R_{Lead} = Dose calibrator reading of sample in lead;
— R_{air} = Dose calibrator reading of sample in air;
— $k = 1x10^6$ (conversion factor relating the ratio R_{Lead} / R_{air} to the emission rate from Tc impurities per MBq of ^{99m}Tc).

The specification requires the acceptance criteria: r <6000.

3.1.4.3. Case 3: Determining the breakthrough of $^{99}Mo/^{99m}Tc$ generators

The aim is to determine the ^{99}Mo content in a $^{99}Mo/^{99m}Tc$ generator eluate. The method is as follows:

Zero the dose calibrator on the ^{99}Mo assay setting;

- Place the eluate vial (or a high activity ^{99m}Tc labelled radiopharmaceutical) in a designated lead shield of known thickness and record the reading in the dose calibrator on the ^{99}Mo ASSAY setting (the ^{99}Mo ASSAY setting applies a correction factor for the partial attenuation of the 740 keV ^{99}Mo photons; the 140 keV ^{99m}Tc photons are completely attenuated);
- Remove the ^{99m}Tc vial from the lead shield and record its activity on the ^{99m}Tc setting;
- Divide the ^{99}Mo reading by the ^{99m}Tc reading to calculate the % ^{99}Mo in ^{99m}Tc value.

The specification establishes by Ph. Eur. and Ph. Int. limit is 0.1% ^{99}Mo in ^{99m}Tc. The USP limit is 0.15 kBq ^{99}Mo per MBq ^{99m}Tc (which is equivalent to 0.15 µCi ^{99}Mo per mCi ^{99m}Tc or 0.015%). Furthermore, the USP specifies that the total amount of ^{99}Mo is no more than 92 kBq (2.5 µCi) per administered dose. Note that the limit applies at the expiration time of the radiopharmaceutical, even though the measurement is normally taken shortly after elution; thus, if the expiry time is 8 h after elution the limit would be 0.04% at elution in the case of the Ph. Eur. specification.

3.1.4.4. Gamma-counting

The aim is to determine the percentage of ^{68}Ge breakthrough in ^{68}Ga labelled radiopharmaceutical batches prepared using $^{68}Ge/^{68}Ga$ generators. The method is as follows:

- The ^{68}Ga labelled radiopharmaceutical samples of known volume will be stored for ≥24 hours to allow for ^{68}Ga radioactivity to decay completely;
- The decayed samples are then analysed in a gamma counter;
- A ^{68}Ge reference source with known activity and calibration date is also analysed in the gamma counter;
- The radioactivity produced by ^{68}Ge present in the product samples will be calculated based on the comparison of the counts produced by the product samples to the counts produced by the reference standard of known ^{68}Ge activity, decay-corrected to the date of analysis;
- The total batch ^{68}Ge activity at EOS will be calculated by multiplying the decayed sample activity by the ratio of total batch volume to the decayed sample volume and then decay corrected to the batch EOS time.

Note that the percentage of ^{68}Ge breakthrough will be determined by dividing the batch activity produced by ^{68}Ge at EOS by the total measured ^{68}Ga batch radioactivity at EOS.

The specification mandates the acceptance criteria for ^{68}Ge breakthrough is ≤0.001%.

3.1.4.5. Gamma-spectrometry

The aim is to determine the amount of radionuclidic gamma emitting impurities. The method is as follow:

- The sample is allowed to decay completely until the gamma peaks present on the gamma spectrum for the radionuclide of interest are either not detectable or present in quantities that allow for detection of other gamma peaks that are associated with radionuclidic impurities that may be present in the product sample;
- The identity of radioactive impurities may be established by examination of the energies of the peaks;
- The content of a specific impurity may also be established via comparison of the signal strength of a particular peak that is associated with impurity radionuclide to the signal strength of a peak produced analysing a sample known to contain a certain amount of impurity;
- This method requires significant expertise in gamma spectrometry and physics and is affected by many factors, such as instrument efficiency and sample geometry. Therefore, unless expertise is present in-house, it is recommended that an outside laboratory specializing in radiation environmental sample analysis is contracted to perform this type of analysis.

The specification varies depending on the radionuclide. For new radionuclides where no reference exists, the value must be established based on gamma spectrometry experimentation.

3.1.5. Radiochemical identity

3.1.5.1. Thin and instant layer chromatography

The aim is to identity a radiolabelled molecule by comparing its retention factor (R_f) with the reference standard R_f. The method is generally in a TLC, iTLC chromatogram, the R_f of the radioactive peak corresponds with of a known reference standard. Radiochemical identity is thus assessed by comparing the R_f from the TLC, iTLC radioactive scanner report with the R_f of the UV spot corresponding to the non-labelled molecule. The R_f is determined as the ratio of the distance from the origin to the spot to the distance from the origin to the solvent front. The specification requires that the R_f of the radiolabelled molecule should be within 10% of the R_f of the reference standard.

3.1.5.2. Size exclusion high performance liquid chromatography

The aim is to confirm the radiochemical identity of the radiolabelled antibody by comparing the product sample peak retention time to the reference standard retention time. The method is as follow:

- A 20 µL aliquot of product sample is loaded into a HPLC system equipped with a 7.8 mm x 30 cm, 3 to 5 µm SEC-HPLC column and using 100 mM sodium citrate/100 mM sodium chloride, pH 6.4, solution mobile phase at a flow rate of 1.0 mL/min;

- The total analysis time is 20 minutes;
- Smaller SEC-HPLC columns may also be used and may produce better visualization of the UV peaks;
- The retention time of the radioactive peak produced during radioactive sample analysis (Figure 1) is then compared to the retention time of the reference standard 280 nm UV peak (Figure 2);
- Agreement in retention times between the product sample peak on the radioactivity detector trace and the standard peak on the UV trace indicates conformance to the radiochemical identity specification.

The reference standard used to establish identity may be either an unmodified protein of known structure or a protein reference standard solution (e.g. Phenomenex, Cat # AL0-3042). A representative copy of radiochemical identity testing is depicted in Figure 1, below.

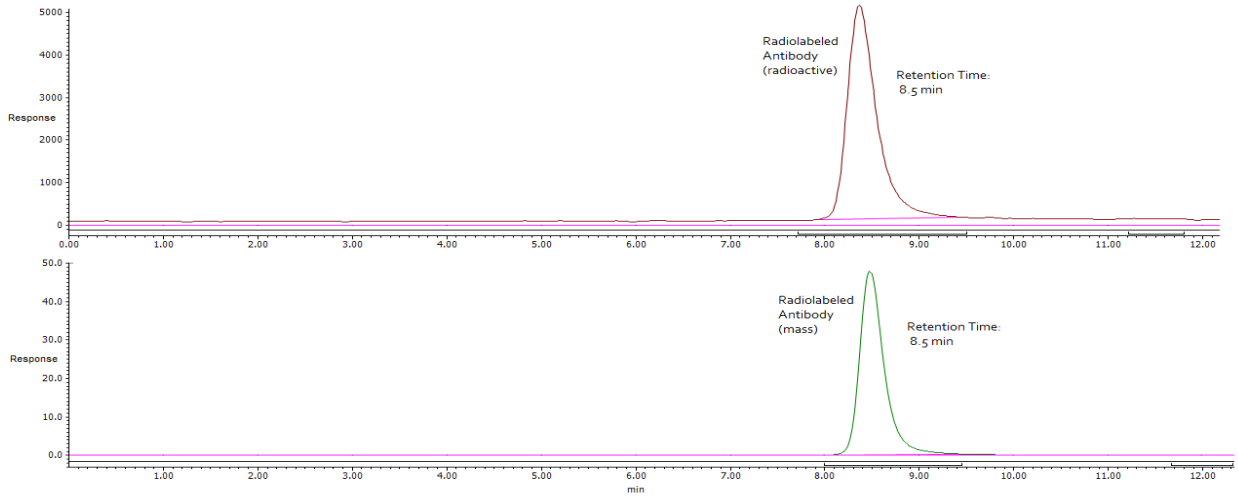

FIG. 1. Monoclonal Antibody Standard Representative Chromatogram.

The specification requires the retention time of the product peak should correspond to the retention time of the reference standard peak. Values of 10 to 15% have been used.

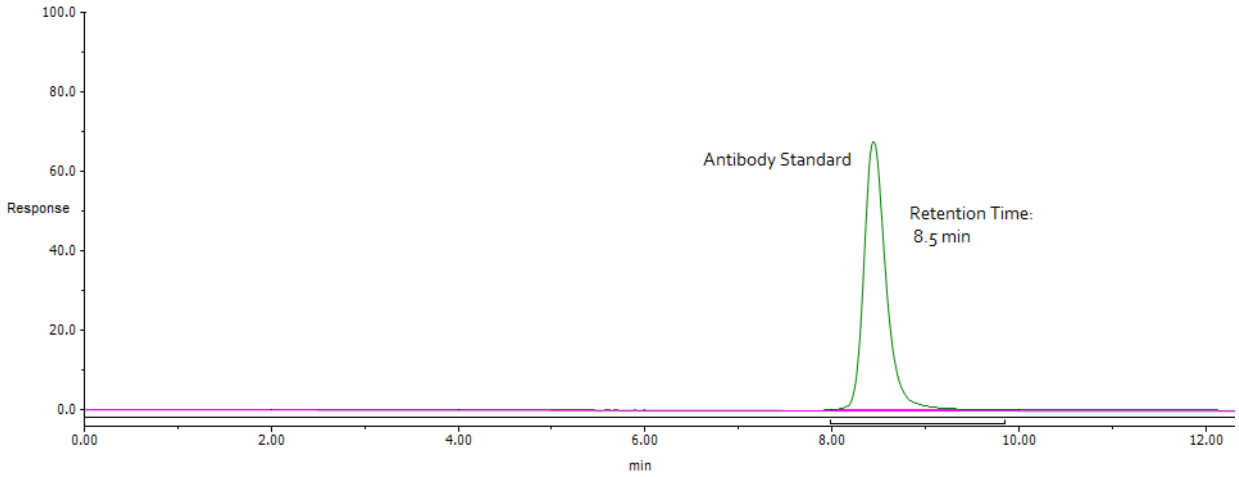

FIG. 1. Monoclonal Antibody Standard Representative Chromatogram (radiopharmaceutical batch).

In FIG. 2 the UV detector, set to 280 nm wavelength, detects a peak at 8.5 minutes. Radioactivity detector trace (not shown) produces no pronounced peaks.

Radioactivity detector trace shows a peak with retention time of 8.5 minutes, which is the same as the retention time of the standard presented in Figure 2, above. This confirms the radiochemical identity of the radiolabelled antibody. UV detector also detects antibody mass as the radiolabelled antibody is passing through the detector, producing a UV peak at 8.5 minutes.

3.1.5.3. High performance liquid chromatography

The aim is to determine the radiochemical identity of a radiopharmaceutical using HPLC. The method is the following:

- Inject a sample of the radiopharmaceutical into a validated HPLC system. For example, in a system equipped with a reverse phase HPLC column, an ultraviolet/visible (UV/Vis) spectrophotometer and a radioactivity detector;
- Record the chromatogram(s) and;
- Identify the radioactive and the UV/Vis (if applicable) peak(s) as the radiopharmaceutical by comparison of the retention time recorded with an authentic sample of the non-labelled derivative as reference.

The specification requires that the radiochromatogram should contain one radioactive peak, which has a retention time compatible (+/- 10%) with the one obtained in the UV chromatogram with a solution of the reference compound.

3.1.5.4. Paper-electrophoresis

The aim is to determine the radiochemical identity of a radiopharmaceutical using paper electrophoresis. The method is as follow:

- Apply a sample of the radiopharmaceutical and a sample of a radioactive reference compound on a wetted suitable paper strip and start the paper electrophoresis by power on the electric current at the defined voltage;
- After the defined runtime the electrophoresis is stopped, and the paper strip is dried;
- By measurement using a TLC scanner, the electrophoretic migration is determined;
- The test is valid only if the radioactive reference moves to the specified distances from the baseline and the sample migrate in a specific ratio.

The specification requires the radio electrophoresis should contain a principal radioactive peak, which has a specific movement ratio compared to the movement of a radioactive reference compound.

3.2. RADIOCHEMICAL PURITY

3.2.1. Chromatographic methods

3.2.1.1.Thin and instant layer chromatography

The aim is to determine the radiochemical purity of a radiopharmaceutical. The method is as follows:

- Apply a sample of the radiopharmaceutical (5 µL or a suitable volume) on a TLC plate/iTLC paper and dry the spot in a stream of air;
- Insert the TLC/iTLC paper into a chamber containing a suitable solvent (mobile phase) which is added a few minutes before into the chamber for the saturation;
- Allow the mobile phase to migrate to the top of the TLC/iTLC paper. The mobile phase level must be below the test spot on the TLC/iTLC paper;
- RCP is established depending on the distribution of components between the stationary (TLC/iTLC paper) and the mobile phase;
- Therefore, the radioactivity distribution is determined by using a radioactivity scanner (plate or paper strip) or by counting small pieces in the case of iTLC (paper).

RCP is defined as the ratio of counts in the product peak (or product counts in the pieces) compared to total counts on the plate / paper. RCP (%) = 100 x (counts in product /total counts on plate or paper). If the RCP is less than the specification (typically 95% or recommended value in related monograph), the batch is rejected.

3.2.1.2.Size Exclusion High Performance Liquid Chromatography

The aim is to provide information on radiolabelled macromolecules protein integrity (i.e. the existence of high mass aggregates or low molecular weight species in protein solutions). The methods refer to the Radiochemical Identity by SEC-HPLC section above for details on how to analyse samples. In order to understand how to interpret a SEC-HPLC chromatogram one needs to know the SEC-HPLC column method of separation, which is depicted in Figure 3, below.

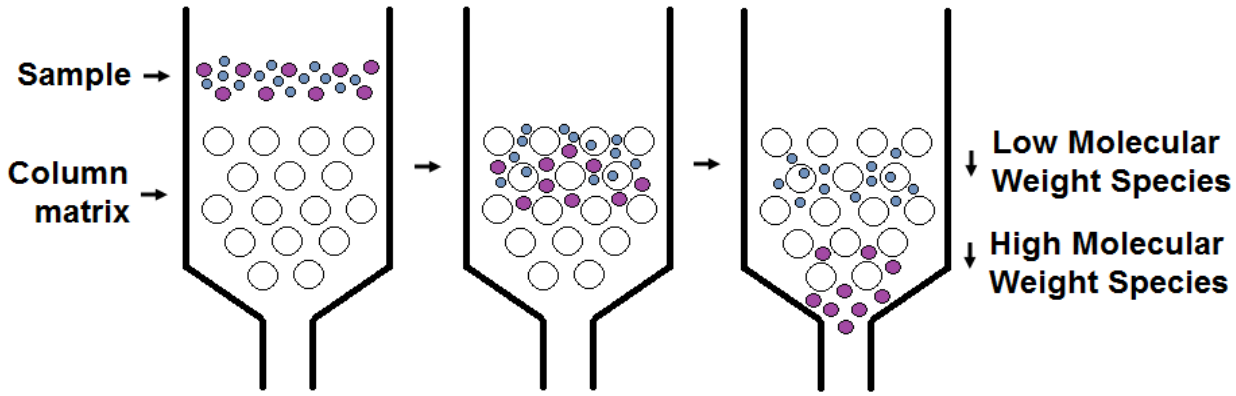

FIG.2. SEC-HPLC Column Method of Separation.

- As the sample components interact with the SEC-HPLC column matrix, larger size molecules such as aggregated proteins (also known as high molecular weight species or HMWS) pass through the column quicker;
- These molecules are then detected by the detectors and produce peaks with relatively shorter retention times;
- Smaller molecules such as pieces of protein (also known as low molecular weight species or LMWS) interact with the column for longer periods of time and produce peaks with relatively longer retention times, once it is eluted from the column and detected by the detectors;
- Therefore, one can use the impurity peak retention times, relative to the known peak retention time, to determine whether the impurity has either higher molecular weight or smaller molecular weight than the molecule of interest. For example, if the radiolabelled IgG antibody produces a peak at 8.5 minutes, any peaks detected prior to 8.5 minutes indicate presence of high molecular weight species (aggregates in some cases) and any peaks present after 8.5 minutes indicate presence of low molecular weight species;
- Integrating the areas under the curve for every peak present on the radioactivity trace and then dividing the area under the curve for the peak of interest by the sum of all peak AUC's and multiplying the result by 100 provides the percent value for the radiochemical purity.

It is product specific and it may vary. Values of ≥80% monomer, ≤10% of high molecular weight species, and ≤10% of low molecular weight species have been used. The values are based on the radioactivity detector signal as formulation may affect the ability to interpret the UV signal if UV absorbing species are present in the final formulation.

3.2.1.3.Solid phase extraction

The aim is to determine the radiochemical purity of a radiopharmaceutical using a solid phase extraction (SPE) cartridge. The method is as follows:

- Condition the SPE cartridge by washing with 5 to 10 mL organic solvent (e.g. ethanol) followed by a similar volume of aqueous solvent (e.g. buffer);

- Place an aliquot of the radiopharmaceutical in the inlet of the SPE;
- Elute the SPE sequentially with 2 to 5 mL volumes of solvents known to selectively elute impurities or the desired compound and collect each in a separate test tube;
- Assay the activities in each of the test tubes and residual activity on the SPE using a dose calibrator (for activities >5 MBq) or a gamma well counter (for much lower activities);
- Calculate the RCP as the % activity in the desired fraction divided by the total activity in all test tubes and the residual activity;
- Note that these directions are for use of a reversed phase SPE and may be slightly different for other types of cartridges.

The specification requires that the % RCP should be greater than the minimum specified in the monograph for the particular radiopharmaceutical.

3.2.1.4.High performance liquid chromatography

The aim is to determine the radiochemical purity of a radiopharmaceutical using HPLC. The method is as follow:

- Inject a sample of the radiopharmaceutical onto a validated HPLC system. For example, a system equipped with a reverse phase HPLC column and a radioactivity detector;
- Record the chromatogram and determine the radiopharmaceutical peak area ratio versus all other detectable peaks of radioactivity, if any.

The specification establishes that the radioactive area corresponding to the radiopharmaceutical should represent at least 95% of total peak areas detectable on the radiochromatogram (95% is a typical value but may vary between monographs).

3.2.1.5.Paper-electrophoresis

The aim is to determine the radiochemical purity of a radiopharmaceutical using paper electrophoresis. The method is as follow:

- Apply a sample of the radiopharmaceutical to a wetted suitable paper strip and start the paper electrophoresis by power on the electric current at the defined voltage;
- After the defined runtime the electrophoresis is stopped, and the paper strip is dried;
- By measurement using a TLC scanner or cut and count technique, the distribution of activity along the strip is determined.

The specification requires that the principal radioactive peak should contain at least the minimum specified percentage of the total activity on the strip.

3.2.2. Enantiomeric purity

The aim is to determine the enantiomeric purity of a radiopharmaceutical that exists in two forms using chiral HPLC. The method is as follows:

- Inject a sample of the radiopharmaceutical into a validated HPLC system, for example a system equipped with a chiral column, Crownpack-CR, radioactivity and UV detector;
- Record the chromatogram and determine the radiopharmaceutical peak area ratio versus L- and -D isomers peaks areas detected by UV.

Note that the preparation may be released for use before completion of the test.

The specification establishes that the radioactive area corresponding to the specified (usually) L-isomer of radiopharmaceutical should represent at least 90% of total radioactive peak areas belonged to both enantiomers.

3.2.2.1.Filtration

Filtration test is required by Ph. Eur. monograph on ^{99m}Tc labelled human serum albumin aggregates. The test does not allow to specifically detect and quantify radiochemical impurities such as colloidal or free pertechnetate, and it only provides information about the amount of activity bound to the albumin aggregates, which is retained on a suitable filter. In principle, it is possible to develop other test to determine radiochemical purity of albumin aggregates, e.g. by TLC.

The aim is to determine the amount of non-filterable radioactivity in preparation of ^{99m}Tc labelled albumin macroaggregates. The method is as follows:

- Draw a sample of at least 0.2 mL from a reconstituted vial of albumin macroaggregates labelled with ^{99m}Tc;
- Place it on the surface of a suitable poly-carbonate membrane filter, with pores diameter of 3 μm;
- Rinse the membrane with 20 mL of saline physiological solution;
- Experimentally determine the radioactivity left on the membrane.

The specification establishes that radioactivity retained on the membrane should be ≥90%.

3.3. CHEMICAL PURITY

3.3.1. Chromatographic methods

3.3.1.1.High performance liquid chromatography

The aim is to determine the chemical purity of a radiopharmaceutical using HPLC. The method is as follows:

- Inject a sample of the radiopharmaceutical onto a validated HPLC system. For example, a system equipped with a reverse phase HPLC column and an ultraviolet/visible (UV/Vis) spectrophotometer;
- Record the chromatogram and determine the radiopharmaceutical peak area ratio versus all other detectable peaks, if any.

The specification demonstrates that the area corresponding to the radiopharmaceutical should represent at least 90% of total peak areas detectable on the chromatogram. Sometimes limits are set for specific anticipated impurities which must be identified by their retention times and quantified using a reference standard.

3.3.1.2.Colorimetric assays (spots tests) UV/VIS

The aim is to determine the chemical purity of a radiopharmaceutical. The method is as follows:

- The colorimetric assays are performed by comparison of the colour intensity obtained for the radiopharmaceutical and a standard solution after deposition on a support (i.e. TLC plate, indicator strip), or after addition of a chromogenic reagent;
- In some cases, the colour may be revealed with a stain (i.e. iodine stain is used for Kryptofix-222);
- The reading of the colour change can either be done directly, or by using a suitable spectrophotometer set at a specific wavelength.

The specification establishes that the acceptance criteria depends on the toxicity of the contaminant (i.e. it is set to 2.2 mg per V for Kryptofix-222, while the maximum limit tolerated for aluminium in sodium [^{99m}Tc] pertechnetate solution is 10 ppm according to USP).

3.3.1.3.Inductively Coupled Plasma-Atomic Emission Spectrometer

The aim is to determine the concentration of trace metal ions in radiolabelling solutions using an Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES), because many trace metal ions drastically reduce the radiolabelling yields with various ligands [5]. The method is as follows: allow the radionuclide of interest (for instance ^{90}Y) to decay completely (>20 half-lives); and analyse the sample by ICP-AES:

- ICP-AES uses inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths that are characteristic of each element when they return to the ground state;
- The intensity of the energy emitted at a particular wavelength is proportional to the concentration of that particular element in the sample being analysed;
- The particular wavelengths characteristic of each element's solution and their corresponding intensities are determined;
- Calibration curves are drawn using different concentrations of reference standards of the elements of interest;
- The concentration of trace metallic impurities in solutions can be determined in relation to the reference standards.

The specification establishes that the limits for few trace metal ions such as Cu, Fe, Zn etc. in ^{90}Y solution is given by manufacturers to be less than 0.1 ppm/GBq on the date of production [5].

3.3.1.4.Inductively Coupled Plasma-Mass Spectrometer

The aim is to determine the concentration of trace metal ions in radiolabelling solutions using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS). It is an analytical technique used for elemental determinations, capable of detecting metals and several non-metals at parts per billion to parts per trillion levels [6]. An ICP-MS combines high temperature inductively coupled plasma with a mass spectrometer. Major components of an ICP-MS are nebulizer, spray chamber, plasma torch, interface and detector. The method is as follows:

- In ICP-MS, the sample (in solution form) is pumped into a nebulizer (with a peristaltic pump), where it is converted into a fine aerosol with argon gas;
- The fine aerosol is then transported into the plasma torch via a sample injector;
- The plasma torch generates positively charged ions which are directed into the mass spectrometer;
- In the mass spectrometer, the ions are separated on the basis of their mass-to-charge ratio and a detector receives an ion signal proportional to the concentration;
- The electronic signal of the detector is processed by the data handling system, and is converted into analyte concentration by calibration with certified reference standards.

The specifications require that the limits for various trace metal ions in radiolabelling solutions are dependent on the particular radioisotope/radiopharmaceutical.

3.3.1.5.Residual solvents by gas chromatography

Residual solvents may arise from the preparation process, where they can be used as a reaction media in various steps that bring the final product. Although they are generally removed during purification steps, residues may be found in the final formulated radiopharmaceutical. Residual solvents are typically determined by gas chromatography (GC), but in principle other methods may be used, provided that they are validated and demonstrate to be suitable for the intended purpose (e.g. in case only class 3 solvents are expected to be present, methods such as loss on drying may be acceptable). Principles, methods and acceptance criteria are established by the International Committee on Harmonization (ICH) [7].

Solvents are classified in three groups depending on their toxicity levels:

(a) Class 1 solvents include for instance benzene or CCl_4, that have a high toxicity and/or carcinogenic solvents, thus it should ideally be avoided;
(b) Class 2 solvents should be limited, as they have intermediate toxicity. In this class belongs acetonitrile, which is one of the most frequently used solvent in PET radiopharmaceutical preparations, especially in ^{18}F radiochemistry;
(c) Class 3 solvents have low toxicity solvents, and they include other typical solvents such as acetone or ethanol. It is often used as an excipient in the final radiopharmaceutical formulation. In this case, it should not be controlled as a residual solvent, and other specifications apply [8].

The aim is to determine the presence of residual solvents in the final radiopharmaceutical product using gas chromatography. The method is as follows:

- Draw a sample from the radiopharmaceutical solution and inject it in a suitable gas chromatographic system. Both direct injection and head-space injection systems are allowed, although the latter is preferred as it provides better reproducibility;
- For the column choice there are two general options: packed column and capillary columns, and even in this case the latter should be preferred, due to their higher number of theoretical plates and resolutions;
- The most frequently used detector is Flame Ionization Detector (FID), which is capable to detect most of the solvents with high sensitivity, but other detectors are acceptable, such as the Thermal Conductivity Detector (TCD);
- Run the sample when the column oven is at the proper temperature;
- Record the chromatogram and determine retention time and peak areas, to be compared with those obtained from a suitable calibration curve.

Please note that often temperature is increased during the analysis, following a user implemented method.

The specifications require that the acceptance criteria are set by ICH guidelines, and they depend on the solvent class as defined above:

(a) For class 3 solvents, a limit of 5,000 ppm (or 50 mg) per day is considered acceptable without further justification;
(b) For class 2 solvents, limits are specifically assigned to each solvent, and the values range from 60 ppm for chloroform to 4880 ppm for N-methyl-pyrrolidone; for acetonitrile the limit is set to 410 ppm;
(c) Class 1 solvent should be avoided, and their limit is generally <10 ppm, but they are generally not used in the preparation of radiopharmaceuticals.

3.3.1.6.Polarography (for kits with ^{99m}Tc)

The aim is to quantify $SnCl_2$ in radiopharmaceutical cold kits [9, 10]. Polarography is an accurate alternative method that can differentiate the oxidation states of Sn(II) and Sn(IV) ions. The method is as follows:

- The polarographic analysis is carried out using 1 s drop time, 50 mV s^{-1} scan rate, -50 mV pulse amplitude, 40 ms pulse time and 10 mV step amplitude;
- To quantify Sn(IV), oxidation of Sn(II) by H_2O_2 was performed standing the cold kit vial at 37 ºC for 5 minutes.

The analytical curves for Sn(II) in 3 mol L^{-1} H_2SO_4 and Sn(IV) in 3 mol L^{-1} HCl were represented by the following equations:

$i(\mu A) = 0.098\ [Sn(II)] + 0.018$ ($r^2 = 0.998$) and;
$i(\mu A) = 0.092\ [Sn(IV)] + 0.016$ ($r^2 = 0.998$), respectively.

The detection limit for:

Sn(II) was 0.21 μg mL^{-1} and for;
Sn(IV) was 0.15 μg mL^{-1}.

In 3 mol L^{-1} H_2SO_4:

- Only Sn(II) produced a polarographic wave with the maximum current in -370 mV;
- Under the same conditions, no current could be determined for Sn(IV);
- In 3 mol L^{-1} HCl, Sn(II) and Sn(IV) were electroactive and the maximum currents of the two waves appeared in -250 and -470 mV;
- Depending on the medium composition, Sn(II) ions in low concentration solutions (< 2.0 10^{-4} mol L^{-1}) are oxidized and the formation of basic complexes takes place above pH 2.00.

The specification requires that the number of stannous ions is a variable (0.03-1.5 mg of $SnCl_2$) although a minimum concentration must be present to guarantee the lyophilized reagent (LR)'s shelf life and for an efficient labelling with ^{99m}Tc.

3.3.2. Specific activity

3.3.2.1.High Performance Liquid Chromatography

The aim is to determine the specific radioactivity of a radiopharmaceutical using HPLC. The method is as follow:

- Inject a sample of the radiopharmaceutical onto a validated HPLC system. For example, a system equipped with a reverse phase HPLC column, an ultraviolet/visible (UV/Vis) spectrophotometer and a radioactivity detector;
- Record the chromatogram(s) based on the radiochromatogram to collect the fraction corresponding of the radiopharmaceutical;
- Determine the mass associated with the radiopharmaceutical by integrating the UV/V's absorbance peak corresponding to the radiopharmaceutical on the UV/Vis chromatogram, and comparing the value to a standard curve relating mass to UV's absorbance;
- Measure the radioactivity of the collected fraction corresponding to the radiopharmaceutical, using for example an ionization chamber;
- Calculate the specific activity by dividing the counted radioactivity by the mass determined.

The Specification requires that the radiopharmaceutical and/or clinical is trial dependent.

3.3.2.2.UV / VIS

The aim is to determine radiolabelled antibody concentration. The method is as follow:

- A sample of radiolabelled solution (diluted to <0.5 mg/mL) is analysed using the UV spectrophotometer, set to 280 nm wavelength;

- The concentration is determined by diving the absorption unit at UV280 by a trastuzumab extinction coefficient for 1 mg/mL antibody solution and;
- Apply the Beer's law for concentration (c=A/εL, c= A/ε when L = 1cm, C; concentration, A; absorbance, ε; extinction coefficient, L; light path length in centimetres).

The specification establishes that ranges depend on the product. Usually the content is less than 10 mg/mL. The potency is for macromolecules only.

3.3.2.3.Immunoreactivity

The aim is to determine the immunoreactive fraction in a radiolabelled antibody drug product. The method is as follow:

- The immunoreactivity of the radiolabelled antibody's final drug product can be assessed according to the method of Lindmo, which extrapolates the binding of the radiolabelled antibody at an infinite excess antigen;
- The method summary is described in Figure 4 below. Briefly, the testing samples are prepared by mixing approximately 25000 cpm radiolabelled antibody in PBS with 0.5% BSA and increasing amounts of antigen expressing cells (0 to 1 x 10^6 cells) in PBS with 0.5% BSA to the total volume of 750 µL;
- The samples are incubated for one hour at ambient temperature with gentle shake and the unbound portion of the radiolabelled protein is removed by centrifugation at 1500 rpm (210 x g) for 5 min at ambient temperature;
- The cell pellets are then washed once with PBS with 0.5% BSA and centrifugation 1500 rpm for 5 min at ambient temperature;
- The cells are then counted in a gamma counter, in an energy window specific to the radionuclide energy, with standards representing the total added radioactivity;
- The data is then plotted using the Lindmo method as the reciprocal of the substrate concentration (x-axis) against the reciprocal of the fraction bound (y-axis);
- The data is then fitted according to a least squares linear regression method and the y interception reciprocates the immunoreactivity.

The specification establishes that it varies with different antibodies and it is usually more than 70%.

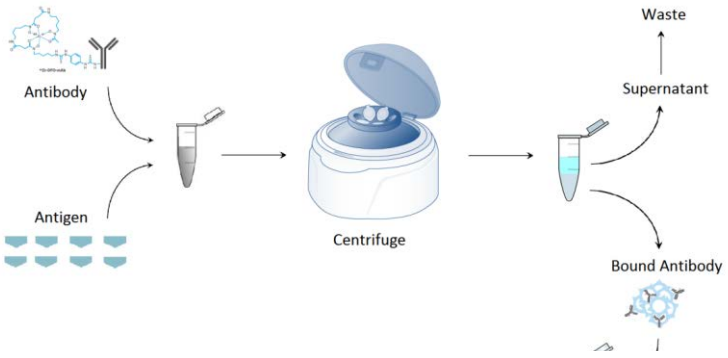

FIG.3. Immunoreactivity Testing using the Lidmo Testing Method

3.4. pH

3.4.1. pH paper

The aim is to determine the pH of a solution by colorimetric evaluation with pH paper. The method is as follows:

- pH papers change colour at a particular pH value (between pH 1 and pH 14). For accurate measurement a narrow-band pH paper can be used;
- Briefly, the pH paper is exposed to the sample solution, and;
- By comparing the paper colour changes to the reference chart, it gives information about the acidity or alkalinity of the solution without counting the hydrogen ions.

The specification establishes that the ideal pH of a radiopharmaceutical is 7.4. However, pH can range first due to the low volume of injection (typically < 10 mL) and also due to the high buffer capacity of the blood. Typically, the range is from 3.5 to 8.5 as exemplified in Annex III.

3.4.2. pH meter

The aim is to determine the pH of a solution (non-radioactive) by using a glass electrode. The method is as follows: prior to sample analysis, remove the wetting cap and inspect the electrode to ensure it is clean, undamaged, and filled with electrolyte solution. If the pH meter is not equipped with a temperature probe, manually adjust the temperature setting on the meter to the temperature of the sample, using the instructions that are normally found in the pH meter user's manual supplied by the pH meter manufacturer. Rinse the electrode with water, but avoid heavy wiping or drying of the electrode probe. Perform at least a two-point calibration of the pH meter probe using fresh samples of pH reference standards. Do not reuse the standards. Detailed instructions on how to perform the calibration are different for different models of pH meters, but can always be found in the user's manual for a specific pH meter model. The actual procedure involves taking a measurement of at least two reference standards with a known pH values, in most cases with pH of 4.0, 7.0 or 10.0. The exact choice of which standards to use depends on the anticipated pH of the solution whose pH will need to be determined.

The anticipated solution sample pH should ideally fall into the range between the two pH reference standards that are used and one of the standards used should be close to the neutral pH of 7.0. Therefore, for more basic solutions, pH 7.0 and pH 10.0 standards would be used. For acidic samples, on the other hand, pH 7.0 and pH 4.0 standards should be used. The purpose of performing the calibration is to ensure that the pH meter response is both linear and accurate. The pH meter specifications that describe linearity and accuracy of the response are often referred to as the slope and the offset at zero point. While the acceptance limits for the slope and the offset at zero point may vary slightly between different pH meter models, the slope of 100±10% and the offset at zero point of <30mV are generally considered to be acceptable. Once calibration has been successfully completed, rinse the electrode probe with water, but do not allow the electrode to dry and avoid heavy wiping. Begin measuring the sample by inserting the electrode probe into the solution sample, ensuring the probe reference junction is submerged in sample solution. Be sure that the solution is stirring slowly (at constant temperature if it is necessary) during measurement and pH adjustment. When the measurement is complete,

remove the electrode from solution and rinse thoroughly with water. Place the electrode probe back it in pH storage buffer.

The specification establishes that if possible, the target radiopharmaceutical pH should be close to the physiologic value of 7.4. However, most radiopharmaceuticals have relatively low volumes (typically < 10 mL). Injecting these solutions does not overwhelm the bodily fluids buffering capacity, allowing or a wider pH range to be used. Typically, a range is from 3.5 to 8.5 can be used, depending on the injection volume, the route of administration, and the injection rate.

3.4. OSMOLALITY

3.4.1. Freezing point depression osmolality measurement

The aim is to determine osmolality (tonicity) of a solution. The method is as follows:

- A 250 µL sample is loaded into a single use sample holder, which is then loaded into an osmometer;
- Once, the START function is initiated, the osmometer lowers a probe into the sample. The probe consists of a temperature probe and a rotating wire which is agitating the sample solution inside the sample holder, preventing it from freezing;
- The sample is cooled by running cooling fluid around the sample holder. Because the sample solution is continuously agitated, it does not freeze, even when the sample temperature goes below the freezing point of the solution. This process is known as supercooling;
- Once the sample is supercooled, the osmometer stops the slow agitation of the sample solution and shocks the sample solution by vibrating the wire in a single powerful jolt. This shock causes an immediate sample solution frozen and crystal formation. During crystal formation, the energy released is given off as heat, which can be detected by the osmometer temperature probe;
- The change in temperature from the supercooled sample point to the moment where crystallization occurs, is then automatically plotted against a regression curve produced by analysing samples of reference standards with known concentrations.

This method relies on correlation between ion content and freezing point depression. Therefore, solutions that may contain non-ion species (e.g. solvents such as ethanol) may affect the results.

The specification for isotonic solution is 290 mOsm/kg. How much a radiopharmaceutical solution can deviate from this value depends on the volume of administration, the injection rate, and the route of administration.

3.4.2. Sodium chloride equivalent

The aim is to calculate the osmolality of a solution. The method is as follows:

- The osmolality of a solution can be calculated from its known components using the sodium chloride equivalent;
- The sodium chloride equivalent is the amount of sodium chloride which will produce the same osmotic effect as one unit of the drug;
- The concentration of each component is multiplied by its sodium chloride equivalent, and the values for each component are added up and compared to an isotonic solution of 0.9% sodium chloride[a].

The specification for isotonic solution is 290 mOsm/kg. How much a radiopharmaceutical solution can deviate from this value depends on the volume of administration, the injection rate, and the route of administration.

3.4.3. Visual inspection

The aim is to determine the colour change and presence of any particulate matter of solution by visual inspection of the sample–batch. The method is as follows: stand behind a lead glass shield, use tongs to hold the test sample (or final product) vial against a light beam, and gently shake it to check for the presence of any particulate matter. For the colour change hold the vial against a white paper and quickly look at the colour of the solution.

The specifications mandates that the solution should be clear, colourless and free from particulate matter. For some radiopharmaceutical solutions, a slight yellow colour in the preparation is also acceptable. Some radiopharmaceuticals are suspensions of particles or colloids.

3.4.4. Radioactive concentration

The aim is to determine the amount of radioactivity per volume of a radiopharmaceutical solution at a certain time. The method consists of the total radioactivity measured in a dose calibrator (double check that the radioisotope setting is correct) at a certain time point, and then the obtained radioactivity value is divided by the total volume of the solution.

The specification establishes that the regulatory agencies use the radioactive concentration at the end-of-synthesis time as a measure of radiopharmaceutical ‘strength’. Validation and stability studies should be carried out at the highest radioactive concentration that is intended to be used in the clinic in order to demonstrate ‘the worst-case possible’ effect of radiolysis on the stability of the radiopharmaceutical.

[a] A table of sodium chloride equivalents is available at: http://rxistsource.blogspot.co.uk/2012/12/table-of-sodium-chloride-equivalents.html

3.4.5. Endotoxin-content

The aim is to detect and/or quantify the presence of bacterial endotoxins (BET) originating from gram-negative bacteria. The method consists of: Gel-clot method or; chromogenic kinetic method with endotoxin testing system (B). Both methods make use of the reaction between BET and limulus amebocyte lysate (LAL), an extract of the horseshoe crab.

3.4.5.1.Gel-clot method

The method is as follows:

Prepare the four different solutions A, B, C and D described below in test tubes;

— Incubate the reaction mixtures at 37 ± 1°C for 60 ± 2 minutes avoiding vibration;
— To test the integrity of the gel, take each tube in turn directly from the incubator and invert it through about 180° in one smooth motion;
— If a firm gel has formed the remains in place upon inversion, record the result as positive.

A result is negative if an intact gel is not formed. The test is considered valid when both replicates of solution B and C are positive, and when those of solution D are negative. Note that sensitivity of the labelled lysate and detection of interfering factors must be assayed to ensure the precision and the validity of the gel-clot test.

TABLE 1. SPECIFICATIONS OF TEST SOLUTIONS FOR ENDOTOXIN TEST

SOLUTION	ENDOTOXIN CONCENTRATION / SOLUTION TO WHICH ENDOTOXIN IS ADDED	NUMBER OF REPLICATES
A	None / Diluted sample solution	2
B	2× / Diluted sample solution	2
C	2× / Water for BET	2
D	None / Water for BET	2

3.4.5.2.Chromogenic kinetic method with endotoxin testing system

The endotoxin testing system performs a duplicate endotoxin test of the sample and a positive control to comply with the regulatory requirements USP BET <85> and Ph. Eur. BET <2.6.14>. The devise is measuring the amount of chromophore released from a chromogenic synthetic peptide by the reaction between endotoxins and the lysate. The analysis is usually performed on a cartridge preloaded with the reagents. The user is only required to add a specific volume of the sample to each well prior to perform the analysis. Testing for interferences and the optimal dilution factor is needed before to run endotoxin measurements on a radiopharmaceutical preparation.

The specification requires that the endotoxin limit for a radiopharmaceutical administered parenterally is specified in IU/mL or IU/V. The specification for the endotoxin-content may

vary depending on the radiopharmaceutical (i.e. a typical value is ≤ 17.5 IU/mL or ≤ 175 IU/injection).

3.5. FILTER INTEGRITY: BUBBLE POINT TEST

Most radiopharmaceuticals are prepared and administered in the form of sterile parenteral solutions. There are 2 methods of sterilization that generally apply to radiopharmaceuticals: sterilization by autoclave and using membrane filters. Despite its higher effectiveness, autoclaving is not very frequently used, as chemical integrity of the labelled molecule is often at risk in the typical sterilization conditions (121°C for at least 15 minutes), and also due to the decay, that may bring to an unacceptable loss of activity. For the above reasons, membrane filtration is often the method of choice, although it does not provide the same safety level as the autoclave does.

Indeed, filters might be damaged and could allow microorganisms (if any) to enter in the final formulation. Furthermore, membranes have a limited bacterial load capability, although bioburden is typically low in radiopharmaceutical preparations and this drawback is generally not of concern. Membranes have an average pore diameter of 0.22 μm; there are several membrane materials that fit with the different possible solutions to be filtered. In case of radiopharmaceuticals, final formulation is often made of saline physiological solution, with or without other excipients (e.g. ethanol, ascorbic buffer, etc.). The latter should be considered, as the test specifications provided by the filter manufacturer usually applies for a certain media, and acceptance criteria may vary depending on the solution composition. Different tests may be performed to test filter integrity, such as diffusion rate, pressure hold or bubble point test, which is the more frequently used due to its simplicity.

Following applicable guidelines for normal, non-radioactive medicinal products, filter integrity should be tested before and after their use [11]; however, considering the specific nature of radiopharmaceuticals, exemptions to the above rule are allowed, and filter integrity is generally tested after their use only, but before the administration of the radiopharmaceutical product to the patients [12, 13].

The aim is to verify the integrity of the membrane filter used in the sterilization of the final radiopharmaceutical formulation. The method is as follows:

- Connect a suitable gas source (e.g. compressed air) to the filter inlet. The gas source should be connected with a suitable pressure gauge to allow the monitoring of the pressure during the test;
- Connect one end of a suitable plastic tube to the filter outlet, while submerging the other end into a container filled with water;
- Depending on the available setup (e.g. whether it is computer or manually controlled), increase the pressure until the ‘bubble point’ indicated in the manufacturer’s instruction is approached;
- Continue to increase the pressure but more slowly, until the surface tension forces exerted by the wetting liquid on the membrane, that keep the pores closed, are counterbalanced: this is the so called bubble point, and the gas is now free to pass through the membrane and bubbles are observed into the water;

– Record the pressure at which bubble formation is detected.

Please note that the test needs to be performed on a wet membrane. If the filter is dry, it should be first rinsed, in aseptic conditions, with the same media expected to be used in the radiopharmaceutical solution.

Bubble points are specific for the intended filters, and are usually indicated in the filter manufacturer information sheet. Detected bubble point pressure should be higher than the above specified pressure. Please consider that, as already said, manufacturer's specifications are valid provided that the proper media are passed through the membrane (e.g. WFI or physiological saline solution). Indeed, it is well known that the addition of a solvent (e.g. ethanol) can affect the interactions between the rinsing media and the membrane, thus changing the experimental bubble point pressure. In such a circumstance, method should be specifically validated for the intended radiopharmaceutical formulation.

3.7. STERILITY: DIRECT INOCULATION

The aim is to evaluate the sterility of a radiopharmaceutical injection by direct inoculation. The method is as follows:

– Inoculate a sample volume of the radiopharmaceutical directly into suitable culture media preferably Fluid Thioglycollate and Soybean-Casein Digest Medium, followed by incubation at 32.5±2.5 °C and 22.5±2.5 °C respectively, for 14 days;
– The sample volume must be representative of the batch and cannot be more than 10% of the total volume of the culture media unless otherwise prescribed;
– At intervals during the incubation period and at its conclusion, examine the media for macroscopic evidence of microbial growth.

Note that Zolle [14] stated that the sterility testing of every batch prepared, although ideal, is unrealistic in practice; and that the testing program should ensure that all different types of product prepared are tested on a regular basis. The product decay for a sufficient period of time to allow a low level of radiation will decrease the sensitivity of the test since the number of any viable organisms in the preparation may decrease on storage.

The specification indicates that If no evidence of microbial growth is found, the product to be examined complies with the test for sterility. If evidence of microbial growth is found, the product to be examined does not comply with the test for sterility, unless it can be clearly demonstrated that the test was invalid for causes unrelated to the product to be examined [15].

The results of a sterility test are necessarily retrospective and as such, constitute a control of production processes [14].

3.8. HANDLING OF OUT-OF-SPECIFICATION RESULTS

An Out-Of-Specification (OOS) event is a situation that arises when the obtained analytical QC test result does not conform to the established specification–acceptance criteria. Encountering an OOS event indicates that either a particular batch of the product does not have acceptable

quality, or that a problem occurred during the QC analysis itself and the obtained result is invalid. In both instances, a documented investigation into the OOS event must be performed in order to determine the possible root cause. Knowing the most likely root causes allows to take corrective steps to minimize the chances of the problem occurring again.

In case of OOS the very first step is to notify the person responsible for the quality of the radiopharmaceutical. The next step is to initiate the documentation of the OOS event. Manufacturers of radiopharmaceuticals with approved marketing authorization normally use specially designed OOS Investigation Report forms, whereas OOS investigations in academic–hospital radiopharmacies may be documented in the comments section of the production batch record. The initiation section of the OOS report normally describes in a few sentences the OOS event itself and immediate actions that were taken.

Once notified, the person responsible for quality control has to perform a documented investigation, review all of the pertinent data, and decide on how to proceed with investigating the possible cause of an OOS. The investigation steps taken and the justification for the ultimate disposition of an OOS event should be documented in sufficient detail to allow another QA person to easily understand what had happened during the OOS event at least two years after the OOS event occurrence. Some of the preliminary investigative steps may include production data review, analytical instrument systems suitability testing review, calculations review, comparison of obtained result to historical data, trending, reference standards check, equipment verification, and instrument calibration status verification.

If clear evidence exists that the OOS event is invalid (e.g. a result of operator error or instrument malfunction), then at least two confirmatory analyses should be performed to demonstrate that the specification in question conforms to the acceptance criteria, unless the half-life of the intended radionuclide is very short (e.g. ^{11}C), in such a case a single analysis may be sufficient. Additional retesting may also be performed in situations where clear evidence that the OOS result is invalid may not be available, but there is some evidence that shows that the original OOS test result may be invalid and performing the retest may prove that (e.g. radio-TLC scanner identifies radioactive peaks in scanner areas where the TLC strip is not physically present, indicating possible contamination). In both cases, the decision to re-test must be based on well documented reasoning that suggests that the obtained OOS may be invalid. Simply retesting in hopes that the re-test results will conform to the acceptance criteria is known as ‘testing into compliance’ and is not permitted. The batch of product should be rejected and not released for patient administration when there is either absence of evidence that demonstrates that the original result is invalid or when the data indicates that the OOS event is a correct non-conformance. The above recommendations may be summarized in the decision tree in Figure 5.

A typical example of using this decision tree is on performing a [^{18}F] FDG radiochemical purity TLC analysis: You obtain a result where the free [^{18}F] fluoride peak is where it is supposed to be on the TLC strip but it represents 11%, and thus the radiochemical purity for [^{18}F] FDG is 89%. The USP acceptance specification is 90%, so this is an OOS. At this point, in order to claim that the OOS is invalid and to re-test for the release of the batch, you, as QA, need some type of evidence that points to the fact that the original OOS may be invalid. In this case, you check everything and that there is no evidence to disprove the validity of the OOS and the batch

should be rejected. Simply retesting in hopes that the second result will pass (e.g. 90.1% purity) in order to release the batch is not allowed in this situation. Of course, the batch could be retested for confirmatory or informational purposes, but not for the purposes of batch release even if the second retesting result is within the acceptance criteria. Now, imagine a different situation where the radiochemical purity for [^{18}F] FDG is still 89%, but you, as QA notice that the free [^{18}F] fluoride peak has a different shape than usual and it is in lower on the TLC strip than usual. Based on this observation, you can reason that this peak may have caused by some other factor such as contamination, for example. So, at this point you do not have clear evidence of operator error or equipment malfunction, but have reason to believe that the OOS may have been invalid. You survey the TLC strip holder plate and the gloves that the operator had used and notice that both are contaminated. So now you have not only a reason to believe, but also supporting evidence that points to the fact that the original OOS result may be invalid. However, you still do not know for sure until you perform the retest and confirm that the true batch result does indeed confirm to the acceptance specification.

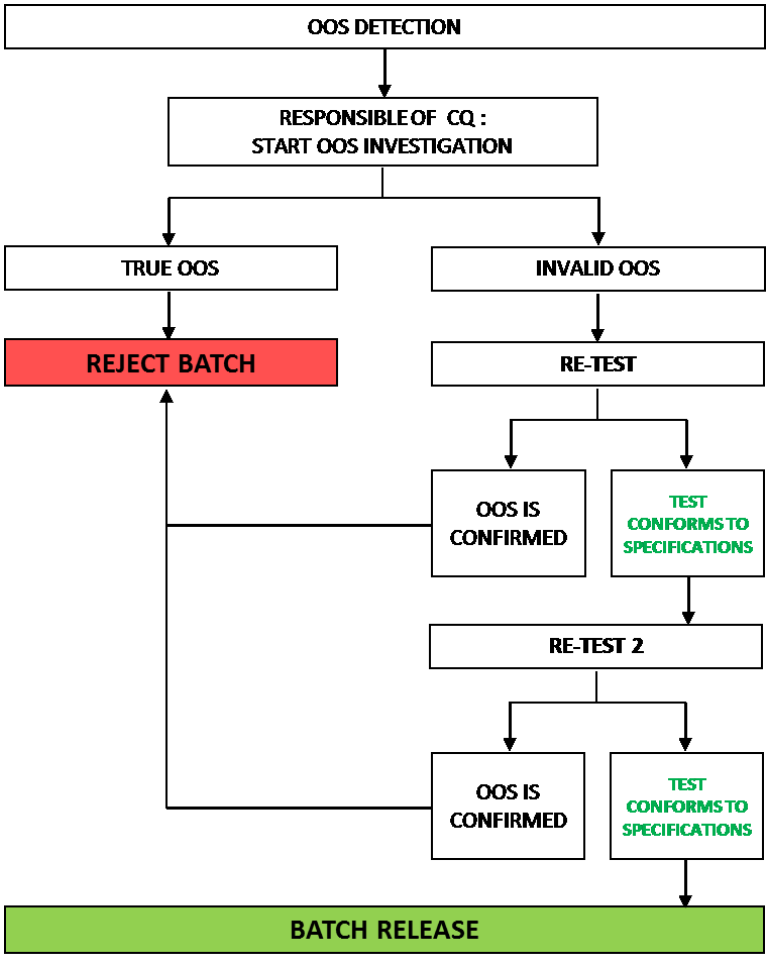

FIG. 4. Decision Tree

The last portion of the OOS event investigation should include an evaluation of the probability of event recurrence, occurrence of negative trends, or possible effects on other batches. This evaluation should be based on the data collected during investigation and any of the corrective and preventive actions that have been implemented subsequent to the OOS event occurrence.

One of the most challenging decisions for a QA person to make during an OOS event is how to proceed immediately after notification of an OOS event occurrence. Decisions and actions to be taken are normally influenced by factors such as the half-life of the radionuclide, the nature of the considered analytical test, and radiation protection issues. In case of short (or even ultra-short) half-life, decisions should be taken immediately, and proposed actions should be compatible with radionuclide decay and with possible radiation hazard to the operators. The one factor that should not influence a QA decision on how to proceed is the pressure from the clinic to deliver the radiopharmaceutical to the patient. Inability to deliver the radiopharmaceutical to the clinic does cause stressful situations in the clinic. The frustration that develops in these situations is often communicated by the clinicians to the QA person who has made a decision not to release the batch. This communication may include increased pressure to release the batch, despite limited understanding of radiopharmaceutical quality or regulatory implications. A QA person must have sufficient independence and should not be influenced by this pressure.

Another important skill that a QA person must possess is to be able to differentiate an invalid result from a true non-conformance result. Invalid results are normally caused by either sampling errors or analytical errors but may be caused by other factors such drifts in instrument response or instrument malfunction. Some examples of analytical errors include under/overestimation of pH due to pH meter electrode insufficient cleaning, HPLC injector carrying contamination from previous analyses (i.e. carryover), air bubble formation in an HPLC column, etc. Sampling errors are typical operator errors such as sampling from wrong QC 'bulk' sample, sampling wrong volumes from the correct bulk vial, improper storage of the QC aliquot to be analysed.

Handling sterility testing OOS events present another unique challenge as the OOS is normally discovered several days after the radiopharmaceutical has been prepared. During this time period, the bacterial growth support properties in the remaining product may change, making valid retesting impossible. In other words, sterility testing may be repeated, but only to obtain investigational data that may help determine the true cause of the OOS event, and not to demonstrate that the original product was sterile. Another important investigative step that should be taken is organism identification at least by genus and species. This service is normally performed by an external laboratory specialized in microbial identification testing. Organism identification allows QA person to establish the most likely source of organism contamination, efficacy of the cleaning agents used, and steps that may need to be taken to prevent recurrence. Other investigative steps may include performing equipment specific or operator specific environmental monitoring data review and trending, equipment checks, and operator observation. The ultimate goal of the sterility OOS event investigation is to determine the root cause so that corrective actions could be taken to minimize the risk of recurrence. The secondary goal may be to determine whether the OOS event occurred due to a true sterility failure (i.e. the final product was not sterile) or due to sample contamination during the test itself. In absence of sufficient supporting data, it should be assumed that the sterility OOS is a true sterility failure. Lastly, since sterility OOS is usually discovered several days after the radiopharmaceutical has been administered to the patient, it is a very good and highly recommended practice to notify the responsible physician as soon as sterility OOS event is discovered, and then again once the results of the investigation become available.

Finally, OOS events should not be confused with deviations. A deviation is any event during the entire manufacturing process (both production and QC) where something is different, or 'deviates,' from the established method or established historical process. Some examples of deviations include radiochemical yield lower than expected (unless the yield is defined as a release specification), any technical problem detected during preparation of the radiopharmaceutical, such as a temporary loss of power during automated synthesis–dispensing, a problem with the air handling system, defects in container, improper labelling, instrument detector malfunction, etc. Deviations, although not being the same as OOS events, may significantly affect analytical results and lead to an OOS event. Therefore, it is important to evaluate deviations on a case by case basis in order to identify any possible negative impact on the quality of the final drug product.

4. EQUIPTMENT AND METHODS

4.1 ISOTOPE CALIBRATOR

4.1.1. Description

An isotope calibrator [16], also called dose calibrator or ionisation chamber, consists of a well into which a vial or syringe containing radioactivity can be lowered. The hollow walls of the chamber contain a gas across which a high voltage is applied. Emissions from the radioactive source will cause ionisation of the gas and a current will flow which is proportional to the amount of radioactivity. The electronics convert the current to a radioactivity measurement (MBq or mCi) via a calibration factor for the particular radionuclide. Three important features of an isotope calibrator are its rapid response (stabilises within a few seconds), linearity over ~6 orders of magnitude, and its output is activity units rather than counts per unit time.

4.1.2. Routine checks

The following verification should be performed daily: high voltage correctly set; display readings correctly; electronics adjusted to zero; low background activity reading; and measurement of long lived check source (^{137}Cs or ^{57}Co) is correct on all relevant calibration settings. The following corroborations should be performed at least annually:

- Accuracy checked by sending a sample to the national radiation metrology institute; reproducibility on repeated measures;
- Linearity through either repeated measurement of a short-lived source over ~10 half-lives or use of attenuators.

For ^{99m}Tc and positron emitters the type of container (glass vial vs plastic syringe) makes little difference; however, measurements of ^{123}I and ^{111}In can be greatly influenced by the container due to their low energy X rays which are attenuated by glass much more than by plastic.

4.1.3. Maintenance

An isotope calibrator requires little maintenance. The most common problem is a dead battery. Serious problems will generally be detected by the accuracy or linearity measurements.

4.2. MULTICHANNEL ANALYSER

4.2.1. Description

The multichannel analyser (MCA), also known as gamma spectrometer, is an instrument used to discriminate and identify radionuclides, based on their characteristic gamma emission energies. Gamma rays emitted by the radionuclides interact with the detector crystal atoms, and their energy is partly converted to light photons, as a consequence of their return to the ground state. Emitted light is then amplified and converted to electric pulses whose energies are proportional to the gamma photo energies. An energy spectrum of the radioactive source may thus be obtained, where energies are represented on the x-axis and amounts of radioactivity on the y-axis. Typical peak shape are Gaussian curves characterized by a 'centroid', which correspond to the peak maximum height and it is used as a reference to assign the peak energy (and, subsequently, to identify the radionuclide), and by a peak width due to the pulse dispersion around the centroid. Detectors include a scintillator element and a photomultiplier which, combined together, convert ionizing/excitation events in electric signals. Due to the high sensitivity of the detectors, samples containing high activity levels should be avoided, as counting efficiency might be strongly affected yielding significantly underestimated results. To this regard, detector deadtime is a useful parameter to be considered, and typically samples with deadtime values > 5% should be discarded or the radioactivity concentration decreased (by dilution, or by time) until a suitable deadtime is obtained. Most frequently used detectors are thallium activated NaI or high purity germanium (HPGe), respectively. Measured energies are typically in the range 0-2000 KeV, which is suitable for the identification of most of the radionuclide used in medicine. NaI(Tl) show higher counting efficiency, and they are suitable in case radioactivity quantification is important.

On the other hand, their resolution is rather poor, in the range 30 to 50 KeV: this means that with a NaI(Tl) detector the identification and quantification of peaks whose difference in energy fall within the above range may not be discriminated. HPGe resolution is much higher, in the range of 1 to 2 KeV, and practically all the possible peaks may be clearly separated. HPGe drawbacks are needed in a cooling system, that may be physical (e.g. using liquid nitrogen) or electrical, the greater space required and, most of all, their costs, which are significantly higher compared with NaI (Tl) detectors. However, their better resolution makes them suitable for the purposes of identification and determination of radionuclidic purity. For these reasons, they are usually preferred. Finally, as said above, detector shielding is critical, and cylinders made of piled-up 50 mm lead rings are frequently used, depending on the intended purpose and the radiation background expected in the QC lab.

4.3.2. Routine checks

MCA need to be periodically calibrated. Two different types of calibration have to be considered: i) energy calibration; ii) efficiency calibration. Energy calibration is aimed to verify that actual emission energies determined by the instrument that are in agreement with the

expected energies. For instance, in case of positron emission radionuclides the typical 511 KeV peak due to the positron annihilation should be observed: if not, calibration and adjustment are necessary. Energy calibration should be performed at least two times a year, or at any time in case evidence of misalignment between actual and expected energies are detected (see above). For the calibration procedure, a suitable multinuclide, or single nuclide, multi-energy sealed source is necessary. Whatever is the selected (and available) source, it is important that the emitted energies may cover the intended measurement range that, as said above, is usually 0-2000 KeV. In case of multinuclide source, ideally the half-lives of the various radionuclides should be long enough to guarantee a suitable duration for the source itself, and they should not be very different from each other, to minimize their unbalanced decay. The calibration source is placed close to the detector for a suitable time (e.g. the time necessary to detect >10,000 counts from the lower activity radionuclide), and the gamma spectrum is thus acquired. Depending on the available software, proper parameters may be changed in order to adjust detector output so as to align with expected energies, if required.

Procedure should be repeated until a satisfactory calibration is obtained. Efficiency calibration is aimed to verify activity quantification accuracy, and may be performed, at least annually, using the same above described calibration source or another suitable source. Details of calibration procedure are depending on the available software, although in general they rely on the adjustment of the detector output, in terms of activity counting in comparison with the source of known activity, which has to be decay corrected.

4.2.3. Maintenance

MCA detectors require little maintenance, except for liquid nitrogen refilling in case of HPGe detectors cooled with the above modality. Most frequent accidents rely on the electrical cooler, which are often very sensitive to power failure.

4.3. THIN-LAYER CHROMATOGRAPHY

4.3.1. Description

A radio TLC scanner comprises a radioactivity detector placed at a fixed distance from a movable, motor driven scanning platform where the radio TLC is positioned [17]. As radioactivity detector, a proportional counter or collimated NaI or scintillation detectors can be used. The platform moves along an axis so that the entire surface is scanned during a single run. The detector is connected to a counting device. The radioactivity distribution on the TLC plate is automatically recorded, and the profile describes peaks having areas proportional to the distances.

4.3.2. Routine checks

A system suitability test is performed by measurement of long lived two spots test strip (e.g.^{137}Cs), which can be obtained from the instrument manufacturer. A demonstration of the limits of detection and the linearity of the detector can be made by a calibration. For calibration spots on a TLC plate were applied covering the radioactivity from 0.1% to 100% of the expected range. The samples are verified of the detector response by integration of the peak. The peak

resolving power can be checked applying 5 µL spots separated by distances increasing from 4 mm to 20 mm in increments. The resolution is given when in the radioactivity profile two spots are clearly separated by a baseline.

4.3.3. Maintenance

A TLC scanner requires no routine maintenance other than recalibration.

4.4. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

4.4.1. Description

An HPLC system basically consists of a chromatography column, a sample injector, high pressure pumps for pumping the solvents (mobile phase) through the column and detectors for detecting the various components of the sample. The sample is introduced into the continuously flowing mobile phase by the sample injector. The mobile phase carries the sample into the chromatography column. The high-pressure pumps push the mobile phase through the column at a specified flow rate (mL/min). Separation of various components in the test sample is affected by the chromatography column which contains the chromatographic packing material. The packing material is called the stationary phase as it is held in place by the column hardware. Depending upon the nature of the radiochemical species to be separated, a variety of chromatography columns are available [such as silica (normal phase) C8, C18 (reversed phase), gel columns, etc.].

HPLC systems may consist of single pumps (for only one solvent) or may have multiple pumps for passing a mixture of pressurised solvents through the column. Each component in the sample interacts slightly differently with the stationary phase, resulting in different flow rates for the different components, leading to their separation as they flow out the column. A particular component exiting the column passes through the detector into a waste container or is collected (in preparative HPLC). The time taken for a particular component to travel through the column to the detector is known as its retention time (R_t). This time is measured from the time at which the sample is injected to the time at which the detector shows a maximum peak height for that component. An analyte which has the least interaction with the stationary phase will exit out of the column faster and will therefore have a shorter Rt. The information from the detector is sent to the computer to generate a chromatogram. Choice of detector is dictated by the chemical species of interest. HPLC systems used for determining the radiochemical purity of radiopharmaceuticals consist of an ultraviolet (UV) or refractive index (RI) detector for identifying the chemical species and a radioactivity detector [consisting of NaI(Tl) detector] for detecting the radioactive species.

4.4.2. Routine checks

Check for any change in the column pressure (from the normal) every time the HPLC is used. Sample retention times may vary if there is a build-up of impurities in the stationary phase, if the equilibration time for the mobile phase is insufficient or due to change in column temperature. Take care to inject samples devoid of particulate matter to prolong the life of the injector and the column. The detector baseline needs to be checked every time the HPLC is used. Any change in the detector baseline would indicate the presence of impurities in the

mobile phase exiting the column. This would require washing the column with the mobile phase to flush out the impurities. Other problems related to the detector include leaks and air bubbles which give rise to spikes or baseline noise on the chromatograms or low sensitivity.

4.4.3. Maintenance

An HPLC system requires routine maintenance. The solvent inlet filters, in-line filters etc. need to be replaced periodically. The life of the various components such as chromatography column, UV lamp, auto-sampler parts, among others, would depend upon various factors such as the extent of use, types of samples being injected, mobile phase, etc.

4.5. GAS CHROMATOGRAPHY

4.5.1. Description

Gas chromatograph (GC) is an instrument that is used to determine the content of solvents in a sample. Once the sample is injected into the heated injector oven, it becomes almost immediately volatile. This vapour is then pushed through the column. The various species present in the sample have different affinities for the column vs the gas that is pushing the sample along through the column. The higher the affinity for the column, the later a specific analyte will come out, resulting in a greater retention time. Once any of the analytes come out from the column, they are pushed into a flame ionization detector (FID). Inside the FID, the analyte is incinerated resulting in the creation of positive charge on the carbons presented in the sample. These changes in charge are then interpreted by the computer and are plotted as a signal on the chromatogram. The higher the carbon content, the higher the signal on the chromatogram. Because different analytes come out of the column at different times due to their interaction with the column, retention time can be used to identify a specific analyte present in the sample. Additionally, the instrument response to a specific analyte can be used to quantify the content of the analyte by comparing the response (i.e. integrated area under the curve for the peak of interest) to the response produced via analysis of reference standard with known concentration of the analyte. For example, the simplest test to determine whether the acetonitrile content in the product sample is less than the specification is to inject the reference standard sample (sample that has ethanol content equal to the specified limit) in triplicate, followed by the product sample injection. If the measured response for the sample analysis is less than the average of the three reference standard sample injections, then the analyte content inside the product batch is less than the limit value.

4.5.2. Routine checks

Injection of the reference standard in triplicate.

4.5.3. Maintenance

Injector septa, injector liners, columns, and gas traps should be changed periodically. The frequency depends on the frequency of instrument use and the samples being analysed.

4.6. PH METER

4.6.1. Description

pH meter is an instrument that measures the hydrogen ion concentration in a solution.

4.6.2. Routine checks

Routine checks should include inspecting the electrode to ensure the electrode is clean, undamaged, and is filled with electrolyte solution. The electrode probe wetting cap should be inspected periodically in order to ensure sufficient levels of the wetting fluid are present. The meter should be calibrated regularly using reference pH buffers as described in Section 3.4 above.

4.6.3. Maintenance

The exact electrode probe maintenance requirements vary as they depend on factors such as the nature of samples being measured and the frequency of use. Some of the maintenance that may need to be performed most often includes refilling of the electrode fluid, rewetting of the electrode probe wet membrane, and cleaning of the probe reference junction. The detailed instructions on how to perform maintenance for a specific model of pH meter can be found in the pH Meter User's Manual specific to a particular model. The frequency of maintenance may also be affected by the factors mentioned above. Therefore, it is recommended that maintenance be performed, at a minimum, when the slope and the offset at zero-point results obtained during the two-point calibration (described above) approach out-of-acceptance range values. In cases where maintenance is ineffective in fixing the electrode probe function, the electrode probe should be replaced.

4.7. OSMOMETER

4.7.1. Description

Osmometer is a non-invasive in-vitro diagnostic system for the measurement of the total osmolality of various aqueous solutions. It provides a measure of contribution of the various solutes that are present in a solution to the overall osmotic pressure of the solution. The osmotic pressure is independent on the chemical nature of the compounds and ions that are dissolved, but it is dependent on the concentration of the various species in the solutions. The measured parameter is an osmolality, which is an estimation of the osmolar concentration and it is proportional to the number of particles per kilogram of solvent. It is expressed as mOsmol/kg (the SI unit is mmol/kg but mOsmol/kg is still widely used).

Osmolality is measured by two types of osmometer: either a freezing point depression osmometer or a vapour pressure depression osmometer. The normal osmolality of extracellular fluid is 280 to 295 mOsmol/kg. The measuring of freezing point depression is most commonly used in the quality control of radiopharmaceuticals quality control with the help of cryoscopic osmometer, for example Osmomat 030. The general mode of operation is to measure the freezing point of pure water and the sample and compare the two measurements. Water has a

freezing point of 0°C and a solution with a saline concentration of 1 Osmol/kg has a freezing point of - 1.858°C.

4.7.2. Routine checks

The following checks should be performed on a daily basis: setting the instrument zero using water (place a sample of water, measure the freezing point and set the display to zero). Also, a new measuring vessel has to be used for each measurement. Calibrate the system with a calibration standard on a periodic basis to ensure that the system is operating in an acceptable manner. Standard calibration solutions containing NaCl with an osmolality of 100, 300, 500, 850 and 2000 mOsmol/kg in ampoules are used. The corresponding NaCl concentrations are provided in the instructions.

4.7.3. Maintenance

Check the power, fan and elevator movement daily for the presence of contamination. The osmolality of one or more reference samples should be measured to ensure that the system is operating properly and the results are within the maximum measuring deviation (error margin). Reference solutions for osmometry should be produced according to national or international pharmaceuticals directives. The reference sample is usually included as a set together with osmometer device. On a monthly basis check the position of the initiation needle, check and adjust the orientation of thermistor probe by means of the adjustment tool, check the corrosion of the cooling nipple. The system should be clean.

4.8. POLAROGRAPHY

4.8.1. Description

Polarography is an electrochemical method of analysis based on the measurement of the current flow resulting from the electrolysis of a solution at a polarizable microelectrode, as a function of an applied voltage [18]. Basic instrumentation for polarography includes: a voltage ramp generator; current-measuring circuitry; a cell with working electrolyte, reference and counter electrodes; and a recorder device. Instruments having DC or pulse-polarographic capabilities are generally quite adequate for stripping application. The working electrode commonly used is the hanging mercury drop electrode. A saturated calomel electrode or a silver–silver chloride electrode serves as the reference, and a platinum wire is commonly employed as the counter electrode. Test specimens containing suitable electrolyte are pipetted into the cell. Dissolved oxygen is removed by bubbling nitrogen through the cell. After deposition, the stirring is discontinued and the solution and electrode are allowed to equilibrate for a short period. The potential is then rapidly scanned anodically. The limiting current is proportional to concentration of the species, while the half wave potential or peak potential identifies the species.

4.8.2. Routine checks

Verify that the mercury is dropping regularly and pipette a suitable electrolyte in the cell and scan the potential to check if the background is low as possible in the region of interest. Use a

solution of Cd or Pb in an acid medium as reference to check if the potential is not dislocated due the malfunction of the reference electrode.

4.8.3. Maintenance

Check if there is corrosion on the metallic contact screws. Use the reservoir with fresh top quality hexadistilled mercury, avoiding air bubbles being trapped into the capillary. Renew the mercury drop and let the capillary end immersed in purified water. The inner solution of the reference electrode should be renewed approximately every 2 weeks.

4.9. PAPER ELECTROPHORESIS

4.9.1. Description

Paper electrophoresis (zone electrophoresis) is a physical method of analysis permitting the separation of compounds that are capable of acquiring electric charge in conducting electrolytes [19–23]. In this medium, ionized particles such as ^{18}F, ^{124}I or ^{99m}Tc, move more or less rapidly under the influence of an electric field. An apparatus for paper electrophoresis contained a chamber for the electrophoresis divided with a diffusion barrier, the two electrodes (anode and cathode), the electrolyte media (e.g. 0.05 M acetate buffer pH 4.5) and a sample holder. As a source of the current, a stabilized voltage power supply with an adjustable output of typical up to 450 VDC at 150 mA should be used.

On a strip of paper (e.g. Whatman 3MM of about 30 cm by 2 cm) the starting point is marked with a pencil. Then the strip is wetted with the electrolyte solution and placed on the sample holder. Now the strip forms an electric bridge between the two electrolyte sub-chambers of anode and cathode. For example, to determine free ^{124}I in a sample, 5 µL of the test solution is placed on the strip with the starting point near the cathode. The apparatus is closed with a lid, and the power supply is switched on. After 15 min at 250 VDC free ^{124}I is migrated about 7 cm towards the anode while uncharged compounds e.g. ^{124}I labelled macromolecules are remaining at the start. The electrophoretic migration on the dried sample strip is evaluated with a radio TLC scanner.

4.9.2. Routine checks

After each start of an electrophoresis check the voltage and the typical current on the instruments of the power supply. A zero value of mA at the ampere meter indicates the electrophoresis is not working.

4.9.3. Maintenance

The electrolyte medium should regularly be replaced. The safety switches at the lid, the electrodes and wires should be inspected for corrosion routinely to prevent an electric hazard.

4.10. ENDOTOXIN TEST

4.10.1 Description

Endotoxin testing systems are small portable devices, usually made of a disposable test cartridge and a reader, which allow endotoxin detection by a kinetic chromogenic assay. Such devices can provide a quantitative result in about 15 minutes, they do not require the preparation of endotoxin standards and use a tiny amount of product to run the analysis. Consequently, they are very practical for the analysis of radiopharmaceutical preparations, especially when dealing with short half-life isotopes.

4.10.2. Routine checks

No routine checks are required. However, it is recommended to run a validation on a cartridge for each new batch of cartridges and LAL Reagent Water (LRW) used for dilutions.

4.10.3. Maintenance

Endotoxin testing systems do not typically require routine maintenance.

4.11. GAMMA COUNTER

4.11.1. Description

An instrument that is capable of quantifying the number of gamma counts in a sample per certain period of time.

4.11.2. Routine checks

Counting of a reference standard with known radioactivity value prior to sample analysis. Periodic energy calibration.

4.11.3. Maintenance

Gamma counters do not typically require routine maintenance.

5. EQUIPMENT AND METHOD QUALIFICATION

Qualification of analytical instrumentation is essential for accurate and precise measurement of analytical data.

5.1. QUALIFICATION OF QUALITY CONTROL EQUIPMENT

Qualification may be defined as “the action of proving and documenting that any premises, systems and equipment are properly installed, and/or work correctly and lead to the expected results” [21]. Qualification is often confused with Validation, but the latter is a broader and

more general concept, and qualification may be considered as a part (the initial stage) of validation. As may be gathered from the above definition, that qualification is typically related to the instrumentation or equipment, while validation apply e.g. to the whole process (process validation) or the analytical methods, that will be described with more details in the next paragraph.

Qualification is a 'step' procedure, that usually begins with the so called User Requirement Specifications (URS), and proceeds with (in the proper order) Design Qualification, Installation Qualification, Operational Qualification, and is completed with Performance Qualification. The above terms and concepts are accepted worldwide, they are very familiar for the radiopharmaceutical industry, and they also apply to the facilities engaged in the 'in-house' preparation of radiopharmaceuticals. As previously described, there is a wide variety of equipment used for quality control, ranging from very simple pH meters to the sophisticated electronic chain underlying the gamma spectrometers, and a detailed description of procedures and acceptance criteria required for the full qualification of every specific instrument that is out of the scope of the present publication. On the contrary, the qualification process will be spilt into its main components, and we will pinpoint both general and specific rules to be considered during the preparation of the documentation, experimental setup design and test execution necessary to carry out the qualification protocol.

It is important to note that all the qualification activities are based on written protocols, which include scope, specifications, references, test and related acceptance criteria, experimental raw data, calculations, deviations (if any, and in case of positive response also preventive or corrective action to be implemented), discussion, summary, conclusion, and a statement about the equipment status (e.g. if the protocol has been successful, the routine use of the intended instrument may begin). Qualification protocols need to be performed by adequately trained personnel; in case of installation and operational qualification, personnel may be provided either by the radiopharmacy or by manufacturer, while performance qualification, as well as user requirement specification and design qualification cannot be outsourced. Finally, under some circumstances, it may be necessary to requalify an equipment. This is usually not a of concern for simple equipment (e.g. pH meters, balances, etc.), which are simply replaced with new units at the end of their working life, while it is applicable in case of more complex technologies, where the replacement or repairing of a component (e.g. a detector) may apply, and require the repetition of all or part of the tests originally performed during initial qualification.

5.1.1. User requirement specification

The qualification of an instrument may begin with the URS, which are aimed to define the technical characteristics of the intended equipment required to evaluate its general performance. User requirements should be clear, concise and verifiable; general requirements such as 'the system should be reliable' should be avoided, as they do not provide an objective and quantitative way to determine whether the system meet or not the established criteria. On the contrary, a statement such as 'the injection volume precision should have a relative standard deviation (RSD) <0.5%' is appropriate, as it allows the instrument characteristic to be experimentally tested. User requirements should be set for parameters/characteristics that are critical for system operations, such as sensitivity for UV detectors, injection volume precision for autosamplers, energy resolution for gamma spectrometers, etc. URS should be described in

a proper document; whose level of detail depends on the design and function expected for the intended instruments. Thus, URS for a pH meter could simply include a description of the equipment and the place of installation, and set the requested accuracy and/or linearity of the electrode response. On the contrary, URS for more sophisticated technologies such as radio-HPLC system or gamma spectrometers, should include more information, such as:

— Scope of the document;
— Description of the equipment, and its intended use, including software;
— Description of the room where the instrument is supposed to be installed, indicating expected environmental conditions;
— Description of the utilities and ancillary systems required by the instrument for its proper functioning (e.g. power supply voltage, number of electrical sockets, gas and their purity, etc.);
— Requirements for software installation (e.g. operating system, version, hard disk space, etc.);
— Requirements for software access, establishing different privileges depending on the users. For instance, laboratory analysts should not be allowed to delete data or to create new methods, and the above functions should be allowed for QC responsible only;
— Requirements for software traceability such as audit trails, which allows to automatically record the actions performed with the system;
— In case the system is equipped with an autosampler, precision of the injected volumes, carry over and number of samples may be considered in URS;
— Precision and accuracy of the HPLC pump flow rate, verifiable collecting and weighing eluent samples;
— Define whether the pump has to work in gradient or isocratic mode;
— If the system is equipped with a column heater, temperature control (e.g. precision, accuracy) should be defined;
— Precision, linearity, sensitivity of detectors, verifiable using appropriate standards;
— URS should set other useful parameters such as background noise;
— Technical documentation to be requested to the manufacturer.

In conclusion, URS may be considered as the first stage in the qualification ‘flowchart’, and it may help in providing a useful basis for requesting a price quotation from the instrument manufacturer. In case the equipment is not commercially available (e.g. it is home-made or custom made), URS is even more important, as it represents the major source of information that lead the user to the design and commissioning of the equipment itself.

5.1.2. Design Qualification

Design Qualification (DQ) aims to verify that the system or instrument has been designed suitably for the intended purpose. Particularly that the design meets the user requirement specification (URS) and complies with all the applicable guidelines and standards. DQ is of particular importance when the equipment or system is homemade or custom designed; for instance, this is the case of HVAC (heating, ventilation and air conditioning systems), which have to be specifically designed by keeping into account the site layout, the number of rooms,

the requested GMP environment classification, etc. Design qualification is of less importance in case of QC equipment, as only commercially available instrumentation is usually of concern.

5.1.3. Factory and site acceptance testing

Factoring Acceptance Testing (FAT) may be particularly useful in case of novel or complex technology, and/or when equipment is bulky and difficult to transport and install. For example, this is the case of shielded hot cells used for the preparation and dispensing of radiopharmaceuticals. Factory testing of a hot cell, based on specifications set in URS or during the DQ step, is very helpful, as failures and deviation (if any) occurred during tests may be easier to fix directly at the factory, where skilled personnel, spare parts, tools, are promptly available, than at the customer site. Site Acceptance Testing (SAT) usually means a repetition of the FAT tests at the customer site, with the addition of specific tests that make use of radioactivity (not available at the factory). For the above reasons, FAT and SAT are often not of concern in case of QC equipment, except for very complex technologies such as GC-MS or LC-MS, that cannot be considered as routine QC instruments, and will not be considered further in the present document.

5.1.4. Installation Qualification

Installation Qualification (IQ) aims to verify that the instrument has been installed correctly, based on the manufacturer's recommendations and/or the approved specifications of the User. It usually includes administrative information, necessary to trace the purchasing process. Thus, price quotation, order, packing list, and formal acceptance report are part of the IQ protocol. The packing list also allows to verify early that the content of the shipped equipment meet the order and URS specifications, if applicable. Manufacturer documentation, such as operating instructions (that usually include information on installation), maintenance user guide, technical characteristics, drawings, schematics, etc. must be collected and reported in the IQ protocol. The above documentation is often used as a basis to set up Standard Operating Procedures, which are part of the quality assurance system. During installation qualification, logbooks must be specifically created for each instrument, that allow the user to report major operations such as failures, preventive and corrective maintenance interventions, calibration procedures, major changes. Specific instrumentation data, such as manufacturer, instrument model, serial number, place of installation, environmental condition (temperature, relative humidity) should be collected. Following the installation of the equipment or system, a schematic of the installed equipment with indication of cables, tubing, pipes, connections should be prepared. Also, a verification of available spare part, if applicable, may be considered as part of IQ.

5.1.5. Operational Qualification

Operational Qualification (OQ) aims to verify that the system or instrument is operating properly, and that the responses of critical components (e.g. sensors) match the expected values and are within the desired range. To do that, OQ requires the availability of reference standards, such as calibration solutions for pH meters, calibrated thermometers, flowmeters or pressure gauges in case sensor output have to be verified, calibrated weighs for analytical balances OQ or, again, substances with known absorbance if UV or DAD detectors are the subject of

qualification. A brief description of the most significant test typically performed during the OQ of the most frequently used QC equipment is described as follows. It has to be noted that some OQ tests, especially in case of simple instruments, may overlap with PQ tests.

5.1.5.1. pH meters

Linearity and reproducibility of the instrument should be verified, using a set of standard buffer solutions that cover the intended pH range (e.g. in the interval 4 to 12); precision may be evaluated by repeating 6 measurements with each of the standard buffer solution and calculating individual relative standard deviation (also known as 'coefficient of variation' CV%), while linearity should be tested taking advantage of the different buffers.

5.1.5.2. Dose calibrators

Precision may be evaluated by repeated measurements using one or two different calibration sources of suitable energy and activity. ^{137}Cs and ^{133}Ba have sufficiently long half-lives that measurement results don't need to be decay corrected. Other tests may include accuracy verification and current or voltage output verification using a calibrated meter. OQ tests for dose calibrators may clearly overlap, in part, with routine checks previously described.

5.1.5.3. Radio-high performance liquid chromatography

UV or Diode array detector (UV / DAD) functionality may be verified through several tests, such as drift/noise test, which measure average noise after 4 to 6 short term tests, or determining wavelength accuracy by measuring UV absorbance after injection of a known standard. Also, a linearity test using suitable dilutions of a reference standard (e.g. caffeine, anthracene), may be considered for OQ. As for HPLC pump, typical OQ tests include flow rate precision test, to be performed by setting a suitable pump flowrate (e.g. 1 mL/min), collecting 5 to 6 samples, weighing the samples using a calibrated balance to determine volumes with sufficient accuracy, and finally calculating CV%. Another useful OQ test is pressure ramp and hold test, that allow to check the pump safety pressure interlocks (eluent flow should stop after a pressure limit is reached) and pump tightness (pressure is monitored for a sufficient time, e.g. 3 min, after the limit has been reached) at the same time. OQ of autosampler is typically performed executing precision and linearity tests, with the repeated injection of reference standard samples and subsequent chromatogram acquisition, peak area determination and CV% and R2 calculations, respectively. Another important test to be performed on autosampler is carry over test, that measures the amount of sample left over in the injection system between two consecutive injections, and it is a measure of the 'auto-cleaning' efficiency of the instrument. Radiochemical, 'flow' HPLC detectors are often tested directly during PQ, as due to their intrinsic nature it's not trivial to establish suitable OQ tests. Finally, acquisition and control software functionality may be verified following tests which are specific for the intended software. However, there are general tests that may be performed, irrespective of the specific software package; they include checks on the different privileges related to different user access credentials, on the archive functions (backup and restore procedures), and audit trails function.

5.1.5.4. Gas-chromatography

Critical components and parameters to be tested during OQ for GC systems are first, the oven temperature control, that may be verified using calibrated thermocouples and checking for accuracy (difference between the temperatures set by the instrument and actual values obtained by the calibrated sensor) and linearity. Second, gas flow rate that requires a calibrated flow meter, calibrated for the same gas routinely used by the instrument; here also accuracy and linearity should be tested, following the same principles already described for the temperature. In case an automated sample injection is included, such as popular head space injection systems, also leak test, to verify the tightness of the system, and volume injection precision should be determined.

5.1.5.5. Radio-thin-layer chromatography scanners

Also, for these kinds of detectors OQ tests may significantly overlap with PQ tests, which will be described with more details in the next section.

5.1.5.6. Multichannel analyser

OQ may include an energy calibration of the instrument, with the aim to verify that detected energies match with expected values. Both mono- and multinuclide calibration sources may be used. Multinuclide or single-nuclide, multi-energy (e.g. 152 Eu) sources should be preferred, so as to check calibration status in a broader energy range. Usually, typical working range of the above instruments is indeed 0 to 2000 KeV. A minimum of 6 acquisitions for each of the selected energy signals, followed by coefficient of variation (CV%) calculation allow for energy calibration determination. Efficiency is another parameter to be considered in OQ, especially when gamma spectrometry is used for quantification purposes. Here also multinuclide sources are ideally suited, as they allow for quantification of radioactivity amount of the various nuclides, provided that they are sufficiently long lived (medium half-life radionuclides might also be used, but errors are higher). The same above described sources may be used to determine accuracy, which is indeed strictly linked with efficiency.

5.1.6. Performance qualification

The goal of Performance Qualification (PQ) is to verify that the system or instrument performs properly and reproducibly in the intended routine conditions set for the specific preparation process, and that they are using approved methods. As already stated above, PQ is the last step in the qualification process, and it has to be performed by the user, following approved methods and procedures and setting experimental conditions that mimic those planned for the routine use of the intended equipment. For this reason, it is sometime possible that tests planned for PQ overlaps with those intended for the validation of analytical methods. A brief description of the most significant tests typically performed during the PQ of the most frequently used QC equipment is described as follows. As already mentioned above, some PQ tests, especially in case of simple instruments, may also overlap with OQ tests.

5.1.6.1. pH meter

There are no significant differences between OQ tests, which were already described in the previous section, and PQ tests. In addition, accuracy may be evaluated, also using standard buffer solution and recording the difference between actual and expected values.

5.1.6.2. Dose calibrators

Accuracy, precision and linearity may be evaluated using a sample of one or more of the most frequently used radionuclides (e.g. ^{99m}Tc). Before to start with the experimental measurements, the proper conversion factors, specific for the desired radioisotopes, have to be selected. Linearity has to be tested by a series of measurements that cover the expected activity range (e.g. 10-1000 MBq); for RSD the calculation necessary to quantitatively determine precision, measured activity values need to be corrected for decay.

5.1.6.3. Radio-high performance liquid chromatography

Linearity and precision should be determined for both 'mass' detectors (e.g. UV, conductivity, electrochemical, etc.) and 'flow' radiochemical detectors. For the former, linearity may be determined preparing and analysing a suitable series of dilution of a representative analyte, with concentrations covering the expected working range, and coefficient of determination R2 should be calculated; typically, a $R2 \geq 0.99$ is considered as the gold standard. Precision may be evaluated following 5 to 6 runs using one of the above samples (typically, the sample with intermediate concentration), and calculating RSD, as usual. For radiochemical detectors, linearity may be checked using a solution containing a representative radionuclide, with a starting radioactive concentration in the same order of the magnitude of the maximum expected routine radioactive concentration. Due to the inherent nature of the radioactivity, and provided that the half-life of the selected radionuclide is sufficiently short, it is not necessary to prepare a series of dilutions, but successive runs may rather be performed using the same starting solution, until the lowest expected working radioactive concentration is reached. Determination of precision does not require further actions, and the same chromatograms obtained following linearity determination may be used, correcting peak areas related to the intended radionuclide based on the decay law, and then calculating RSD as usual.

5.1.6.4. Gas-chromatography

Here also PQ may include determination of precision and accuracy following the same general principles already described for HPLC. As for the sample to be used, this will depend on the intended use; one of the most frequent application of GC in the QC of radiopharmaceuticals is the determination of residual solvents; in this case, the sample could be represented by a solution of one (or more) of the solvents expected to potentially contaminate the final radiopharmaceutical solution.

5.1.6.5. Radio-thin-layer chromatography scanners

PQ tests are very similar to those already described for HPLC flow radiochemical detectors, and linearity and precision should be determined by using a solution with a starting radioactive concentration in the same order of magnitude of the maximum expected routine radioactive concentration. Linearity should be evaluated by analysing the same radioactive solution at 5 to 6 different times, selected taking into account the working activity range; precision could be determined quickly by placing on the same TLC sheet 4 to 5 spots, using a capillary or preferably a micropipette, at a suitable distance between two adjacent spots, and determining the spot activities in a single run.

5.1.6.6. Multichannel analyser

MCA are typically used for identifying expected radionuclide(s), for which quantification is not required and energy calibration performed during OQ (and as a routine calibration check) is sufficient. But MCA are also used for radionuclidic purity determination, and in this case quantification may be necessary. Thus, PQ of gamma spectrometers may include linearity, precision and accuracy checks by using samples of one or more of the radionuclides which are expected to be analysed. The same principles already depicted for the other radioactivity detectors apply.

5.2. VALIDATION OF ANALYTICAL METHODS

As stated by the general reference document Validation of Analytical Procedures, text and methodology, issued by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), "the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose*"*[24]. To validate an analytical method, the following characteristics may be considered: accuracy, linearity, precision (repeatability and intermediate precision), specificity, limit of detection (LOD), limit of quantitation (LOQ), range and robustness. Looking at the above list of parameters, it is easy to understand that validation of an analytical method may be not be trivial, requiring a considerable amount of time and human resources; so, it is important to establish when a validation is strictly necessary. Analytical methods do not need to be validated when they are included in a pharmacopoeia monograph. For instance, in the 'General Notices' of the European Pharmacopoeia it is stated that "The test methods given in monographs and general chapters have been validated in accordance with accepted scientific practice and current recommendations on analytical validation. Unless otherwise stated in the monograph or general chapter, validation of the test methods by the analyst is not required" [25]. This does not mean that a pharmacopoeial method may be implemented without any preliminary testing and verification, and at least the most critical parameters should be verified, depending on the intended method (e.g. linearity and accuracy). Full validation is usually required when at least one of the following situations applies:

— When the analytical method is not included in a pharmacopoeia;
— When it is described in a pharmacopoeia monograph, but it's used outside the scope and application of the monograph;

— When the analytical method is newly designed and developed (before its introduction into routine use);
— In case of significant changes in the equipment (e.g. a detector is replaced with a new one);
— Whenever the method is changed, and the change is outside the original scope of the method;
— When QC trends indicate that results are changing with time (out of trends);
— When in-house developed methods are supposed to provide a better response compared with pharmacopoeial methods.

ICH text on validation of analytical methods provides a useful guidance that may be summarized in Table 2.

TABLE 2. ANALYTICAL METHODS AND VALIDATION CRITERIA

TYPE OF ANALYTICAL PROCEDURE Characteristics[a]	IDENTIFICATION	TESTING FOR IMPURITIES Quantitat. Limit		ASSAY - Dissolution (measurement only) - Content/potency
Accuracy	-	+	-	+
Precision				
– Repeatability	-	+	-	+
– Interim. Precision	-	+ (1)	-	+ (1)
Specificity (2)	+	+	+	+
Detection Limit	-	- (3)	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

[a] (-) Signifies that this characteristic is not normally evaluated;
(+) signifies that this characteristic is normally evaluated
(a) In cases where reproducibility has been performed, intermediate precision is not needed;
(b) Lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s);
(c) May be needed in some cases.

In addition to ICH guidelines, there is a wide variety of publications that may provide useful guidance, such as the document issued by FDA 'Analytical procedures and method validation for the drugs and biologics'[26], industry guidelines and dedicated books [27]. The above references are particularly suited for the validation of chromatographic methods such as HPLC with mass detectors or GC, while for the validation of radioanalytical methods the proposed tests and methodology are not always applicable, and adaption is often required.

Analytical procedures are classified in four main types, depending on the intended substance to be analysed (e.g. the desired product or an impurity), or whether the tests are aimed to identify or to quantitatively measure the active substance. In case of radiopharmaceuticals, identification

is confirmed with two combined tests. The structural identity of the molecule is often confirmed with chromatographic methods, by comparison of the retention time of the radioactive peak obtained following the analysis of the labelled product with the retention time of the peak obtained analysing a suitable 'cold' reference standard (e.g. [^{19}F] FDG for [^{18}F] FDG or ^{12}C for ^{11}C. This is not always applicable, as there are radionuclides (e.g. ^{99m}Tc) that do not have any stable isotopes, but the above principle may still apply, using the cold precursor as the reference compound. Gamma spectrometry is also a mandatory identification test, and gamma emissions of the sample to be analysed are compared with known emission energy(ies) of the desired radionuclide. Other identification tests are possible, depending on the selected radiopharmaceuticals, but the two above indicated analyses are the most frequently used. For identification tests, ICH guidelines prompt for verification of specificity that may be performed preparing a reference solution containing the desired cold counterpart of the radiolabelled compound and, if available, other substance which are expected to be present in the preparation to be examined (e.g. known impurities). Specificity is then quantified through the 'resolution factor', which measures the ability of the method to distinguish between two potentially adjacent peaks. Determination of specificity in case of gamma spectrometry test may be more difficult, as suitable standard of radioisotopic impurities may not be easily available. However, this hurdle may sometime be overcome by using sealed radioactive reference sources, provided that their radionuclides have suitable energies.

Limit of detection (LOD) and of quantitation (LOQ) are two characteristics that should normally be evaluated for impurities, if they are known and available. There are different methods to determine LOD, based on visual evaluation, on signal-to-noise ratio or using response and an appropriate formula. In case of radiopharmaceuticals, the above methods are applicable to the 'cold' part of the molecule, while for the radioactive moiety it may be difficult to have suitable radiolabelled impurities to be used for LOD evaluation; thus, often LOD is determined on the desired radiopharmaceutical product, as a mean to evaluate the LOD of the radioactivity detector in order to establish the minimum activity to be sampled and analysed to have a meaningful response from the instrument. Methods for LOQ evaluation are similar to those listed for LOD, except that in case the signal-to-noise approach is selected, the minimum concentration of the intended analyte needs to be verified by determining the precision at that concentration (i.e., it has to be demonstrated that the minimum concentration can be reliably quantitated). Again, LOQ determination is trivial in case of 'cold' samples, while in case of radioactively labelled impurities is often not applicable; it is rather determined on the desired final product, provided that an absolute quantitation is requested. Indeed, radiochemical detectors coupled with HPLC or TLC are usually used to evaluate radiochemical purity as the ratio between the peak area of the intended radiolabelled compound and the sum total of all the radioactive detected peaks, rather than as a true determination of radioactivity. Even in case of gamma spectrometry, due to its inherent technical characteristics, LOQ is evaluated ad Minimum Detectable Activity (MDA) which is a parameter that may (slightly) change every measurement and it is dependent on several factors such as geometry, background, etc.

The other validation characteristics (precision, linearity, accuracy, range, robustness) apply both to the quantitation of impurities and of the desired product. As already mentioned above, in case of chromatographic methods such as GC or HPLC with mass detectors, the above parameters may be evaluated following the suggestions of ICH guidelines and other literature references. For the determination of linearity and precision, procedures are very similar to those

described in the previous paragraph dedicated to the qualification of equipment of the present document, and linearity may be checked by using a solution containing the desired radiopharmaceutical, with a starting radioactive concentration in the same order of magnitude of the maximum expected routine radioactive concentration. As mentioned above, it is not necessary to prepare a series of dilutions, but successive runs may rather be performed using the same starting solution, until the lowest expected working radioactive concentration is reached. Determination of precision does not require further experimental actions, and the same chromatograms obtained following linearity determination may be used, correcting peak areas related to the intended radionuclide based on the decay law, and then calculating RSD as usual. Range is determined during the execution of the above tests, while accuracy may be easily evaluated in case of determination of radionuclidic purity using gamma spectrometry, which provide a genuine quantitative response, while it is more troublesome when radio-HPLC or radio-TLC methods for the determination of radiochemical purity are under assessment, as those instruments usually do not provide a true quantitative determination of radioactivity. Finally, robustness is a characteristic which is typically evaluated with mass detectors, but the obtained results then may apply to the radiochemical detectors as well. Robustness "show the reliability of an analysis with respect to deliberate variation in method parameters"[24]. Examples are variations in HPLC eluent composition, HPLC eluent flow, GC gas flow, GC oven temperature, etc. Of course, variations have to be reasonably modest (e.g. 1.1 mL/min vs 1.0 mL/min), to mimic a potential deviation in the normal functioning of the intended instrument or unintentional mistakes by operators. The robustness of the analytical method is then evaluated by measuring the difference in response and by calculating the impact with the Anova test.

An analytical method should be revalidated in case of:

i) Changes in the radiopharmaceutical preparation process that may affect the quality of the final products. Examples of such changes are represented by the modification of the selected precursor, or by changes in reaction parameters (e.g. temperature and reaction time), when purification components are replaced by different ones (e.g. alumina cartridges are replaced by ion exchange cartridges) or the purification method is changed (e.g. HPLC vs SPE);
ii) Changes in the composition of the final product. An example of such changes is a variation in radioactive concentration that could potentially increase radiolysis and related growth of radiolabelled impurities;
iii) Significant changes in analytical procedure. Examples of such changes are the replacement of existing HPLC column with a new one with a different stationary phase, or the replacement of a detector.

5.3. DAILY SUITABILITY TESTING

A daily suitability test is a test on an analytical instrument prior to the test sample analysis (usually with a reference standard) that assures that the instrument is fit for the intended analysis and will produce a valid result. The exact testing method depends on the instrument being used.

6. SAFETY CONSIDERATIONS

6.1 CHEMICAL SAFETY, RADIATION SAFETY

Safety should be a top priority in any laboratory. In addition to chemical safety requirements applicable to any laboratory where flammable, corrosive, or toxic chemicals and/or gases are stored, laboratories producing radiopharmaceuticals have to implement measures to minimize the risk of unnecessary worker radiation exposure and contamination. Quality Control (QC) sample handling should be performed in a shielded fume hood. The thickness of shielding depends on the type and the amount of the radionuclide being handled. additionally, QC equipment waste collection should also be shielded, and vented if necessary. Special precautions such as wearing personal dosimetry devices, wearing proper personal protective equipment, and following procedures that ensure that radiation exposure is as low as reasonably achievable are critical. A special consideration should be made for radionuclides used for radiotherapy applications. Generally, these nuclides have lower energies, requiring less shielding thickness. However, longer half-life, much higher linear energy deposition, and more difficult detection of contamination (in case of alpha emitters) make working with chemicals very hazardous. Extra precautions that minimize chances of surface contamination and human ingestion or inhalation must be taken when working with these compounds.

6.2 LABORATORY REQUIREMENTS

Laboratory requirements for quality control depend on several factors:

— Complexity and variety of the required tests: the easier setting up may be represented by a ‘traditional’ nuclear medicine department involved in the preparation of ^{99m}Tc labelling kit only. On the contrary, the QCs of extemporaneous preparations are usually more complex, requiring more sophisticated instrumentation, etc.
— The expected workload of the facility (e.g. the number of radiopharmaceuticals to be tested, the testing frequency, etc.).

The regulatory framework: highly regulated framework is typical for commercial manufacturers and, in general, of GMP inspected site. The complexity of QC test, the need for frequent QC related test (e.g. system suitability test, validation and revalidation tests) and the requested high level of traceability prompt for using, as much as possible, dedicated instrumentation, and may hamper the use of, for example, the same HPLC equipment for the analysis of multiple radiopharmaceuticals.

Room dedicated to quality control should thus be suitable for the intended purposes and the characteristics of the Facility. QC laboratories do not need to be classified following GMP (or ISO, or other applicable standards), and they should comply with requisites for radiation protection only. Room dedicated to quality control should thus be suitable for the intended purposes and the characteristics of the facility. To this regard, it has to be noted that the radiological risk is often underestimated while designing QC labs, probably due to the typically low activity of the samples used to perform the analytical tests. But the difficulty to adequately shield instruments such as HPLC or GC may nonetheless significantly increase the radiation hazard to the personnel. In case of a simple setting-up (see above), only small instrumentation

such as pH meter (if any), analytical balance, and radio-TLC scanner are of concern, and a small room could house all the necessary equipment, together with a safety cabinet for TLC solvent storage. When a higher-level arrangement applies (e.g. for extemporaneous preparations), in addition to the above mentioned small equipment, including safety cabinet for solvent (and sometimes acids/bases) storage, instruments such as radio-HPLC, GC and gamma-spectrometer have to be housed, and requested available room may be much higher. Typical laboratory workbench may be used to locate most of the QC instruments, but in case some shielding is requested (e.g. it could be necessary to place some lead to protect operators from gamma radiation coming from HPLC column), the maximum load capacity of the workbench should be considered. Manipulation of the QC samples should ideally be performed in shielded hood, whose front side may be open, except for a sliding shielding with lead glass that allow the operators to protect head (and especially the eyes) and chest. If for any reasons (e.g. economical, or due to insufficient room) is not possible to immediately install such a shielded hood, at least a suitably shielded area, made of lead bricks and possibly with lead glass, should be in place (see Figure 6), to allow the operators to safely handle the 'bulk' QC sample and prepare the various aliquots to be used for the intended tests.

FIG. 5. Example of an inexpensive lead-shielded area for the manipulation of QC samples.

The above specifications may not always apply to gamma spectrometers to reduce the radiation background to a minimum level, whose detectors are highly sensitive and require heavy and bulky dedicated shielding. In such a case, if the shielding is not available or is not sufficient, it might be necessary to locate gamma spectrometer in a different room, where no other radioactivity manipulation takes place.

QC sampling is a very important and critical operation, which poses different challenges. Here are some of the most important aspects to be considered:

— QC samples should be representative of the whole content of the intended radiopharmaceutical product;
— The amount of the sample should be sufficient to perform all the required QC tests;
— QC samples should be stored to prevent contamination and to not alter physico-chemical and microbiological properties of the sample itself. In other words, QC results should not be affected by improper storage of the QC samples;
— Containers used to store samples should be of sufficient volume, of proper shape (to allow easy and efficient sample withdrawal) and of suitable material, to avoid the release of

undesired contaminants in the radiopharmaceutical formulation and/or to minimize possible loss of activity due to interaction between the products–excipients and the container material itself.

QC samples may be prepared during automated dispensing of the final product, in case the latter is fractionated in multiple vials–containers, or they may be withdrawn (e.g. using a syringe) from the single vial containing the desired product after formulation step. QC samples should not be obtained before the radiopharmaceutical product has been fully collected into the final container. For example, in principle it should be technically possible to connect, through a 3-way valve, the container for QC sample to the final product transfer line in an automated radiosynthesis system, but this kind of sampling operation should be avoided. Whatever are the preferred sampling procedures, they should always be conducted aseptically. Currently, commercially available dispensing systems are designed to ensure a working area in class 'A' – GMP (or ISO-5), and sampling may be performed automatically or remotely (e.g. using tele-pliers) or even manually, through suitable gloves, provided that the manipulations are compatible with radiation protection principles. In case such an equipment is not available, a laminar flow cabinet could be used, provided that the vial containing the final product may be safely recovered from the product preparation hot cell and that it is then placed under the laminar flow into a suitably shielded container. The worst case is the withdrawal of a QC sample from a vial placed in the preparation hot cell, that usually do not provide laminar flow and class 'A' environment (although often guarantee class B–C). This may be of concern in small facilities, where sophisticated and expensive equipment may not be available. Sampling should be performed anyway trying to reduce as much as possible product exposure to the surrounding environment, for instance using sterile, pyrogen free single use materials (syringes, needles, plastic tube, if any, etc.).

6.3 STAFF TRAINING REQUIREMENTS

The aim is to assure control over the radiopharmaceutical manufacturing process by enabling the operators to perform the activities related to the manufacturing, preparation, dispensing, as well as the quality control and quality assurance of radiopharmaceuticals in compliance with approved Standard Operating Procedures (SOP's).

Staff training may be performed in several different formats that include didactic instruction, read and understand, physical observation by the trainer, and instructional videos, a teleconference, a workshop, or a validation study to demonstrate operator's ability to comply with the training requirements. Most often, the training is carried out in the 'read and understand' and didactic instruction format. In the commercial manufacturing setting, staff training is normally performed by members of the centralized QA department, with help from the local facility QA person. In the academic setting where radiopharmaceuticals for clinical trials are produced, staff training is normally performed by the QA person or another subject matter expert. Some manufacturers (both commercial and academic) may rely on additional trainee competency testing to ensure that the trainee has understood the training material. The exact training design may be flexible, but it is important to design it in a way so that the training material and the delivery of information allows the operator to easily understand and retain the training content. Also, in addition to the regulatory requirements, facility specific operations and design may need to be considered when deciding on the exact raining requirements. This

requirement applies especially to manufacturers producing radiopharmaceuticals for clinical trials. Oftentimes in this setting, the manufacturing process may be so new that applicable regulations may not exist yet and the local QA person must rely on the scientific evidence, and on the regulatory feedback available at the time to design an appropriate process. Regardless of the training format chosen, staff training must always be documented so that the training record could be made available during regulatory inspections.

Staff retraining requirements also vary between various manufacturers. Retraining can be classified into two main categories: for-cause retraining and refresher retraining. For-cause retraining is normally performed either when the SOP is changed or as part of the corrective and preventive action designed to minimize the chances of the problem occurring again. For example, lab QA may decide to retrain the QC operator on how to perform a particular QC analysis because the operator recently has made several mistakes when performing the analysis. Refresher retraining is performed at certain time intervals, regardless of whether the cause for retraining exists. Commercial manufacturers often choose a set of procedures that all staff must be retrained on an annual basis. This type of refresher training is possible in this setting because the staff is normally dedicated to producing one to two agents using an established process which does not change significantly from year to the next. Therefore, the content of the training also does not change meaningfully. Other manufactures may choose to perform refresher training at longer time intervals such as every two or three years, depending on the feedback from the regulatory agencies. In an academic setting where agents for clinical trials are produced, the continuously changing processes associated with early stage drug development make implementation of the refresher training extremely challenging. Therefore, only for-cause retraining is recommended in this setting. However, if both radiopharmaceuticals with marketing authorization and clinical trials agents are made in the same academic setting, then the more stringent refresher training requirements may apply to the manufacture of the radiopharmaceutical with marketing authorization.

In practice, it is very important to plan the training schedule carefully and then stick to the planned schedule. Operational factors such as clinical production schedule, trainee availability, and trainer availability often make getting all the staff trained at the same time difficult. Therefore, trainers should develop a training schedule, possibly involving multiple training sessions, that will ensure successful training of the entire team. It is also important that the trainer is a current subject matter expert on the training content. There may be times when an expert consultant may need to used expertise if a particular matter is not available onsite. In addition to simply conveying the information to the trainee, the trainer should also be engaging and able to explain in detail the basis as well as the importance of a particular requirement or process. This type of training normally results in the trainee being more willing to comply with the training requirements, which ultimately contributes to the training program success.

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ANNEX I: RADIONUCLIDES INCLUDED IN THIS PUBLICATION

Radionuclides included within this document are listed in the table below, together with half-life and mode of decay, including energy of the particle or photon.

TABLE I–1. DECAY PROPERTIES OF VARIOUS RADIOISOTOPES USED IN RADIOPHARMACEUTICAL PRODUCTION

RADIONUCLIDE	$T_{1/2}$	DISINTEGRATION (ABUNDANCE)	MAX ENERGY
^{11}C	20.38 min	β^+ (99.8%)	Emax β^+ 961 keV
^{18}F	109.8 min	β^+ (96.9%)	Emax β^+ 635 keV
^{64}Cu	12.70 h	β^+ (18%) / β^-(39%) / EC (43%)	Emax β^+ 653 keV
^{68}Ga	67.7 min	β^+ (89%)	Emax β^+ 1899 keV
^{89}Zr	3.3 d	β^+ (23%)	Emax β^+ 897 keV
^{90}Y	64.1 h	β^- (100%)	Emax β^- 2.28 MeV
^{99}Mo	65.98 h	β^- (82.2%)	Emax β^- 1214 keV
^{99m}Tc	6.01 h	IT (89%)	E ɣ 140 keV
^{111}In	2.80 d	EC (100%)	E ɣ 171 / 255 keV
^{124}I	4.18 d	β^+ (23%)	Emax β^+ 2135 keV
^{177}Lu	6.73 d	β^- (99.9%)	Emax β^- 498 keV
^{213}Bi[a]	45.6 min	α	Eα 8.4 MeV E_α 440 keV (26.1 % emission probability)
^{223}Ra[b]	11.4 d	α	Eα 5.64 MeV
^{225}Ac[c]	9.9 d	α	Eα 5.5 MeV

[a] ^{213}Bi is a mixed alpha / beta emitter with a half-life of 45.6 min. It mainly decays via beta emission to the ultra-short lived, pure alpha emitter ^{213}Po ($T_{1/2}$ = 4.2 µs, Eα= 8.4 MeV) with a branching ratio of 97.8%). The remaining 2.2 % of ^{213}Bi decays lead to ^{209}Tl via alpha particle emission (E_α= 5.5 MeV, 0.16%, E_α= 5.9 MeV, 2.01 %). Therefore, ^{213}Bi itself can be practically considered as an α emitter.

[b] ^{223}Ra is an alpha emitting radioisotope that decays via 7 daughter nuclides before it stabilizes as ^{207}Pb. During the decay of each ^{223}Ra, 4 α particles and 2 electrons (β particles) are emitted.

[c] The predominant decay path of ^{225}Ac yields net 4 alpha particles with a large cumulative energy of 28 MeV and 2 beta disintegrations of $E_{\alpha,max}$= 1.6 MeV and $E_{\alpha,max}$= 0.6 MeV

ANNEX II: QUALITY CONTROL EXAMPLES FOR STARTING RADIOACTIVE ISOTOPES

II-1. QUALITY CONTROL OF ^{68}Ga OBTAINED FROM A ^{68}Ge/^{68}Ga GENERATOR

^{68}Ga [$t_{1/2}$=67.7 min, $E_{\beta+}$max = 1.92 MeV (89%)], a positron emitting radioisotope is conveniently availability from ^{68}Ge/^{68}Ga generator by decay of the parent ^{68}Ge ($t_{1/2}$=270.95 days), as seen in Table II-1. ^{68}Ga labelled somatostatin receptor-avid peptides (e.g. [^{68}Ga] DOTATOC) are routinely used for PET imaging of neuroendocrine tumours.

TABLE II–1. QUALITY CONTROL TESTS FOR ^{68}GA FROM ^{68}GE/^{68}GA GENERATOR ELUTED WITH DILUTE HYDROCHLORIC ACID

TESTS	METHODS	SPECIFICATION OF ^{68}Ga[a]
Appearance	Visual examination	Clear colourless solution
pH	pH indicator strip	Maximum 2
Radionuclide identity	Follow the decay pattern of ^{68}Ga[b]	Half-life 62 to 74 min
Radionuclidic purity	Analysis of decayed sample using HPGe detector coupled to a MCA[c]	Minimum 99.9 % of the total radioactivity as ^{68}Ga
^{68}Ge breakthrough	Analysis of decayed sample using HPGe detector coupled to a MCA[d]	<0.001 %
Radiochemical purity	Paper chromatography using 10 mM EDTA as mobile phase [e]	Minimum 95 % of the total radioactivity due to ^{68}Ga(III)
Chemical purity	ICP-AES/ICP-MS [f]	Fe: 10 µg/GBq Zn: 10 µg/GBq
Bacterial Endotoxin Content	LAL test	≤ 175 EU/Total volume

[a] ^{68}Ga chloride solution for radiolabelling. European Pharmacopoeia Monograph No. 2464:

[b] **Identity**: Place a small aliquot of [^{68}Ga] $GaCl_3$ (in a test tube) in a well type NaI (Tl) scintillation counter. Record the counts at fixed intervals of time, setting appropriate energy window for detecting the 511 keV γ radiations of ^{68}Ga. Note down the counts and the time of counting. Plot the decay curve [Time on X axis vs. Counts (in log scale) on Y axis]. Determine the half-life of the ^{68}Ga sample from the slope of the decay curve.

[c] **Radionuclidic purity**: Allow the $^{68}GaCl_3$ eluted from the generator to decay for 48 hours. Analyse the decayed sample using an HPGe detector coupled to a multi-channel analyser (MCA) for the presence of γ emitting impurities.

[d] **^{68}Ge breakthrough**: As ^{68}Ge decays exclusively by electron capture to ^{68}Ga, the presence of ^{68}Ge impurity in ^{68}Ga eluate cannot be directly determined by γ spectroscopy. Allow the $^{68}GaCl_3$ eluted from the generator to decay for 48 hours. Analyse the decayed ^{68}Ga sample using an HPGe detector coupled to a MCA. Measure the 511 keV γ radiations from the ^{68}Ga daughter, which corresponds to the ^{68}Ge impurity present in the sample

[e] **Radiochemical purity**: An aliquot of the $^{68}GaCl_3$ eluted from the generator be analysed by paper chromatography on a Whatman™ 3 mm chromatography paper (12 x 1 cm) using 10 mM EDTA as mobile phase. In this system, ^{68}Ga(III) moves towards the solvent front (R_f = 0.9-1.0) while colloidal and non-cationic ^{68}Ga species remain close to the origin.

[f] **Chemical purity**: Presence of trace metallic impurities in the $^{68}GaCl_3$ solution can be quantified by ICP-AES analysis of a decayed sample. Calibration curves for the trace metal ions of interest (eg. Fe, Zn) are obtained using standard solutions containing known concentration of these trace metal ions.

II-2. QUALITY CONTROL OF ^{68}Ga PRODUCED BY CYCLOTRON

^{68}Ga can be produced by small size biomedical cyclotrons by proton irradiation of ^{68}Zn targets [II-1, II-2]. After irradiation, ^{68}Ga is recovered into a concentrated HCl solution and loaded onto a cation exchange resin [II-3]. After several washing steps, ^{68}Ga is eluted from the column by using dilute hydrochloric acid, and the eluate is directly transferred on a column containing DGA resin. The second column is rinsed, and ^{68}Ga is eluted with water.

TABLE II-2. THE LIST OF THE TESTS PERFORMED, METHODS AND SPECIFICATIONS (ACCEPTANCE CRITERIA) RECOMMENDED FOR CYCLOTRON PRODUCED ^{68}Ga. NOTE: STERILITY TESTING IS PERFORMED AS A POST-RELEASED CONTROL

TESTS	METHODS	SPECIFICATIONS
Appearance of the solution	Visual examination	Clear and colourless solution Free from visible particulates
pH	pH indicator strip	Maximum 2
Radionuclidic identity	Half-life determination	Half-life between 65 and 71 minutes
Radionuclidic purity	HPGe γ spectrometry	Minimum 99.9 % of the total radioactivity as ^{68}Ga
Radiochemical identity	Cation-exchange HPLC[a]	t_R ± 10% (comparison with standard)
Radiochemical purity		≥ 95.0%
Chemical purity	ICP-MS	Fe: ≤ 10 ppm Cu: ≤ 10 ppm Ni: ≤ 10 ppm Zn: ≤ 10 ppm
Bacterial endotoxin content	LAL test	≤ 175 EU/injection
Sterility	Direct inoculation	Sterile

[a] Chromatography methods:

Equipment:	Dionex ion analysis HPLC system
Column:	IonPac CS5A analytical column (l = 250 cm, ∅ = 4 mm, for cations) – Dionex
Mobile phase:	Dionex MetPac eluent
Flow rate:	1.2 mL/min
Injection:	10 to 50 µL
Run time:	10 min
Detection:	Radioactivity
R_t value:	^{68}Ga: 2.6 min

II.3. QUALITY CONTROL OF ^{90}Y OBTAINED FROM A ^{90}Sr/^{90}Y GENERATOR

^{90}Y ($t_{1/2}$ = 64.1 h), a pure β^- emitter ($E_{\beta\text{-max}}$ = 2.28 MeV), is a therapeutic radionuclide available from ^{90}Sr/^{90}Y generators by decay of the long-lived parent ^{90}Sr ($t_{1/2}$=28.8 years), and its QC tests are seen in table II-3.

TABLE II–3. QUALITY CONTROL TESTS FOR ^{90}Y OBTAINED FROM ^{90}Sr/^{90}Y GENERATOR.

TESTS	METHODS	SPECIFICATIONS
Appearance	Visual inspection	Clear colourless solution, free of particulate matter
pH	pH indicator strip	1.0 to 1.5
Identity	Liquid scintillation counting	Corresponds to ^{90}Y
γ emitting impurities	Analysis of decayed sample using HPGe detector coupled to a MCA [a]	< 1×10^{-4} Bq/Bq of ^{90}Y
^{90}Sr breakthrough	Chromatography using cellulose phosphate paper [b]	< 1×10^{-5} Bq ^{90}Sr/Bq of ^{90}Y
Radiochemical purity	DTPA complexation[c]	> 99 % of ^{90}Y as Y^{3+}
Metal impurities	Polarography DTPA complexation	Cd: 1 µg/mL[d] Cu: 1 µg/mL Fe: 10 µg/mL Pb: 5 µg/mL Zn: 5 µg/mL Better than 95 % complexation of ^{90}Y[e]
Sterility	Direct inoculation	Sterile
Bacterial Endotoxin Content	LAL test	25 IU/mL

[a] γ emitting impurities. Allow the $^{90}YCl_3$ eluted from the generator to decay completely (> 20 half-lives). Analyse the decayed sample using an HPGe detector coupled to a MCA for the presence of γ emitting impurities.

[b] ^{90}Sr breakthrough. The USP monograph for [^{90}Y] Ibritumomab tiuxetan injection (Zevalin) describes a chromatography technique using Whatman cellulose phosphate paper for estimation of ^{90}Sr content in $^{90}YCl_3$. A Sr/Y carrier solution containing 0.34 mg of $YCl_3.6H_2O$ and 0.30 mg of $SrCl_2.6H_2O$ per mL of 0.1 N HCl is prepared. About 50 µL of this solution is applied at the origin of a 20×2 cm cellulose phosphate chromatographic strip and allowed to dry. 5 µL of the $^{90}YCl_3$ solution is applied at the origin and the chromatogram is developed using 3 N HCl as the developing solvent, until the solvent migrates to 15 cm mark. It is then allowed to dry. The strip is cut at the 8cm mark and solvent front is placed in a liquid scintillation cocktail and counted in a liquid scintillation counter for presence of ^{90}Sr. See also USP monograph. ^{90}Y Ibritumomab tiuxetan injection can be found in the Pharmacopeia.

[c] Radiochemical purity. An aliquot of the $^{90}YCl_3$ eluted from the ^{90}Sr–^{90}Y generator be analysed by paper chromatography on a Whatman™ 3 mm chromatography paper (12×1 cm) using 10 mM DTPA as mobile phase. In this system, ^{90}Y(III) moves towards the solvent front (R_f= 0.9-1.0) while colloidal ^{90}Y species remain close to the origin.

[d] The presence of trace metal ions in the $^{90}YCl_3$ solution can be also assessed by complexing 74 kBq of ^{90}Y sample solution with DTPA solution containing 25 picomoles of DTPA, as seen in http://www.ezag.com/fileadmin/ezag/user-uploads/radiopharma/radiopharma/7132-0024_90Yttrium_Chloride_Solution.pdf.

[e] Metal impurities. Presence of trace metallic impurities in the $^{90}YCl_3$ solution can be quantified by carrying out polarography of decayed ^{90}Y sample samples.

II-4. QUALITY CONTROL OF ^{99m}Tc PRODUCED BY CYCLOTRON

Direct production of ^{99m}Tc with biomedical cyclotron (16 to 24 MeV) via the ^{100}Mo (p,2n) ^{99m}Tc reaction is a promising alternative to generator based ^{99m}Tc [31]. After irradiation, the target is dissolved with hydrogen peroxide and the [^{99m}Tc] pertechnetate is purified via a solid phase extraction by using cross linked polyethylene glycol resin [32]. Final elution with a 0.9% sodium chloride solution is providing the [^{99m}Tc] pertechnetate. The quality of the cyclotron produced ^{99m}Tc (CPTc) is highly influenced by the isotopic composition of the target material, the irradiation conditions, and time of injection after end of bombardment [33]. Formation of

Tc impurities by (p, n), (p,2n), and (p,3n) reactions on the molybdenum isotopes (i.e. $^{92\text{-}98}$Mo) contained in the target material cannot be avoided and cannot be segregated from ^{99m}Tc [II–7]. Their contribution may have a detrimental effect on the effective dose to the patient and the image quality [II–8]. Globally, the quality controls performed for cyclotron produced technetium are identical to the quality controls defined for generator produced ^{99m}Tc in the pharmacopoeia monographs. The main difference results in the quantification of the Tc impurities is to limit the dose increase due to their presence to an acceptable limit [II–9]. The list of the tests performed, methods and specifications (acceptance criteria) are shown in Table II-4, as prepared by TRIUMF (Canada's national laboratory for particle and nuclear physics and accelerator-based science), Vancouver, Canada.

TABLE II–4. REQUIRED TESTS PERFORMED FOR CYCLOTRON PRODUCED ^{99m}Tc BATCHES (NOTE:STERILITY TESTING IS PERFORMED AS A POST-RELEASED CONTROL)

TESTS	METHODS	SPECIFICATIONS
Appearance of the solution	Visual examination	Clear and colourless solution Free from visible particulates
pH	pH indicator strip	4.5 to 7.5
Radionuclidic identity	Half-life determination	Half-life between 5.72 and 6.32 hours
Radionuclidic purity [II–10]	Modified Molybdenum shield assay[a]	Isotopes other than ^{99m}Tc contribute to less than 6000 emissions/sec / MBq of ^{99m}Tc.
Radiochemical identity	TLC-radiometric[b]	R_f = 0.8 to 1.0
Radiochemical purity		≥95.0%
Chemical impurity	Aluminium: colorimetric assay [c]	≤10 µg/mL
	Hydrogen peroxide: colorimetric assay [c]	≤50 µg/mL
	Molybdenum colorimetric assay [c]	≤30 µg/mL
Radioactivity concentration	Ionization chamber	≤27.8 GBq/mL
Bacterial endotoxin content	LAL test	≤175 EU/injection
Sterility	Direct inoculation	Sterile

[a] Modified Molybdenum shield assay method: Set the dose calibrator to the ^{99m}Tc setting and perform a measurement with a sample of the sodium [^{99m}Tc] pertechnetate, which is no less than 0.5 GBq. The measurement is performed without the molybdenum assay shield and is recorded as R_{air}. Make a second measurement with the same sample enclosed in the molybdenum assay shield. The reading is recorded as R_{Lead}. The emission rate from the impurities per MBq of ^{99m}Tc is calculated according to the method previously described in the section about the quality controls related to the radionuclides (see Section 3.1).

[b] TLC-radiometric method: perform the test as it is described in the section below for the Quality Control of ^{99m}Tc produced by ^{99}Mo/^{99m}Tc generator.

[c] Colorimetric assays methods:

(a) **Aluminium**: Pipet 15 µL of the sodium [^{99m}Tc] pertechnetate and place it on the indicator paper of a Biodex Tec-Control Aluminium Breakthru Kit. Using the standard's dropper, place a drop of the aluminium standard on the indicator paper next to the sample spot (the drops must be the same size). Compare the colour intensity of the two red spots formed. If the sample spot is less intense than the standard, then the sodium [^{99m}Tc] pertechnetate contains less than 10 ppm of aluminium.

(b) **Hydrogen peroxide**: Place one drop of the sodium [^{99m}Tc] pertechnetate and one drop of a hydrogen peroxide standard solution (10 ppm) on a Quantofix Peroxide test stick. After 5 seconds, compare the colour of each spot with the colour scale provided. In the presence of H_2O_2, the test paper turns blue and the limit of detection is 1 ppm.

(c) **Molybdenum**: Prior to the analysis the following solutions must be prepared:

i) solution (A) is a 3-mM solution of C18mimBr in 0.1 M chloroacetic acid;

ii) solution (B) is a 1mM pyrogallol red solution in EtOH/water (v/v, 1:1), and

iii) a 100-ppm standard solution of Mo(VI). Working standards of Mo(VI) at 5, 10, 15, 20, 25, and 30 ppm concentration are prepared by dilution of the standard solution of Mo(VI) with 0.1 M chloroacetic acid.

Then, colorimetric standards corresponding to each concentration are prepared by mixing 100 µL of solution (A), 100 µL of solution (B), 15 µL of the corresponding working standard, and 15 µL of saline solution. The sodium [^{99m}Tc] pertechnetate is analysed by comparing the colour obtained 8 minutes after mixing 100 µL of solution (A), 100 µL of solution (B), 15 µL of 0.1 M chloroacetic acid, and 15 µL of the sodium [^{99m}Tc] pertechnetate to the colorimetric standards. The limit of detection is 5 ppm saline solution and the concentration of Mo(VI) is not greater than 30 ppm.

Note that the filter integrity test shall be done >50 psi performed for each batch but as a production control.

II-5. QUALITY CONTROL OF ^{99m}Tc OBTAINED FROM A ^{99}Mo/^{99m}Tc GENERATOR

^{99m}Tc is a radioactive nuclide formed by the radioactive decay of ^{99}Mo, which is a radioactive isotope of molybdenum and may be formed by the neutron bombardment of ^{98}Mo or as a product of uranium fission. The ^{99}Mo/^{99m}Tc generator is constructed with alumina (Al_2O_3) loaded in a plastic or glass column. The ^{99}Mo radioactivity is adsorbed on alumina in the chemical form MoO_4^{-2} (molybdate). The technetium is obtained as a [^{99m}Tc] pertechnetate anion ($^{99m}TcO_4^-$) by elution of the ^{99}Mo/^{99m}Tc generator with a 0.9% sodium chloride solution. TABLE II-5 demonstrates required tests performed for routinely produced ^{99}Mo/^{99m}Tc generator batches. Sterility testing of the eluates is performed on the same day of production albeit it is a post-release control as the results are ready after 14 days of incubation.

TABLE II-5. QUALITY CONTROL TESTS FOR ROUTINELY PRODUCED ^{99}Mo/^{99m}Tc GENERATOR BATCHES

TESTS	METHODS	SPECIFICATIONS
Appearance of the solution	Visual examination	Clear, colourless solution
pH	pH paper	4.5 to 7.5 [II-11, II-12]; 4.0 to 8.0[II-13]
Radionuclide identification	γ spectrometry	γ photons of 140 keV
Radiochemical purity	Paper chromatography[a]	≥95.0%
Radionuclidic purity	Ionization chamber	The amount of ^{99}Mo is not greater than 0.15 kBq/MBq (µCi/mCi) of ^{99m}Tc per administered dose in the injection, at the time of administration.

	Measurement in a High Pure Germanium Detector (γ spectrometry)	^{131}I and ^{103}Ru: not more than 0.05 kBq/MBq of ^{99m}Tc at the time of administration. ^{89}Sr: not more than 0.0006 kBq/MBq of ^{99m}Tc ^{90}Sr: not more than 0.00006 kBq/MBq of ^{99m}TC All other radionuclidic impurities: not more than 0.01% of all other beta and gamma emitters is present at the time of administration. Not more than 0.001 Bq of gross alpha impurity per 1 MBq of ^{99m}Tc [II-11].
Chemical impurity: Aluminium [b]	Spectrophotometry (1) Colorimetry (2)	≤10 μg/mL [II-12]; ≤5 μg/mL [II-13]
Radioactivity measurement	Ionization chamber	Measurement of syringe for injection
Bacterial endotoxin content	LAL test	≤175 IU/injection
Sterility	Direct inoculation	Sterile

[a] **Chromatography methods:**

Solid phase:	Chromatographic paper.
Mobile phase:	Acetone: 2N Hydrochloric acid (80:20 V/V) [II-11] or Water: Methanol (20:80 V/V) [II-12] [II-13].
Method:	Place a spot of the radiopharmaceutical near the bottom of the chromatographic paper strip. Place the strip in a chamber or tank and allow the mobile phase to migrate up the strip until it nears the end. Remove the strip and allow it to dry.
Analysis:	Analyse the distribution of the activity on the strip, for each mobile phase cut the strip into 1 cm segments and measure the activity of each segment in a gamma counter.
R_f values:	^{99m}Tc has an R_f value of about 0.90 [II-11] and 0.6 [II-12] [II-13], respectively.

[b] **Aluminum:**

Pipet 10 mL of Aluminum Standard Solution (2 ppm) into each of two 50 mL volumetric flasks. To each flask add 3 drops of methyl orange TS and 2 drops of 6 N ammonium hydroxide, then add 0.5 N hydrochloric acid, dropwise, until the solution turns red. To one flask add 25 mL of sodium thioglycolate TS, and to the other flask add 1 mL of edetate disodium TS. To each flask add 5 mL of eriochrome cyanine TS and 5 mL of acetate buffer TS, and add water to volume. Immediately determine the absorbance of the solution containing sodium thioglycolate TS at the wavelength of maximum absorbance at about 535 nm, with a suitable spectrophotometer, using the solution containing the edetate disodium TS as a blank. Repeat the procedure using two 1.0 mL aliquots of Injection. Calculate the quantity, in μg per mL, of aluminium in the Injection taken by the formula: $20(T_u / T_s)$, in which T_u and T_s are the absorbances of the solution from the Injection and the solution containing aluminium standard, respectively. The concentration of aluminium ion in the Injection is not greater than 10 μg per mL [II-11].

Dilute 1 mL of the injection to be examined to 2.5 mL with water R. Mix 2 mL of the resulting solution and 1 mL of acetate buffer, pH 4.6, TS in a test tube of about 12 mm in internal diameter. Add 0.05 mL of a 10 g L^{-1} solution of chromazurol SR. Allow to stand for 3 minutes. The colour of the solution is not more intense than that of an aluminium standard (2 ppm Al) TS prepared in the same manner. The concentration of aluminium ion in the injection is not more than 5 ppm [II-13].

II-6. QUALITY CONTROL OF ^{177}Lu PRODUCED BY A REACTOR

The therapeutic radionuclide ^{177}Lu ($t_{1/2}$=6.73 d), a β^- emitter ($E_{\beta\text{-max}}$ = 2.28 MeV) with major γ emissions of 113 keV and 208 keV, is produced by neutron capture reaction ^{176}Lu (n, γ) ^{177}Lu. The target Lu_2O_3 (^{176}Lu enriched) is irradiated and dissolved in hydrochloric acid to form a $^{177}LuCl_3$ solution. After irradiation, the target is decayed for 3 days for reducing the activity of ^{176m}Lu ($t_{1/2}$=3.66 h) produced by a side reaction [II-14]. The irradiation time should not too be

long to avoid an enhancement of the long-lived isomer ^{177m}Lu ($t_{1/2}$=160.4 d). Both ^{177}Lu and ^{177m}Lu decays to the stable ^{177}Hf.

An indirect method to produce ^{177}Lu performs on the radiation of enriched ^{176}Yb by the reactions ^{176}Yb (n, γ) ^{177}Yb, which decays with $t_{1/2}$=1.91 h to ^{177}Lu. The target material Yb is separated from ^{177}Lu by multistep solid phase or liquid extraction process [II-15]. ^{177}Lu produced via ^{176}Yb path contains no-carrier-Lu and no ^{177m}Lu as an impurity. Several QC tests from ^{177}Lu are shown in Table II-6.

TABLE II-6. QUALITY CONTROL TESTS FOR ^{177}Lu PREPARED VIA BOTH PATHWAYS [II-16].

TESTS	METHODS	SPECIFICATIONS
Appearance	Visual inspection	Clear colourless solution
pH	pH indicator strip	1–2
Identity	γ spectrometry, TLC[b]	γ photons of 113 keV and 208 keV
Specific activity	ICP-AES[a]	> 2,5 GBq/mg (Lu^{3+}: < 0.4 mg/GBq)
Metal impurities	ICP-AES[a]	Cu: ≤ 1,0 µg/GBq Fe: ≤0,5 µg/GBq Pb: ≤ 0,5 µg/GBq Zn: ≤ 1,0 µg/GBq
Radionuclide purity	γ spectrometry	^{177}Lu: > 99.9 % ^{177m}Lu (impurity): ≤ 0.1 % ^{175}Yb (impurity): ≤ 0.07 % Other impurities: ≤ 0.01 %
Radiochemical purity	TLC[b]	>99 % of ^{177}Lu as Lu^{3+}
Sterility	Direct inoculation	Sterile
Bacterial Endotoxin Content	LAL test	<25 IU/mL

[a] Prepare a ^{177}Lu solution with 50 MBq/mL and determine the metal ions by inductively coupled plasma-atomic emission spectrometry (ICP-AES).
[b] $^{177}LuCl_3$ is analysed by paper chromatography on Varian ITLC SG using saline adjusted to pH 2.3 with hydrochloric acid as mobile phase. R_f values: $^{177}LuCl_3$ = 0.4-0.7; reference: ^{177}Lu-DTPA > 0.9.

II-7. QUALITY CONTROL OF ^{213}Bi OBTAINED FROM A ^{225}Ac/^{213}Bi GENERATOR

^{225}Ac [$t_{1/2}$ = 9.9 d] can be produced by radiochemical separation from a ^{229}Th source or via cyclotrons by proton irradiation of ^{226}Ra targets (^{226}Ra- (p,2n) ^{225}Ac) [II-17 - II-20] and can be loaded on a generator [II-21]. ^{213}Bi [$t_{1/2}$=45.6 min, E_α = 8.4 MeV and E_γ= 440 keV, 26.1 % emission probability) is eluted from the column by using a 0.1 mol hydrochloric acid/sodium iodate solution into solution containing buffer and ascorbic acid. The buffer is depending on the ^{213}Bi chelating agent. Sodium acetate buffer (4 M) is recommended for CHX-DTPA ((p-SCN-Bz)-cyclohexyldiethyIenetriaminpentaacaticacid) and TRIS (2-Amino-2-

(hydroxymethyl) propane-1,3-diol, 2M) for DOTA (1,4,7,10 tetraazacyclododecane-1,4,7,10-tetraacetic acid) chelators.

Table II-7 demonstrates the recommended quality control tests for ^{213}Bi as prepared at the European Commission, Joint Research Centre, Directorate G. Nuclear Safety and Security, Karlsruhe, Germany. Note that sterility and bacterial endotoxin testing is performed as a post-released control for the final labelled radiopharmaceutical. The ICP-MS analysis is usually performed randomly in an indirect manner, deduced from previous testing of generators of identical type.

TABLE II-7. QUALITY CONTROL TESTS ARE RECOMMENDED FOR ^{213}Bi

TESTS	METHODS	SPECIFICATIONS
Appearance of the solution	Visual examination	Clear and colourless solution Free from visible particles
pH	pH indicator strip	5.3 to 5.7 for sodium acetate buffer 8.5 to 9.0 for TRIS buffer
Radionuclidic identity	Half-life determination	Half-life between 43 to 50 minutes
Radionuclidic purity	HPGe γ spectrometry	Minimum 99.9 % of the total radioactivity as ^{213}Bi
Chemical purity	ICP-MS	Sum of non-radioactive cations < 1 µg/mL
Bacterial endotoxin content	LAL test	≤ 175 EU/injection
Sterility	Direct inoculation	Sterile

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ANNEX III: REPRESENTATIVE EXAMPLES OF QUALITY CONTROL OF RADIOPHARMACEUTICALS

III-1. [^{11}C] PIB

PIB is labelled with ^{11}C ($T_{1/2}$ = 20.38 min) at its methylamino group from the corresponding nor-derivative (desmethyl-PIB, 2-(4'-aminophenyl)-6-hydroxybenzothiazole) as precursor for labelling and the methylation reagent [^{11}C] methyl triflate (the latter prepared in three chemical steps from cyclotron-produced [^{11}C] carbon dioxide), based on published procedures [III-1] with slight modifications and using a TRACERLab® FX C Pro synthesizer (GEMS). [^{11}C] PIB is then purified by HPLC and formulated as an 10% ethanolic saline (0.9% aq. NaCl) solution after SepPak® cartridge-based removal of the HPLC solvents and sterile filtration on a Millex® GV 0.22 μm filter (Millipore®). Figure III-1 shows the production route for [^{11}C] PIB as prepared at CEA, SHFJ (academic research centre), Orsay, France.

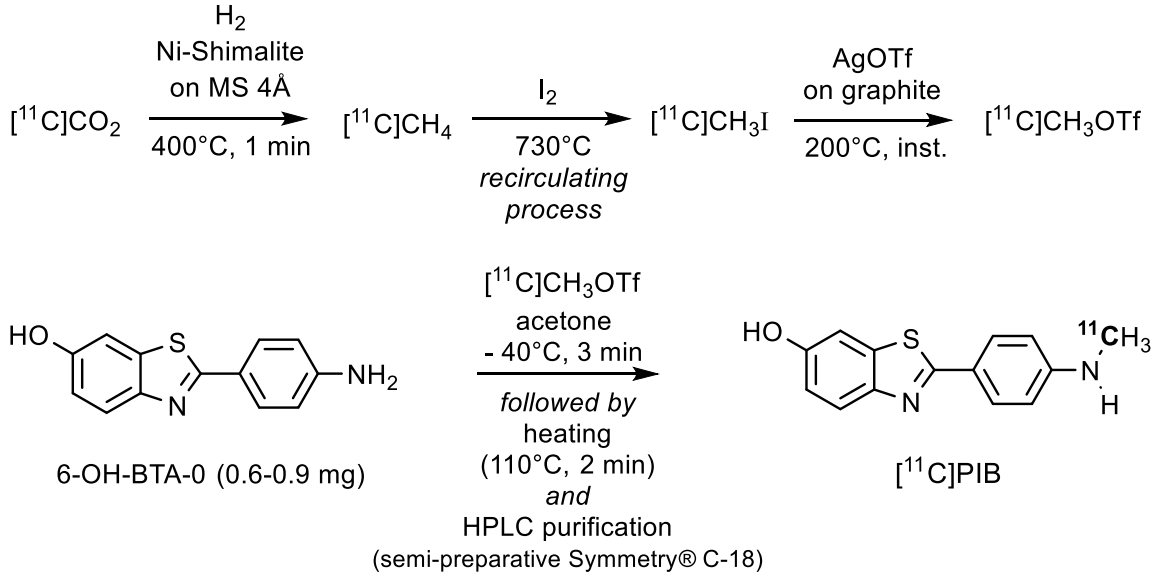

FIG. III-1. Production route for [^{11}C] PIB

TABLE III-1 demonstrates tests performed for routinely produced [^{11}C] PIB batches. Note that residual solvents quantification, bacterial endotoxin determination and sterility testing are performed as post-released controls.

TABLE III-1. QUALITY CONTROL TESTS FOR [^{11}C] PIB BATCHES

TESTS	METHODS	SPECIFICATIONS
Appearance of the solution	Visual examination	Clear, colourless solution
pH	pH paper	4.5 to 8.5
Radiochemical identification	Reverse-phase HPLC with UV/radioactivity detectors[a]	t_R ± 10% (comparison with standard)

Radiochemical purity	Reverse-phase HPLC with radioactivity detector [a]	≥ 95.0%
Specific activity determination	Reverse-phase HPLC with UV detector/ ionization chamber	≥ 7.07 GBq/µmol (≤ 13.4 µg of PIB / injection (10 mL)
Chemical impurity: 6-OH-BTA-0 level	Reverse-phase HPLC with UV detector	≤ 1.34 µg/injection (10 mL)
Radioactivity measurement	Ionization chamber	Measurement of syringe for injection
Residual solvent acetone acetonitrile	Gas Chromatography with static head space	 ≤ 50.0 mg/injection (10 mL) ≤ 4.1 mg/injection (10 mL)
Bacterial endotoxin content	LAL test	≤ 50 IU/injection (10 mL)
Sterility	Direct inoculation	Sterile

[a] Chromatography methods:

Equipment:	Alliance 2690 – Waters
Column:	Analytical Symmetry ® C-18, (l = 0.05 m, ∅ = 4.6 mm, 3.5 µm) – Waters
Temperature:	30°C
	Mobil phase: Solvent A / Solvent B: 50/50 (v/v) [Solvent A: water containing low-UV PIC® B7 reagent 980/20 (v/v) (low-UV PIC® B7 reagent: methanol (18- 22%), heptane sulfonic acid-sodium salts (4-6%), phosphate buffer solution (3-7%), water (65-75%), pH 3, Waters); Solvent B: water/acetonitrile 30/70 (v/v) containing low-UV PIC® B7 reagent (980/20 (v/v))]
Flow rate:	2.0 mL / min
Injection:	10 to 50 µL
Run time:	6 min
Detection:	Radioactivity detector (LB 509 – Berthold) and UV spectrophotometer at γ: 230 nm (996 Photodiode Array Detector – Waters)
Integrator:	Empower pro – Waters
R_t value:	[^{11}C] PIB: 2.77 min

The filter integrity test shall be done >50 psi performed for each batch but as a production control.

In addition to the tests described in the table above for routinely produced [^{11}C] PIB batches, the following tests shown in Table III-3 are also performed in validation batches, as part of the IMP dossier.

TABLE III-3. VALIDATION TESTS FOR [^{11}C] PIB BATCHES

TESTS	METHODS	SPECIFICATIONS
Radiochemical identification	γ spectrometry	γ photons of 511 keV
Chemical identification	UV-spectroscopic analysis post-HPLC (UV-profile and γ_{max} determination)	Superimposable to reference spectrum
Radiochemical purity	Half-life measurement	Half-life: 19.9 to 20.9 min

The following test also performed before sterile filtration on aliquots of [^{11}C] PIB batches dedicated to validation.

TABLE III-4. VALIDATION TESTS FOR STERILE FILTRATION OF [^{11}C] PIB BATCHES

TESTS	METHODS	SPECIFICATIONS
Bioburden	Membrane filtration test	< 1 CFU/mL

III-2. [^{11}C] METHIONINE FOR CLINICAL TRIALS

[^{11}C] MET is labelled with ^{11}C ($T_{1/2}$ = 20.38 min) via *S*-^{11}C-methylation of L-homocysteine thiolactone hydrochloride in the presence of sodium hydroxide as a base using a published method [III-2] with slight modifications [III-3]. Methylating agent, [^{11}C]CH3I, is produced by classical 'wet method' via the reduction of in-target produced [^{11}C] carbon dioxide with lithium aluminium hydride. The formed [^{11}C] methanol is reacted with hydroiodic acid at 120oC; the formed [^{11}C]CH3I is transferred by nitrogen gas flow (15 to 20 mL/min) onto tC18 cartridge (Waters) preloaded with the solution of L-homocysteine thiolactone hydrochloride (2.5 mg in 0.2 mL of 0.5 M NaOH in EtOH: H_2O 35/65 by volume). On-line [^{11}C] methylation takes place at room temperature. The product is eluted from tC18 Plus cartridge by passing of 0.05M sodium dihydrophospate (6 mL, PharmGrade) following on line-purification on the C18 Plus cartridge (Waters) connected in a series. The solution is collected in the sterile receiving vial preloaded with 0.07 M sodium monohydrophosphate (4 mL, PharmGrade) and the content is purged by nitrogen flow to get rid of non-reacted [^{11}C]CH_3I. It is transferred by nitrogen flow through Millex® GV 0.22 µm filter (Millipore®) into sterile injection vial. Figure III-2 shows the synthetic Route to produce [^{11}C] MET ((2s)-2-amino-4-([^{11}c] methylsulphanyl) butanoic acid, l-[methyl-^{11}c] methionine, [^{11}c] met), as an extemporary prepared at the Institute of Human Brain (IHB RAS, academic research centre) in St.-Petersburg, Russia.

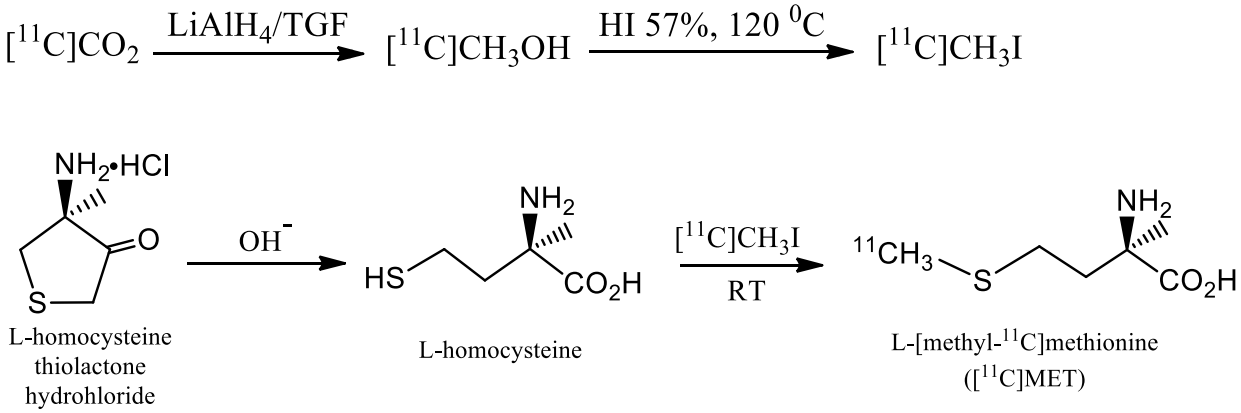

FIG. III-2. Synthetic Route for Production of [^{11}C]MET

TABLE III-5 demonstrates the required tests performed for routinely produced [^{11}C] MET batches. Note that enantiomeric purity, residual solvents quantification, chemical impurities, osmolality, bacterial endotoxin determination and sterility testing are performed as post-released controls and tested in every 10th run, based on retrospective data analysis of more than 10,000 batches and risk assessment.

TABLE III-5. REQUIRED TESTS FOR ROUTINELY PRODUCED [^{11}C] MET BATCHES

TESTS	METHODS	SPECIFICATIONS
Appearance of the solution	Visual examination	Clear, colourless solution
pH	pH paper or potentiometry	4.5 to 8.5
Radiochemical identification	Cation exchange HPLC with UV/radioactivity detectors[a]	t_R ± 10% (comparison with standard)
Radiochemical purity	Cation exchange HPLC with UV/radioactivity detectors [a]	Not less than 95.0%
Enantiomeric purity	Chiral HPLC with UV/radioactivity detectors [b]	Not less than 90.0%
Activity per volume determination	Radiometry–isotopic calibrator	185 to 740 MBq/mL
Chemical impurity: L-homocysteine thiolactone hydrochloride	Cation exchange HPLC with UV detector	Not more than 0.06 mg/mL
Chemical impurity: L-homocysteine	Cation exchange HPLC with UV detector	Not more than 0.2 mg/mL
Chemical impurity: L-methionine	Cation exchange HPLC with UV detector	Not more than 0.2 mg/mL
Residual solvents: ethanol	Gas Chromatography (Varian 3400)	Not more than 8 mg/mL
Osmolality	Osmometry	250-300 mOsmol/kg
Bacterial endotoxin content	LAL test	Less than 20 IU/mL
Sterility	Direct inoculation	Sterile
Filter integrity test	Bubble point	> 50 psi

[a] **Chromatography methods**: Radiochemical purity, Identity, Chemical Impurities

Equipment: HPLC system, Gilson
Column: Zorbax SCX (l = 0.25 m, ∅ = 4.6 mm, 5 µm), Zorbax
Temperature: Room temperature
Mobile phase: 0.01 M NaH_2PO_4 (pH 3,0
Flow rate: 1.0 mL / min
Injection: 20 µL
Run time: 25 min
Detection: Radioactivity detector Beckman-170 and Gilson-116 UV detector at λ: 220 nm
Integration: Multichrom software, Ampersend, Russian Federation
R_t value: [^{11}C] MET: 5.93 min;
D,L methionine: 5.43 min;
D,L homocysteine: 4.12 min;
D,L homocysteine thiolactone: 20.08 min

[b] **Chromatography methods**: Enantiomeric purity

Equipment: HPLC system, Gilson
Column: Crownpack-CR (l = 0.15 m, ∅ = 4 mm, 5 µm), Daicel
Temperature: Room temperature
Mobile phase: $HClO_4$ (pH 2,0)
Flow rate: 0.8 mL/min

Injection:	20 µL
Run time:	8 min
Detection:	Radioactivity detector Beckman-170 and Gilson-116 UV detector at λ: 220 nm
Integrator:	Multichrom software, Ampersend, Russian Federation
R_t value:	D-[^{11}C] MET: 3.15 min; L-[^{11}C] MET methionine: 4.62 min.

In addition to the tests described in the table above for routinely produced [^{11}C] MET batches, the following tests shown in Table III-6 are also performed in validation batches, as part of the registration dossier.

TABLE III-6. RADIONUCLIDE PURITY ASSAY FOR [^{11}C] MET

TESTS	METHODS	SPECIFICATIONS
Radionuclidic purity	Half-life measurement γ spectrometry	Half-life: 19.9 – 20.9 min γ photons of 511 keV

III-3. [^{18}F] FLT FOR CLINICAL TRIALS

[^{18}F] FLT (also known as deoxy-3'[^{18}f] fluoro-thymidine) by adaptation of the method originally proposed by Grierson et al. [III-4]. ^{18}F is obtained via the ^{18}O (p, n)^{18}F nuclear reaction by irradiating an enriched [^{18}O] H2O target. At the end of the bombardment, the solution containing ^{18}F is transferred to the automated radiosynthesis device (GE Tracerlab Fx-Fn Pro) and the radionuclide is purified by loading and subsequent elution on a QMA cartridge. After evaporation of residual water, 10 mg of the 3-N-boc-5'-O-dimethoxytrityl-3'-O-nosyl-thymidine dissolved in 1 mL of acetonitrile are added to the reaction vial, and allowed to react with ^{18}F for 8 min at 100°C. At the end of the nucleophilic substitution reaction, 0.3 mL of 1M HCl are added and hydrolysis reaction take place at 105°C for 6 min, and then the resulting intermediate solution reaction mixture is neutralized with 1 mL of 2N sodium acetate. The reaction mixture is then diluted with 2.3 mL of WFI and submitted to semi-preparative HPLC purification, whose conditions are as follow: column: Nucleosil C18, 250x10 mm, 7 µ; flow: 9 mL/min; UV detector: 254 nm; gamma detector; mobile phase: WFI/ethanol 95 / 5. The desired product elutes at 32 min, and the fraction is directly passed through the membrane filter for sterilization and collection into the final glass vial. Thus, Formulation is corresponding to the HPLC mobile phase. The whole process lasts approximately 80 min, and the average radiochemical yield of [^{18}F] FLT is 10%, not decay corrected. A reaction schematic is showed in the following Figure III-3.

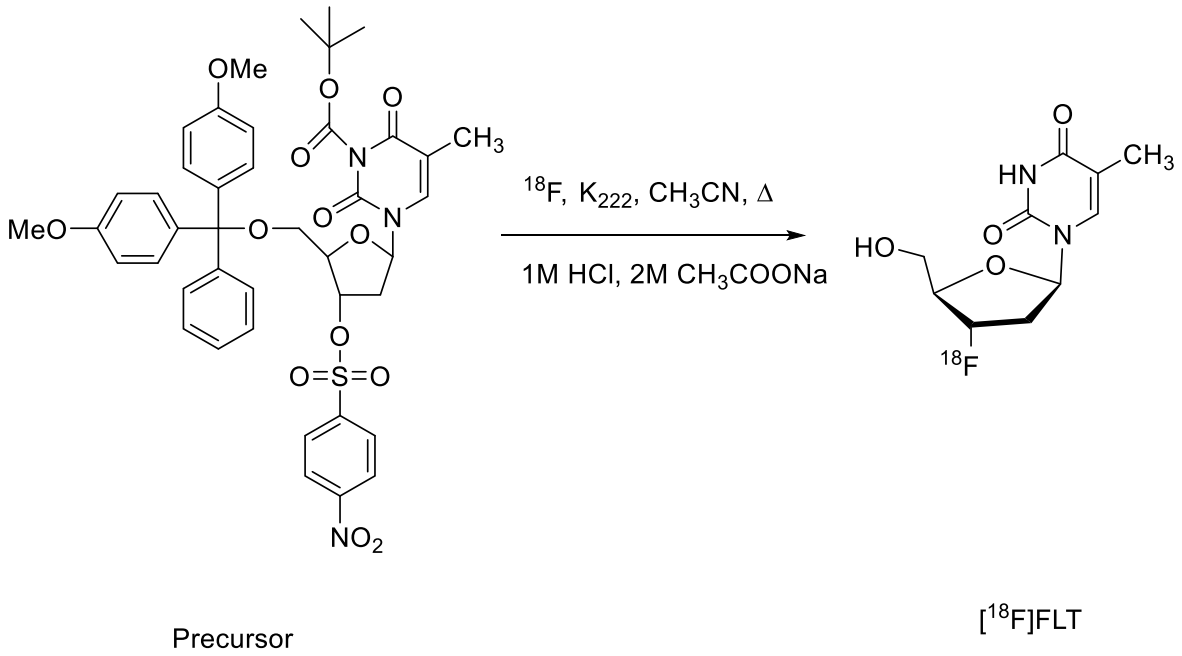

FIG. III-3. Synthetic Route for Production of [^{18}F] FLT

In the following Table III-7, the different [^{18}F] FLT batches QC tests are shown as prepared at the Tecnomed Foundation / Nuclear Medicine Department of Hospital San Gerardo, in Monza, Italy

TABLE III-7. QUALITY CONTROL TESTS PERFORMED FOR ROUTINELY PRODUCED [^{18}F] FLT BATCHES

TESTS	METHODS	SPECIFICATIONS
pH	pH meter (potentiometry)	4.5 to 8.5
Appearance	Visual examination	Clear, colourless solution
Identification	gamma spectrometry with NaI(Tl) 511 KeV	511 ± 10 KeV
Identification	HPLC with UV detector[a]	Retention time of the main radioactive peak is approximately the same of the peak obtained with a [^{18}F] FLT standard
Residual solvents: acetonitrile	Gas chromatography [b]	≤ 4.1 mg/V
Residual solvents: acetone	Gas chromatography [b]	≤ 50 mg/V
Excipients: ethanol	Gas chromatography [b]	≤ 300 mg/V
Chemical purity: FLT	HPLC with UV detector [b]	≤ 6.1 μg/V
Chemical purity: stavudine	HPLC with UV detector [b]	≤ 1.5 μg/V
Chemical purity: K222	Spot test	≤ 2.2 mg/V
Other impurities	HPLC with UV detector [a]	≤ 0.1 mg/V each; 0.5 mg/V the sum of impurities

Radiochemical purity	HPLC with radiochemical detector [a]	≥ 95% [^{18}F] FLT
Half-life	Dose calibrator	105 - 115 min
Filter integrity: sterilizing filter	Bubble point test	≥35 psi
Filter integrity: vent filter	Bubble point test	≥50 psi

[a] HPLC method:

Equipment:	Perikin Elmer 200 series
Column:	Waters Xterra RP, (250x4.6 mm, 5 µm) Mobil phase: acetonitrile/water10/90 (v/v)
Flow rate:	1.0 mL/min
Injection:	10 to 50 µL
Run time:	15 min
Detection:	Radioactivity detector (Bioscan flow count) and UV spectrophotometer at λ: 267 nm (996 Photodiode Array Detector – Waters)
Software:	Totalchrom workstation
R_t value:	[^{18}F] FLT: 8 min

[b] GC method:

Equipment:	Perkin Elmer Clarus 500 GC
Column:	capillary column 30 m, 0.32 mm, 1 m, stationary phase: cyanopropylphenyl polysyloxane
Flow rate:	1.0 mL / min
Injection:	head-space Turbomatrix
Run time:	8 min
Injector temp.:	150°C
Detector temp.:	200°C
Oven temp. ramp:	45°C / 2 min 45 to 90°: T = 15°C/min 90 to 125° T = 30°C/min 125°C / 4 min
Helium flow:	1 mL/min
Air flow:	450 mL/min
Hydrogen flow:	45 mL/min
Split:	5 mL/min
Detection:	Flame Ionization Detector
Software:	Totalchrom workstation
R_t value:	ethanol: 3.8 min acetone: 4.1 min acetonitrile: 4.4 min

In addition to the tests described in the table above for routinely produced [^{18}F] FLT batches, Table III-8 demonstrates required tests performed routinely after batch release:

TABLE III-8. ROUTINELY PERFORMED TESTS AFTER [^{18}F] FLT BATCH RELEASE

TESTS	METHODS	SPECIFICATIONS
Radionuclidic purity	Gamma spectrometry with NaI(Tl) detector	F-18 > 99.9%
Sterility	Membrane filtration test	Sterile
Bacterial endotoxins	PTS Charles River	< 175 IU/V

The following test shown in Table III-9 is also performed as a process test every 6 months.

TABLE III-9. BIOBURDEN TEST

TESTS	METHODS	SPECIFICATIONS
Bioburden	Membrane filtration test	< 10 CFU/100 mL

III-4. [^{18}F] FDG

Please refer to: Cyclotron produced radionuclides: guidance on facility design and production of [^{18}F] fluorodeoxyglucose ([^{18}F] FDG). IAEA radioisotopes and radiopharmaceuticals, series no. 3., International Atomic Energy Agency, Vienna, 2012.

III-5. [^{64}Cu] ATSM FOR CLINICAL TRIALS

The complex is prepared by adding 10 µg of H_2ATSM dissolved in 10 µL DMSO to a solution of [^{64}Cu] copper chloride [diacetyl-bis(n4-methylthiosemicarbazone)] in 0.1 M HCl buffered with 1 M sodium acetate. The reaction mixture is loaded onto a conditioned C_{18} solid phase extraction (SPE) cartridge and the impurities are washed off with 10 mL sterile water for injection. The product is eluted with a minimal volume of ethanol and diluted with 20 volumes of saline containing 1 mg/mL ascorbic acid. Finally, the product undergoes sterile filtration on an 0.22 µm membrane filter.

Table III-10 demonstrates required quality control tests for routinely produced batches, as prepared at the PET Centre in King's College London, UK. Note that residual solvents quantification, bacterial endotoxin determination and sterility testing are performed as post-released controls.

TABLE III-10. QUALITY CONTROL TESTS PERFORMED FOR ROUTINELY PRODUCED [^{64}Cu] ATSM

TESTS	METHODS	SPECIFICATIONS
Appearance of the solution	Visual examination	Clear, colourless to pale yellow solution
pH	pH paper	4.5 to 8.0
Radionuclide identity	High resolution γ spectroscopy	Peaks only at 511 and 1345 keV
Radiochemical purity	Thin layer chromatography[a]	≥ 95.0%
Filter integrity	Bubble point test	> 50 psi
Radioactivity measurement	Ionization chamber	Measurement of syringe for injection
Solvent analysis	Gas chromatography	≤10% v/v ethanol
Bacterial endotoxin content	LAL test	≤ 175 IU/injection
Sterility	Direct inoculation	Sterile

[a] **Chromatography methods:**

Solid phase: Silica gel on glass or plastic
Mobile phase: Ethyl acetate

Method:	Place a spot of the radiopharmaceutical near the bottom of TLC plate and allow the spot to dry. Place the plate in a tank containing ethyl acetate and allow the mobile phase to migrate up the strip until it nears the end. Remove the strip and allow it to dry.
Analysis:	Analyse the distribution of activity on the strip using a radiochromatogram scanner or phosphor imager.
R_f values:	[^{64}Cu] ATSM: $R_f \sim 1$; [^{64}Cu] chloride: $R_f \sim 0$

III-6. [^{68}Ga] DOTATOC USING DOTATOC COLD KITS

^{68}Ga labelled somatostatin receptor-avid peptides such as [^{68}Ga] DOTATOC ([^{68}Ga] DOTA-Tyr^3-Octreotide) are used for PET imaging of neuroendocrine tumours. A kit labelling procedure has been standardized for use in conjunction with ^{68}Ge/^{68}Ga generators (without use of an automated module) for the preparation of [^{68}Ga] DOTATOC. The procedure for kit formulation of DOTATOC, radiolabelling with ^{68}Ga (eluted in 0.1 N HCl or in 0.05 N HCl from ^{68}Ge/^{68}Ga generators) and the quality control methods are given below.

III-6.1. Kit formulation of DOTATOC (20 kit vials per batch)

1 mg (lyophilized powder) of DOTATOC (DOTA-Tyr3-Octreotide) is reconstituted in HPLC grade water to make a 1 mg/mL solution. 8 mL of 0.5 M Sodium acetate solution is prepared in sterile HPLC grade water. The peptide solution (1 mL) is added to the 0.5 M sodium acetate solution (8 mL) to form the final stock solution (9 mL). The stock solution is filtered through a 0.22 µm PVDF filter (33 mm, Millipore) and dispensed into sterile 10 mL glass vials resulting in a peptide concentration of 50 µg per vial. The vials are immediately frozen with liquid nitrogen and loaded into the freeze dryer with a shelf temperature of -50°C and freeze drying carried out for 4 h. Subsequently, the vials are vacuum sealed under sterile conditions and stored at -20° C until use. Hydrochloric acid of strength 0.1 N (or 0.05 N HCl, depending on the type of generator used) is also prepared using suprapur HCl and sterile HPLC grade water, dispensed aseptically and provided along with the kits for elution of ^{68}Ga from the ^{68}Ge/^{68}Ga generator.

III-6.2. ^{68}Ga labelling of DOTATOC kits

One DOTATOC kit vial is thawed to room temperature. 1.5 mL of $^{68}GaCl_3$ (370 MBq) eluted in 0.1 N HCl from the generator or 3 mL of $^{68}GaCl_3$ (370 MBq) eluted in 0.05 N HCl from the generator is added to the kit vial. Reaction carried out at 90°C in a water bath for 10 minutes. The reaction vial is allowed to cool for 5 minutes and filtered using 0.22 µm filter (using a shielded syringe) into another sterile vial containing 3 mL of sterile saline. The radiosynthesis for [^{68}Ga] DOTATOC is shown in Figure III-4 below.

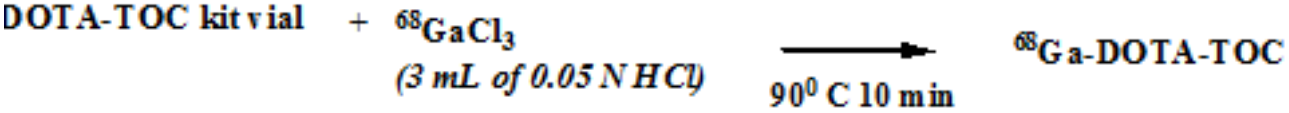

FIG. III-4. Radio synthesis steps for [^{68}Ga] DOTATOC.

Table III-10 demonstrates the required tests performed for determining the quality of [68Ga] DOTATOC cold kits as prepared at the Isotope Production & Applications Division, BARC, in Trombay, Mumbai, India. Note that the cold kits must pass all the tests prior to use.

TABLE III-10. QUALITY CONTROL TEST PERFORMED FOR DOTATOC COLD KIT

TEST	METHOD	SPECIFICATIONS
Appearance	Visual examination	White powder
Sterility test	Direct inoculation	Sterile
Bacterial endotoxin content	LAL test	< 175 EU/total volume

Table III-11 demonstrates the tests performed for determining the quality of [^{68}Ga] DOTATOC. Note that test for ^{68}Ge breakthrough, bacterial endotoxin test and sterility test are performed post-release.

TABLE III-11. QUALITY CONTROL TESTS PERFORMED FOR [^{68}Ga] DOTATOC

TEST	METHOD	SPECIFICATIONS
Appearance of the solution	Visual examination	Clear, colourless solution
pH	pH paper	4.0 to 8.0
^{68}Ge breakthrough	Analysis of decayed sample using HPGe detector coupled to a MCA [a]	< 0.001 % [c]
Radiochemical purity	ITLC/Paper Chromatography[b] or HPLC[c]	≥ 95 % of [^{68}Ga] DOTATOC
Radioactivity measurement	Dose calibrator	Measurement of injection syringe
Bacterial endotoxin content	LAL test	< 175 EU/total volume
Sterility	Direct inoculation	Sterile

[a] **Instant Thin Layer Chromatography/Paper chromatography method**

Solid phase:	ITLC-SG strip/Whatman 3 mm chromatography paper (12×1 cm)
Mobile phase:	1:1 (v/v) ratio of 1 M ammonium acetate and methanol
Method:	Spot ~ 5 µL of the test sample near the bottom of the chromatography strip. Place the strip in a test tube containing the mobile phase and allow the mobile phase to migrate up the strip until it nears the end. Remove the strip and allow it to dry.
Analysis:	Analyse the distribution of radioactivity on the chromatographic paper strip using a radiochromatogram scanner or cut the strip into 1 cm segments and measure the activity in a dose calibrator or gamma counter

R_f values: In this system, free as well as colloidal ^{68}Ga remain close to the origin (R_f= 0.0-0.1) while [^{68}Ga] DOTATOC migrates to the solvent front (R_f= 0.8-1.0).

[b] **HPLC method**

Equipment:	JASCO HPLC system
Column:	C18 reverse phase
Temperature:	25°C
Mobil phase	Gradient elution of Water (A) and Acetonitrile (B), both containing 0.1 % Trifluoroacetic acid (0-4 min 5 % B, 4-20 min 5-95 % B, 20-30 min 95-5 % B)

Flow rate:	1.0 mL/min
Injection:	10 to 20 µL
Run time:	30 min
Detection:	Radioactivity detector (Raytest) and UV detector at 280 nm (JASCO, Japan)
Integrator:	GINASTAR Software (Raytest, Germany)
R_t value	[^{68}Ga] Ga-DOTATOC – 18.3 min (±10 %); ^{68}Ga(III) – 3.3 min (±10 %)

[c] ^{68}Ge breakthrough. As ^{68}Ge decays exclusively by electron capture to ^{68}Ga, the presence of ^{68}Ge impurity in ^{68}Ga eluate cannot be directly determined by γ spectroscopy. Allow the $^{68}GaCl_3$ eluted from the generator to decay for 48 hours. Analyse the decayed ^{68}Ga sample using an HPGe detector coupled to a MCA. Measure the 511 keV γ radiations from the ^{68}Ga daughter, which correspond to the ^{68}Ge impurity present in the sample.

III-7. [^{89}Zr] DFO-TRASTUZUMAB, FOR CLINICAL TRIALS

[^{89}Zr] DFO-Trastuzumab (^{89}Zr labelled antibody using Dfo) is made by conjugation of p-SCN-Desferrioxamine (DFO) to Trastuzumab (Herceptin, Genentech), a humanized IgG1 monoclonal antibody targeting human epidermal growth factor receptor 2 (HER2), followed by radiolabelling with ^{89}Zr. Radiolabelling involves first neutralizing the [^{89}Zr] oxalate with 2M sodium carbonate, followed by 1M ammonium acetate to ensure the radionuclide reaction mixture pH is within 6.8 to 7.2 range. Then, 3 mg of the DFO- trastuzumab (dissolved in 1M ammonium acetate) is added to the reaction mixture and allowed to react at ambient temperature for 50 minutes. Following incubation, the reaction mixture is loaded onto the desalting gel column (P-6 gel or PD-10 column can be used) The product is eluted form the column with a solution of ~4 mg/mL gentisic acid, ~0.2M sodium acetate, and ~5% w: v of HSA, USP. The conjugation and subsequent radiolabelling process are depicted in Figure III-5. In addition, the QC tests performed for [^{89}Zr] DFO-Trastuzumab in Table III-12, are shown as prepared at the MSK Radiochemistry and Molecular Imaging Probes Core Facility in New York, USA

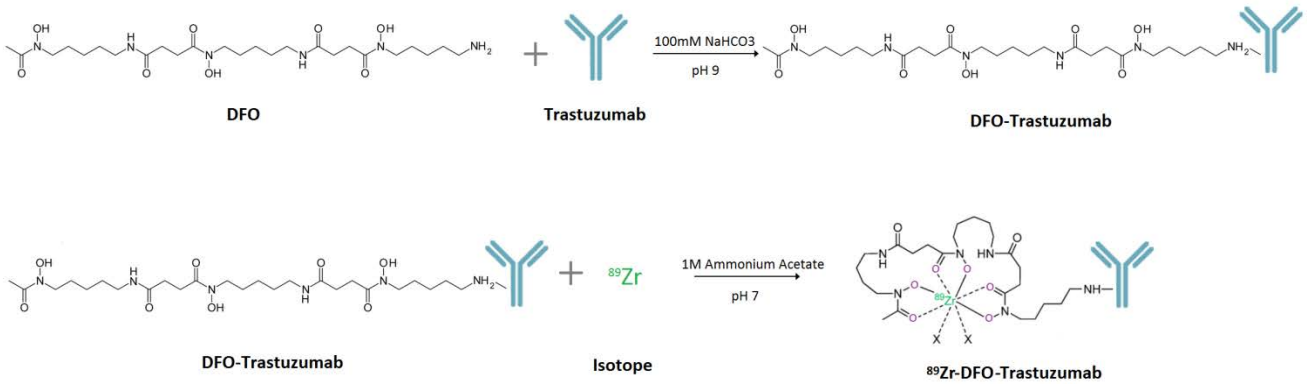

FIG. III-5. Steps Involved in the Preparation of [^{89}Zr] DFO-Trastuzumab

TABLE III-12. QUALITY CONTROL TEST PERFORMED FOR [^{89}Zr] DFO-TRASTUZUMAB

TESTS	METHODS	SPECIFICATIONS
Appearance of the solution	Visual examination	Clear, colourless to amber solution
pH	pH paper	4.0 to 8.0
Radionuclide identity	High resolution γ spectroscopy	Gamma peaks only at 511 and 909 keV
Radiochemical purity	Thin layer chromatography[a]	≥ 95.0%
Filter integrity	Bubble point test	> 50 psi
Radioactivity measurement	Ionization chamber	Measurement of syringe for injection
Bacterial endotoxin content	LAL test	≤ 175 IU / injection
Sterility	Direct inoculation	Sterile

[a] **Chromatography methods:**

Solid phase:	Silica gel strips
Mobile phase:	10 mM EDTA
Method:	Place a spot of the radiopharmaceutical near the bottom of TLC plate and allow the spot to dry. Place the plate in a tank containing 10 mM EDTA and allow the mobile phase to migrate up the strip until it nears the end. Remove the strip and allow it to dry.
Analysis:	Analyse the distribution of activity on the strip using a radio TLC scanner.
R_f values:	[^{89}Zr] DFO-Trastuzumab: R_f ~0; ^{89}Zr: R_f ~1

III-8 [^{90}Y] DOTA-RITUXIMAB FOR TREATMENT OF NON-HODGKIN'S LYMPHOMA

The anti CD20 antibody Rituximab is radiolabelled with ^{90}Y after conjugating it with the bifunctional chelator for isothiocyanato benzyl 1,4,7,10-tetra aza cyclododecane tetra acetic acid (p-SCN-Bn-DOTA), as seen in Figure III-6.

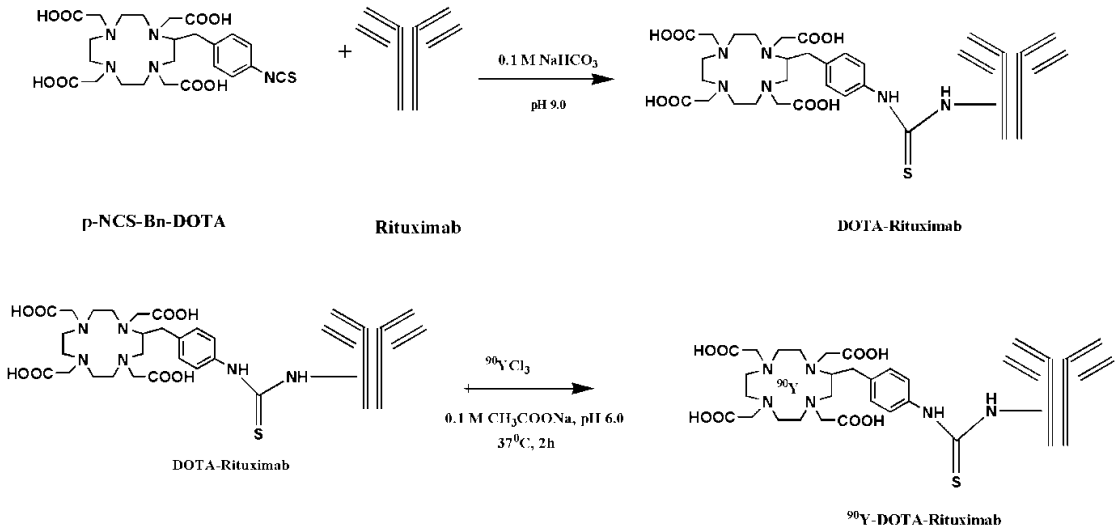

FIG. III-6. A Schematic Overview for the Preparation of [^{90}Y] labelled Antibody

III-8.1. Conjugation of Rituximab with p-SCN-Bn-DOTA

Rituximab is conjugated with p-SCN-Bn-DOTA at 10:1 molar ratio of p-SCN-Bn-DOTA to antibody [III-6]. A 2-mL aliquot of Rituximab (10 mg/mL) is concentrated to 1 mL using AMICON ultra centrifugal filter (MWCO 10,000 Da) by centrifuging at 3000 rpm for 30 minutes. pH is adjusted to 9.0 with 0.1 M sodium bicarbonate. ~ 1 mg of p-SCN-Bn-DOTA is added to Rituximab solution and the reaction mixture incubated at room temperature (25°C) for 2 h followed by incubation overnight at 4°C. The reaction mixture is then centrifuged to remove free p-SCN-Bn-DOTA. Buffer exchange into 0.1 M sodium acetate (pH 6.0) and complete removal of free p-SCN-Bn-DOTA is achieved by repeated washings with 0.1 M sodium acetate. The protein concentration of the conjugate is determined by Lowry's method [III-7]. The average number of DOTA molecules per Rituximab molecule is determined by carrying out spectroscopic assay using Cu(II)-Arsenazo complex [III-8, III-9].

III-8.2. Radiolabelling of DOTA-Rituximab conjugate with ^{90}Y

DOTA-Rituximab conjugate (3 mg) is taken in 0.5 mL of 0.1 M sodium acetate (pH 6.0) to which 1.48 GBq (40 mCi) of ^{90}Y chloride (in minimum volume of 0.1 N HCl) added. The radiolabelling reaction is carried out at pH 6.0 for 2 h at 37°C. Purification of [^{90}Y] Y-DOTA-Rituximab reaction mixture is carried out on a PD-10 column using 0.1 M sodium acetate (pH 6.0) for elution. Radiochemical purity of ^{90}Y-DOTA-Rituximab is determined by performing size exclusion HPLC on a TSK G3000SWXL gel column with SWXL guard column using 0.05 M phosphate buffer, pH 6.8 as mobile phase at a flow rate of 0.6 mL/min).

TABLE III-13 demonstrates required tests for determining the quality of ^{90}Y-DOTA-Rituximab As prepared at the Isotope Production & Applications Division, BARC, Trombay, in Mumbai, India. Note that bacterial endotoxin test and sterility test are performed post-release.

TABLE III-13. QUALITY CONTROL TESTS REQUIRED FOR ^{90}Y-DOTA-RITUXIMAB

TESTS	METHODS	SPECIFICATIONS
Appearance of the solution	Visual examination	Clear, colourless solution
pH	pH paper	6.0 to 8.0
No. of DOTA per antibody molecule	Cu(II)-Arsenazo assay	Not more than six DOTA molecules per antibody molecule
Radiochemical purity	Size exclusion HPLC[a]	≥ 95.0 % (R_t ± 10 %) Peak of [^{90}Y] DOTA-Rituximab super-imposable on UV of cold (non-radioactive) Rituximab
In vitro cell binding	Cell binding studies in CD20 expressing Raji/Daudi cells[b]	Specific binding to CD20 expressing cells; inhibition in binding of [^{90}Y] Y-DOTA-Rituximab when co-incubated with cold Rituximab
In vivo biodistribution study	Biodistribution in lymphoma bearing mice	High tumour uptake
Radioactivity measurement	Dose calibrator	Measurement of injection syringe

Bacterial endotoxin content	LAL test	≤ 50 IU/injection (10 mL)
Sterility	Direct inoculation	Sterile

[a] **Chromatography method:**

Equipment:	JASCO HPLC system {(having UV detector (JASCO, Japan) and NaI(Tl) Radioactive detector (Raytest, Germany)}
Column:	TSK G3000SWXL gel column with SWXL guard column
Temperature:	25°C
Mobil phase	Isocratic elution using 0.05 M phosphate buffer, pH 6.8
Flow rate:	0.6 mL/min
Injection:	10 to 20 µL
Run time:	30 min
Detection:	Radioactivity detector (Raytest) and UV detector at 280 nm (JASCO, Japan)
Integrator:	GINASTAR Software (Raytest, Germany)

R_t value [^{90}Y] Y-DOTA-Rituximab: 15.0 min

[b] **In vitro cell binding studies of ^{90}Y-DOTA-Rituximab**

Raji cells which express CD20 antigen on their surface are used for in vitro cell binding studies. Cells are grown to confluence in RPMI medium containing 10 % fetal bovine serum. $2x10^6$ cells (i.e. 2×10^7 cells/mL) are incubated with [^{90}Y] DOTA-Rituximab (0.7 nM) for 2 h at 37°C. Cells are then washed twice with 1 mL of 0.05 M phosphate buffer (pH 7.4) and centrifuged at 2000 rpm for 20 min at room temperature. Supernatant is aspirated and radioactivity associated with pellet is counted. Non-specific binding was determined by blank studies wherein the same number of cells is incubated with [^{90}Y] DOTA-Rituximab along with 100 nM of cold Rituximab under identical conditions. The immunoreactivity of [^{90}Y] DOTA-Rituximab is determined by Lindmo's method [III-9].

III-9. [^{99m}Tc] MDP ([^{99m}Tc] TECHNETIUM MEDRONATE) FOR ROUTINE USE

Technetium (^{99m}Tc) (medronate complex injection is a sterile solution of sodium methylene diphosphonate (sodium medronate) that is complexed with ^{99m}Tc at a maximum activity of 9250 MBq (250 mCi). For the synthesis of ^{99m}Tc labelled molecules, the technetium must be reduced to lower oxidation states. The sodium medronate preparation contains a stannous salt and stabilizing agents. Other formulations may also contain chelating, filling and antioxidizing agents as well as antimicrobial preservatives and buffers. The medronate binds directly to the technetium atom as seen in Figure III-7.

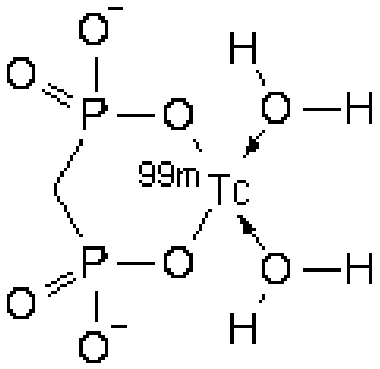

FIG. III-7. $CH_6O_8P_2{}^{99m}Tc$ (proposed structure)

The following tests are performed routinely for [^{99m}Tc] MDP batches as prepared at the Radiopharmacy Centre, IPEN-CNEN/SP, in Brazil. It is essential to control pH and radiochemical purity on every batch.

TABLE III-14. QUALITY CONTROL TESTS FOR [^{99m}Tc] MDP BATCHES

TESTS	METHODS	SPECIFICATIONS
Appearance of the solution	Visual examination	Clear, colourless solution
pH	pH paper	4.0 - 7.8 [III-8]
Radioactivity measurement	Ionization chamber	Measurement of syringe for injection
Radiochemical purity	Paper chromatography[a]	≥ 90.0% [III-8]

[a] **Paper chromatography methods**:

Solid phase:	Whatman 3MM chromatographic paper
Mobile phase:	*System A* – Sodium chloride solution (0.9 g in 100 mL) *System B* – Acetone
Method:	Place a spot of the radiopharmaceutical near the bottom of a Whatman 3MM chromatographic paper strip. Place the strip in a chamber or tank and allow the mobile phase to migrate up the strip until it nears the end. Remove the strip and allow it to dry.
Analysis:	Analyse the distribution of activity on the chromatographic paper strip, for *System A* (12.5 cm x 1.5 cm paper strip) cut into 1 cm segments and measure the activity of each segment in a gamma counter; for *System B* (8 cm x 1 cm paper strip), cut the strip at R_f 0.5 and measure the activity in the two portions in a gamma counter. The sum of the percentage of radioactivity at the origin in *System A* plus the percentage of radioactivity at the solvent front in *System B* is not greater than 10.0%.
R_f values:	*System A* – Hydrolysed ^{99m}Tc and technetium-tin colloid are located at the origin (R_F 0 to 0.1) *System B* – Free pertechnetate is located at the solvent front.

Filter integrity test is also performed for each routinely produced MDP batch using bubble point test (> 50 psi)

In addition to the tests described in the table above, the following tests are performed by the manufacturer as prepared at the Radiopharmacy Centre, IPEN-CNEN/SP in Brazil.

TABLE III-16. QUALITY CONTROL TESTS PERFORMED BY MANUFACTURER

TESTS	METHODS	SPECIFICATIONS
Radionuclidic purity	Ionization chamber	See [^{99m}Tc] Sodium Pertechnetate generator eluate
Radionuclidic identification	γ spectrometry	γ photons of 140 keV
Bacterial endotoxin content	LAL Test	≤ 175 IU/injection
Sterility	Membrane filtration	Sterile

Biological distribution	USP method as recommended in the [^{99m}Tc] MDP monograph	% radioactivity ≤ 5.0 in the liver, % radioactivity ≤ 5.0 in the kidneys, ≥ 1.0 in the femur

The bioburden test is also performed before sterile filtration on aliquots as a process control for every batch.

TABLE III-17. BIOBURDEN TEST

TESTS	METHODS	SPECIFICATIONS
Bioburden	Membrane filtration	No limit specified as a process control

III-10. [^{111}In] PENTETREOTIDE

[^{111}In] Pentetreotide is prepared (also known as [^{111}In] DTPA-octreotide) by adding [^{111}In] indium chloride ($T_{1/2}$ = 67 h) to a kit containing 10 µg pentetreotide and other excipients. The complex forms at room temperature by chelation. After the complex has formed the product can be diluted with saline if desired. In order to maximize the recovery upon transfer of [^{111}In] indium chloride, a 7cm spinal needle is supplied so that the liquid can be withdrawn without the need to invert the vial which could result in loss of volume on glass surfaces and the stopper. However, residence time in the needle must be short to avoid the HCl vehicle leaching iron from the needle; iron could compete with [^{111}In] chloride for labelling.

TABLE III-18 demonstrates the required tests performed routinely for [^{111}In] pentetreotide batches, as prepared at Guy's and St Thomas' Hospital, in London, UK. However, since this is a licensed product is prepared from precursors with a marketing authorization, it is not essential to control pH and radiochemical purity on every batch.

TABLE III-18. QUALITY CONTROL TESTS FOR [^{111}In] PENTETREOTIDE

TESTS	METHODS	SPECIFICATIONS
Appearance of the solution	Visual examination	Clear, colourless solution
pH	pH paper	3.8 to 4.3
Radioactivity measurement	Ionization chamber	Measurement of syringe for injection
Radiochemical purity	Thin-layer chromatography[a]	≥ 98.0%

[a] **Chromatography methods:**

Solid phase:	Silica gel embedded fibre glass (ITLC-SG)
Mobile phase:	Citrate buffer, 0.1 M, pH 5
Method:	Place a spot of the radiopharmaceutical near the bottom of an ITLC strip. Place the strip in a tube or tank and allow the mobile phase to migrate up the strip until it nears the end. Remove the strip and allow it to dry.
Analysis:	Analyse the distribution of activity on the strip using a radiochromatogram scanner or phosphor imager, or cut the strip at R_f 0.5 and measure the activity in the two portions in a dose calibrator or gamma counter.
R_f values:	[^{111}In] Pentetreotide, $R_f \sim 0$; [^{111}In] chloride, $R_f \sim 1$

In addition to the tests described in the table above, other tests are performed by the manufacturer as shown in Table III-19.

TABLE III-19. QUALITY CONTROL TESTS PERFORMED BY MANUFACTURER

TESTS	METHODS	SPECIFICATIONS
Radionuclidic purity	γ spectrometry	^{111}In, >99.0% other gamma emitters, <0.1% ^{114m}In, <500 Bq per MBq ^{111}In
Radiochemical identification	γ spectrometry	γ photons of 172 and 247 keV
Bacterial endotoxin content	Ph. Eur. method	≤ 175 IU/injection
Sterility	Direct inoculation	Sterile

III-11. [^{124}I] MIBG (N.C.A.) FOR CLINICAL TRIALS

[^{124}I] MIBG (N.C.A.) [^{124}I] metaiodobenzylguanidine) is labelled with ^{124}I ($T_{1/2}$ = 4.2 days) by oxidative radioiodo-destannylation of the precursor *N,N*'-bis(t-butoxycarbonyl)-3-(trimethylstannyl)benzylguanidine (bis-BOC-mTMSBG) [III-10]. The automatization using a GRP-Module (Scintomics) and sterile cassettes is established slightly adopted as described [III-11]. ^{124}I solution in 0.1 M NaOH is added to the precursor (0.2 mg, ~ 0.4 µmol) dissolved in acetic acid. The labelling is initiated by the addition of an aq. solution of chloramine-T (60 µg). In a second step, the BOC protecting groups are removed by heating. After naturalization with 2M NaOH, the reaction mixture is loaded onto a conditioned C_{18} solid phase extraction (SPE) cartridge. The cartridge is washed with water and [^{124}I] MIBG is eluted with 2 mL ethanol. After dilution with 14 mL PBS buffer the product undergoes sterile filtration on a 0.22 µm membrane filter. This process is shown in Figure III-8, below.

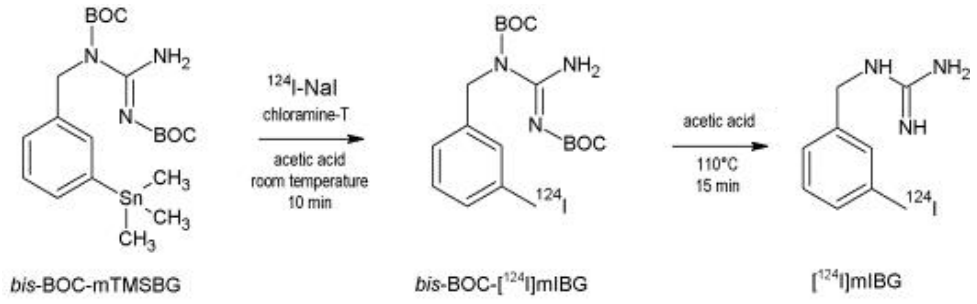

FIG. III-8. Synthesis Route for Preparation of [^{124}I] MIBG

List of the tests performed, methods and specifications (acceptance criteria) are shown in Table III- 20 as prepared by the Division of Nuclear Medicine, Medical University of Graz in Austria. These tests are performed for routinely produced batches. Note that solvents analysis, bacterial endotoxin determination and sterility testing are performed as post-released controls.

TABLE III-20. QUALITY CONTROL TESTS FOR [^{124}I] MIBG

TESTS	METHODS	SPECIFICATIONS
Appearance of the solution	Visual examination	Clear, colourless solution
pH	pH paper	3.5 to 8.0
Radionuclide identification	γ spectrometry	γ photons of 511 keV and 603 keV
Radiochemical identification	Reverse-phase HPLC with UV/radioactivity detectors [a]	t_R ± 10% (comparison with standard)
Radiochemical purity	Reverse-phase HPLC with UV/radioactivity detector [a]	≥ 95.0%
Radioactivity measurement	Ionization chamber	Measurement of syringe for injection
Solvent analyses	Gas chromatography	≤ 10% v/v ethanol
Bacterial endotoxin content	LAL test (Ph. Eur.)	≤ 175 IU/injection(V)
Sterility	Direct inoculation (Ph. Ph eur.)	Sterile
Filter integrity	Bubble point test	> 50 psi

[a] **Chromatography methods:**

Equipment:	Agilent 1260
Column:	Zorbax SB aqu C-18 (*l* = 0.25 m, ∅ = 4.6 mm, 5 µm) – Agilent
Temperature:	RT
Mobile phase:	0.05M phosphate buffer pH 4.5 / methanol, 60/40 (v/v),
Flow rate:	1.0 mL/min
Injection:	20 µL
Run time:	20 min
Detection:	Radioactivity detector (Raytest Gabi) and UV spectrophotometer (Agilent VWD) at λ: 270 nm
Integrator:	Raytest Gina
R_t value:	[^{124}I] MIBG: 8.3 min

In addition to the tests described in the table above for routinely produced [^{124}I] MIBG batches, the following tests are also performed in validation batches, as part of the IMP dossier (Table III-21), also as prepared by the Division of Nuclear Medicine, Medical University of Graz in Austria.

TABLE III-21. VALIDATION TESTS FOR [^{124}I] MIBG BATCHES

TESTS	METHODS	SPECIFICATIONS
Radionuclide purity	γ spectrometry after decay of ^{124}I	^{125}I: < 0.5 % or as from the manufacture of ^{124}I for labelling specified
Specific activity	Reverse-phase HPLC with UV/radioactivity detectors	Minimum 10 MBq ^{124}I per mg MIBG[a]
Chemical impurity: Trimetyltin chloride	Reverse-phase HPLC with UV detector	< 0.5 mg/injection (V)[b]

[a] The new synthesis route provides a no carrier added [^{124}I] MIBG in contrast to the conventional isotope exchange method. Therefore, the limit of the specific activity (carrier MIBG) described in Ph. Eur. monograph of [^{123}I] MIBG [III-12] can theoretically never go below, but should tested in the validation batches.
[b] Due to the low mass of used precursor the established limit of trimethyltin chloride at Ph. Eur. [III-13] could theoretically never reached, but should tested in the validation batches.

III.12 [^{177}Lu] DOTATATE

[^{177}Lu] DOTATATE (also known as Lu-dota-octreotate, lu-dota^{0}-tyr^{3}-octreotate*)* is prepared by adding up to 9 GBq no-carrier-added [^{177}Lu] lutetium chloride (EndolucinBeta, ITG) ($T_{1/2}$ = 6.71 d) to a kit containing 100 µg DOTATATE and 50 mg ascorbic acid (Polatom). The complex forms by chelation when the kit is heated at 90 to 100°C for 10 to 20 min. After the complex has formed the product can be diluted with saline if desired. Carrier added [^{177}Lu] lutetium chloride may be used (e.g. Lumark, AAA) but the activity limit may be lower or the quantity of peptide higher.

Table III-22 demonstrates the required tests performed routinely for [^{177}Lu] DOTATATE batches prior to release, as prepared for research use at a centre in Guy's and St Thomas' Hospital in London, UK.

TABLE III-22. QUALITY CONTROL TEST FOR [^{177}Lu] DOTATATE BATCHES

TESTS	METHODS	SPECIFICATIONS
Appearance of the solution	Visual examination	Clear, colourless or pale yellow solution
pH	pH paper	4.5 to 8.5
Radioactivity measurement	Ionization chamber	Report activity
Radionuclide incorporation	Thin-layer chromatography[a] [a]	≥ 97%

[a] **Chromatography methods:**

Solid phase:	Silica gel embedded fibre glass (ITLC-SG)
Mobile phase:	Citrate buffer, 0.1 M, pH 5
Method:	Place a spot of the radiopharmaceutical near the bottom of an ITLC strip. Place the strip in a tube or tank and allow the mobile phase to migrate up the strip until it nears the end. Remove the strip and allow it to dry.
Analysis:	Analyse the distribution of activity on the strip using a radiochromatogram scanner or phosphor imager, or cut the strip at R_f 0.5 and measure the activity in the two portions in a dose calibrator or gamma counter.
Rf values:	[^{177}Lu] DOTATATE, R_f ~0; [^{177}Lu] LuCl, R_f ~1

Table III-23 demonstrates the required tests to be performed by the commercial manufacturer, also as prepared for research use at a centre in Guy's and St Thomas' Hospital in London, UK.

TABLE III-23. QUALITY CONTROL TESTS FOR [^{177}Lu] DOTATATE BATCHES PERFORMED BY MANUFACTURER

TESTS	METHODS	SPECIFICATIONS
Radiochemical purity	HPLC	≥ 97%
Chemical purity	HPLC	≥ 90%
Specific activity	Calculation	> 53 GBq/µmol
Filter integrity test	Bubble point	>3.45 bar
Bacterial endotoxin content	Ph. Eur. method	≤ 175 IU/injection
^{177}Lu identity via half-life[a]	Dose calibrator	6.37 - 7.05 d (6.71 d ± 5%)
Sterility [a]	Direct inoculation	Sterile

[a] These tests performed post release

[^{177}Lu] DOTATATE is prepared by adding up to 185 GBq [^{177}Lu] lutetium chloride (Lumark, IDB Radiopharmacy, The Netherlands) ($T_{1/2}$ = 6.71 d) to a kit containing sufficient volume of 400 µg mL^{-1} DOTA-TYR^3-OCTREOTATE in gentisic acid/ascorbate solution (Apotheek Erasmus). The complex forms by chelation when the kit is heated at 83±2 ºC for 30 min. After cooling, 0.5-1.0 mL of a 4 mg mL^{-1} pentetic acid solution in saline is added. The product can be diluted with saline if desired.

Table III-24 demonstrates required tests to be performed routinely for [^{177}Lu] DOTATATE batches prior to release, as prepared at the Radiopharmacy Centre, IPEN-CNEN/SP, in Brazil.

TABLE III-24. QUALITY CONTROL TEST FOR [^{177}Lu] DOTATATE BATCHES

TESTS	METHODS	SPECIFICATIONS
Appearance of the solution	Visual examination	Clear, colourless or pale yellow solution
pH	pH paper	4.0 to 5.5
Radioactivity measurement	Ionization chamber	Report activity
Radiochemical purity	Thin-layer chromatography [a]	≥ 95%
Specific activity	Calculation	15 to 40 MBq/µg DOTATATE
Filter integrity test	Bubble point	> 3.45 bar
Bacterial endotoxin content	Ph. Eur. method	≤ 175 IU / injection
Sterility[b]	Direct inoculation	Sterile

[a] **Chromatography method:**

- Solid phase: Silica gel embedded fibre glass (ITLC-SG) or silica gel 60 aluminium (TLC-SG)
- Mobile phase: Citrate buffer, 0.1 M, pH 5.5
- Method: Place a spot of the radiopharmaceutical near the bottom of an ITLC or a TLC

— strip. Place the strip in a tube or tank and allow the mobile phase to migrate up the strip until it nears the end. Remove the strip and allow it to dry.
— Analysis: Analyse the distribution of activity on the strip using a radiochromatogram
— scanner or cut the strip into 1 cm pieces and measure the activity in a gamma counter.
— R_f values: [^{177}Lu] DOTATATE, R_f ~0 – 0.3; [^{177}Lu] LuCl, R_f ~1.0

[b] Post release results

III.13 [^{213}Bi] DOTA-PEPTIDES

^{213}Bi is eluted as BiI_4^-/BiI_5^{2-} ion from an ^{225}Ac/^{213}Bi generator using 1.4 mL 0.1 M HCl/0.1 M NaI. The eluate is added to a microwave vial containing 350 µL 2M TRIS buffer, 100 µL 20% ascorbic acid and 15 µL of 2 mg/m: DOTATOC solution. The reaction mixture is heated to 95 °C for 5 min using a microwave synthesizer and subsequently cooled to <50 °C using pressurized air. Quality control is performed by instant thin layer chromatography with 0.05 M citric acid, pH 5, as solvent. Under these conditions, unbound ^{213}Bi moves with the solvent front (Rf=1), while [^{213}Bi] DOTATOC remains on the bottom of the strip (Rf=0). For example, [^{213}Bi] DOTATOC for clinical trials or experimental patient treatment. Radiochemical purity is determined by measuring the activity of the 440 keV gamma emission of ^{213}Bi on the upper and lower part of the ITLC strip in an automated gamma counter using an energy window of 400 to 500 keV. Specific activity is typically 80 MBq/nmol peptides at the time of injection. Before injection, of the final formulation a sterile filtration has to be performed [III-14].

Table III-24 demonstrates the required tests performed routinely for [^{213}Bi] DOTATOC batches, as prepared at the European Commission, Joint Research Centre, Directorate G - Nuclear Safety and Security, Karlsruhe, Germany. Note that sterility and bacterial endotoxin testing is performed as a post-released control.

TABLE III- 25. QUALITY CONTROL TEST FOR [^{213}Bi] DOTATOC

TESTS	METHODS	SPECIFICATIONS
Appearance of the solution	Visual examination	Clear, colourless to pale yellow solution
pH	pH paper	8.5 to 9.0
Radioactivity measurement	Ionization chamber	Measurement of syringe for injection
Radiochemical purity	Thin-layer chromatography [a]	≥ 95.0%
Bacterial endotoxin content	Ph. Eur. method	≤ 175 IU/injection
Sterility	Direct inoculation	Sterile

[a] **Chromatography methods:**

Solid phase:	Silica gel embedded fibre glass (ITLC-SG)
Mobile phase:	Citrate buffer, 0.05 M, pH 5
Method:	Place a spot of the radiopharmaceutical near the bottom of an ITLC strip and put the strip in a tube to allow the mobile phase to migrate up the strip until it nears the end.
Analysis:	Analyse the distribution of activity on the strip using a radiochromatogram scanner or phosphor imager, or cut the strip at R_f 0.5 and measure the activity in the two portions in a gamma counter.

R_f values: [^{213}Bi]DOTATOC, $R_f \sim 0$; ^{213}Bi, $R_f \sim 1$

III.14. [^{213}Bi] DTPA-ANTIBODIES

^{213}Bi is eluted as BiI_4^-/BiI_5^{2-} ion from an ^{225}Ac/^{213}Bi generator using 1.4 mL 0.1 M HCl/0.1 M NaI. The eluate is added to 280 µL 2M ammonium acetate buffer (pH 5.3) and 118 µL 20% ascorbic acid. The pH should be 5.5. The Anti-EGFR-MAb (100 µg; matuzumab, Merck) conjugated with the ^{213}Bi-chelating agent SCN-CHX-A-DTPA is incubated for 5 min at room temperature. Purification of ^{213}Bi-DTPA-antiEGFR reaction mixture is carried out on a PD 6 column using PBS for elution. Quality control is performed by instant thin-layer chromatography with 0.05 M citric acid, pH 5, as solvent. The final pH of the formulation is adjusted to 7.4 and the sterility is ensured via sterile filtration.

Table III-25 demonstrates the required tests to performed routinely for [^{213}Bi] DTPA-anti EGFR batches as prepared at the European Commission, Joint Research Centre, Directorate G - Nuclear Safety and Security, Karlsruhe, Germany. Note that sterility and bacterial endotoxin testing is performed as a post-released control.

TABLE III-26. QUALITY CONTROL TESTS FOR [^{213}Bi] DTPA-ANTIBODIES

TESTS	METHODS	SPECIFICATIONS
Appearance of the solution	Visual examination	Clear, colourless solution
pH	pH paper	7.1 to 7.7
Radioactivity measurement	Ionization chamber	Measurement of syringe for injection
Radiochemical purity	Thin-layer chromatography[a]	≥ 95.0%
Bacterial endotoxin content	Ph. Eur. method	≤ 175 IU/injection
Sterility	Direct inoculation	Sterile

[a] **Chromatography methods:**

- Solid phase: Silica gel embedded fibre glass (ITLC-SG)
- Mobile phase: Citrate buffer, 0.05 M, pH 5
- Method: Place a spot of the radiopharmaceutical near the bottom of an ITLC strip and
- put the strip in a tube to allow the mobile phase to migrate up the strip until it nears the end.
- Analysis: Analyse the distribution of activity on the strip using a radiochromatogram
- scanner or phosphor imager, or cut the strip at R_f 0.5 and measure the activity in the two portions in a gamma counter.
- R_f values: [^{213}Bi] DTPA-antiEGFR, $R_f \sim 0$; ^{213}Bi, $R_f \sim 1$

III.15 XOFIGO® ([^{223}Ra] $RaCl_2$)

Xofigo® ([^{223}Ra] $RaCl_2$) is a ready to use alpha particle emitting radioactive therapeutic agent, centrally produced (industrial scale), and is indicated for the treatment of patients with castration resistant prostate cancer, symptomatic bone metastases and no known visceral metastatic disease. Xofigo is supplied in single-use vials containing 6 mL of solution at a concentration of 1 000 kBq/mL (27 microcurie/mL) with a total radioactivity of 6 000 kBq /

vial (162 microcurie/vial) at the reference date (the volume to be administered to a given patient should be calculated using the patient's body weight (kg), dosage level 50 kBq/kg body weight or 1.35 microcurie / kg body weight) and the radioactivity concentration of the product (1 000 kBq/mL; 27 microcurie/mL at the reference date) decay corrected to the date of administration [III-15].

List of the tests performed, methods and specifications (acceptance criteria) is shown in Table III- 26, as supplied by Bayer HealthCare Pharmaceutical Incs., for therapeutic use. Note that parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever the solution and container permit. Xofigo is a ready-to-use solution and should not be diluted or mixed with any solutions. Each vial is for single use only.

TABLE III-27. QUALITY CONTROL TESTS FOR [^{223}Ra] $RaCl_2$

TESTS	METHODS	SPECIFICATIONS
Appearance of the solution	Visual examination	Clear, colourless solution
Radioactivity measurement	Ionisation chamber	Measurement of syringe for injection

III.16 [^{225}Ac] DOTA-PEPTIDES

For radiolabelling of [^{225}Ac] DOTA-PSMA-617, an aliquot of ^{225}Ac stock solution is added to a microwave vial containing 0.1 M TRIS buffer (pH 9) and an appropriate amount of DOTA-PSMA-617 stock solution. The reaction mixture is heated to 95°C for 5 min using a microwave synthesizer. Quality control is performed by instant thin-layer chromatography (ITLC-SG) with 0.05 M citric acid (pH 5) as the solvent. After development, the chromatography strip is stored for at least 1 h until radiochemical equilibrium is obtained between ^{225}Ac ($T_{½}$ = 9.9 d) and its daughter nuclide ^{221}Fr ($T_{½}$ = 4.8 min). Subsequently, radiochemical purity is determined by measuring the activity of the 218 keV γ emission of ^{221}Fr on the upper and lower parts of the strip using high-resolution γ spectrometry. After synthesis, an aliquot of ascorbic acid is added to the reaction mixture (to minimize radiolytic degradation of [^{225}Ac] DOTA-PSMA-617) together with an aliquot of diethylenetriaminepentaacetic acid (to scavenge free radiometals). The final pH of the formulation is adjusted to 7.4 and the sterility is ensured via sterile filtration [III-16].

Table III- 27 demonstrates the required tests done routinely for [^{225}Ac] DOTA-PSMA-617 batches, as prepared at the European Commission, Joint Research Centre, Directorate G - Nuclear Safety and Security in Karlsruhe, Germany. Note that sterility and bacterial endotoxin testing is performed as a post-released control.

TABLE III- 28. QUALITY CONTROL TESTS FOR [^{225}Ac] DOTA-PEPTIDES

TESTS	METHODS	SPECIFICATIONS
Appearance of the solution	Visual examination	Clear, colourless solution
pH	pH paper	7.1 to 7.7
Radioactivity measurement	Ionization chamber	Measurement of syringe for injection

Radiochemical purity	Thin-layer chromatography[a]	≥ 95.0%
Bacterial endotoxin content	Ph. Eur. method	≤ 175 IU/injection
Sterility	Direct inoculation	Sterile

[a] **Chromatography methods:**

Solid phase:	Silica gel embedded fibre glass (ITLC-SG)
Mobile phase:	Citrate buffer, 0.05 M, pH 5
Method:	Place a spot of the radiopharmaceutical near the bottom of an ITLC strip and put the strip in a tube to allow the mobile phase to migrate up the strip until it nears the end.
Analysis:	Analyse the distribution of activity on the strip using a radiochromatogram scanner or phosphor imager, or cut the strip at R_f 0.5 and measure the activity in the two portions in a gamma counter.
R_f values:	[^{225}Ac] DOTA-PSMA-617, $R_f \sim 0$; ^{225}Ac, $R_f \sim 1$

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ANNEX IV: STATUS OF THE EXISTING LEGISLATION RELATED TO RADIOPHARMACEUTICALS: BINDING DOCUMENTS AND GUIDELINES

A section based on the participants of this technical meeting

Radiopharmaceuticals are highly regulated products. They are radiolabelled molecules, and as such are controlled both as medicinal products and as radioactive substances. To make things even more complex, some of these controls are international (e.g. transport regulations) while others are specific to individual countries (e.g. pharmaceutical regulations).

IV-1. EUROPE (EU)

In the EU, two sources of legislation apply: 'drug quality regulations' (such as Good Manufacturing Practice (GMP), initially developed for regulating the large scale and centralized manufacturing of active substances) and 'safety regulations' (radiations laws, toxicology data). Specific regulations regarding the manufacturing site as such may also be applicable.

Private manufacturing companies, as well as public research centres, hospitals or internal radiopharmacies are confronted to a 'jungle' of legislation: directives, regulations (binding documents) and guidelines, guidance (non-binding documents), which may be classified with respect to the radiopharmaceutical status [IV-1]:

- Radiopharmaceuticals with a marketing authorization;
- Radiopharmaceuticals to be used in clinical trials (CT);
- Unlicensed radiopharmaceuticals extemporaneously (just before use) prepared, not for CT.

IV-1.1. Binding documents that apply to all radiopharmaceuticals

European Pharmacopoeia

- General monograph (Ph. Eur. general monograph 0125 [01/2014:0125 - European Pharmacopoeia 8.0] [IV-2]
- Specific monographs (~ 65 individual radiopharmaceuticals available).

IV-1.2. Documents that specifically apply to radiopharmaceuticals with market authorization

IV-1.2.1. Binding documents

European Pharmacopoeia (see above), plus:

Council Directive 89/343/EEC (Radiopharmaceuticals Directive) (came into force from 1992) [IV-3]. Radiopharmaceuticals prepared at the time of use from authorized precursors, generators and kits were exempted from this directive.

Directive 2001/83/EC (community code on medicinal products for human use), which provides requirements for industrially produced medicinal products for human use to be placed on the market [IV-4].

As in Directive 89/343/EEC, marketing authorization is required for radionuclide generators, radionuclide precursors and kits, but not for radiopharmaceuticals prepared from authorized components at the time of use. In general, Directive 2001/83/EC does not apply to products prepared in a pharmacy in accordance with a medical prescription (magistral formula) or prescriptions of a pharmacopoeia (officinal formula) to be supplied directly to individual patients or to medicinal products intended for research in clinical trials (to which Directive 2001/20/EC applies, see the section Binding documents for radiopharmaceuticals to be used in clinical trials).

Directive 2003/63/EC presented a revised version of Annex 1 to Directive 2001/83/EC [IV-5]. In Part III (particular medicinal products), section 2, special requirements for the contents of the application dossier are mentioned, such as the description of the manufacturing method and the structure of the radiolabelled compound and specifications and testing methods of radionuclide and radiochemical purity, specific activity, stability and radiation dosimetry.

Directive 2004/27/EC provides an amendment to Directive 2001/83/EC but contains no major alterations with respect to radiopharmaceuticals [IV-6].

IV-1.2.2. Guidance documents

Annex 13 of the GMP guidelines (Investigational Medicinal Products) [IMP dossier has to be developed: specifications for starting materials, intermediates, bulk and finished products; manufacturing process (formula and instructions); packaging and labelling; quality control, storage and release] [IV-7].

European Medicines Agency Guideline on Radiopharmaceuticals (EMA Radiopharmaceuticals) [IV-8].

IV-1.3. Documents that specifically apply to radiopharmaceuticals to be used in clinical trials

IV-1.3.1. Binding documents

European Pharmacopoeia (see above), plus:

(a) EU directives:

Directive 2001/20/EC (Clinical trial directive) → Dictates the pharmaceutical requirements for investigational medicinal products (IMPs), such as manufacturing authorization and the employment of a qualified person [IV-9].

Directive 2003/94/EC (→ GMP directives). The Commission Directive 2003/94/EC of 8 October 2003 establishes the principles and guidelines of good manufacturing practice in

respect of medicinal products for human use and investigational medicinal products for human use [IV-10].

Directive 2005/28/EC (Good Clinical Practice and requirements for manufacturing authorization) lays down 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 [IV-11].

(b) EU regulations:

Regulation (EU) No 536/2014 (entered into force on June 16, 2014, but should be applied not earlier than October 2018) [IV-12, IV-13].

IV-1.3.2. Guidance documents

Annex 13 of the GMP guidelines (Investigational Medicinal products): IMP dossier must be developed: specifications for starting materials, intermediates, bulk and finished products; manufacturing process (formula and instructions); packaging and labelling; quality control, storage and release [IV-7] .

EC Guidance IMP/NIMP [IV-14].

European Medicines Agency Guideline IMP versus NIMP (→ IMP dossier) [IV-15].

European Medicines Agency Guideline 'First-in-human clinical trials' [IV-16].

IV-1.4. Documents that specifically apply to extemporaneously prepared radiopharmaceuticals

IV-1.4.1. Binding documents

European Pharmacopoeia (see above)

IV-1.4.2. Guidance documents

European Pharmacopoeia: general chapter 5.19 'Extemporaneous preparation of radiopharmaceutical preparations' [IV-17].

PIC/S guide (Interpretation of GMP issues for small scale preparation of medicinal products) [IV-18].

EANM guidance on current Good Radiopharmacy Practice [IV-19, IV-20].

Note that rules for the extemporaneously preparation of radiopharmaceuticals are under the responsibility of the individual member states (see sections below).

IV-1.5. Recent legislation developments

(a) Regulation (EU) No 536/2014**:** entered into force on June 16, 2014, but should be applied not earlier than October 2018 [IV-12] [IV-13].

In this new regulation, manufacturing authorization and compliance to GMP will not be required for diagnostic radiopharmaceuticals used in clinical trials and for IMPs prepared before use (magistral formula and officinal formula according to 2001/83/EC). Instead, GMP like requirements will be applicable, which are supervised by the national competent authorities. Furthermore, the general labelling requirements are not applicable to radiopharmaceuticals used as IMPs or auxiliary medicinal products (AMPs, formerly known as NIMPs) [IV-21].

(b) A new General Chapter for the Ph. Eur. has been recently published

In 5.19 Extemporaneous preparation of radiopharmaceutical preparations [IV-17]. This chapter will not be applicable for industrial producers, nor for the preparation of IMPs, but instead it will be applicable for preparation in radiopharmacies. It aims to provide minimal requirements for kit based preparations, PET radiopharmaceuticals and radiolabelled blood cells. Being a General Chapter, it will not be mandatory, unless it is mentioned in a monograph. It is remarkable that cross-reference is made to 'PIC/S PE 010' and the 'EANM Guidelines on Good Radiopharmacy Practice', which are nonbinding documents as well. This document tries to link the differences between European countries and standard radiopharmacy practice.

IV-1.6. France

IV-1.6.1. Nuclear medicine specifications

Most of the nuclear medicine departments in France are in public institutions (about 85%) and equipped with a radiopharmacy dedicated at least to the preparation of ^{99m}Tc labelled radiopharmaceuticals. Taken all together, about 450 SPECT tomographs and over 400 PET tomographs are installed over the territory, for about 1 300 000 scans per year (75% SPECT, 25% PET). For SPECT, bone scintigraphy (using for example [^{99m}Tc] TECEOS®, or [^{99m}Tc] OSTEOCIS®) and cardiac scintigraphy (using for example [^{99m}Tc] CARDIOLITE ®, or [^{99m}Tc] STAMICIS ®) represent the majority of the examinations (over 90%) whereas brain scintigraphy ([^{123}I] DATSCAN for example) remains rare (<5%). PET-scans are most often performed with the ^{18}F labelled radiopharmaceutical [^{18}F] FDG (>95%) and particularly in the field of oncology (metastasis and secondary tumour detection and staging, therapeutic efficacy). For the latter radiopharmaceutical, as well as for a few other ^{18}F labelled ones (see table in the next section), their daily delivery in the nuclear medicine departments depends on a significant number of (private) radiopharmaceutical companies - CIS Bio International, Advanced Accelerator Applications (AAA), Cyclopharma, PETNET solutions - operating a total of 20 manufacturing sites (data from May 2016) geographically distributed over the French territory, and strongly competing in terms of distribution network and manufacturing cost. Additionally, a few academic research centres (a non-exhaustive list includes the CEA-SHFJ (Orsay, 25 km south of Paris), CEA-CYCERON (Caen), CERMEP (Lyon), CYRCÉ (Strasbourg), CERRP (Tours)) are also equipped with (or have direct access to) a cyclotron, producing thus other ^{18}F labelled molecules as well as ^{11}C labelled ones. Some facilities also started with the preparation

of ^{68}Ga labelled radiopharmaceuticals ([^{68}Ga] DOTATATE for example) and are as such equipped with ^{68}Ge/^{68}Ga generators.

IV-1.6.2. Legislation and regulatory authorities

France is part of the European Union; EU pharmaceutical legislation applies to the manufacturing and quality control of radiopharmaceuticals. As such, the whole arsenal of European pharmacopeia (general and specific monographs), EU directives and regulations but also guidelines and directions are applicable (see also section above on EU legislation), with the following three independent, but highly cross-linked agencies, responsible for their control and application, sharing both radiation protection aspects and pharmaceutical aspects:

(a) Nuclear Safety Authority

The Nuclear Safety Authority (ASN, Autorité de sûreté nucléaire) is more particularly in charge of nuclear safety and radiation protection aspects linked to radiopharmaceutical manufacturing. Depending on the radiopharmaceutical used, the following authorizations may be required:

- AUTO/RN/FABCYC: "Demande d'autorisation de détenir et d'utiliser un accélérateur de particules de type cyclotron et demande d'autorisation de fabriquer, de détenir et d'utiliser des radionucléides émetteurs de positons et des produits en contenant" [IV-22] (licence authorizing to hold and use a cyclotron-like particle accelerator and authorizing to manufacture, hold and use positron-emitting radionuclides and products containing them);
- AUTO/MED/MEDNU: "Demande d'autorisation de détention et d'utilisation de radionucléides et d'appareils électriques émettant des rayonnements ionisants pour une activité de médecine nucléaire et/ou de diagnostic in vitro incluant la recherche biomédicale" [IV-22] (licence authorizing to hold and use radionuclides and electric devices emitting ionizing radiation for a nuclear medicine activity, and/or in vitro diagnostic including biomedical research*);*
- AUTO/RN/DISTR: "Demande d'autorisation de distribuer, d'importer ou d'exporter (dans le cadre de la distribution) des radionucléides, des produits ou dispositifs en contenant dans le domaine industriel, medical ou de la recherche" [IV-22] (licence authorizing to distribute or import radionuclides and products or devices containing them for use in industrial domain, medical domain or research).

(b) Regional Health Agency

The Regional Health Agency (ARS, Agence Régionale de Santé) oversees the delivery of site authorizations for the pharmacies (non-commercial sites) within hospitals (Pharmacie à Usage Intérieur (PUI)). When the use and/or manufacturing of radiopharmaceuticals is concerned, the following specific and so-called 'optional activities' are mandatory [IV-23]:

- "Préparation des médicaments radiopharmaceutiques" *(*preparation of radiopharmaceuticals*)* [IV-23];

- "Préparations rendues nécessaires par les expérimentations ou essais des médicaments mentionnés aux articles L. 5126-11 et L. 5126-12" (preparations required by experimentations or trials of drugs as described in articles L. 5126-11 et L. 5126-12 [of the Code de la Santé Publique -see below) [IV-23].

(c) National Agency for The Security of The Drugs and Health Products

The National Agency for The Security of The Drugs and Health Products (ANSM, Agence Nationale de Sécurité du Médicament et des produits de santé). More particularly in charge of the drug aspect of the radiopharmaceuticals [IV-24], including the reviewing of all Investigational Medicinal Product dossiers (IMPD, 'Dossier Médicament Expérimental' (DME) in France). This dossier (DME) is the breakdown of the EU IMPD, and as such, also includes information related to chemical and pharmaceutical quality (Part I in which the radiopharmaceutical and its preparation QC are exhaustively described), as well as non-clinical data related to pharmacology, pharmacokinetics, dosimetry and toxicology (Part II), and finally description of the clinical trial (Part III).

From a regulatory point of view, legislation linked to health aspects in France, including the ones relative to radiopharmaceuticals, is reported within the French 'Code de la Santé Publique' (compilation of public health laws and rules [IV-25]). Distinct sections are described, depending on the use of the radiopharmaceutical and its status. As for UE, three types of radiopharmaceuticals are considered:

(i) Unlicensed radiopharmaceuticals extemporaneously prepared, not for clinical trials

In this case, the radiopharmaceutical is considered a 'Préparation magistrale' (magistral preparation), and an IMP-like dossier (DME, see above)) is not required. Manufacturing of the radiopharmaceutical has to follow the 'Bonnes Pratiques de Préparation (BPP) magistrales, officinales et hospitalières' (good practices for magistral, hospital or officinal preparations) [Article L5121-1].

(ii) Radiopharmaceuticals to be used in clinical trials

In this case, the radiopharmaceutical is considered a 'médicament radiopharmaceutique' (radiopharmaceutical drug), and an IMP-like dossier (DME) is required. Manufacturing of the radiopharmaceutical has to follow both the BPP the 'Bonnes Pratiques de Fabrication (BPF) des médicaments à usage humain' (good manufacturing practices (GMP) for drug used in human) [Article L5111-1]. When used within clinical trials, the radiopharmaceutical and the protocol using it have also to be evaluated by local Ethic Committees.

(iii) Radiopharmaceuticals with marketing authorization

In this case, the radiopharmaceutical is provided with an 'Autorisation de Mise sur le Marché (AMM)' (marketing authorization) and is manufactured by a radiopharmaceutical company (under full GMP). Also, in this case radiopharmacies are no longer authorized to manufacture these radiopharmaceuticals. The table below summarizes the situation in France (data from May 2016) concerning licensed ^{18}F labelled (PET) radiopharmaceuticals.

TABLE IV-2. LICENSED ^{18}F LABELLED (PET) RADIOPHARMACEUTICALS IN FRANCE (May 2016)

DCI	TRADENAME	MA HOLDER	COMMERCIALIZED IN FRANCE
[^{18}F]Fludésoxyglucose	Efdegé®	IASON Gmbh	IASON Gmbh
	Fludésoxyglucose [^{18}F]-IBA®	IBA PHARMA	IBA, CIS bio Int.
	Glucotep®	Cyclopharma	Cyclopharma
	Gluscan®	AAA	AAA
	Metatrace FDG®	PETNET solutions	PETNET solutions
[^{18}F]Fluorodopa	Dopacis®	CIS bio Int.	CIS bio int.
	IASOdopa®	IASON Gmbh	AAA
	DOPAVIEW®	AAA	AAA
[^{18}F]Fluorocholine	IASOCholine®	IASON Gmbh	AAA
	no trade name yet	CIS bio Int.	CIS bio Int.
Fluorure [^{18}F] de sodium	Cisnaf®	CIS bio Int.	CIS bio Int.
	Iasoflu®	IASON Gmbh	AAA
	NaF MetaTrace®	PETNET solutions	PETNET solutions
[^{18}F]Florbetaben	Neuraceq®	Piramal Imaging Ltd	CIS bio int.
[^{18}F]Florbetapir	Amyvid®	Eli Lilly	AAA, Cyclopharma, PETNET solutions
[^{18}F]Flutemetamol	VisaMyl®	GE Healthcare	AAA
[^{18}F]Fluoroethyltyrosine	IASOglio®	IASON Gmbh	IASON Gmbh

a

[a] To this list may be added today [^{18}F]FLT ([^{18}F]Fluorothymidine) and [^{18}F]MISO ([^{18}F]Fluoromizonidazole), produced with the status of « Autorisation Temporaire d'Utilisation » (ATU). Coming soon also with a marketing authorization (MA) in France, [^{18}F]FES ([^{18}F]Fluorooestradiol, Cyclopharma) and [^{18}F]Fluciclovine ([^{18}F]FACBC, anti-1-amino-3-[^{18}F]fluorocyclobutane-1-carboxylic acid, Blue Earth Diagnostics Ltd. and PETNET sol).

IV-1.6.3. Quality control frequency, tests and the qualified person

For all radiopharmaceuticals (licensed radiopharmaceuticals, radiopharmaceuticals used in clinical trials, or extemporaneously prepared radiopharmaceuticals not for clinical trials), QC is required on every batch. QC most often comprises pre- and post-release tests (radionuclide half-life dependent) and includes in addition to pH measurement and visual inspection, at least radiochemical and nucleic purity determination and identity, radioactive concentration determination, residual impurities measurements (especially residual solvent testing) as well as endotoxin content and sterility assessments. Specifications, limits and acceptance criteria should be set for each tested parameter. Annex III provides a representative example, the specifications, limits and acceptance criteria for a ^{11}C labelled (PET) radiopharmaceutical ([^{11}C] PIB), produced for a clinical trial in France. The qualified person (QP) in France –in charge of all manufacturing aspects thus including QC– is the radiopharmacist.

IV-1.7. United Kingdom

IV-1.7.1. Nuclear medicine specifications

There are ~200 nuclear medicine departments in the UK for a population of 65 million, most of them in public hospitals. There are ~500 gamma cameras and ~60 PET scanners, which is lower than the European average. In total there are~600 000 nuclear medicine procedures and ~100 000 PET scans annually. The most common nuclear medicine procedures are bone scintigraphy, lung ventilation/perfusion scans, and myocardial perfusion imaging. PET scans are most often (>95%) performed in the field of oncology with most of these utilising [^{18}F] FDG. Two hospitals use the ^{82}Sr / ^{82}Rb generator for myocardial perfusion PET and a small but increasing number of centres have access to ^{68}Ga labelled peptides. Radiopharmaceuticals labelled with ^{99m}Tc are provided by ~100 radiopharmacies, about half of them are run by hospital pharmacies and the other half operate under a Specials manufacturing licence (see below) which allows them to sell to other institutions. Currently, there is only 1 commercial radiopharmacy operating. Most of the [^{18}F] FDG is supplied from 5 commercial cyclotrons run by Alliance Medical Imaging and PETNET Solutions. There are ~8 cyclotrons in the public sector that mainly produce radiopharmaceuticals for research.

IV-1.7.2. Legislation and regulatory authorities

As the UK is part of the European Union, EU pharmaceutical legislation applies to manufacturing under GMP and quality control of radiopharmaceuticals. Preparation of radiopharmaceuticals in hospitals can be performed under one of two frameworks: a manufacturing licence or practice of pharmacy.

The Specials Manufacturing licence is issued and inspected by the Medicines and Healthcare Products Regulatory Agency (MHRA). There must be a named production manager and quality controller, both of whom must be approved by the MHRA even though there are not firm criteria. These roles are not limited to pharmacists. Products prepared under a Specials licence may be sold to other institutions. ^{99m}Tc labelled preparations are performed under this licence, as is the manufacture of non-[^{18}F] FDG PET tracers ([^{18}F] FDG must be produced under a full manufacturing licence).

The practice of pharmacy is carried out under the Section 10 exemption to the Medicines Act 1968. Preparation must be under the supervision of a pharmacist who then releases the products on prescription. Since 1997 these operations have been inspected by the regional National Health Service (NHS) quality assurance specialists accompanied by an external radiopharmacy expert. The standards are essentially the same as required for a licence but the enforcement is different.

The following agencies regulate the practice of radiopharmacy and nuclear medicine in the UK:

— Medicines and Healthcare Products Regulatory Agency (MHRA): approves drugs for marketing; issues licences and inspects manufacturing facilities;
— Administration of Radioactive Substances Advisory Committee (ARSAC): concerned with radiation exposure to patients and issues certificates to physicians allowing them

to perform nuclear medicine procedures, if they have adequate training and experience, and if the appropriate infrastructure is available, including radiopharmacy and radiation protection;
- Environment Agency (EA): issue permits for holding and disposing radioactive materials;
- Health and Safety Executive (HSE): concerned with radiation safety practices affecting staff;
- Office of Nuclear Regulation (ONR): transport and security of radioactive materials.

From a regulatory point of view, three types of radiopharmaceuticals are considered:

(a) Radiopharmaceuticals extemporaneously prepared, not for clinical trials

As stated above, these radiopharmaceuticals (whether SPECT or PET, hospital or commercial radiopharmacy/cyclotron) must be prepared either under a Specials manufacturing licence or practice of pharmacy. In addition to a range of ^{99m}Tc labelled products this would include [^{18}F] fluoride, [^{18}F] fluoromethyl- or fluoroethylcholine, and ^{68}Ga labelled peptides.

(b) Radiopharmaceuticals to be used in clinical trials

In this case, the radiopharmaceutical is considered an Investigational Medicinal Product (IMP) and manufacturing must be performed under an IMP licence with release by a Qualified Person (QP); the definition of QP is more restrictive in the UK than in the rest of Europe. An IMP Dossier is required and the radiopharmaceutical and the trial protocol have to be evaluated by the local Ethics Committees. When the EU Clinical Trials Regulations 2014 are implemented, clinical trials of diagnostic radiopharmaceuticals in hospitals will be exempt from these requirements.

(c) Radiopharmaceuticals with marketing authorization

In this case, the radiopharmaceutical has a Marketing Authorization issued by the MHRA or European Medicines Agency (EMA) and is manufactured by a radiopharmaceutical company (or PET cyclotron unit) under full GMP including release by a QP. This category would include ^{99}Mo/^{99m}Tc generators, kits for labelling with ^{99m}Tc, ^{131}I and ^{111}In labelled products. PET agents include [^{18}F]FDG, [^{18}F]florbetapir, [^{18}F]florbetaben, and [^{18}F]flutemetamol.

IV-1.7.3. Quality control frequency, tests and the qualified person

For radiopharmaceuticals with a marketing authorization, IMPs used in clinical trials, or extemporaneously prepared radiopharmaceuticals using open procedures, QC is required on every batch. QC most often comprises pre- and post-release tests (radionuclide half-life dependent) and includes in addition to pH measurement and visual inspection, at least radiochemical and nucleic purity determination and identity, radioactive concentration determination, residual impurities measurements (especially residual solvent testing) as well as endotoxin content and sterility assessments. Specifications, limits and acceptance criteria should be set for each tested parameter. For products with a marketing authorization and IMPs, release must be performed by a QP. Under a Specials licence there is a releasing officer who

does not need to be a QP. Products prepared from licensed generators and kits using closed procedures under aseptic conditions do not require full QC before use as long as this practice has been supported by a risk assessment.

IV-1.8. Germany

IV-1.8.1. Nuclear medicine specifications

In Germany, more than 600 gamma cameras and 80 PET / CT scanners are installed in public or private hospitals. Nuclear medicine procedures are increasingly performed as cross-sectional imaging like SPECT/CT and PET/CT. The most common radioisotope for SPECT is ^{99m}Tc. Due to the limitations in the supply of ^{99}Mo the frequency of thyroid scans with ^{123}I has increased as well as the use of [^{18}F] fluoride PET as a substitute for conventional bone scans. Most PET scans are performed by use of [^{18}F] compounds, e.g. [^{18}F] FDG, [^{18}F] DOPA. ^{18}F is produced in commercial cyclotron operating companies as well as in cyclotrons owned by hospitals. PET examinations using ^{68}Ga are increasing in recent years due to the availability of ^{68}Ge/^{68}Ga generators, appropriate syntheses platforms and newly developed radio tracer compounds, e.g. [^{68}Ga] DOTATOC, [^{68}Ga] PSMA.

In the therapeutic field radiopharmaceuticals based on ^{131}I, ^{90}Y, ^{223}Ra, ^{177}Lu are used in hospitals and are prepared either by commercial suppliers or in-house by the hospital radiopharmacy.

IV-1.8.2. Legislation and regulatory authorities

Germany as a member of the European Union adapted the existing European directives into national laws and ordinances. The legal basis for operation of radiochemical–radiopharmaceutical laboratory is the German radiation protection ordinance ("Strahlenschutzverordnung (StrlSchV) vom 20. Juli 2001 (BGBl. I S. 1714; 2002 I S. 1459), die zuletzt durch Artikel 5 der Verordnung vom 11. Dezember 2014 (BGBl. I S. 2010) geändert worden ist"). 'Strahlenschutz in der Medizin - Richtlinie zur Strahlenschutzverordnung (StrlSchV)' (Nuclear medicine aspects are also regulated by the directive for radiation protection in nuclear medicine) [vom 26. Mai 2011 (GMBl. 2011, Nr. 44-47, S. 867), zuletzt geändert durch RdSchr. des BMUB vom 11.Juli 2014 (GMBl. 2014, Nr. 49, S. 1020)]. For the manufacturing of pharmaceuticals, it is regulated by the German law on handling pharmaceuticals (Arzneimittelgesetz (AMG) in der Fassung der Bekanntmachung vom 12. Dezember 2005 (BGBl. I S. 3394), das durch Artikel 3 des Gesetzes vom 4. April 2016 (BGBl. I S. 569) geändert worden ist). §13 of AMG define the framework for manufacturing pharmaceuticals. The requirements concerning the quality of pharmaceuticals and the conditions for manufacturing (GMP-conditions) are regulated in the ordinance on the production of pharmaceuticals and active pharmaceutical ingredients [Arzneimittel- und Wirkstoffherstellungsverordnung vom 3. November 2006 (BGBl. I S. 2523), die zuletzt durch Artikel 1 der Verordnung vom 28. Oktober 2014 (BGBl. I S. 1655) geändert worden ist]. Special requirements for the production of radiopharmaceuticals are regulated in the ordinance about radiopharmaceutical drugs or drugs using ionising radiation [Verordnung über radioaktive oder mit ionisierenden Strahlen behandelte Arzneimittel (AMRadV) in der Fassung der Bekanntmachung vom 19. January 2007 (BGBl. I S. 48)]. § 2 give the framework for the

production of radiopharmaceuticals in smaller scale and for in-house radionuclide generator operation and in-house production of radiopharmaceuticals via kit formulation according to the manufacturing instruction provided by the kit manufacturer.

The following federal institutions are involved in the regulation of handling of radioactivity and radiopharmaceuticals:

— 'Bundesamt für Strahlenschutz (BfS)' (Federal Office for Radiation Protection) is an independent federal higher authority within the portfolio of the Federal Ministry of Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety;
— 'Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM)' (Federal Institute for Drugs and Medical Devices) is an independent federal higher authority within the portfolio of the Federal Ministry of Health.

IV-1.8.3. Quality control frequency, tests and the qualified person

The preparation of radiopharmaceuticals must be performed in accordance with the Pharmacopoeia with QC on every batch. In general, the QC includes at least pH measurement and visual inspection, radiochemical and nucleic purity determination and identity, radioactive concentration determination, residual impurities measurements as well as endotoxin content and sterility assessments as defined in the SOP. Preparations in hospitals without a monograph in the Pharmacopoeia should be performed following the latest state of the scientific and technical knowledge that can be released following the 'four eyes principle' by a radiopharmacist/radiochemist or by a nuclear medicine physician. The QP should have an appropriate training (e.g. post graduate course 'Specialisation in Radiopharmacy') and working experience in the field.

IV-1.9. Austria

IV-1.9.1. Nuclear medicine specifications

In Austria there are about 60 nuclear medicine institutions for 8 million citizens. About 30 are situated in a nuclear medicine department of a public hospital. About 30 are mainly small, private operated institutions or assigned to a private hospital. Most of these are operating one gamma camera or SPECT and offer only examinations with available commercial SPECT or ^{99m}Tc labelled radiopharmaceuticals. About 20 of the nuclear medicine institutions equipped with PET scanners, three of them are privately operated. The major SPECT applications are thyroid, bone, and cardiac scintigraphy. More than 90% of all PET examinations performed with [^{18}F] FDG. In addition, [^{18}F] DOPA, [^{18}F] NaF and [^{18}F] fluoromethylcholine are commercially available as approved drugs. ^{18}F labelled radiopharmaceuticals are supplied daily from 3 private operated cyclotron production sites situated in Austria. IASON operates two private cyclotron sites housed in hospitals at Linz and Klagenfurt. The third private cyclotron site is located at the Research Centre Seibersdorf. The only cyclotron site which is operated by the state is at the University Hospital of Vienna (AKH). This site only serves PET radiopharmaceuticals in-house. A rising trend can be observed in the use of ^{68}Ga labelled

radiopharmaceuticals. In the meantime, more than five nuclear medicine hospital departments provide PET scans with in-house prepared [^{68}Ga] DOTATOC and [^{68}Ga] PSMA.

IV-1.9.2. Legislation and regulatory authorities

As Austria is a member of the European Union, the EU pharmaceutical legislation for manufacture and quality control of radiopharmaceuticals are applied. Generally, radiopharmaceuticals in hospitals can be prepared by a manufacturing licence or under the framework of pharmacy practice, but that allows only an in-house application. The Austrian regulatory responsibilities in the preparation of the radiopharmaceutical are divided into the radiation protection aspects and the pharmaceutical aspects.

The responsibility for the radiation protection aspects is subject to the Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management. The European EURATOM directives are implemented in Austria with the 'Strahlenschutzgesetz' (Radiation Protection Law) and the affiliated 'Strahlenschutzverordnung' (General Radiation Protection Ordinance). Each application of radiation requires construction permission and operation permission. At first instance, the state government is responsible for licences and inspections. For complex radiation system e.g. accelerators, the responsibility is taken over by the Austrian Federal Ministry.

Pharmaceutical aspects fall within the competence of the Federal Ministry of Health and the associated Austrian Federal Office for Safety in Health Care (BASG) as well as the Austrian Agency for Health and Food Safety (AGES). The quality of medicines including radiopharmaceuticals is covered by the Austrian Drug Law (Arzneimittelgesetz, AMG). All manufactured drugs or active ingredients and their quality must comply with current standards in science, particularly in the quality requirements of the European Pharmacopoeia. The quality requirements for the production and quality control of medicines according to European directives EUDRALEX are implemented in the Austrian Regulation on Operating Instructions for Medical Products (Arzneimittelbetriebsordnung, AMBO), called 'Austrian GMP Directive'.

— Bundesamt für Sicherheit im Gesundheitswesen (BASG): it issues manufacturing licenses, market authorizations for drugs, permission of clinical trials, approval of a qualified person for a licensed facility.
— Agentur für Gesundheit und Ernährungssicherheit (AGES): approval of facilities for manufacturing licenses and pharmaceutical inspections.

(a) Unlicensed radiopharmaceuticals extemporaneously prepared, not for clinical trials

For a magistral preparation of a radiopharmaceutical under the prescription of a nuclear physician and direct, immediate use (in-house only) the hospital radiopharmacy is exempted from a manufacturing licence. However, every preparation must fulfil pharmacy practices and the Pharmacopeia. If an extemporaneous preparation is delivered outside a manufacturing licence and full compliance with GMP is needed.

(b) Radiopharmaceuticals to be used in clinical trials

If a radiopharmaceutical is prepared as an Investigational Medicinal Product (IMP), a manufacturing licence is necessary with the release of a Qualified Person (QP). Since the EU Clinical Trials Regulations 2014 are implemented, clinical trials of diagnostic radiopharmaceuticals in hospitals are exempt from this requirement, but an IMP dossier is mandatory. The radiopharmaceutical and the trial protocol have to be evaluated by local Ethic Committees and approved by competent authority BASG.

(c) Radiopharmaceuticals with marketing authorization

Radiopharmaceuticals with marketing authorization must be prepared under a manufacturing licence with full GMP, including the release by a QP. In Austria, only PET radiopharmaceuticals e.g. [^{18}F] FDG, [^{18}F]DOPA, [^{18}F] fluoromethylcholine, [^{18}F] NaF, [^{18}F] florbetaben, [^{18}F] flutemetamol are manufactured under a marketing authorisation, which is issued by the European Medicines Agency (EMA) or by the competent authority BASG. If a radiopharmaceutical is described in a monograph of the European Pharmacopeia but not frequently used the competent authority, BASG, can decide after a request that no marketing authorisation for a commercial supply in Austria is required.

IV-1.9.3. Quality control frequency, tests and the qualified person

All preparations of radiopharmaceuticals (licensed radiopharmaceuticals, radiopharmaceuticals used in clinical trials, or extemporaneously prepared radiopharmaceuticals) must be performed by the Pharmacopoeia with QC on every batch. Particular attention should take for a routine test of the microbiological purity. Preparations from licensed generators and licensed kits using closed procedures under aseptic conditions do not require full QC as long as they are carried out according to the SPC. Extemporaneous preparations in hospitals can be released by a radiopharmacist or by a nuclear medicine physician. Radiopharmaceuticals that are prepared under a manufacturing licence (licensed radiopharmaceuticals or for clinical trials), the release must be performed by a certificated QP. The competent authority awards the certificate to a pharmacist who has two years of practice in a GMP laboratory. Then a holder of the QP certificate is authorized to release all kinds of pharmaceuticals. Physicians, veterinarians, chemists, and biologists can also perform the function of a QP when they complete the post gradual course ‘Pharmaceutical Quality Management’ at the University of Vienna.

IV-1.10. Italy

IV-1.10.1. Nuclear medicine specifications

Currently, there are over 250 nuclear medicine departments in Italy, for a population of 60 million, where around 65% of them are in public hospitals, while the others are in private institutions. Approximately 50% of NM departments include PET/CT scanners. Classic nuclear medicine examinations are 650 000 a year, while ~200 000 PET a year are performed. Over 50% of the nuclear medicine departments are engaged in the therapy with radiopharmaceuticals, although most of them make use of commercially available, ‘ready-to-use’ radiopharmaceuticals, and only a few centres are involved in their preparation ‘on site’. About

35% of SPECT procedures are bone scintigraphy, followed by a 25% of myocardial perfusion imaging. ^{99m}Tc labelled radiopharmaceuticals are prepared within nuclear medicine departments by specifically trained technicians, under the responsibility of the nuclear medicine doctor. PET scans are most often (>95%) performed in the field of oncology with most of these utilising [^{18}F] FDG; remaining PET examinations are represented by myocardial perfusion studies with [^{13}N] NH_3, and PET brain scans again with [^{18}F] FDG and, more recently with the 'new wave' of radiopharmaceuticals for β-amyloid plaque imaging. An increasing number of Institutes (about 20, to date) are currently equipped for the preparation of ^{68}Ga labelled peptides, in particular [^{68}Ga] DOTATOC. PET radiopharmaceuticals, including those prepared using generator produced radionuclides, may be prepared, for internal use, by nuclear medicine radiopharmacies. However, due to their enhanced complexity, preparations are performed under the responsibility of highly trained personnel. There are 38 cyclotrons in Italy, 26 are included in public or private hospitals, while 12 are owned and operated by commercial manufacturers. Due to specific national legislation, it is currently allowed in Italy for hospital radiopharmacies to prepare and distribute [^{18}F]FDG [IV-26]. Although, a significant proportion of the overall [^{18}F] FDG doses (ca 70%) are distributed by commercial manufacturers, which are based in 12 production sites located throughout the country, with the capability to cover most of the Italian regions.

IV-1.10.2. Legislation and regulatory authorities

The European Union pharmaceutical legislation apply to the manufacturing and quality control of licensed radiopharmaceuticals[a] . In particular, Directive 2001/83/EC set the standard format for documentation, including chapters dedicated to the QC of both Drug Substance (or active substance) and Drug Product (or finished product). The preparation of radiopharmaceuticals in Nuclear Medicine or University hospital departments is regulated by specific local rules, named 'Norme di Buona Preparazione dei Radiofarmaci in Medicina Nucleare' (NBP-MN), which are based on the same general principles of GMP but with adaptations to the specific case of radiopharmaceuticals[b]. NBP-MN apply to the preparation of radiopharmaceuticals for internal use only, and they are also suitable for the preparation of investigational radiopharmaceuticals, provided that they are used in non-profit clinical trials. Although legislation will be adapted after the new EU 'Regulation on clinical trials on medicinal products for human use' n. 536/2014 will finally become applicable, which is currently expected by the year 2018. NBP-MN rules have been approved in 2005, but their practical application started in 2011. There are not specific requirements for education and training of personnel, including responsibility for release, except for a general statement on adequate training and scientific expertise. Most of those responsible for release are pharmacists, but also chemists, biologists and even nuclear medicine doctors are appointed. The manipulation of unsealed radioactive source is also subject to radiation protection legislation, and the EU directives 89/618/Euratom, 90/641/Euratom, 92/3/Euratom and 96/29/Euratom, updated by directive 2013/59/Euratom, have been translated into national legislation through the Decree Law n. 230/1995, which still provides the general

[a] A list of the concerned EU Directives may be found at http://ec.europa.eu/health/documents/eudralex/vol-1/index_en.htm

[b] It can be found at (https://www.aimn.it/lex/NBP_Radiofarmaci.pdf)

framework for all the aspects concerned with radioactivity manipulation and radiation protection, including authorization procedures that are, in turn, divided into two main categories:

- Cat. A authorization, which is required for facilities hosting particle accelerators such as cyclotrons, and include Category B authorization;
- Cat. B authorization, which is of concern for nuclear medicine departments.

The Institutions involved in the handling of radioactivity and radiopharmaceuticals preparation are:

- 'Istituto Superiore per la Protezione e la Ricerca Ambientale I.S.P.R.A.' (National Institute for research and environmental protection), to which the request for Category A authorization should be addressed; this kind of licence is usually released after the documentation of the Applicant is assessed and Site inspected, while other Ministries are involved in the Cat. A authorization, such as Ministry of Health for the evaluation of radiation impact on population, and the Home Fire Protection Department for the general safety concerns. ISPRA, together with other Ministries, is also involved in the release of the authorization necessary to transport radioactive materials, including radiopharmaceuticals; moreover, a periodic report of the transport, with data related to the amount of radioactivity transported, and details of the transport themselves (date, time, route, means of transport) have to be sent to ISPRA by the interested carriers;
- Local commissions on radioprotection, which are responsible for Category B authorization release;
- 'Agenzia Italiana del Farmaco A.I.F.A.' (Italian Medicine Agency), to which request for marketing authorization (MA) have to be submitted and, following EU legislation, release MA after production site inspection and dossier assessment. AIFA is also responsible for the evaluation of Investigational Medicinal Product Dossiers (IMPDs) submitted in case of (radio)pharmaceuticals are to be used in clinical trials;
- 'Istituto Superiore di Sanità I.S.S.' (National Institute of Health): for phase I clinical trials, AIFA outsources the dossiers assessment to ISS, although it keeps the full responsibility for authorization;
- Commercial manufacturers have also to deal with local chambers of commerce, for specific commercial licence required in order to put radiopharmaceutical (and also precursors for radiolabelling) on the market.

From a regulatory point of view, three types of radiopharmaceuticals are considered:

(a) Radiopharmaceuticals extemporaneously prepared, not for clinical trials

They include PET, SPECT and also therapeutic radiopharmaceuticals, that may be prepared, as stated above, in nuclear medicine departments under the umbrella of national rules NBP-MN.

(b) Radiopharmaceuticals for diagnostic/therapeutic to be used in clinical trials

In this case, the radiopharmaceutical is often (but not always, the RP status being dependent on several factors) considered an Investigational Medicinal Product (IMP); preparation and quality assurance aspects are covered, again, by national rules NBP-MN, provided that the radiopharmaceuticals are used in not-for-profit studies. In case of investigational radiopharmaceuticals, compliance with parts of Annex 13 of GMP, such as the need for a Product Specification File (PSF), is also requested. In case of sponsored clinical trials, standard EU legislation apply, and manufacturing must be performed under GMP. Notwithstanding for the clinical trial status, an IMP Dossier is required and the radiopharmaceutical and the trial protocol must be evaluated by local Ethic Committees.

(c) Radiopharmaceuticals with marketing authorization

In this case, depending on the desired authorization pathway (e.g. whether MA is requested for the country of concern only or for the whole EU through a centralized procedure) the commercialization of radiopharmaceuticals require a Marketing Authorization, which is issued by AIFA or EMA, and is manufactured by a radiopharmaceutical company under full GMP, including release by a QP. This category would include, amongst others, ^{99}Mo/^{99m}Tc generators, kits for labelling with ^{99m}Tc, PET RPs, etc. A list of the PET radiopharmaceuticals with a MA in Italy is presented in Table IV-4.

TABLE IV.3. PET-RADIOPHARMACEUTICALS WITH A MA IN ITALY

DCI	TRADENAME (MA HOLDER)
[^{18}F] FDG	EFDEGE (IASON GMBH)
[^{18}F] FDG	STERIPET (GE HEALTHCARE S.R.L.)
[^{18}F]FDG	FLUODEOSSIGLUCOSIO [^{18}F] IBA (IBA PHARMA S.A.)
[^{18}F]FDG	GLUCOMPET (A.C.O.M. - ADVANCED CENTER ONCOLOGY MACERATA - S.P.A.)
[^{18}F]FDG	GLUSCAN (ADVANCED ACCELERATOR APPLICATIONS)
[^{18}F]FLUORODOPA	FLUORODOPA CIS BIO (IBA MOLECULAR ITALY S.R.L.)
[^{18}F]FLUORODOPA	FLUORODOPA (IASON IASON GMBH)
[^{18}F]fluorbetapir	AMYVID (ELI LILLY NEDERLAND B.V.)
[^{18}F]fluorbetaben	NEURACEQ (TEGELER DEUTSCHLAND)
[^{18}F]flutemetamol	VIZAMYL (GE HEALTHCARE LTD)
[^{18}F]fluorocholine	PCOLINA (IASON GMBH)
[^{18}F]sodium fluoride	IASONFLUORIDE (IASON GMBH)
^{68}Ge/^{68}Ga generator	GERMANIO CLORURO (^{68}Ge)/GALLIO CLORURO (^{68}Ga) GALLIAPHARM (ECKERT & ZIEGLER RADIOPH GMBH)

Besides the PET radiopharmaceuticals listed above, another most popular SPECT radiopharmaceuticals, such as ^{99}Mo/^{99m}Tc and ^{68}Ge/^{68}Ga generators, and kit for most of the typical SPECT diagnostic imaging agents, have a MA, as well as a few RPs labelled with ^{123}I and ^{111}In. Finally, licensed therapeutic radiopharmaceuticals such as ^{131}I labelled capsules, ^{90}Y

labelled Zevalin™, Xofigo™ ([^{223}Ra]$RaCl_2$), other ^{90}Y labelled radiopharmaceuticals and more, are available on the Italian market.

IV-1.10.3. Quality control frequency, tests and the qualified person

National rules NBP-MN do not state for specific educational requirements for QC personnel, including QC responsibility, except for a general statement on adequate training and scientific expertise. Briefly, the NBP-MN chapter dedicated to quality control states that:

- QCs are required on every batch, before release, except for tests that may be completed post-release (e.g. sterility and radionuclidic purity);
- Specifications, limits and acceptance criteria should be set for each tested parameter;
- QC testing programme should be summarized in a general document and SOPs describing analytical methods and use, calibration and maintenance of the equipment should be in place;
- Personnel involved in QC should be different from that involved in preparation of RPs;
- Results should be summarized in a suitable certificate of analysis (CoA);
- Where applicable, 'in-process' controls should be performed;
- QC tests whose duration is not compatible with the radionuclide half-life, may be performed after release (see above);
- Retention samples should be collected for every batch;
- In case of investigational radiopharmaceuticals, it is acceptable a partial validation, provided that at least LOQ and specificity are addressed;
- Out of specifications (OOS) should be carefully investigated and a decision should be taken by the responsible person;
- Specific QC on ^{99}Mo/^{99m}Tc generators (once per generator) are stated: i) elution yield, ii) ^{99}Mo breakthrough, iii) aluminium ions.

Specifications and acceptance criteria for licensed radiopharmaceuticals (e.g.^{99m}Tc labelled kit) are defined by the respective 'Summary of Product Characteristics' (SPC) or Ph. Eur. monographs, while for extemporaneous preparations they may be found in dedicated or general European Pharmacopoeia monographs (e.g. Monograph n. 0125 Radiopharmaceutical Preparations). In case a monograph is not available, other sources such as relevant international guidelines (e.g. EANM, IAEA) or scientific literature may be of help in defining specifications. Analytical methods should be validated:

i) In case an Ph. Eur. monograph is not available;
ii) In case of newly developed methods;
iii) When methods or equipment undergo significant changes, or;
iv) If analytical result trends prompt for possible method failure/problems.

As for the facilities, no special requirements are set for the QC labs; no classification following Annex 1 – GMP is required, and only general principles of radiation protection apply. Finally, QC equipment dedicated to the routine QC of radiopharmaceuticals should be qualified following general principles described in Annex 15 – GMP (IQ, OQ, PQ). Typically, QC for

^{99m}Tc labelled kits include pH measurement, visual inspection and radiochemical purity (using radio-TLC); QC on ^{99m}Tc/^{99}Mo generators include early radionuclidic purity test to check for ^{99}Mo breakthrough, elution yield and aluminium ions. QC on PET radiopharmaceuticals, and more generally on extemporaneous preparations, are more complex and, in addition to the previously cited test, also radiochemical purity with HPLC, radionuclidic purity with NaI(Tl) or (preferably) HPGe detectors, residual solvent, as well as endotoxin content and sterility, are assessed.

IV-2. NORTH AMERICA

IV-2.1 USA

IV-2.1.1. Nuclear medicine specifications

The current USA population is estimated at 324 million, according to the United States Census Bureau. Over 17 million nuclear medicine procedures are performed in the United States every year, with nuclear cardiology procedures accounting for more than 50% of those procedures. More than 1.5 million PET procedures are performed in the US each year, with more than 90% of PET and PET/CT procedures performed are for cancer diagnosis applications. There are approximately 1600 PET imaging facilities in the United States, with more than 84% of the facilities having PET/CT systems [IV-27].

IV-2.1.2. Legislation and regulatory authorities

Similar to the traditional pharmaceutical manufacturing, radiopharmaceutical production in the United States is federally regulated and the standards are enforced by the United States Food and Drug Administration.

(a) Kit or generator based radiopharmaceuticals extemporaneously prepared

Preparation of these types of radiopharmaceuticals is normally performed under the supervision of a state-licensed nuclear pharmacist, using FDA approved components (i.e. kits), in a state registered nuclear pharmacy. The process usually involves a simple mixing of the kit reagents and the radionuclide. It is important to note that even though the radiolabelling process falls under the practice of pharmacy, the kit used for radiolabelling must be FDA approved (i.e. requires marketing authorization). Furthermore, a pharmacist must follow FDA approved radiolabelling and QC testing protocol described by kit official documentation. Because the practice of radiolabelling kits falls under the practice of nuclear pharmacy compounding, local state boards of pharmacy may also inspect pharmacies performing nuclear pharmacy compounding. Preparation of FDA approved kit components (i.e. the radionuclide or radionuclide generator, the precursor, and drug components) is regulated by the FDA issued United States Code of Federal Regulations [IV-28]. These regulations are the same as for non-radioactive pharmaceutical preparations.). These regulations are the same as for non-radioactive pharmaceutical preparations.

(b) Radiopharmaceuticals to be used in clinical trials

Manufacture of Positron Emission Tomography (PET) radiopharmaceuticals used for FDA approved diagnostic indications are regulated by PET drug class specific regulation called '21CFR 212 Current Good Manufacturing Practice for Positron Emission Tomography Drugs', recognizing the special nature of these agents. These agents require preparation from raw materials – a complex process that involves radionuclide production, incorporation, drug purification, and formulation. This type of radiopharmaceutical preparation falls under the practice of manufacturing and is governed entirely by the FDA. The exact QC testing requirements for these agents are intentionally not defined in detail in order to provide flexibility to the manufacturers of different PET drugs. The regulation currently affects manufacture of PET agents requiring a marketing authorization: [^{18}F]FDG, [^{18}F]NaF, [^{18}F]Fluciclovine, [^{13}N]NH_3, [^{11}C]choline, [^{68}Ga]DOTATATE, [^{18}F]Florbetapir, [^{18}F]Florbetaben and [^{18}F]Flutemetamol. The regulation does specify that QC has to be performed for materials acceptance, in-process if applicable, and on the final drug product. It also requires that the QC methods be validated for accuracy, sensitivity, precision, and specificity.

Similar to manufacture of the kits that are subsequently radiolabelled in nuclear pharmacies, manufacture of FDA approved radiopharmaceuticals with relatively long half-lives (mostly radiotherapy pharmaceuticals such as [^{223}Ra]Radium Chloride or[^{131}I]Sodium Iodide) is governed by the FDA- issued United States Code of Federal Regulations [IV-28]. Usually, a central manufacturing facility makes a large batch of radiopharmaceuticals, and distributes smaller sub-batches to local nuclear pharmacies. Patient unit dose preparation and dispensing are then performed under the practice of nuclear pharmacy compounding.). Usually, a central manufacturing facility makes a large batch of radiopharmaceuticals, and distributes smaller sub-batches to local nuclear pharmacies. Patient unit dose preparation and dispensing are then performed under the practice of nuclear pharmacy compounding.

(c) Radiopharmaceuticals with marketing authorization

The manufacture of radiopharmaceuticals produced for clinical trial investigations is governed by the FDA regulation titled '21 CFR 312 Investigational New Drug Application'. The regulation requires investigators to perform studies to gather enough data in order to reasonably estimate the expected agent safety and behaviour in human subjects, as well as to establish agent manufacturing controls that allow the investigators to reliably produce the investigational agent of acceptable quality. The obtained data is then compiled into a document called the Investigational New Drug Application, or IND. The IND application is submitted to the FDA for a 30-day review period. During the 30-day review period, FDA and the investigators communicate in order to address any deficiencies or concerns related to the IND application. If no major deficiencies exist at the end of the 30-day review period, the FDA allows the investigators to proceed with the clinical trial. If deficiencies or concerns from the FDA do exist at the end of the 30-day review period, the FDA may put the application on hold until the concerns are addressed by the investigators.

The exact QC requirements for new investigational agents are intentionally not clearly defined in the regulations. Instead, the QC testing commitments, as well as the drug acceptance criteria,

are described in the section of the IND application called 'Chemistry, Manufacturing, and Controls', or CMC. The QC control testing proposed by the subject-matter experts in the CMC is based on the preclinical and agent manufacturing development data. The FDA reviews the proposal during the review period. The final QC testing is based on the consensus between the investigators and the regulatory agency. This mechanism offers many advantages, main one being the flexibility that allows QC testing to be made specific to a specific agent. Investigational radiopharmaceuticals come in many different forms: different radionuclides, different carrier molecules, different formulation, different stability profiles, and different routes of administration. Due to this variability, each investigational agent may require a different set of QC tests. Ability to establish agent specific QC testing requirements addresses this challenge. Furthermore, QC specifications and the analytical methods that are initially set during first-in-human investigations may change as additional drug manufacturing data is obtained and the agent is better characterized. Relying on the CMC mechanism, these changes can be easily implemented via submission of a CMC amendment to the FDA.

IV-2.1.3. Quality control frequency, tests and the qualified person

Every batch of radiopharmaceutical manufactured for human use must undergo radiopharmaceutical QC testing specific to the drug. Even though QC testing varies depending on the radiopharmaceutical being tested, in general, tests such as radiochemical purity and identity, chemical impurities (i.e. residual solvents and K-222), radionuclidic identity, endotoxin content, visual inspection, pH, bubble point, and radioactivity measurement should be performed. Sterility testing is preformed post release, and must be performed on every batch. Periodically additional QC testing in the form of radionuclidic purity determination (i.e. trace radionuclide analysis gamma spectroscopy) and osmolality determination may be performed. Specific activity determination (applicable mostly to agents used for neurology applications) may also need to be determined, but only if the agent localization kinetics may be affected by the specific activity. Lastly, radiolabelled antibody immunoreactivity determination is performed to make sure that the antibody has not lost its ability to bind to the antigen due to modification process (i.e. conjugation and subsequent radionuclide incorporation).

The role of the person responsible for quality (also known as a qualified person in the EU) varies depending on the type of the radiopharmaceutical being produced and the manufacturing facility setting. Also, unlike some European countries where professional training such as being a pharmacist is a requirement to become a QP, there are no defined professional training requirements that exist in the US. Rather, the focus is made on responsible person's training and expertise in the area. In nuclear pharmacies, pharmacists are normally responsible for the quality assurance of the radiopharmaceuticals being produced. In PET drug producing facilities, trained radiopharmacists are also normally responsible for manufacturing quality assurance. In non-PET radiopharmaceutical manufacturing facilities, a radiochemist, a medical physicist, or a radiopharmacist usually functions as a QA responsible person.

A special consideration should be made with regards to training qualified persons involved in quality assurance of agents used in clinical trials, especially in the first-in-human trials. Agents manufactured for routine clinical normally used have established standards which are based on a large amount of collected manufacturing data. The role of the qualified person in this case is to assure adherence to these established standards. Investigational agents, on the other hand,

may not have these standards due to lack of sufficient supporting data as well as the unique nature of these new agents. The responsibilities of qualified persons, in this case, are expanded to include analytical method development and to establish new agent acceptance specifications. The amount and the type of QC testing should be sufficient to reasonably demonstrate that the quality of the manufactured agent will not cause unnecessary harm to the patient and will allow for accurate evaluation of the investigational agent. Deciding on how much QC testing is sufficient is a critical function for a qualified person involved in investigational agent manufacture. On one hand, insufficient QC may increase the risk of patient harm or negatively affect the accuracy of estimating agent's efficacy. On the other hand, performing redundant QC testing that is not supported by sound scientific evidence available at the time will require unnecessary additional time and resources, which may make the entire investigation cost prohibitive. The resources may not be available due to limited funding as well as the unwillingness of the investigators to financially invest into agents that statistically have low chance of progressing from clinical trials to marketing authorization. Therefore, qualified persons must be a subject matter expert and must work very closely with the clinicians, researchers, and the regulatory agency experts in order to make an appropriate decision.

IV-2.2 Canada

IV-2.2.1. Nuclear medicine specifications

In Canada, there are ~274 medical facilities performing diagnostic or therapeutic nuclear medicine procedures for a population of 35 million. Most of the nuclear medicine facilities are publicly-funded. There are 478 gamma cameras (264 SPECT and 214 SPECT-CT) and most of the 1 480 000 million nuclear medicine procedures are bone scintigraphy, lung ventilation/perfusion scans, and myocardial perfusion imaging. 60% of the nuclear medicine centres are supplied with radiopharmaceuticals from centralized radiopharmacies, while the others are relying on trained and certified technologists for the preparation of the radiopharmaceuticals. 49 PET scanners (47 PET-CT and 2 PET-MRI) are available in Canada for a total of ~77,000 PET scans annually. PET scans are mostly used for oncologic applications (>98%) and most of them are performed with [^{18}F]FDG. [^{18}F]FDG is available as the following approved products: CanTrace™, FluGlucoScan™, GLUCOVISION™ and GLUDEF™.

IV-2.2.2. Legislation and regulatory authorities

With the exception of Positron Emitting Radiopharmaceuticals (PERs), the preparation of radiopharmaceuticals is considered a compounding activity, which falls under the practice of pharmacy. General chapter <797> of the US Pharmacopeia is defining the standards that apply specifically to compounding of sterile pharmaceuticals, including radiopharmaceuticals. USP<797> covers the requirements in premises, equipment, personnel, aseptic techniques, manufacturing and quality controls. The majority of the radiopharmacies in Canada are still not compliant with USP<797>. Although implementation of USP<797> is seen as a way to improve safety, the regulation authorities and nuclear medicine professionals are still debating on how it could be implement in a cost-effective manner. It is considered that full adoption of USP<797> would have serious consequences on healthcare costs, reducing access and increasing waiting times for procedures; nevertheless, radiopharmacies in Canada have

consistently demonstrated an excellent safety record with respect to product preparation and quality control.

In Canada, regulatory oversight of pharmacy operations is a provincial responsibility. However, to date, professional pharmacy associations in Canada have not included the preparation of radiopharmaceuticals as part of their regulatory oversight, and Health Canada has yet to step into this role. This may be due to their limited expertise in radiation safety and radiopharmaceutical quality assurance.

The following agencies regulate the practice of radiopharmacy and nuclear medicine in Canada:

- Health Canada is the Medicines and Healthcare Products Regulatory Agency, which is responsible for the approval of drugs and to maximize their safety and efficacy;
- Canadian Nuclear Safety Commission (CNSC) provides regulations and guidance documents related to radiation safety requirements.

From a regulatory point of view, three types of radiopharmaceuticals are considered:

(a) Radiopharmaceuticals extemporaneously prepared, not for clinical trials:

The extemporaneous preparation of radiopharmaceuticals is considered a compounding activity, which falls under the practice of pharmacy. Thus, this activity is regulated by the General chapter <797> of the US Pharmacopeia. Complementary information is provided by Health Canada in the Annex 3 of the Current Edition of the Good Manufacturing Practices Guidelines – Schedule C Drugs (GUI-0026).

(b) Preparation of PET Radiopharmaceuticals

Preparation of Positron Emitting Radiopharmaceuticals (PERs) is an Establishment License activity in Canada, and therefore it falls under GMP guidelines, rather than compounding. Production of PERs is covered by the following documents: Good Manufacturing Practices (GMP) Guidelines (GUI-0001), Good Manufacturing Practices for Positron Emitting Radiopharmaceuticals (Guide-0071) and Guidance on Drug Establishment Licenses and Drug Establishment Licensing Fees (GUI-0002). Manufacturing sites with a Drug Establishment License undergo periodic inspections by Health Canada.

(c) Radiopharmaceuticals to be used in clinical trials

In this case, the radiopharmaceutical is considered an Investigational Medicinal Product (IMP). An annex (GUI-0036) of the Good Manufacturing Practices Guidelines is specifically referring to the drugs used in clinical trials. For PERs, additional documentation is available: a) Guidance Policy on the Use of PERs in Basic Research (Policy-0053), and b) a Guidance Document on the Preparation of Applications for Authorization of PERs for use in Basic Clinical Research Studies.

IV-2.2.3. Quality control frequency, tests and the qualified person

All radiopharmaceuticals (extemporaneous preparation, PERs, radiopharmaceuticals in clinical trials) for human applications must undergo QC testing on every batch. While special attention on microbiological purity (endotoxin content and sterility assessments) is required, pre- and post-release QC tests (radionuclide half-life dependent) often include: pH measurement, visual inspection, radiochemical and nucleic purity determination and identity, radioactive concentration determination, residual impurities measurements (especially residual solvent testing). Specifications, limits and acceptance criteria for each tested parameter can be found in Pharmacopoeia monographs or manufacturer's instructions. However, if such document isn't available, they can be set by the person responsible for quality based on the knowledge of the manufacturing process.

The role of the person responsible for quality varies depending on the type of the radiopharmaceutical being produced and the manufacturing facility setting. The extemporaneous preparation of radiopharmaceuticals is an activity that falls under the 'practice of medicine', with the Nuclear Medicine Physician ultimately responsible for the quality of the radiopharmaceutical. However, if the radiopharmaceuticals are under a Drug Establishment License (e.g. preparation by centralized radiopharmacies, PERs and IMPs), then operation must be supervised by a qualified radiopharmacist trained in Good Manufacturing Practices. Release must be performed by the radiopharmacist.

IV-3. SOUTH AMERICA

IV-3.1 Brazil

IV-3.1.1. Nuclear medicine specifications

Brazil operates about 380 nuclear medicine departments. Most of them are private institutions (about 95%) and equipped with a radiopharmacy dedicated at least to the preparation of ^{99m}Tc labelled radiopharmaceuticals. About 300 gamma cameras, 100 SPECT tomographs and over 112 PET tomographs are installed over the territory, for about 2 300 000 scans per year (90% SPECT, 10% PET). For SPECT, bone scintigraphy (using for example [^{99m}Tc]MDP, and cardiac scintigraphy (using for example [^{99m}Tc]MIBI), represent the majority of the examinations (over 90%). PET-scans are most often performed with the ^{18}F labelled radiopharmaceutical [^{18}F]FDG (>95%) and particularly in the field of oncology (metastasis and secondary tumour detection and staging, therapeutic efficacy). For [^{18}F]FDG, there are around 12 manufacturing sites geographically distributed over the Brazilian territory. Additionally, a few academic research centres (Clinics Hospital of the University of São Paulo in São Paulo, Institute for Brain Studies in Porto Alegre – South of Brazil) are also equipped with (or have direct access to) a cyclotron, producing thus other ^{18}F labelled molecules as well as ^{11}C labelled ones. Some facilities equipped with ^{68}Ge/^{68}Ga generators have started with the preparation of ^{68}Ga labelled radiopharmaceuticals ([^{68}Ga] DOTATATE for example, that is used for diagnostic of neuroendocrine tumours).

IV-3.1.2. Legislation and regulatory authorities

The directives of the National Health Surveillance Agency (ANVISA) are applied to the manufacturing and quality control of radiopharmaceuticals in Brazil. As such, the whole arsenal of Brazilian pharmacopeia (general and specific monographs), ANVISA directives and regulations but also guidelines and guidance are applicable for control and application of radiopharmaceuticals in nuclear medicine, taking into account both radiation protection aspects and pharmaceutical aspects:

(a) 'Comissão Nacional de Energia Nuclear CNEN' (National Nuclear Energy Commission)

It is the nuclear safety agency in charge of nuclear safety and radiation protection aspects linked to radiopharmaceutical manufacturing and radiation management:

Directive CNEN-NN-3.01: Basic Guidelines for Radiation Protection; establishes the requirements for radiation protection from individual exposure to ionizing radiation.

Directive CNEN-NN 6.02: Licensing radioactive facilities; provides licence for radioactive facilities using sealed sources, non-sealed sources of ionizing radiation generating equipment and radioactive facilities to produce radioisotopes, either by cyclotron-produced or reactor-produced radionuclides.

(b) 'Agência Nacional de Vigilância Sanitária National ANVISA' (Health Surveillance Agency national agency for the security of the drugs and health products)

It is responsible for the control and authorization for the usage and commercialization of radiopharmaceuticals [IV-29]. It plays a key role in the regulation of the radiopharmaceutical market in Brazil by analysing and reviewing all 'Investigational Medicinal Product dossiers *(*IMPD)'. This dossier includes information related to chemical and pharmaceutical quality (the radiopharmaceutical and its preparation / QC related assays), as well as non-clinical data related to pharmacology, pharmacokinetics, dosimetry and toxicology, and finally description of the clinical trial.

Directive 2010/17: Good Manufacturing Practices for Pharmaceuticals; drug manufacturers must comply with this directive in all operations involved in the manufacture of pharmaceuticals, including drugs for clinical trials.

Directive 2009/63: Good Manufacturing Practices for Radiopharmaceuticals; applies to the preparation of radiopharmaceuticals in hospital radiopharmacies, centralized radiopharmacies, radiopharmaceutical manufacturing, preparation and production of radiopharmaceuticals in PET Centres. The compliance with Directive 2010/17 is also required for radiopharmaceuticals when applicable.

Directive 2009/64: Registration of Radiopharmaceuticals; This directive aims to establish minimum requirements for radiopharmaceuticals' registration in Brazil to ensure their quality, safety and efficacy. It applies to: ready-to-use radiopharmaceuticals; non-radioactive

components for labelling with a radionuclide; radionuclides, including radionuclide generators. There are three situations for radiopharmaceutical registration:

(i) New or innovative radiopharmaceutical: it is composed of at least one active drug, being the first to describe a new mechanism of action or the first to have proven efficacy, safety and quality at ANVISA;
(ii) Radiopharmaceutical: it contains the same active ingredients, has the same concentration, dosage form, route of administration, dosage and therapeutic indication of the new or innovative radiopharmaceutical, or of the established use radiopharmaceutical registered at ANVISA;
(iii) It may only differ in characteristics related to size and shape of product, shelf life, packaging, labelling, excipients and vehicle; it must always be identified by trade name or brand;
(iv) Radiopharmaceutical of established use: new or innovative radiopharmaceutical that is commercialized a long time in the country, and has several published studies that proves its efficacy and safety.

TABLE IV-4. LICENSED RADIOPHARMACEUTICALS IN BRAZIL (data on May 2016)

DCI	TRADENAME	MA HOLDER	COMMERCIALIZED IN BRAZIL
[18F]Fludeoxyglucose	Radioglic®	CDTN-CNEN	CDTN-CNEN
[Ra 223]Radium Dichloride	Xofigo®	Bayer HealthCare	Bayer HealthCare

Directive 2008/38: Installation and Operation of Nuclear Medicine Services in vivo (including Hospital Radiopharmacy); it establishes the requirements and sanitary control parameters for the operation of nuclear medicine services aiming to protect patients' health, involved professionals and the public. Every nuclear medicine service must have an operating authorization issued by the National Nuclear Energy Commission and be licensed by the local health authority.

(c) National Health Surveillance Agency (ANVISA) and regional health agencies

In charge of inspections of radiopharmaceutical commercial manufacturers (GMP certification) and hospital radiopharmacies (non-commercial sites) where radiopharmaceuticals are prepared, manipulated and dispensed.

IV-3.1.3. Quality control frequency, tests and the qualified person

For all radiopharmaceuticals (licensed radiopharmaceuticals, radiopharmaceuticals used in clinical trials, or extemporaneously prepared radiopharmaceuticals not for clinical trials), QC are required on every batch. QC most often comprises pre- and post-release tests (radionuclide half-life dependent) and includes beside pH measurement and visual inspection, at least radiochemical and radionuclidic purity determination and identity, radioactive concentration determination, residual impurities measurements (especially residual solvent testing) as well as

endotoxin content and sterility testing. Specifications, limits and acceptance criteria should be set for each tested parameter. The qualified person (QP) in Brazil in charge of all manufacturing aspects thus including QC is the radiopharmacist.

IV-4. ASIA AND RUSSIA

IV-4.1 Russia

IV-4.1.1. Nuclear medicine specifications

There are ~ 220 nuclear medicine departments in the Russian Federation, most of them are located in public hospitals. The list of diagnostic equipment includes ~ 250 gamma cameras (100 are planar), some of them are very old (from 1980s). In recent years, 100 SPECT cameras and 50 SPECT/CT have been installed. Most of the diagnostic procedures are based on ^{99m}Tc labelled radiopharmaceuticals. The ^{99}Mo/^{99m}Tc generators are available from two Russian manufacturers; the production facilities are reconstructed for GMP standards in 2015. Central processing extraction-type generators for ^{99m}Tc are intensively used in St. Petersburg and Tomsk (Siberia). The reagent kits for ^{99m}Tc labelled radiopharmaceuticals are produced by the Russian manufacturer 'Diamed', Moscow, according to GMP requirements. A number of ^{123}I-based radiopharmaceuticals are available including sodium [^{123}I]iodide (caps and i.v. solution), sodium *o*-[^{123}I]iodohippurate, [^{123}I]MIBG, [^{123}I]BMIPP fatty acid. Other SPECT agents such as [^{67}Ga]$GaCl_3$, [^{111}In]$InCl_3$ (for diagnostic use only) and [^{201}Tl]TlCl have found limited application. For SPECT and planar scintigraphy the majority of examinations are presented by kidney function (40%) following bone scans (30%) and thyroid (13%).

PET studies were started in 1991 in St. Petersburg and later in Moscow with proven clinical impact, especially in oncology. At present PET/CT is recognized by the Russian Ministry of Health, Rosatom and Rosnano State corporations along with other government organizations as the most significant nuclear imaging modality. Within the State Oncology program of 2009 to 2013 a number of PET state projects have been initiated and supported by the government. They are usually located in and constitute part of the base infrastructure in regional oncological centres (Cheljabinsk, Magnitogorsk, Hanti-Mansisk, Chabarovsk, Kazan). At present 17 PET/cyclotrons facilities and 12 PET units are functioning with over 45 PET and PET/CT camera under operation. Two centralized production facilities (partnership between government and private company) supplied [^{18}F]FDG on commercial basis. PET-scans are most often performed with the ^{18}F labelled radiopharmaceutical [^{18}F]FDG and particularly in the field of oncology and neurology with a limited application in cardiac PET. In addition to [^{18}F]FDG, several radiotracers such as L-[methyl-^{11}C]methionine, N-[methyl-^{11}C]choline, [^{11}C]butyrate, [^{18}F]FLT, [^{18}F]FET, [^{18}F] MISO, [^{13}N]NH_3, [^{15}O]H_2O are used in different PET centres. Recently ^{68}Ge/^{68}Ga generator (ZAO "Cyclotron", Obninsk) has been registered in Russia as a medical device following introduction of [^{68}Ga] DOTATATE as a first peptide-based radiotracer. Also, ^{82}Sr/^{82}Rb generator is available from Russian manufacturers but has not find wide interest.

IV-4.1.2. Legislation and regulatory authorities

Production of radiopharmaceuticals in Russia is governed by Federal low №61-FL, 12.04.2010 (Drug Law). In addition, the requirements for setting up the process of production and quality control of pharmaceuticals (cGMP equivalent) were issued by the Ministry of Industry and Trade in 2013 and are regulating the production process. In 2015, the Directive for extemporaneous preparation of radiopharmaceuticals (on site preparation without marketing authorization) was issued by the Ministry of Health. The latest XIII edition of the State Pharmacopoeia of the Russian Federation includes the revised version of General Monograph on "Radiopharmaceutical Preparations". There are no individual monographs for radiopharmaceuticals or radioactive precursors and there is no working group for the preparation of these kinds of individual monographs. The work on elaborating the first individual monograph on [^{18}F]FDG has started. The monograph is closely associated with the Ph. Eur. 8.0. Fludeoxyglucose (^{18}F) injection. The harmonization process between the State Pharmacopoeia of the Russian Federation, the Ph. Eur., and USP was initiated by the Ministry of Health, is now in progress. According to the Order of the Government of the Russian Federation, the activities on the elaboration, revision and adoption of the general chapters and monographs of the State Pharmacopoeia of the Russian Federation are delegated to the Ministry of Health of the Russian Federation. MoH in collaboration with its own scientific related organization and external institutions elaborates new and revises existing monographs. Once a draft monograph or a general chapter is received by the MoH, it is posted on the MoH website for public discussion. The deadline for discussion is 30 days. Afterwards a special working group, the Board of the State Pharmacopoeia, performs the expert evaluation of a general chapter or a monograph taking into account the comments and observations received during the public discussion. The Board consists of scientists, representatives of the pharmaceutical industry, experts of the scientific centres, etc. After the finalization of a monograph or a general chapter, it is included in the current edition of the Russian State Pharmacopoeia.

The following agencies regulate the practice of radiopharmacy and nuclear medicine in Russia:

- MoH (Ministry of Health of Russian Federation) regulates Marketing authorization and elaborates the State Pharmacopoeia of the Russian Federation**;**
- SCEEMP (Scientific Centre for Expert Evaluation of Medicinal Products) regulates Marketing authorization and elaborates the State Pharmacopoeia of the Russian Federation under guidance of the MoH. The SCEEMP is also a Testing Centre for Evaluation of Medicinal Products Quality that is regularly inspected by WHO;
- MIaT (Ministry of Industry and Trade) issues the license for radiopharmaceuticals production and control GMP requirements;
- ROSTECHNADZOR (Federal Environmental, Industrial and Nuclear Supervision Service of Russian Federation) issues permits and licenses for holding and disposing radioactive materials and sources.

From a regulatory point of view, two types of radiopharmaceuticals are considered:

(a) Radiopharmaceuticals extemporaneously prepared

The registration is not required for the Radiopharmaceuticals that are prepared in medicinal organizations in the accordance to the guidelines established by the federal executive organ; directive for Extemporaneous preparation of radiopharmaceuticals in medicinal organizations on-site, issued by Ministry of Health, №211n from 27.04.2015. Every manufacturer elaborates its own 'Drug Individual monograph' with the reference to General Monograph "Radiopharmaceutical preparation" and related Monographs of the State Pharmacopoeia of Russian Federation (Sterility, Bacterial Endotoxin etc.) as well as Registration Dossier. All documentation must be approved by the Ethic committee and Hospital/Institute authorities. Almost all the PET radiopharmaceuticals in Russian Federation are extemporaneously prepared.

(b) Radiopharmaceuticals with marketing authorization

In this case, the radiopharmaceutical has a Marketing Authorization issued by the MoH**.** Every manufacturer elaborates its own 'Drug Individual monograph' with the reference to General Monograph 'Radiopharmaceutical preparation' and related Monographs of the State Pharmacopoeia of Russian Federation (Sterility, Bacterial Endotoxin etc.) as well as Registration Dossier. In this case all the documentation must be approved by MoH after expertise of QC procedures by the experts of the SCEEMP and based on their conclusion. Among PET radiopharmaceuticals only [^{18}F] FDG has marketing authorization (two PET centres in St.-Petersburg and one in Chabarovsk).

IV-4.1.3. Quality control frequency, tests and the qualified person

For radiopharmaceuticals with a marketing authorization or extemporaneously prepared radiopharmaceuticals using open procedures, QC is required on every batch. QC most often comprises pre- and post-release tests (radionuclide half-life dependent) and includes beside pH measurement and visual inspection, at least radiochemical and radionuclidic purity, chemical purity, identity, radioactive concentration determination, residual solvents testing as well as endotoxin content and sterility assessments. For extemporaneously prepared radiopharmaceuticals the endotoxin and sterility tests are usually performed for every 10th batch.

IV-4.2 Turkey

IV-.4.2.1. Legislation and regulatory authorities

In Turkey, hospitals can be divided into two general categories: state-owned and privately operated. Approximately 300 hospitals have nuclear medicine departments and 116 of them PET scan capability. There are ~205 gamma cameras and ~120 PET scanners over Turkey, and in total there are ~230,000 PET scans annually performed. The most common 'classic' nuclear medicine procedures are bone scintigraphy, lung ventilation/perfusion scans, and myocardial perfusion imaging. ^{99m}Tc Radiopharmaceuticals are prepared within nuclear medicine

departments by specifically trained technicians, under the responsibility of the nuclear medicine doctors. There are two 'Molly generator' producers in Turkey: Eczacıbaşı Monrol and Kamrusepa. Eczacıbaşı Monrol is also producing freeze-dried ^{99m}Tc labelled kits (MIBI, MDP, DMSA, DTPA, ECD). In addition, distributor companies must be licensed to import SPECT and therapeutic radiopharmaceuticals.

PET scans are most often (>95%) performed in the field of oncology with most of these utilising [^{18}F]FDG and [^{18}F]NaF. Majority of the cyclotrons (13 cyclotrons in total) are installed in Istanbul, Ankara, İzmir and south of Turkey and run by private companies. Most of [^{18}F]FDG doses are supplied from commercial cyclotrons run by Eczacıbaşı Monrol, Moltek and Medicheck. Additionally, two state-owned university hospitals (Ankara University and Hacettepe University) are in the process of building PET drug (labelled with ^{11}C or ^{18}F) production facilities. These radiopharmaceutical production facilities are expected to become operational in 2017. In the past six years, there also has been a significant and evident increase in the preparation and use of both in-house-produced of PET radiopharmaceuticals, as well as therapeutic radiopharmaceuticals. Currently, there are 25 state and private hospitals that offer ^{68}Ga labelled and ^{177}Lu labelled radiopharmaceuticals for clinical use. Most of these radiopharmaceuticals are produced using cassette-based automated radiopharmaceutical synthesizers.

IV-.4.2.2. Legislation and regulatory authorities

The Turkish regulatory responsibilities in the preparation of radiopharmaceuticals are divided into the pharmaceutical aspects and the radiation protection aspects. Turkish Medicines and Medical Devices Agency (TITCK), a section of the Turkish Ministry of Health, regulates all pharmaceutical manufacturing in Turkey, including manufacturing of radiopharmaceuticals. Pharmaceutical (radiopharmaceutical) licensing procedures are governed by the national 'Regulation on Licensing of Human Medicinal Products' adapted from the European Commission Directive 2001/83/EC for human medicinal products. Pharmaceutical (radiopharmaceutical) manufacturing procedures are governed by the national 'Regulation on Manufacturing Plants of Medicinal Products for Human Use' adapted from European Commission Directive 91/356/EEC and Directive 2001/83/EC. As such, pharmaceuticals (radiopharmaceuticals) must be manufactured under GMP and controlled for quality in compliance with the general and individual monographs of related preparations' directives in the European Pharmacopeia.

The Turkish Atomic Energy Authority (TAEK) regulates radioisotope production, quality control, production scale up, and distribution. Licensure by TAEK serves as authorization for governmental or private bodies or persons who possess, use, import or export, transport, store, and trade both radioactive materials and radiation equipment. TAEK also provides oversight with regards to radiation protection.

Generally, radiopharmaceuticals in hospitals can be prepared by a special manufacturing (or preparing) licence but that allows only an in-house application. The manufacturing licence is issued and inspected by both TITCK and TAEK. Therefore, radiopharmaceutical manufacturers and radiopharmacy laboratories in hospitals have to comply with the regulatory requirements implemented by these agencies.

From a regulatory point of view, three types of radiopharmaceuticals are considered:

(a) Radiopharmaceuticals extemporaneously prepared, not for clinical trials

Very short half-life radiopharmaceuticals need to be prepared extemporaneously 'in house' i.e. in the hospital where they are used shortly after preparation. A major difference to other pharmaceuticals prepared in hospital pharmacies lies in the specific environment, where radioactive drugs must be handled in hospitals where they are prepared under a special manufacturing licence. In addition to a range of ^{99m}Tc labelled products this would include ^{68}Ga and ^{177}Lu labelled peptides or small molecules, and radiopharmaceuticals labelled with ^{18}F.

(b) Radiopharmaceuticals to be used in clinical trials

In this case, the radiopharmaceutical is considered an Investigational Medicinal Product (IMP) and manufacturing must be performed under an IMP licence with release by a Qualified Person (QP); and the radiopharmaceutical and the trial protocol must be evaluated by the local Ethic Committees. Investigational Medicinal Product (IMP) are used in the hospitals without marketing authorization for clinical trials and non-officially approved indications.

(c) Radiopharmaceuticals with marketing authorization

In this case, the radiopharmaceutical has a Marketing Authorization issued by TİTCK and is manufactured by a radiopharmaceutical company (or PET cyclotron unit) under full GMP, including release by a Qualified Person (QP). During marketing authorization of the application process, the national authority (i.e. TİTCK) evaluates the product in terms of its quality, safety and efficacy. This category would include ^{99}Mo/^{99m}Tc generators, kits for labelling with ^{99m}Tc and ^{131}I, and ^{111}In labelled products. PET agents include [^{18}F]FDG, [^{18}F]NaF.

IV-4.2.3. Quality control frequency, tests and the qualified person

Larger state-owned hospitals, especially university hospitals, are better equipped than the private hospitals, when it comes to nuclear medicine applications. Additionally, quality control of radiopharmaceuticals is better controlled in the state-owned hospitals. The manufacturing controls and the quality control testing used in preparation of radiopharmaceuticals are in compliance with both the general and individual monographs and published sources. Radiopharmaceuticals prepared from licensed generators and kits by using closed procedures under aseptic conditions do not require full QC before use as long as this practice has been supported by a risk assessment.

Radiopharmaceuticals used in clinical trials, or extemporaneously prepared radiopharmaceuticals not for clinical trials, full QC is required. QC most often comprises pre- and post-release tests (radionuclide half-life dependent) and includes identification, pH measurement, visual inspection, chemical impurity test, radiochemical purity test, radionuclidic purity, residual impurity test, (especially residual solvent testing) as well as bacterial endotoxin content and sterility assessments. Specifications, limits and acceptance criteria should be set for each tested parameter. These radiopharmaceuticals must be released by a QP under responsibility of the medical doctor.

IV-4.3. India

IV-4.3.1. Nuclear medicine specifications

There are about 220 nuclear medicine centres spread across 42 cities of India which use gamma cameras, SPECT or SPECT/CT systems and about 120 PET centres mainly located in the metropolitan cities of India, catering to a population of about 1.25 billion. The Board of Radioisotope and Technology (BRIT), an independent unit of the Department of Atomic Energy (DAE) under the Government of India was the sole manufacturer of Radiopharmaceuticals in India till recently since its inception in 1989. Six regional centres of BRIT spread across the length and breadth of India serve as centralized units for distribution of various radiopharmaceuticals and cold kits for preparation ^{99m}TC labelled radiopharmaceuticals. ^{99m}Tc still remains the widely used diagnostic radioisotope in India. BRIT supplies ^{90}Mo/^{99m}Tc generators and 15 kit based formulations for preparation of ^{99m}Tc labelled radiopharmaceuticals. Ready to use therapeutic and diagnostic radiopharmaceuticals supplied by BRIT include ^{131}I capsules (both diagnostics and therapeutic doses), [^{131}I]MIBG Injection (both diagnostic and therapeutic), [^{153}Sm]EDTMP Injection, Samarium [^{32}P]phosphate colloidal injection, [^{14}C]urea capsules, [^{177}Lu]$LuCl_3$ for preparation of [^{177}Lu]DOTATATE etc. The first medical cyclotron (16 MeV) of India set up by DAE at the Radiation Medicine Centre, Mumbai in October 2002, is operated by BRIT. ~1 Ci of [^{18}F]FDG is supplied to hospitals around Mumbai daily. The successful installation and operational experience of medical cyclotron has revolutionised the nuclear medicine scenario in India and has given impetus to growth in the field. Currently, there are about 15 medical cyclotrons installed in India, which the majority are owned by semi-government institutions and private companies. Although [^{18}F]FDG is the most commonly used PET radiopharmaceutical, other PET radiopharmaceuticals are also used in the clinics which include [^{18}F]Fluoride, [^{18}F]MISO, [^{18}F]FLT, [^{13}N]NH_3 etc. The commercial availability of ^{68}Ge/^{68}Ga generators and automated modules for synthesis of ^{68}Ga labelled peptides has led to the availability of ^{68}Ga labelled peptides such as [^{68}Ga]DOTATOC and [^{68}Ga]PSMA in the metropolitan cities.

IV-.4.3.2. Legislation and regulatory authorities

Radiopharmaceuticals are classified as drugs in India under Section 3b of the Drugs & Cosmetics (D&C) Act, 1940. Chapter III of the Drugs & Cosmetics Act, 1940 deals with the importation of drugs and cosmetics, while Chapter IV deal with the manufacture, sale and distribution of drugs and cosmetics in India. The Government of India as per GSR 926 dated 24th June 1977 (Published in the Gazette of India, Part II Sec 3(i) No 29 dated July 27,1977) exempted radiopharmaceuticals from the application of many provisions of Chapter IV of the Act and Rules, based on the unique nature of radiopharmaceuticals in comparison to conventional drugs/pharmaceuticals. However, the importation of radiopharmaceuticals continues to be covered under Chapter III of the Drugs & Cosmetics Act, 1940 and the rules there under. For import of radiopharmaceuticals, the customer/user has to obtain a No Objection Certificate (NOC) issued by the Atomic Energy Regulatory Board (AERB), the radiological regulatory authority of the Government of India, in order to comply with the radiological safety requirements [IV-30, IV-31].

Self-regulation policy is practiced by DAE for production and supply of radiopharmaceuticals. DAE has therefore constituted an oversight peer review experts group called 'Radiopharmaceutical Committee (RPC)' for approving radiopharmaceuticals produced by units under it (including BRIT). The past decade has seen a significant increase in the import of radioisotopes/radiopharmaceuticals into India and the setting up of facilities for production and supply of radiopharmaceuticals by private companies. Hence, in order to ensure safety of radiopharmaceuticals administered in patients as well as to bring in regulations in harmony with the globally existing regulations, an Expert Committee was constituted in 2010 by the Indian Pharmacopoeia Commission (IPC), an Autonomous Institute of the Ministry of Health and Family Welfare, Government of India to review and process monographs of radiopharmaceuticals for incorporation in Indian Pharmacopoeia (IP). The Expert Committee initiated the process for inclusion of radiopharmaceutical monographs in Indian Pharmacopoeia. The terms of references of DAE-RPC include inter-alia extending professional expertise and support to Drug Controller General of India (DCGI), Indian Pharmacopoeia Commission (IPC) and other government agencies towards further strengthening the system of regulation for radiopharmaceuticals in India. Due to the joint efforts of RPC and IPC, for the first time, a General Chapter on radiopharmaceuticals [IV-32] and 19 Radiopharmaceutical Monographs were included in the Indian Pharmacopoeia 2014 (IP-2014) [IV-33]. Subsequently, ten more monographs were included in Addendum 2015 to IP-2014 [IV-34] and 3 monographs are included in Addendum 2016 of IP-2014 [IV-35]. The product specifications, quality standards and testing procedures cited in IP monographs have legal status under the second schedule of D&C Act, 1940 and Rules 1945 there under, and are applicable to imported, manufactured for sale, stocked or exhibited drugs for sale or distribution in India.

TABLE IV-5. RADIOPHARMACEUTICALS INCLUDED IN IP-2014 [IV-33]

S.NO.	RADIOPHARMACEUTICAL
1.	(^{18}F)Fluoro deoxy glucose injection
2.	(^{131}I) Meta-iodobenzyl guanidine injection for
3.	(^{131}I) Meta-Iodobenzyl guanidine injection for
4.	Samarium (^{153}Sm) ethylene diamine tetramethylene
5.	(^{18}F) Sodium fluoride injection
6.	(^{131}I) Sodium iodide capsules for diagnostic use,
7.	(^{131}I) Sodium iodide capsules for therapeutic use
8.	(^{131}I) Sodium iodide solution
9.	(^{99m}Tc) Sodium pertechnetate injection (Fission)
10.	(^{99m}Tc) Sodium pertechnetate injection (Non-fission)
11.	Sodium phosphate (^{32}P) injection
12.	Technetium (^{99m}Tc) DMSA injection
13.	Technetium (^{99m}Tc) DTPA Injection
14.	Technetium (^{99m}Tc) EC injection
15.	Technetium (^{99m}Tc) ECD injection
16.	Technetium (^{99m}Tc) glucoheptonate injection
17.	Technetium (^{99m}Tc) mebrofenin injection
18.	Technetium (^{99m}Tc) medronate complex injection
19.	Technetium (^{99m}Tc) MIBI injection

TABLE IV-6. RADIOPHARMACEUTICALS INCLUDED IN ADDENDUM 2015 OF IP [IV-34]

S.NO.	RADIOPHARMACEUTICAL
1.	Gallium citrate (^{67}Ga) injection
2.	Strontium (^{89}Sr) chloride injection
3.	Technetium (^{99m}Tc) colloidal rhenium sulfide injection
4.	Technetium (^{99m}Tc) exametazime injection
5.	Technetium (^{99m}Tc) HYNIC-TOC injection
6.	Technetium (^{99m}Tc) macrosalb injection
7.	Technetium (^{99m}Tc) mertiatide injection
8.	Technetium (^{99m}Tc) tetrofosmin complex injection
9.	Technetium (^{99m}Tc) trodat injection
10.	Urea (^{14}C) capsules

TABLE IV-7. RADIOPHARMACEUTICALS INCLUDED IN ADDENDUM 2016 OF IP [IV-35]

S.NO.	RADIOPHARMACEUTICAL
1.	Samarium phosphate ^{32}P colloidal injection
2.	Technetium (^{99m}Tc) labeled human serum albumin nanocolloid injection
3.	Thallous (^{201}Tl) chloride injection

IV-4.3.3. Quality control frequency, tests and the qualified person

All the radiopharmaceuticals (approved radiopharmaceuticals, radiopharmaceuticals used in clinical trials or extemporaneously prepared radiopharmaceuticals not for clinical trials), should comply with the quality criteria specified in the monographs and follow the mandatory QC tests to be carried out on every batch produced. The QC testing required to be carried out before release of the product at hospital radiopharmacy is the responsibility of technologists who have been trained in nuclear medicine and radiopharmacy practices. The quality of ready-to-use radiopharmaceuticals, radionuclide generators, and other products such as cold kits that are used for preparation of the radiopharmaceuticals should be tested for each batch produced as per approved quality control manuals at manufacturer's site. The quality control manual should include validated procedures specifying details of standard methods with specifications of instruments, materials, frequency of test schedule along with the limits, and acceptance criteria for each tested parameter. Quality control tests of radiopharmaceuticals commonly comprises visual inspection, pH measurement, estimation of radionuclide identity, radionuclidic purity and radiochemical purity, radioactive concentration, estimation of residual solvents if any, and determination of pharmaceutical purity by sterility testing and by determination of bacterial endotoxins. Parametric release of products is permitted for certain radiopharmaceuticals, especially for PET radiopharmaceuticals prepared with radionuclides of short or very short

physical half-life such as ^{18}F, ^{11}C, ^{68}Ga wherein certain QC tests such as sterility are completed post-release of the product.

Currently, although legal documents for hospital radiopharmacy guidelines and regulations are not available in India, most of the hospital radiopharmacies have skilled technical and scientific manpower. There are more than 800 members of Society of Nuclear Medicine India (SNMI) which include nuclear medicine physicians, radiation physicists, nuclear medicine technologists and radiopharmaceutical scientists working in the field of radiopharmaceuticals and nuclear medicine. DAE also conducts training courses in nuclear medicine for physicians (DRM) and technologist (DMRIT) to provide knowledgeable and skilled manpower in order to ensure safe preparation and use of radiopharmaceuticals.

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GLOSSARY

Additional definitions may be found, for example, in the European Pharmacopoeia (Ph. Eur.)

These documents are part of the regulatory framework and are therefore mandatory. Regulations are directly enforceable and do not need to be translated into national legislation, whereas directives have to be translated and then implemented in the national legislation.

Chemical purity: The proportion of the preparation that is in the specified chemical form regardless of the presence of radioactivity.

Good Manufacturing Practice (GMP): A set of rules that a radiopharmaceutical manufacturer commits to, in order to demonstrate that the drug can be consistently made with controls that conform to the established acceptance criteria, and that the process is documented, clear, and traceable.

No-carrier-added: Radioactive preparations in which no stable isotopes of the same element (carrier) are either detectable, expected to be present, or intentionally added during the manufacture or processing.

Non-binding documents (e.g. guidelines and guidance): These documents are recommendations only for implementing the legislation (e.g. guidelines provide non-mandatory guidance for effective implementation of directives).

Out of specification: An event that occurs when the obtained QC test result does not conform to the predefined acceptance value.

Quality Assurance (QA): A set of predefined and implemented procedures or rules that, when followed, assures that the chances of compromising any portion of the operational process are minimized. QA relates to the "quality by design" concept and may be applicable to any part of the process (i.e. production, QC, sterility assurance, validation, etc.)

Quality Control (QC): A set of predefined tests which, if completed successfully, demonstrate that the batch specifications conform to the pre-defined acceptance specifications. QC testing is related to analytical methods performed on the final batch of the product's cycle.

Radioactivity concentration: The measured radioactivity of the radionuclide per unit volume of the solution in which the radionuclide is present.

Radiochemical purity (RCP): The percentage of the stated radiopharmaceutical chemical species in relation to the total radiolabelled species present in a batch of product, including any radioactive impurities that may be associated with the manufacturing process. Radiochemical purity is assessed by a variety of analytical techniques such as liquid chromatography, paper chromatography, thin-layer chromatography and electrophoresis.

Radionuclide: Any energetically unstable element emitting ionizing radiation.

Radionuclidic purity: The proportion of the activity of the radionuclide concerned to the total radioactivity of the radiopharmaceutical. Radionuclidic impurities are depending on the radionuclide and the route of production. The relevant potential impurities are listed with their limits in the individual monograph.

Radiopharmaceutical: Radiolabelled macromolecules and low molecular weight molecules, diagnostic radiopharmaceuticals and therapeutic radiopharmaceuticals.

Standard operating procedures (SOP): SOP is a controlled document that describes how a certain operation should be performed. SOPs ensure control and traceability of all the processes.

Specific activity: The activity of the radionuclide per mass of the radionuclide or other isotopes of the same element present in a sample. The term "specific activity" is also sometimes used to describe the measure of radioactivity per total mass of the carrier molecule that is present in the sample. For example, mCi of [^{18}F] MISO per µL of [^{19}F] MISO or mCi of [^{89}Zr] DFO-Trastuzumab per mg of DFO-Trastuzumab/Trastuzumab that is present in the radiolabelled batch.

System suitability test: A test on analytical instrument prior to test sample analysis (usually with a reference standard) that assures that the instrument is fit for the intended analysis and will produce a valid result.

Vector or Carrier: The active pharmaceutical ingredient molecule that is responsible for the localization of the radiopharmaceutical at the intended target.

ABBREVIATIONS

BET	Bacterial Endotoxins Test
[^{11}C] METHIONINE or [^{11}C] MET	(2s)-2-amino-4-([^{11}c] methylsulphanyl) butanoic acid, l-[methyl-^{11}c] methionine
[^{11}C] PIB	Desmethyl-PIB, 2-(4'-aminophenyl)-6-hydroxybenzothiazole
[^{124}I] MIBG	[^{124}I] metaiodobenzylguanidine
[^{18}F] FDG	Deoxy-2[^{18}F] Fluoro-D-Glucose
[^{18}F] NaF	^{18}F-Sodium Fluoride
[^{64}Cu] ATSM	[^{64}Cu] Copper(II)-diacetyl-bis(N(4)-methylthiosemicarbazone
[^{68}Ga] DOTATOC	[^{68}Ga] DOTA-Tyr3-Octreotide
[^{89}Zr] DFO-Trastuzumab	[^{89}Zr] Zirconnium-desferrioxamine B- Trastuzumab
[^{99m}Tc] MDP	[^{99m}Tc] technetium medronate
CoA	Certificate of Analysis
CPTc	Cyclotron Produced ^{99m}Tc
CT	Clinical Trials
DQ	Design Qualification
EOS	End of Separation
EU	European Union
FAT	Factoring Acceptance Testing
FDA	Food and Drug Administration of the United States of America
FID	Flame Ionization Detector
FID	Flame ionization detector
GC	Gas chromatograph
GMP	Good Manufacturing Practice
HPLC	High Performance Liquid Chromatography
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICP-AES	Inductively Coupled Plasma-Atomic Emission Spectrometer
ICP-MS	Inductively Coupled Plasma - Mass Spectrometer
IP	Indian Pharmacopoeia
IQ	Installation Qualification
ITLC	Instant layer chromatography
Kryptofix-222	4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane
LAL	Limulus Amebocyte Lysate
LOD	Limit of detection

LOQ	Limit of quantitation
MA	Marketing Authorization
MCA	Multichannel analyser
OOS	Out-Of-Specification
OQ	Operational Qualification
PERs	Positron Emitting Radiopharmaceuticals
PET	Positron emission tomography
Ph. Eur	European Pharmacopoeia
PQ	Performance Qualification
QA	Quality Assurance
QC	Quality Control
RCP	Radiochemical Purity
RSD	Relative Standard Deviation
SEC–HPLC	Size Exclusion Chromatography-High Performance Liquid Chromatography
SOP	Standard Operating Procedures
SPC	Summary of Product Characteristics
SPE	Solid phase extraction
SPECT	Single-photon emission computed tomography
TCD	Thermal Conductivity Detector
TLC	Thin layer chromatography
URS	User Requirement Specifications
USP	United States Pharmacopeia
UV/VIS	Ultraviolet–visible spectroscopy

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