IMPLEMENTATION OF GUIDELINES ON HOSPITAL RADIOPHARMACY IN LOW-INCOME SETTINGS

by
Fany Pricile Ekoume

Dissertation presented for the degree of
Doctor of Philosophy
in the Faculty of Medicine and Health Sciences at
Stellenbosch University

Supervisor: Prof Sietske Margarete Rubow

Co-supervisor: Dr Hendrikus Hessel Boersma

December 2020

Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or part submitted it for obtaining a qualification.

This dissertation includes three original papers submitted to peer-reviewed journals (one of these papers was accepted) for publication and two papers ready for submission. The development and writing of the papers were the principal responsibility of myself and for each of the papers a declaration is included in the dissertation indicating the nature and extent of the contributions of co-authors.

Fany Pricile Ekoume

December 2020

Summary

Although Radiopharmacy is more than 50 years old, it is still in a stage of rapid development. This dissertation focuses on quality issues in radiopharmacies in developing countries. Guidelines for radiopharmacy practice in many countries prescribe complex facilities, especially air handling units, and extensive quality assurance and documentation requirements. In developing countries, these guidelines are currently not always met. In numerous countries in Africa, enforcement of the international guidelines would lead to closure of radiopharmacies, and consequently, loss of Nuclear Medicine services. The question arises what the consequences of not meeting the requirements of the guidelines are, and if practice can be improved without major expenditure.

This study considered certain aspects of Good Radiopharmacy Practice (GRP) recommendations and collected information from both a relatively well-equipped facility at Tygerberg Hospital (TBH) in South Africa, and a more basic radiopharmacy facility at Yaoundé General Hospital in Cameroon (YGH) to investigate the conditions that will ensure safe and effective products. Factors assessed include efficacy and microbial safety of the radiopharmaceuticals, with some comparison to a state-of-the-art Good Manufacturing Practice (GMP) compliant radiopharmacy at the University Medical Centre Groningen (UMCG) in the Netherlands.

An adapted version of the Quality Management Audits in Nuclear Medicine (QUANUM) tool, tailored for the radiopharmacy context, was used to determine the status of practice in the two African radiopharmacies. Once the current situation and product quality in these radiopharmacies was determined, basic, low-cost interventions to minimise deficiencies were implemented at YGH and the effects of the interventions were assessed. Where the necessary level of safety and efficacy could not be met with currently available systems despite interventions, this was reported.

The efficacy of radiopharmaceuticals depends on their radiochemical purity. As lack of validation of analytical methods was one of the shortcomings noted in the YGH audit, experimentally validating a cost-effective radiochromatography method to be used at YGH was the first step of corrective actions implemented.

As the provision of clean air and maintenance of air handling systems and equipment require a large budget, special emphasis was placed in three further chapters of the dissertation on assessment of microbial contamination of products, and measures to ensure sterility of products. At YGH, we reached better control of microbiological air quality. This was achieved by the implementation of simple microbiological air sampling methods, and subsequent introduction of hygienic and procedural improvements. Sterility testing of SPECT radiopharmaceuticals showed a low contamination rate at both TBH and YGH. Nevertheless, preparing radiopharmaceuticals in a well-maintained laminar air flow cabinet is recommended in order to reduce the risk of contamination of products by airborne microorganisms.

The serious consequences that could arise from not meeting GRP requirements, include transmission of microbial infection to patients or administering radiochemically impure products. This dissertation presents the first work evaluating an affordable approach of the implementation of GRP in sub-Saharan Africa. It is highly recommended to all radiopharmacies in the developing world to adapt GRP in their context and to implement an optimised quality assurance programme, striving for continuous improvement.

Opsomming

Alhoewel Radiofarmasie al meer as 50 jaar bestaan is daar steeds vinnige ontwikkeling op dié gebied. Hierdie verhandeling fokus op vraagstukke ten opsigte van gehaltebeheer in radiofarmasie in ontwikkelende lande. In baie lande vereis riglyne vir radiofarmasiepraktyk komplekse fasiliteite, veral lugversorgingseenhede, en uitgebreide gehaltebeheer en dokumentasie. Hierdie riglyne word tans in ontwikkelende lande nie altyd nagekom nie. In talle lande in Afrika sou afdwing van internasionale riglyne tot sluiting van radiofarmasiefasiliteite lei, en as gevolg daarvan tot verlies van Kerngeneeskunde dienste. Die vraag ontstaan tot watter gevolge dit lei as riglyne se vereistes nie nagekom word nie, en of praktyk sonder groot onkostes verbeter kan word.

Hierdie werk bestudeer 'n aantal aspekte van aanbevelings t.o.v. Goeie Radiofarmasiepraktyk (Engels: Good Radiopharmacy Practice (GRP)) en versamel inligting van beide 'n relatief goed toegeruste fasiliteit by Tygerberg Hospitaal (TBH) in Suid-Afrika, en 'n meer basiese fasiliteit by Yaoundé General Hospital (YGH) in Kameroen, om ondersoek in te stel na die omstandighede wat nodig is om veilige en effektiewe produkte te verseker. Faktore wat beoordeel word sluit effektiwiteit en mikrobiologiese veiligheid van radiofarmaseutika in. Vergelyking word ook getref met 'n moderne radiofarmasie eenheid by die Universiteits Mediese Sentrum Groningen (UMCG), Nederland, wat aan vereistes vir Goeie Vervaardigingspraktyk (Engels: Good Manufacturing Practice (GMP)) voldoen.

Die "Quality Management Audits in Nuclear Medicine" (QUANUM) hulpmiddel is aangepas om spesifiek radiofarmasie omstandighede te oudit en vervolgens gebruik om die stand van praktyk in twee radiofarmasie eenhede in Afrika te beoordeel. Nadat vasgestel is wat die huidge omstandighede en produkgehalte in die eenhede is, is basiese, lae-koste veranderinge by YGH toegepas om tekortkominge te verminder. Die effek van die veranderinge is vervolgens beoordeel. Waar die nodige vlak van veiligheid en effektiwiteit na veranderinge steeds nie bereik kon word nie, is dit aan hospitaalbestuur gerapporteer.

Die effektiwiteit van radiofarmaseutika hang van hul radiochemiese suiwerheid af. Aangesien 'n gebrek aan validasie van analitiese metodes een van die tekortkominge in die YGH oudit was, is 'n koste-

effektiewe radiochromatografie metode vir gebruik by YGH eksperimenteel gevalideer as eerste stap van regstellende aksies.

Die vereistes om skoon lug te voorsien en die onderhoud van lugvoorsieningsisteme kan baie duur wees. Om hierdie rede is daar in drie verdere hoofstukke van die verhandeling baie klem gelê op beoordeling van mikrobiologiese kontaminasie van radiofarmaseutika en maatreëls om steriliteit van produkte te verseker. By YGH kon die mikrobiologiese gehalte van die lug aansienlik verbeter word deur toepassing van eenvoudige mikrobiologiese lugtoetsing, gevolg deur inwerkingstelling van verbeterings t.o.v. higiëne en prosedures. Steriliteitstoetsing van radiofarmaseutika vir enkelfotonemissietomografie (SPECT) het lae vlakke van mikrobiologiese kontaminasie van radiofarmaseutika by YGH en TBH getoon. Ten spyte van dié bevinding word aanbeveel dat radiofarmaseutika in 'n laminêre vloeikabinet wat korrek in stand gehou word, voorberei word om die risiko van mikrobiologiese kontaminasie van produkte te vermider.

Die ernstige gevolge wat uit nie-nakoming van GRP vereistes kan spruit, sluit oordrag van infeksies aan pasiënte of toediening van radiochemies onsuiwer produkte in. Hierdie verhandeling is die eerste beoordeling van 'n bekostigbare benadering tot toepassing van GRP in Afrika suid van die Sahara. Dit word sterk aanbeveel dat alle radiofarmasie eenhede in die ontwikkelende wêreld GRP in hulle konteks aanpas en toepas en om 'n optimale gehaltebeheerprogram in te stel, met 'n gedurige strewe na verbetering.

Acknowledgements

I would like to express sincere thanks to several people without whom this thesis would have been impossible.

Prof Sietske Rubow, my supervisor, was always at hand to provide guidance and assistance, no matter how heavy her workload. She has provided sterling mentorship and friendship for which I am immensely grateful.

Dr Hendrikus Boersma made more valuable contributions than can be listed here. He kept things light and provided sage advice throughout. I keenly appreciate how lucky I was to have him as a co-supervisor.

Prof Faustin Dong à Zok made available the radiopharmacy unit at Yaoundé General Hospital and provided valuable advice.

Thanks for all the team working at the radiopharmacies of Yaoundé General Hospital, Tygerberg Hospital and the University Medical Centre Groningen for the time and effort they have put into collecting data. I would like to extend my gratitude to Prof Annare Ellmann and Prof Jan Pruim without whom this work could not have been done.

I would finally like to acknowledge my family for their love and constant support over the past few years.

Dedication

This dissertation is dedicated to the memory of my father, Reverend Josué Ekoume

Table of Contents

List of Abbreviations

Most abbreviations have been defined in the text when used for the first time. A full list of all abbreviations used in this thesis appears below.

ANOVA   Analysis of variance

CAPA   Corrective and preventive action

CC   Closed cabinet

CV   Coefficient of variation

cps   counts per second

DMSA   Dimercaptosuccinic acid

FDA   Food and Drug Administration

FT   Fingertip testing

GMP   Good manufacturing practice

GRP   Good Radiopharmacy Practice

Gram+   Gram positive

HMDP   Hydroxymethylene diphosphonate

HPLC   High-Performance Liquid Chromatography

IAEA   International Atomic Energy Agency

ICH   International Council for Harmonisation

ISO   International Organization for Standardization

ITLC-SG   instant thin layer chromatography- silica gel

keV   kilo electron volt

LAFC   Laminar airflow cabinet

LoC	Level of conformance
LOQ	Limit of quantitation
MFT	Media fill testing
MIBI	Methoxyisobutylisonitrile
PET	Positron Emission Tomography
QA	Quality Assurance
QC	Quality Control
QUANUM	Quality Management Audits in Nuclear Medicine
RCP	Radiochemical Purity
RP	Radiopharmaceutical
RSD	Relative Standard Deviation
SPECT	Single Photon Emission Computed Tomography
TLC	Thin Layer Chromatography
TBH	Tygerberg Hospital
TSA	Tryptic soy agar
TSB	Tryptic soy broth
UMCG	University Medical Centre Groningen
UPLC	Ultra Performance Liquid Chromatography
USP	United States Pharmacopeia
YGH	Yaoundé General Hospital

List of Tables

List of Figures

Overview of Authors' Contributions

Chapter 2: Implementation of a Quality Management System: Self-assessments in a Sub-Saharan Radiopharmacy (p13-27)

Declaration by candidate

With regards to the article presented in chapter 2, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Literature review, study design, data collection, processing and analysis, manuscript review	70

The following co-authors have contributed to the article presented in chapter 2 as detailed below:

	e-mail address	Nature of contribution	Extent of contribution (%)
Sietske M Rubow	smr@sun.ac.za	Manuscript review	15
Hendrikus H Boersma	h.h.boersma@umcg.nl	Manuscript review	10
Faustin Dong à Zok	dongazok@yahoo.fr	Manuscript review	5

(Declaration with signature in possession of candidate and supervisor.)

Fany Pricile Ekoume
Date: January 2020

Declaration by co-authors

The undersigned hereby confirm that

1.   The declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors in the specified chapters/articles.
2.   No other authors contributed to the specified chapters/articles beside those specified above, and
3.   Potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in the specified chapters/ articles of this dissertation.

Signature	Institutional affiliation	Date
Prof Faustin Dong à Zok	University of Yaoundé I Faculty on Medicine and Biomedical Sciences Department of Biophysics, Medical Imaging and radiotherapy	January 2020
Dr Hendrikus H Boersma	University Medical Centre Groningen. Department of Nuclear Medicine and Molecular Imaging/ Department of Clinical Pharmacy and Pharmacology	January 2020
Prof Sietske M Rubow	Stellenbosch University Faculty of Medicine and Health Sciences, Division of Nuclear Medicine	January 2020

Chapter 3: Validation of a cost-effective alternative for a radiochromatography method for use in a developing country (p28-52)

Declaration by candidate

With regards to the article presented in Chapter 3, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Literature review, study design, data collection, processing and analysis, manuscript review	70

The following co-authors have contributed to the article presented in chapter 3 as detailed below:

Hendrikus H Boersma	h.h.boersma@umcg.nl	Manuscript review, study design	15
Sietske M Rubow	smr@sun.ac.za	Manuscript review, study design	10
Faustin Dong à Zok	dongazok@yahoo.fr	Manuscript review	5

(Declaration with signature in possession of candidate and supervisor.)

Fany Pricile Ekoume
Date: January 2020

Declaration by co-authors

The undersigned hereby confirm that

1. The declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors in the specified chapters/articles.
2. No other authors contributed to the specified chapters/articles beside those specified above, and
3. Potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in the specified chapters/ articles of this dissertation.

Signature	Institutional affiliation	Date
Prof Faustin Dong à Zok	University of Yaoundé I Faculty on Medicine and Biomedical Sciences Department of Biophysics, Medical Imaging and radiotherapy	January 2020
Dr Hendrikus H Boersma	University Medical Centre Groningen. Department of Nuclear Medicine and Molecular Imaging/ Department of Clinical Pharmacy and Pharmacology	January 2020
Prof Sietske M Rubow	Stellenbosch University Faculty of Medicine and Health Sciences, Division of Nuclear Medicine	January 2020

Chapter 4: Implementation of air quality monitoring in a low-income radiopharmacy unit (p53-67)

Declaration by candidate

With regards to the article presented in chapter 4, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Literature review, data collection, processing and analysis, manuscript	75

The following co-authors have contributed to the article presented in chapter 4 as detailed below:

Hendrikus H Boersma	h.h.boersma@umcg.nl	Manuscript review, study design, data collection	15
Sietske M Rubow	smr@sun.ac.za	Manuscript review, study design, data collection	10

(Declaration with signature in possession of candidate and supervisor.)

Fany Pricile Ekoume
Date: January 2020

Declaration by co-authors

The undersigned hereby confirm that

1. The declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors in the specified chapters/articles.
2. No other authors contributed to the specified chapters/articles beside those specified above, and
3. Potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in the specified chapters/ articles of this dissertation.

Signature	Institutional affiliation	Date
Dr Hendrikus H Boersma	University Medical Centre Groningen. Department of Nuclear Medicine and Molecular Imaging/ Department of Clinical Pharmacy and Pharmacology	January 2020
Prof Sietske M Rubow	Stellenbosch University Faculty of Medicine and Health Sciences, Division of Nuclear Medicine	January 2020

Chapter 5: A comparative study of passive air sampling in different radiopharmacies (p68-80)

Declaration by candidate

With regards to the article presented in chapter 5, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Literature review, data collection, processing and analysis, manuscript	75

The following co-authors have contributed to the article presented in chapter 5 as detailed below:

Hendrikus H Boersma	h.h.boersma@umcg.nl	Manuscript review, study design, data collection	15
Sietske M Rubow	smr@sun.ac.za	Manuscript review, study design, data collection	10

(Declaration with signature in possession of candidate and supervisor.)

Fany Pricile Ekoume
Date: January 2020

Declaration by co-authors

The undersigned hereby confirm that

1. The declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors in the specified chapters/articles.
2. No other authors contributed to the specified chapters/articles beside those specified above, and
3. Potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in the specified chapters/ articles of this dissertation.

Signature	Institutional affiliation	Date
Dr Hendrikus H Boersma	University Medical Centre Groningen. Department of Nuclear Medicine and Molecular Imaging/ Department of Clinical Pharmacy and Pharmacology	January 2020
Prof Sietske M Rubow	Stellenbosch University Faculty of Medicine and Health Sciences, Division of Nuclear Medicine	January 2020

Chapter 6: Evaluation of aspects of practice in two African radiopharmacies (p81-99)

Declaration by candidate

With regards to the article presented in chapter 5, the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Literature review, data collection, processing and analysis, manuscript review	70

The following co-authors have contributed to the article presented in chapter 5 as detailed below:

Sietske M Rubow	smr@sun.ac.za	Manuscript review, study design, data collection	15
Hendrikus H Boersma	h.h.boersma@umcg.nl	Manuscript review, study design data collection	15

(Declaration with signature in possession of candidate and supervisor.)

Fany Pricile Ekoume
Date: January 2020

Declaration by co-authors

The undersigned hereby confirm that

1.   The declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors in the specified chapters/articles.
2.   No other authors contributed to the specified chapters/articles beside those specified above, and
3.   Potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in the specified chapters/ articles of this dissertation.

Signature	Institutional affiliation	Date
Dr Hendrikus H Boersma	University Medical Centre Groningen. Department of Nuclear Medicine and Molecular Imaging/ Department of Clinical Pharmacy and Pharmacology	January 2020
Prof Sietske M Rubow	Stellenbosch University Faculty of Medicine and Health Sciences, Division of Nuclear Medicine	January 2020

Chapter 1

Introduction

Background information

With the current expansion of Nuclear Medicine practice in the world, including African countries (Dondi et al. 2011), it is important to find out how and if high quality safe radiopharmaceuticals can be prepared in low income radiopharmacy units where the environment and the equipment do not meet requirements of Good Radiopharmacy Practice (GRP) guidelines. On the one hand, developing countries need Nuclear Medicine services, and should not be deprived of them because their radiopharmacies cannot meet very high standards. On the other hand, the people of developing countries should not be exposed to unsafe radiopharmaceuticals due to deficient radiopharmacy practice.

Clinical use of radiopharmaceuticals is associated with risk deriving from radiation exposure and possible contamination during handling by chemical, biological and microbiological impurities (IAEA 2008, European Commission 2008). Another aspect that could affect use of radiopharmaceuticals is the possibility of procedural errors. Therefore, their preparation and use are regulated by a number of directives, regulations and rules that cover all aspects of radiopharmacy and that must be followed to ensure and prove the quality of products (Guilloteau et al. 2007, Hesslewood 1990). Technetium-99m (Tc-99m) radiopharmaceuticals are prepared from licenced sterile generator eluates and kits and are classified under operational level 2 in the IAEA's *Operational Guidance on Hospital Radiopharmacy*. Several PET radiopharmaceuticals are produced on-site with complex synthesis reactions and multi-step procedures. The risk of contamination during handling such products is higher. For PET radiopharmaceuticals, recommendations for facilities and procedures are therefore more rigorous, and they are classified under the operational level 3 (IAEA 2008). The basic aim of all pharmacy practice guidelines and regulations, including Good Manufacturing Practice

1

(GMP) and Good Radiopharmacy Practice (GRP) guidelines, is to ensure the safety and efficacy of the product. Guidelines are frequently adapted or rephrased into rules to fit a radiopharmacy's specific setting.

Among the main aspects that must be considered when planning a new unit or evaluating an established radiopharmacy, are the facility to accommodate the unit, the layout and the equipment. Self-inspection of the radiopharmacy unit can reflect the standard at which it operates and help to evaluate the conformance to required specifications (IAEA 2008).

Facility, layout and equipment

The best location for a hospital radiopharmacy unit is within or near the Nuclear Medicine Department first of all for containment/ radiation safety reasons, and secondly because of the close relationship between the two units. The design mostly depends on the size and type of work in the unit, with a basic prerequisite of a clean and safe environment regarding dust, microorganisms and radioactivity (Owunwanne et al. 1995, Saha 2010). The unit should be organized in such a way that the risk of cross contamination (i.e. contamination of one product with another) is minimised (Callahan et al. 2007).

Facility layout should not only encompass the primary requirements of GRP and radiation protection associated with product handling, but should also enhance the flow of materials and people, and integrate the structural elements necessary to achieve these objectives. In this respect, application of controlled access in certain areas, interlocks, segregation, and pass-through boxes should be integrated in a building's design, along with the type of structural materials appropriate to meet a facility's objectives (IAEA 2012). For example, ideally, the lay-out should enable unidirectional circulation of people, raw material and final product. All items should enter or leave the cleanest areas through transfer hatches, rather than carrying them from one area to other. In a radiopharmacy located in a Nuclear Medicine unit, it would be optimal to have the dispensing area adjacent or close to the injection area.

The radiopharmacy should be divided to provide a separate administrative area equipped adequately with manual and electronic devices for soft and hard copies of documents, data and records. The entrance of the

2

radiopharmacy should be through a gowning area. In addition to this, a separate reception area should provide sufficient space for receipt of products. Where applicable, it is recommended that before release for use, incoming products should be segregated and placed in a designated area under quarantine to be inspected, sampled and tested before definitive approval and storage for use (Elsinga et al. 2010). Radiolabelling of blood cells must preferably be performed in a well separated clean area different to the kit dispensing area (Hesslewood 1990). Separate shielded areas for generators and waste should be available (Saha 2010). The finishing and layout must provide floors and walls that are easy to clean and decontaminate. There should be separation between low and high activity areas. If needed, there could also be separation between areas for long and short living radionuclides, including separation of radioactive waste containers. Furthermore, the rooms should have sufficient light, and adequate temperature and humidity control.

As microbiological contamination can arise from particles in circulation and personnel movements, including talking, sneezing, and coughing, particle-free air supply is deemed essential to ensure sterile preparations. The underlying philosophy of the requirement to control the particulate contamination for parenterally administered radiopharmaceuticals is that reducing the particle count level lowers the chance of microbial contamination in the final product (Hesslewood 1990). A clean air environment plays an important role in reducing particulate contamination. For this reason, almost all the guidelines and directives available stipulate that preparation of kits and elution of Tc-99m generators should be done in a laminar air flow cabinet (LAFC). Appropriate procedures for the disinfection of materials and equipment being transferred into the aseptic work area should be available (Elsinga et al. 2010). Consideration must be also given to the environment in which such an LAFC is placed, as the room should be provided with filtered air, and controlled temperature and humidity. A prescribed number of air changes should be maintained. It is recommended to have pressure differentials between cleaner and less clean areas in order to prevent inflow of dirty air into a clean environment (Saha 2010, Elsinga et al. 2010).

3

In contrast to the recommendations described above, work stations on an open bench or an LAFC without any maintenance program and in uncontrolled surroundings are found in many radiopharmacies in developing countries[1]. The reality in many radiopharmacies is that they have been built as laboratories without any consideration of the adequate design required for GRP.

Quality Assurance

Quality assurance is a structured, document-based system aiming to demonstrate compliance to accepted and prescribed standards. In a Radiopharmacy, it comprises the total process of preparation of a radiopharmaceutical for patients, involving correct radiolabelling, quality control, i.e. testing of equipment and radiopharmaceuticals, dispensing and record keeping (Callahan et al. 2007). All equipment used in the radiopharmacy should be qualified before its use, including installation, operational and performance qualification. Documents tracing all these steps should be kept (Norenberg et al. 2010). All equipment, including dose calibrators, radiation contamination monitors, LAFCs, isolators, etc. should be checked and maintained on a regular basis with a maintenance logbook (Busemann Sokole and Britten 2015).

Product quality control is also mandatory. The quality of radiopharmaceuticals should be verified after receipt of a new batch and prior to administration to patients. Radionuclidic purity (for example absence of molybdenum-99 in technetium-99m) of the preparation is mandatory for quality images without unnecessary irradiation of the patient. Radiochemical purity testing is carried out to ensure that the radionuclide is present in the desired chemical form. It is most often performed by thin layer chromatography. The presence of chemical impurities in the preparation should also be checked before the administration of the product (Sharma 2012, Elsinga et al. 2010). Sterility of radiopharmaceuticals should also be verified, preferably using a compendial method; although the test results may only become available

---

[1] A brief questionnaire was sent to radiopharmacy units in several African countries. Out of those that responded, 50 % currently (April 2020) do not have a laminar air flow cabinet or closed cabinet for Tc-99m preparation and dispensing.

after the useful shelf-life of the products. To meet the requirements from different guidelines, the validity of all analytical methods should be proven following a pre-established validation protocol (Saha 2010), or be derived from a compendial method, which has been proven to be adequate to operate without validation. In the latter case, limited testing of the method will be sufficient.

To provide patients with optimal and safe care, Nuclear Medicine professionals, including personnel involved in preparing and dispensing radiopharmaceuticals, should be adequately trained. Each radiopharmacy unit should operate under the supervision of a responsible person with specific training in radiopharmacy. In many developing countries this recommendation is not met (IAEA 2008, Dondi et al. 2011).

Documentation and collection of recorded data are integral and key components of GRP. Therefore, data from all processes and procedures having direct influence on the quality of products should be collected as evidence and should be available as records. The aim of documentation is to provide and ensure an audit trail of each operation that takes place in the radiopharmacy unit (IAEA 2008). A systematic verification of the entire unit should be done by self-inspection in order to identify serious out of specification situations. Subsequent initialisation of urgent corrective actions and recommendation of actions should lead to improvements in the overall functioning of the department (IAEA 2008, Elsinga et al. 2010, Solanki and Dondi 2008).

For many reasons, which will differ according to settings of different radiopharmacies, the recommendations summarised above may be only partially met or even not met at all. Obstacles may be lack of finances, lack of training, lack of technical and engineering support, or environmental constraints like irregular power supply.

Important factors contributing to adherence to recommendations will be the facility, the equipment available in the unit, the availability of trained staff and the quality management system (De Roo et al. 2015).

Problems with the implementation of recommended techniques in hospital radiopharmacy are not unique to low income units. A hospital in France reported frequent occurrences of biocontamination of working areas (Maia et al. 2008). More stringent hygiene guidelines have also been implemented in radiopharmacy unit of Louis-Mourier Hospital in France after evidence of contamination of the working environment by microorganisms (Duez et al. 2009). These two examples illustrate the importance of quality assurance programmes where radiopharmaceuticals are prepared.

One of the main issues remains the practicability of the requirements, including the feasibility of recommended guidelines and recommendations in practice. If recommended requirements are not met, it is important to know to which extent the deviation affects the quality and efficacy of the product and the safety of the patient or the working environment. This study focuses on some essential aspects of the existing guidelines. It addresses the status of radiopharmacy practice in Africa and considers the requirements to provide safe radiopharmaceuticals for patient administration as well as safe working environments. As most hospital radiopharmacies in Africa are limited to work at IAEA Operational Level 2, this work focuses on Tc-99m radiopharmaceuticals prepared from commercial kits.

Research questions

The work addresses the following research questions:

1) Which aspects of Good Practice can be improved for radiopharmacies in low-income countries?

2) Can the radiochemical quality of radiopharmaceuticals prepared for patients be reliably tested in developing countries?

3) What is the microbial safety level for the production of SPECT radiopharmaceuticals in the two radiopharmacies in Africa that were included in this study?

Two African radiopharmacies at TBH and YGH are used as examples in the current dissertation and a GMP-compliant facility at UMCG is used as reference site. To clarify the real situation in each unit, the properties of the three radiopharmacies are described in table A.1 in the Addendum.

The societal value of the current study is that it evaluates the impact of not being able to conform to GRP guidelines in developing countries.

Purpose of the proposed research

The **objective of this study** is to evaluate the implementation of some aspects of Good Radiopharmacy Practice guidelines in two radiopharmacies in Africa, and the effect that current practice in these facilities has on product quality and safety.

Certain aspects of existing international guidelines for good radiopharmacy practice are investigated in the two radiopharmacy units. Methods used at the University Medical Centre Groningen (UMCG) radiopharmacy, which operates under GMP conditions, are used where possible and UMCG is used as an example of a facility that does meet requirements.

Framework and overall design of the study

This study addresses two main themes within radiopharmacy practice.

1. Efficacy of products prepared, using radiochemical purity as indicator

2. The effect of facilities and equipment that fail to provide the recommended level of air cleanliness on microbiological aspects of product safety.

The two themes are each developed as a specific objective which contributes to the main objective of the study by its impact or contribution on good radiopharmacy practice.

Specific objective 1: To evaluate the operational standard and the conformance to required specifications at the YGH radiopharmacy

Specific objective 2: To validate the radioactivity detection method used for radiochemical purity of radiopharmaceuticals used at YGH

Specific objective 3: To evaluate the air quality in the radiopharmacies at TBH and at YGH

Specific objective 4: To evaluate the rate of microbial contamination of radiopharmaceuticals at TBH and YGH as well as the aseptic skills of operators at YGH

Brief overview of the five papers

A description with self-audits of the three radiopharmacies included in this study is presented in the addendum of the dissertation (tables A.1 to A.8 and figure A.1).

The research is presented as a series of five articles, of which one has been published in the *EJNMMI Radiopharmacy and Chemistry.*

The topics of the five articles are:

1) *Implementation of a Quality Management System: Self-assessments in a Sub-Saharan Radiopharmacy*:

This paper describes a prospective evaluation of the implementation of quality management in a sub-Saharan radiopharmacy via two self-assessments.

2) *Validation of a cost-effective alternative for a radiochromatography method to be used in a developing country*:

A validation of the method used for quantifying the distribution of radioactivity during radiochemical purity determination of products is presented. The paper addresses the need for a simple and affordable instrument for reading of radiochromatograms.

The next three articles describe the implementation and evaluation of some aspects of good practice relating to microbial safety in two African radiopharmacies.

3) *Implementation of air quality monitoring in a low-income radiopharmacy unit*

Paper 3 presents the prospective implementation of air quality monitoring, using passive air sampling by settle plate exposure at YGH radiopharmacy. After a baseline study, some corrective actions are introduced and evaluated during a further monitoring period to evaluate the impact of changes.

4) *A comparative study of passive air sampling in different radiopharmacies*

This paper presents a comparison of air quality monitoring results in the radiopharmacies at YGH and TBH and considers approaches to address shortcomings.

5) *Evaluation of aspects of practice in two African radiopharmacies*

The last paper describes the evaluation of aseptic skills of staff at YGH by media fill and fingertip testing. It also reviews the sterility test results of radiopharmaceuticals prepared at TBH and YGH and compares them with those of the GMP-compliant radiopharmacy at UMCG.

All studies were conducted with approval of the Stellenbosch University Health Research Ethics Committee (ref: S17/05/097).

References Chapter 1

Busemann Sokole E, Britten A. Routine quality control recommendation for nuclear medicine instrumentation. European Journal of Nuclear Medicine and Molecular Imaging 2010;37:662-671

Callahan RJ, Chilton HM, Ponto JA, Swanson DP et al. Procedure guideline for the use of radiopharmaceuticals 4.0. Journal of Nuclear Medicine Technology 2007;35(4):272-275

De Roo FM, Hilgenrink K, Kosterink JGW, Luurtsema G, Woerdenbag HJ, and Boersma HH. Tracer application in cardiovascular imaging: A triple jump. In Slart RHJA et al., editors. Autonomic Innervation of the Heart: Role of Molecular Imaging. Berlin Heidelberg: Springer-Verlag; 2015. pp. 145-150

Dondi M, Kashyap R, Paez D, Pascual T et al. Trends in Nuclear Medicine in developing countries. Journal of Nuclear Medicine 2011;52:16-23

Duez C, Pons-Kerjean N, Paycha F, Callanquin M. Mise en œuvre de procédure d'hygiène et de contrôles physique et microbiologiques d'environnement au sein d'unité de radiopharmacy. Annales Pharmaceutiques Françaises 2009;67:419-426

Elsinga P, Todde S, Penuelas I, Meyer G et al. Guidance on current good radiopharmacy practice (cGRPP) for small-scale preparation of radiopharmaceuticals. European Journal of Nuclear Medicine and Molecular Imaging 2010;37(5):1049-1062

European Commission: EudraLex - The Rules Governing Medicinal Products in the European Union. Volume 4: EU Guidelines to Good Manufacturing Practice. Annex 1: Manufacture of Sterile Medicinal Products. 2008. https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2008_11_25_gmp-an1_en.pdf [Accessed 6 August 2019]

Guilloteau D, Vergote J, Maia S. Importance of Radiopharmacy in Hospital Practice: Application to Alzheimer and Parkinson's disease exploration. FABAD Journal of Pharmaceutical Sciences 2007;32:41-48

Hesslewood SR. Microbial and radiation monitoring in the radiopharmacy. In Sampson CB (ed.). Textbook of radiopharmacy Theory and Practice. Amsterdam: Gordon and Breach Science Publishers; 1990. pp. 177-182

IAEA. Cyclotron produced radionuclides: Guidance on facility design and production of [^{18}F]Fluorodeoxyglucose (FDG). Vienna: International Atomic Energy Agency; 2012. p. 97

IAEA. Operational Guidance on Hospital Radiopharmacy, A safe and Effective Approach. Vienna : International Atomic Energy Agency; 2008. pp. 28-30

Maia S, Nicol B, Rouleau A, Guilloteau D et al. Contamination microbiologique en radiopharmacie : problématiques et mise en œuvre de contrôles dans le cadre d'une démarche qualité. Pharm Hosp 2008;43(172):11-17

Norenberg JP, Petry NA and Schwarz S. Operation of a Radiopharmacy for a Clinical Trial. Seminars in Nuclear Medicine 2010;40:347-356

Owunwanne A, Patel M and Sadek S. The Handbook of Radiopharmaceuticals (1sted.). London: Chapman & Hall Medical; 1995. pp. 191-198

Saha GB. Fundamentals of Nuclear Pharmacy (5th ed.). New York: Springer-Verlag; 2004. pp. 153-173

Sharma A, Sharma R. Validation of analytical procedures: A comparison of ICH vs. Pharmacopoeia (USP) vs. FDA. International Research Journal of Pharmacy 2012;3(6):39-42

Solanki K and Dondi M. Quality Management Audits in Nuclear Medicine Practices. Vienna: International Atomic Energy Agency; 2008

Chapter 2

Implementation of a Quality Management System:

Self-assessments in a Sub-Saharan Radiopharmacy

Fany P Ekoume[1, 3], Hendrikus H Boersma[2], Faustin Dong à Zok[1], Sietske M Rubow[3]

[1]Yaoundé General Hospital, Cameroon; [2] University Medical Centre Groningen, The Netherlands;

[3]Stellenbosch University, Tygerberg, South Africa

Abstract

Appropriate standards of good practice in Nuclear Medicine and Radiopharmacy are essential, not only in developed countries. The Yaoundé General Hospital radiopharmacy unit has started to implement aspects of good practice and self-assessments in 2017. This study compares the outcome of audits conducted before and after implementation of some aspects of Good Radiopharmacy Practice in order to improve the safety and efficacy of radiopharmaceuticals prepared in the radiopharmacy.

Methods: Based on tools published by the International Atomic Energy Agency, self-assessments of the unit were performed to evaluate the level of compliance to good practice, to identify the areas of non-conformance, and to monitor the effect of changes after the introduction of corrective actions.

Aspects reviewed for conformity with standards were staffing, facilities, purchase of materials, dispensing protocols, preparation protocols, quality control/quality assurance, and waste management. The change of

status of compliance and the level of compliance of each requirement were evaluated and Wilcoxon signed rank tests were used with P<0.05 considered significant.

Results: There were non-conformities in all items in the checklist. Initially, the lowest compliance percentage was found with dispensing protocols (20%). Most of the components show significant improvement.

Conclusion: Implementation of the action plan after initial self-assessment, and the outcome of a follow-up self-audit show improvement of the quality of practice at the Yaoundé General Hospital radiopharmacy. Optimized control and documentation and further improvement of the facility are recommended to address the remaining risks.

Keywords: Self-assessment, quality management system, radiopharmacy, sub-Saharan Africa

Introduction

Organisations implement quality management systems in order to improve the efficiency and effectiveness of performance, and to fulfil regulatory requirements. Auditing may be a requirement for accreditation of Nuclear Medicine departments (Jarrit 2004; Mirzaei et al. 2011); and is regarded as an essential tool in the modern health care system (Begum et al. 2016). An organizational audit process for Nuclear Medicine departments was developed by the British Nuclear Medicine Society for both external and internal audits (Jarrit 2004). In 2008, the International Atomic Energy Agency (IAEA) published guidelines for hospital radiopharmacy practice which included lists of questions for self-audits (IAEA 2008a). The IAEA also introduced an auditing system, referred to as Quality Management Audit in Nuclear Medicine (QUANUM), aiming to support annual systematic audits in clinical Nuclear Medicine (IAEA 2008b). The second edition of the QUANUM manual introduced radar plots and a five-step scoring system which facilitates comparison with earlier audits (IAEA 2015).

Many Nuclear Medicine units and radiopharmacies strive to upgrade their quality management systems, aiming at harmonization and standardization to meet internationally recommended Good Practice guidelines as far as possible. All areas of an organisation's activities are generally reviewed with a standardized scheme with systemic review questionnaires, defined minimum requirements, and well-defined conformance criteria and report formats (Begum et al. 2016). Guidelines for radiopharmacy practice have been published, amongst others by the IAEA, to assist with standardization of radiopharmacies (IAEA 2008a, IAEA 2008b). In developing countries, it is not always possible to meet all good radiopharmacy practice requirements. There is therefore a risk that radiopharmaceuticals prepared or compounded in such sub-optimal facilities may not meet the required safety and efficacy standards. Like many other sub-Saharan African radiopharmacies, Yaoundé General Hospital (YGH) does not have a suitable facility and all recommended equipment for optimal Good Radiopharmacy Practice (GRP) to be achieved. The risk of microbial contamination and presence of radiochemical impurities in the products can lead to transmission of infections to patients, or to poor image quality which could hinder diagnoses and cause unnecessary

15

irradiation. Regular self-audits of the radiopharmacy will promote better control and can help to reduce the risks related to radiopharmaceuticals provided by the radiopharmacy.

YGH radiopharmacy is located within the Nuclear Medicine department with facilities for preparation of SPECT radiopharmaceuticals and low dose iodine-131 therapy. The radiopharmacy works at operational level 2a as defined in the Operational Guidance on Hospital Radiopharmacy (IAEA 2008a). As illustrated in table 2.1, all operators working in the unit have previously received basic training in general radiopharmacy during their nuclear medicine training. Following the trend of implementing good standards of practice in Nuclear Medicine and Radiopharmacy, the YGH radiopharmacy unit introduced several aspects of good practice in 2017. To efficiently focus on relevant aspects, an initial self-assessment was conducted. An action plan was organized with a number of activities, including but not limited to, the development of awareness of the QUANUM tool among staff, development and upgrading of standard operating procedures (SOPs), and improving microbiological and radiation safety in the radiopharmacy. This was followed by another self-audit to evaluate the effect of actions.

The objective of this study is to compare the outcome of self-assessments conducted at YGH before and after implementation of aspects of Good Radiopharmacy Practice (GRP), in order to ensure safety and efficacy of radiopharmaceuticals.

Table 2.1 The status of the YGH Radiopharmacy unit during the study period

Components	Status
Staff and training	Responsible person: MSc Radiopharmacy
	1 Nuclear Medicine technician with six months training in a well-equipped unit
	1 Scientist with a Bachelor's degree in Biology with 4 months training in a well-equipped unit
	1 Scientist with a Bachelor's degree in Biology and European course in radiopharmacy level 1
	1 Chief nurse with 3 months training in a well-established unit in Europe
Equipment	1 glove box (not clean air)
	1 fume hood
	1 laminar air flow cabinet
	1 shielded dispensing area
	1 dose calibrator
	3 radiation contamination monitors
Radionuclides	^{99m}Tc, ^{131}I
Radiopharmaceuticals	MIBI (methoxyisobutylisonitrile), DTPA (diethylene triamine penta acetic acid), HMDP (hydroxy methylene diphosphonate), Nanocolloid (colloidal rhenium sulphide), DMSA (dimercaptosuccinic acid)

Methods

Based on the existing tools from the Operational Guidance on Hospital Radiopharmacy (IAEA 2008a), the QUANUM process (IAEA 2008b; IAEA 2015; Dondi et al. 2017; Dondi et al. 2018) and the status of the unit during the period of study as presented in table 2.1, self-assessments of the unit were performed to evaluate the level of compliance to good practice and to identify areas of non-conformance. The audit questions are shown in the Addendum, tables A.2 to A.8. After a first self-assessment (pre-implementation) to identify problems, realistically achievable and affordable corrective actions were introduced. These included validation of the method to test radiochemical purity (RCP), updating procedures and processes to recommended standard operating procedure (SOP) format, implementation of passive air sampling, sterility testing and media fill testing, staff training, rearrangement of items in the unit aiming for the reduction of contamination in the compounding area, and acquisition of sterile laboratory coats and shoes (easy to clean) dedicated to wear only in the radiopharmacy. A second self-assessment was conducted after completion of corrective actions to monitor the effect of changes.

Components relating to the production of radiopharmaceuticals were reviewed for conformity with standards described in the IAEA guidance (IAEA 2008a). Aspects reviewed were staffing, facilities, purchase of materials, dispensing protocols, preparation protocols, Quality Control/Quality Assurance (QC/QA), and waste. Depending on the level of adherence to each recommended standard, the level of conformance (LoC) was graded as follows: 0 when the component was absent in the unit; 1 when the component was planned; 2 when the component was partially implemented; 3 if the component was largely implemented, and 4 when it was fully implemented. Items scoring 0, 1, or 2 are considered non-compliant. Those that scored 3 and 4 are conforming. The sum of all grades for individual requirements or criteria within a component was expressed as a percentage of the maximum possible score for that component. Depending on the impact the non-conformities may have on the environment, on daily practice or on safety, the IAEA guidelines (IAEA 2008a; IAEA 2008b) classify them as critical, requiring immediate action, or major shortcomings, requiring corrective action within 6 months.

Scores of self-assessments for all components from the checklist before and after intervention were compared and visualised using radar plots. The change of status of compliance and the level of compliance of each requirement were evaluated using a non-parametric Wilcoxon signed rank test with P<0.05 considered significant.

Results

The scoring and compliance status of the YGH radiopharmacy are summarised in table 2.2. The level of conformance (LoC) percentages of each component differed greatly before and after some aspects of Good Practice were implemented (figure 2.1). This difference was significant for most components, but the number of questions in three components was too low to reliably prove significance of the results. Table 2.3 summarises the severity of non-conformances and also lists examples of the interventions that led to the improved status of radiopharmacy practice.
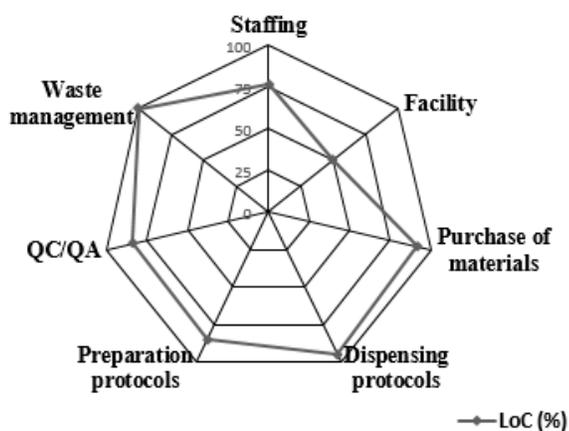
Figure 2.1 Radar plots showing improvement after implementation of some aspects of GRP

Table 2.2 Scoring and compliance status before and after intervention

Component	Staffing	Facility	Purchases	Dispensing protocols	Preparation protocols	QC/QA	Waste management	Total
No of Applicable questions	13	12	6	5	5	31	3	75
Maximum achievable score	52	48	24	20	20	124	12	300
Total score before intervention	29	9	11	8	11	36	8	112
LoC before intervention	56%	19%	46%	40%	55%	29%	67%	37%
Total score after invention	40	24	22	19	17	104	12	238
LoC after intervention	77%	50%	92%	95%	85%	84%	100%	79%
Wilcoxon signed rank test*	P<0.001	P<0.001	P<0.001			P<0.001		

*before vs after intervention

20

Table 2.3 Severity of non-conformance and interventions

	No of non-conformities before / after intervention		Examples of interventions
	Critical	Major	
Staffing	3 / 1	2 / 0	• Staff training • Introduced annual performance review to check competence
Facility	4 / 2	5 / 2	• Introduced evaluation LAF cabinets • Installed transfer hatch • Rearrangement of room lay-out
Purchase of materials	1 / 0	2 / 0	• Improved record keeping
Dispensing protocols	2 / 0	1 / 0	• Introduced SOPs
Preparation protocols	1 / 0	2 / 0	• Improved SOPs and records
QC/QA	5 / 1	16 / 2	• Improved SOPs and records • Validated RCP method • Microbiological QC • Sterile coat and shoes for radiopharmacy use only
Waste management	1 / 0	0 / 0	• Improved record keeping

Discussion

Assuming that a unit working with good practices is more likely to have good outcomes (Mirzaei et al. 2011), we assumed that upgrading our procedures according to GRP standards could improve our radiopharmacy's performance. De Paula et al. recently did an adaption of the QUANUM tool for auditing Brazilian Nuclear Medicine and argued that existing audit tools should be adapted to fit local regulations (De Paula et al. 2018). Audit tools should be reviewed to meet changes in Nuclear Medicine equipment and practice (Jarrit 2004). Using the QUANUM audit tool to evaluate 25 Nuclear Medicine units, improvement in standards of quality in production and use of radiopharmaceuticals could be shown in all audited departments (Dondi et al. 2018). However, the QUANUM system (Dondi et al. 2017) was designed primarily to evaluate Nuclear Medicine centres. As more extended questions on quality assurance and quality control were needed to address microbial safety and efficacy of radiopharmaceuticals, we adapted the QUANUM model to specifically address the quality of work in our radiopharmacy, by focussing in more depth on the different components of radiopharmacy practice, using information from the Operational Guidance on Hospital Radiopharmacy (IAEA 2008a). With this modified tool, the main areas of weakness could be identified and the positive impact of our corrective action plan could be demonstrated. The radar plots adopted from QUANUM were valuable to illustrate the original status and improvements for staff members.

The evaluation in the current work was twofold: firstly, we focused on the change in the total score for each component (% values shown in radar plots), and secondly on the change in the level of conformance. When both score 3 and score 4 are regarded as conforming, it is clear that a component with many requirements that achieved individual grades of 3, and only a few graded 4, will be seen as conforming, even though further improvement would be required to completely meet international recommendations.

The radar plot and the table from the initial self-assessment reveal non-conformities in all items audited in the checklist. In the first audit, staffing, preparation protocols, and waste management already present compliance percentages above 50%. This can be ascribed to the fact that all operators working in the unit have basic training in the field. The radiation protection agency in Cameroon organises regular

educational sessions in radiation protection including radioactive waste management. Staff working with unsealed radioactive sources should renew their radiation protection certificates every 2 years.

The follow up self-assessment shows improvement for all components, with significant differences in level of compliance before and after the implementation of corrective actions for aspects related to staffing, facilities, purchase of materials and QC/QA.

The number of non-conformities drops to zero after corrective action for the purchase of materials, the dispensing protocols, the preparation protocols and waste management. The problems identified in purchase of materials consisted mostly of poor record keeping (e.g. not keeping record of all details). This was addressed through staff training on the importance and relevance of recording the information. The dispensing and preparation protocols were initially not very well structured. They were re-written in SOP format with step-by-step details. As mentioned, staff were already well aware of waste management principles at the time of the first audit. In this case introduction of better shielded waste containers led to an improved score.

QC/QA is now much better implemented. If radiopharmacy staff pay close attention to details of QC/QA procedures, this improvement may be sustained or even improved. A critical non-conformity that could not be immediately addressed is the absence of staff or external technicians who are able to calibrate equipment. This is now addressed by a plan for training a medical physicist to work in the unit.

Additional action is needed to upgrade the facility and equipment. Service and maintenance of equipment should be improved, but there are no qualified technicians and spare parts in the country. After the second audit the hospital recruited a maintenance engineer who will be trained to address this problem. Items were rearranged in the unit to allow smooth and unidirectional transfer of materials from the less clean area to cleaner areas. Sterile coats dedicated for wear only in the radiopharmacy and shoes reserved for wear in the radiopharmacy were provided. Adequate instructions were introduced to reduce staff movement and traffic in the radiopharmacy rooms. A hatch was installed for product transfer from the dispensing area to the injection room. The design of the rooms is however not optimal yet. Funds for major building alterations like the installation of a suitable HVAC system are lacking.

Recommendations were made to authorities in this regard. Frequent interruptions of electrical power supply also pose a challenge. Due to these problems, two critical and two major non-conformities remain for the facility component.

Despite lack of funding and other practical problems, we achieved satisfying results. The overall LoC for the radiopharmacy was 37% before and 79% after implementing changes. Although we have thus reached more than the 70% which is regarded as good level of practice in the QUANUM audits of Nuclear Medicine departments (Dondi et al. 2017), the nature of risks associated with radiopharmaceuticals justifies setting a higher goal. As shown in table 1, the unit is made of 2 rooms with QC area included in the preparation room. A shielded laminar air flow cabinet or isolator placed in a purpose-designed cleanroom with HEPA filtered air, reserved for compounding or preparation and dispensing of radiopharmaceuticals, with a separate room for QC, would be optimal. A softwall or modular hardwall cleanroom within a bigger room may possibly be an option. If the room and air supply cannot be altered, strict protocols to minimise the risk of microbiological contamination and stringent monitoring are essential. Regular assessments will also motivate staff to continue implementing good practice.

A limitation of the current study is that the outcomes are based on only two audits. The study was designed to first audit the radiopharmacy to evaluate if any improvements in the radiopharmacy were needed. A post implementation audit was done after corrective actions but no long term or longitudinal evaluations were planned at that time. More follow up audits of the unit are required to ascertain if the improvement found in the study can be sustained for a longer period.

Conclusion

Implementation of an action plan after initial self-assessment, and the outcome of a follow-up self-audit in the current study show improvement of the quality of practice at the YGH radiopharmacy, with both scores and compliance status revealing positive changes. Further optimized control and documentation and more improvement of the facility are recommended to address the remaining risks.

What is already known on this topic

- Self-audits are regarded as an essential tool in Quality Management and are expected in GMP-compliant radiopharmacies in developed countries but is still not performed in many African radiopharmacies due to lack of skills.

- A detailed questionnaire on radiopharmacy practice was published by the IAEA in 2008.

- The IAEA developed a tool for auditing Nuclear Medicine practice, called QUANUM.

What this study adds

- This is the first work publishing an evaluation of the implementation of GRP in a sub-Saharan radiopharmacy.

- This study adapted the QUANUM tool to focus on radiopharmacy, using an IAEA radiopharmacy questionnaire. This adapted tool can be easily implemented in any radiopharmacy in developing countries.

- The study describes several easy, inexpensive steps that can improve radiopharmacy practice in low-income countries.

Declarations for publication

Contributors

Manuscript: Fany Pricile Ekoume

Manuscript review: All authors

Supervisors: Sietske M Rubow, Hendrikus H Boersma

Conflict of interest

None

Acknowledgements

References Chapter 2

Begum SMF, Begum F and Hasan M. Regular quality management audit in nuclear medicine services - A dynamic approach of self-improvement. Bangladesh Journal of Nuclear Medicine 2016;19(1):52-55

De Paula V, Sà L, Pinheiro M, Lima G, Andrade E, Magalhaes L, Loureiro M. Adaptation of the Quanum platform for internal audits in nuclear medicine in Brazil. Brazilian Journal of Radiation Sciences 2018;6(2A):1-9

Dondi M, Paez D, Torres L, Marengo M, Bischof Delaloye A, Solanki K et al. Implementation of quality system in Nuclear Medicine: Why it matters. An outcome analysis (Quality Management audits in Nuclear Medicine part III). Seminars in Nuclear Medicine 2018;48:299-306

Dondi M, Torres L, Marengo M, Massardo T, Mishani E, Van Zyl Ellmann A et al. Comprehensive auditing in nuclear medicine trough the International Atomic Energy Agency quality management audits in nuclear medicine program. Part 2: Analysis of results. Seminars in Nuclear Medicine 2017;47:687-693

IAEA. Operational Guidance on Hospital Radiopharmacy, A safe and effective approach. Vienna: International Atomic Energy Agency; 2008a

IAEA. Quality Management Audits in Nuclear Medicine Practices. Vienna: IAEA International Atomic Energy Agency; 2008b

IAEA. Quality Management Audits in Nuclear Medicine Practices Second Edition. International Atomic Energy Agency Human Health series No 33. Vienna: IAEA; 2015

Jarritt P, Perkins A and Woods S. Audit of nuclear medicine scientific and technical standards. Nuclear Medicine Communications 2004;25:771-775

Mirzaei S, Maffioli L, Hilson A. Clinical audit in nuclear medicine. European Journal of Nuclear Medicine and Molecular Imaging 2011;38(1):3-4

Chapter 3

Validation of a cost-effective alternative for a radiochromatography method to be used in a developing country

Fany P Ekoume[1, 3], Hendrikus H Boersma[2], Faustin Dong A Zok[1], Sietske M Rubow[3]

[1]Yaoundé General Hospital, Cameroon; [2]University Medical Centre Groningen, The Netherlands; [3]Stellenbosch University, South Africa

Abstract

Introduction: The radiochemical purity (RCP) of technetium-99m labelled radiopharmaceuticals (RP) is important to ensure optimal scintigraphic image quality. In low-income settings, it may not be possible to use compendial analytical methods or expensive equipment for radiochemical purity analysis. All radiochemical analysis methods should however be validated against compendial or otherwise proven methods. To ensure the efficacy of RP prepared at Yaoundé General Hospital (YGH) Cameroon, this study cross-validated a cost-effective routine chromatographic method using a simple survey meter technique. A GMP-compliant method used at the University Medical Centre Groningen (UMCG), the Netherlands was used as the comparator.

Methods: Sestamibi, HMDP and DMSA kits currently used at YGH were reconstituted at UMCG with about 2000 MBq of freshly eluted sodium pertechnetate as described by the manufacturer, and spiked with eluate of the same generator to obtain a range of impurity concentrations. Samples of technetium-99m RP were spotted on 1x10 cm iTLC-SG strips and developed in appropriate mobile

---

phases. Each strip was first scanned on the chromatogram-scanner used at the UMCG (standard method), and immediately thereafter the strip was cut in two pieces and radioactivity from each portion was counted with a small survey meter from YGH. The percentage RCP for each TLC strip was calculated using both counting methods. Internationally recommended validation parameters and acceptance criteria were used. Student's paired t-test or ANOVA were used with 'no significant difference' designated at a 95% confidence-interval (P≥0.05). Linearity of the survey meter was determined for Tc-99m. Readings obtained with the survey meter were also plotted against the scanner results.

Results and Discussion: The proposed method proved to be accurate (CV of mean RCP <2), precise (RSD <2%), linear (slope close to 1, r^2 ≥0.99) within the RCP range of approximately 80% to 100%, and robust (P>0.05). LOD and LOQ were determined for the survey meter. Specificity depends on chemical separation. As we were validating the suitability of a method to quantify radioactivity, specificity was not included in the validation parameters.

Conclusion: The proposed method compared well with the standard-method and is suitable as a reliable low-cost method for limited resource settings.

Key words: Radiochemical method, comparison method, quality control, developing countries

Introduction

1. Importance of method validation

Nuclear Medicine needs optimal quality radiopharmaceuticals for accurate diagnosis and therapy of diseases (IAEA 2008; Dondi 2011). As the use of Nuclear Medicine increases in developing countries, including those in sub-Saharan Africa, so does the need for radiopharmaceuticals. With the increasing demand for radiopharmaceuticals, it is of paramount importance to ensure that only safe and effective products are administered to patients (IAEA 2016). Even though most radiopharmaceuticals in sub-Saharan Africa are prepared in small scale units, quality assurance programs should be implemented (Elsinga et al. 2010). Internationally, guidelines for Good Radiopharmacy Practice recommend that only validated analytical methods should be used to evaluate product quality before patient use. Quality assurance (QA) in the production of radiopharmaceuticals ensures that they are of sufficient quality for their intended purpose; it reduces the possibility of producing substandard products by adhering to specified procedures and protocols (Vincenti et al. 2016). Analytical Quality Control (QC) methods form part of quality assurance, helping to assess the quality of the product. According to Ajay et al., validation of analytical methods provides evidence that the method is suitable for it intended use, and thus helps to ensure provision of safe and effective final products (Ajay et al. 2012). The consequence of using unvalidated non-compendial methods could result in the administration of unknown substances, poor quality images and/or an unnecessary radiation exposure of patients (Amin et al. 2011; Vincenti et al. 2016).

2. The principle of validation of quantitative analytical methods

Validation of an analytical procedure is the process to ensure that the performance characteristics of the procedure adopted in a particular laboratory meet the requirements for the intended analytical application (The United States Pharmacopeial Convention<1225> 2008). In radiopharmacy, one of the most frequently used analytical criteria is radiochemical purity (RCP) of the product. The radiochemical purity of a preparation is the fraction of the total radioactivity in the desired chemical form of the radiopharmaceutical. During the labelling process, radiochemical impurities may be present as a result

of incomplete radiolabelling or decomposition products due to the presence of oxidizing or reducing agents, radiolysis or change of temperature and pH (Saha 2010; Millar et al. 2009; Loveless 2009; Mambilima 2016).

RCP can be assessed by various analytical techniques, for example paper, thin layer, or liquid chromatography and electrophoresis. Radiochromatography involves the separation of components in solution (depending on their affinity with the chromatography materials), and the measurement of the distribution of radioactivity on the chromatogram (Saha 2010).

International guidelines describe validation parameters and acceptance criteria for validation of analytical methods, including accuracy, precision, intermediate precision, linearity, range, specificity, robustness, detection limit and quantitation limit (The United States Pharmacopeial Convention <1225> 2008; EDQM 2018).

3. Validation studies of radiochemical analysis methods in literature

In the literature, analytical methods' performance characteristics for both SPECT (Single Photon Emission Computed Tomography) and PET (Positron Emission Tomography) radiopharmaceuticals differ slightly from one study to another. The parameters recommended by the ICH (International Council for Harmonisation, 2006), namely accuracy, precision, linearity and range, specificity, robustness and limit of quantitation (LOQ), are frequently used, especially for HPLC (High-Performance Liquid Chromatography) methods. However, it should be noted that radio-TLC and radio-HPLC express RCP as a ratio between counts or areas, without requiring absolute quantitative measurement of either the product or impurities. In this scenario, accuracy and LOQ, which both address quantitative measurement, may not be relevant for radio-HPLC or radio-TLC (Todde et al. 2014), or may need a different approach than in the case of chemical analyses.

Mihon et al. mention the ICH parameters for the HPLC determination of identity and RCP of [^{18}F]NaF (Mihon et al. 2016). Leonardi et al. use these parameters in the validation of a paper chromatography method as an alternative for determination of the RCP of [^{18}F]NaF (Leonardi et al. 2012). Seetharaman et al. (2006) validate their simplified solid phase extraction method for determining the RCP of

31

$[^{99m}Tc]Tc\text{-}MAG_3$ by determining specificity, linearity, and robustness. They compare their method against a standard method to evaluate accuracy and precision. Acceptance criteria for analytical method validation may require adjustments according to the type of equipment: radio-HPLC, radio-UPLC (Ultra Performance Liquid Chromatography), radio-TLC (Thin Layer Chromatography), or gamma spectrometry (Todde et al. 2017). Besides performance characteristics related to the method of analysis, analytical method validation could differ regarding experimental details such as mobile phase, stationary phase, flow rate, and wavelength of UV detectors (Todde et al. 2017).

Several studies describe the development of alternative chromatography procedures to replace 'gold-standard' methods for reasons including simplified technical handling and more rapid processing times. Faria et al. in 2015 studied an alternative chromatographic system for the quality control of MIBI where they evaluated different solvents to optimize separation efficiency, reproducibility, and analysis time (Faria et al. in 2015). This was, however, not a full validation study as defined in documents of normative character: ISO (International Organization for Standardization), ICH, FDA (Food and Drug Administration) (Krause 2002; The United States Pharmacopeial Convention <1225> 2008), with performance characteristics evaluated against acceptance criteria from references. Luebke et al. (2000) also evaluated an alternative testing method for the radiochemical purity determination of MIBI but did not include all parameters recommended in guidelines (The United States Pharmacopeial Convention <1225> 2008). Something similar was done for ^{99m}Tc-Annexin A5, using a binding assay instead of radio-TLC for determination of radiochemical purity as a release method (Boersma et al. 2004).

A variety of methods for quantification of distribution of radioactivity on radio-TLC plates are described in literature. Decristoforo and Zolle provide an overview of seven methods used for ^{99m}Tc radiopharmaceuticals, including cutting and counting in a scintillation counter, chromatogram scanning, analysis by linear analyzer and phosphor-imager autoradiography (Decristoforo et al. 2007). The methods differ regarding sensitivity, resolution and linearity, but differences in time required per analysis and the cost of equipment of each of these methods are considerable, making their adoption in resource-poor developing countries challenging. At Yaoundé General Hospital in Cameroon (YGH) there is no chromatogram scanner or more advanced equipment with which the distribution of

radioactivity or chromatograms of radiopharmaceuticals can be analyzed. An alternative method was therefore sought, namely counting sections of the chromatography strips corresponding to the distribution of the different radiochemical species with a contamination monitor available in the Nuclear Medicine department at YGH. Prior to employing it in daily practice, this proposed method had to be validated. The work described herein was performed to validate a cost-effective method for quantifying the distribution of radioactivity on chromatography strips using a RadEye B20 contamination monitor (available at YGH), and comparing it to the already validated method routinely used at the UMCG in the Netherlands.

Materials and methods

1. Materials for product preparation and chromatography

All experiments were performed at the UMCG radiopharmacy in the Netherlands. SPECT radiopharmaceutical kits Stamicis (2-methoxyisobutylisonitrile: MIBI), Osteocis (hydroxymethylene diphosphonate: HMDP), and Renocis (dimercaptosuccinic acid: DMSA), all from Curium, France, currently used for nuclear medicine imaging at YGH, were used for this study. Technetium-99m (^{99m}Tc) was obtained from an Ultra-Technekow generator (Mallinckrodt Medical BV, Netherlands). TLC scanning was performed using a VCS-103 scanner (Comecer S.P.A., Italy) used at UMCG. A calibrated RadEye B20 multipurpose counter (Thermo Fisher Scientific Messtechnik, Germany) was brought from YGH and used at UMCG for the comparative experiments. Chromatography was done using iTLC-SG sheets (Varian) with butan-2-one (methyl ethyl ketone) (Fluka) or 0.9 % saline solution (B Braun) as mobile phases.

2. Sample preparation

Freshly eluted technetium-99m sodium pertechnetate ($[^{99m}$Tc]NaTcO$_4$) was used to prepare each radiopharmaceutical following the kit manufacturer's instructions or summary of product characteristics (Osteocis 3mg 2016; Renocis 2010; Stamicis 2016). Technetium-99m-radiopharmaceutical kits ($[^{99m}$Tc]Tc-HMDP, $[^{99m}$Tc]Tc-MIBI and $[^{99m}$Tc]Tc-DMSA) each from a single lot were prepared and spiked with adequate quantities of sodium pertechnetate in order to reach different RCP values to

33

evaluate. The radiochemical purity aimed for in the spiked products ranged between approximately 80% and 100%.

Each of the cold kits included in this study was reconstituted with approximately 2000 MBq [^{99m}Tc]NaTcO$_4$] read with the dose calibrator (VDC 404 Veenstra, Joure).

3. Chromatography of radiopharmaceuticals

A drop of the same Tc-99m eluate used for kit reconstitution (and to spike the radiopharmaceuticals) was used to run a control analysis under the same conditions as those of the radiopharmaceutical samples.

Five µl of [^{99m}Tc]Tc-RP samples was spotted on 10 cm iTLC-SG strips. For HMDP and DMSA, the strips were then developed in methyl ethyl ketone as mobile phase, and for MIBI, the strips were developed in NaCl 0.9%. Under these conditions, [^{99m}Tc]Tc-HMDP, [^{99m}Tc]Tc-DMSA and [^{99m}Tc]Tc-MIBI remained at the origin and the impurity (pertechnetate) migrated with the solvent front. After developing, each strip was first scanned on the Veenstra VCS-203 TLC scanner using a low energy collimator with energy window range from 135 to 145 keV. The scan time was 60 seconds and background counts were subtracted. Within 5 minutes thereafter the strip was cut in 2 pieces at 4 cm from the origin. The radioactivity from each portion of the strip was counted for 1 minute with the RadEye B 20 counter which is a multipurpose survey meter with gamma energy range from 17 keV to 3 MeV (measurement range from 0 to 500 kcps). Each strip was placed flat in the bottom of a 10 cm deep container and the counter placed at the top of the container to ensure that counting geometry was the same for all samples. Each count rate reading was recorded with background count rate subtracted. From these values, the percentage of radiochemical purity of each strip was calculated.

4. Validation studies

The methods for validation of determination of RCP of the current study followed international guidelines from the United States Pharmacopeia (The United States Pharmacopeial Convention <1225> 2008), and the EDQM Guide (2018). Parameters used include accuracy, precision repeatability, intermediate precision, linearity, assay range, method robustness, limit of detection (LOD) and limit of

quantitation. Note that specificity of chromatographic methods depends on the chemical separation of compound and impurities. In the validation of the radioactive counting method, this parameter is thus not relevant.

4.1. Accuracy

Mean RCP values from three replicates of each sample were determined as well as the coefficient of variation (CV). The accuracy criteria for the radiopharmaceutical component were met when the mean of the standard method differed by not more than 1.5% from the mean value of the proposed method.

4.2. Repeatability

The repeatability precision was assessed with five replicates of a spiked sample and the relative standard deviation (RSD) was calculated.

4.3. Intermediate precision

The intermediate precision was determined by repeating at two different time points (3 h between the tests), the chromatography of 5 replicates of spiked samples performed by 2 different operators, quantifying distribution of activity on the strips using the two different counting systems. Mean RCP values and RSD were calculated. The statistical evaluation by ANOVA of the complete data set where results are grouped by each operator, each time point and instrument were analyzed with acceptance criteria stating no significant difference at 95% CI ($P \geq 0.05$).

4.4. Linearity

To test the linearity, the response of the survey meter to Tc-99m was evaluated. A 5 MBq point source of Tc-99m was repeatedly counted over a period of 27 hours. The measured count rate was plotted against the calculated activity of the point source. In addition, chromatography of three replicates from each spiked sample were performed and the chromatograms were read with both the scanner and the counter for RCP determination as described above. The linearity results include the equation from the linear regression obtained from a plot of % RCP determined with the counter (proposed method) against the RCP values determined with the scanner (the validated control method of the GMP compliant

radiopharmacy), its slope, the correlation coefficient and Y intercept. As acceptance criteria, the slope should be close to 1, and $r^2 \geq 0.99$

4.5. Robustness

The robustness of the chromatography counting method with the RadEye B20 counter was evaluated by comparing the variation of count rates of five replicates of a spiked sample of MIBI. The pairs of strip sections were read with the counter in slightly different positions, i.e. one in the normal reading position and the other section with the counter at an angle differing 5° from the normal position (figure 3.1). Mean RCP from each set of counts and RSD were calculated. ANOVA was used to check if there were any significant differences between the groups. Acceptance criteria are RSD<5% and $P \geq 0.05$.
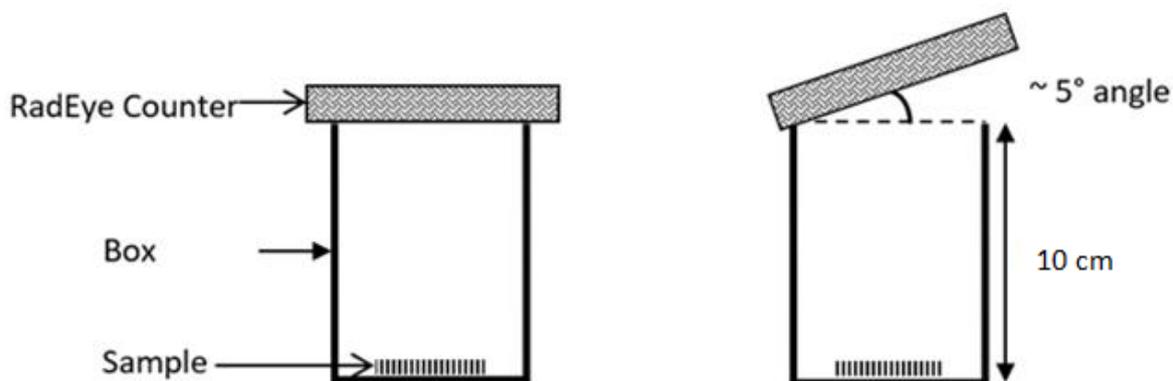
Figure 3.1 Illustration of the robustness test methodology
(reading with counter at normal position and at 5° angle)

4.6. Limit of detection and limit and quantitation

The limit of detection of the RadEye B20 counter was evaluated by counting a blank (strip with no analyte) twenty times with the counter and calculating the mean of blank counts plus 3 times the standard deviation of the blank. The limit of quantitation was calculated as the mean of blank counts plus 10 times the standard deviation of the blank (EDQM 2018).

Results

Complete results are shown in tables and figures.

1. Accuracy

Samples with RCP between 78% and 100%, with activities between 2 and 3 MBq were spotted on the TLC plates. The mean RCP of triplicate analyses at each sample RCP level from reading of both instruments for the 3 radiopharmaceuticals are shown in table 3.1.

Table 3.1 Accuracy of the proposed method for the 3 compounds

| DMSA %RCP | | | MIBI %RCP | | | HMDP %RCP | | |
| Mean ± CV | | | Mean ± CV | | | Mean ± CV | | |
Scanner	Counter	Diff	Scanner	Counter	Diff	Scanner	Counter	Diff
99.7±0.1	99.5±0.1	0.2	99.3±0.4	99.2±0.1	0.1	99.9±0.0	99.4±0.2	0.5
99.2±1.1	99.2±0.1	0.0	98.5±0.4	98.8±0.7	0.3	97.8±1.0	98.0±1.0	0.2
92.0±1.7	91.8±1.1	0.2	91.3±3.7	90.6±3.0	0.7	96.6±1.0	95.2±0.9	1.4
87.9±1.6	88.1±1.3	0.2	84.7±0.6	86.0±2.0	1.3	87.1±1.2	86.28±1.1	0.8
82.9±0.8	81.9±1.5	1.0	78.9±5.0	78.4±7.0	0.5	82.0±0. 6	82.07±0.8	0.1

N=3; Diff: difference between RCP from scanner and counter for each product; CV: coefficient of variation

2. Repeatability precision

The RSD for 5 replicates of spiked samples for the three products from both devices varied between 0.6% and 1.7%. For the impurity values, the RSD ranged from 0.5 to 6.1 (table 3.2).

Table 3.2 Accuracy of the proposed method for the 3 compounds

| | DMSA | | MIBI | | HMDP | |
| | % RCP Mean ± RSD | | % RCP Mean ± RSD | | % RCP Mean ± RSD | |
	Scanner	Counter	Scanner	Counter	Scanner	Counter
RCP	83.1±0.6	83.4±0.8	79.7±1.7	80.9±1.4	82. 0±0.7	82.0±0.6
Impurity	16.8±3.6	16.6±4.2	19.1±4.3	19.02±6.1	18. 0±3.1	17.9±0.5

n=5; RSD: relative standard deviation

3. Intermediate precision

Results from the method performed by different operators at different time points for the three products gave RCP range from 78.3% to 83.4%, impurity ranged from 16.8% to 23.3%, highest RSD value 6.1 with P values between 0.2 and 11.8 (ANOVA) (table 3.3A and 3.3B).

Table 3.3A Intermediate Precision results: Different Operators

(n=5, acceptance criteria P≥0.05)

Spiked samples	Device	Operator 1 Mean %RCP±RSD	Operator 2 Mean %RCP±RSD	ANOVA (P)
DMSA	Scanner RCP	83.1±0.6	81.6±0.8	3.1
	Scanner Imp	16.8±3.6	18.1±2.9	0.7
	Counter RCP	83.4±0.8	82.5±0.7	0.5
	Counter Imp	16.6±4.2	17.4±3.7	0.5
MIBI	Scanner RCP	79.7±1.7	79.8±1.0	1.1
	Scanner Imp	19.1±4.3	19.6±4.9	0.6
	Counter RCP	80.9±1.4	80.3±0.7	0.8
	Counter Imp	19.02±6.1	19.6±2.9	0.8
HMDP	Scanner RCP	82.0±0.7	81.6±0.9	0.4
	Scanner Imp	18.0±3.1	18.0±4.7	0.4
	Counter RCP	82.4±0.6	82.3±0.5	0.2
	Counter Imp	17.9±0.5	17.6±3.1	0.2

Table 3.3B Intermediate Precision results: Effect of Time

(acceptance criteria ANOVA P≥0.05)

Spiked samples	Device	Time 1 mean %RCP±RSD	Time 2 mean %RCP±RSD	P
DMSA	Scanner RCP	83.1±0.6	78.32±0.8	5.8
	Scanner Imp	18.8±3.6	20.12±4.5	3.2
	Counter RCP	83.4±0.8	76.7±1.4	11.8
	Counter Imp	16.6±4.2	23.3±4.9	11.8
MIBI	Scanner RCP	79.7±1.7	77.2±1.1	2.6
	Scanner Imp	19.1±4.3	22.6±4.5	3.4
	Counter RCP	80.9±1.4	78.2±0.6	2.5
	Counter Imp	19.0±6.1	21.7±2.3	2.5
HMDP	Scanner RCP	82.0±0.7	79.1±1.1	2.5
	Scanner Imp	18.0±3.1	19.9±4.4	1.3
	Counter RCP	82.04±0.6	78.3±0.9	3.8
	Counter Imp	17.9±0.5	21.6±3.4	3.8

Note: Same operator at different times; n=5; Imp: impurity

4. Linearity

The response of the counter to Tc-99m activity ranging from 0.2 MBq to 5 MBq is shown in figure 2.2. The regression curves for values obtained with the survey meter against the scanner results for each of the radiopharmaceuticals are shown in figure 3. All values of r^2 were above 0.99 and all slopes close to 1.
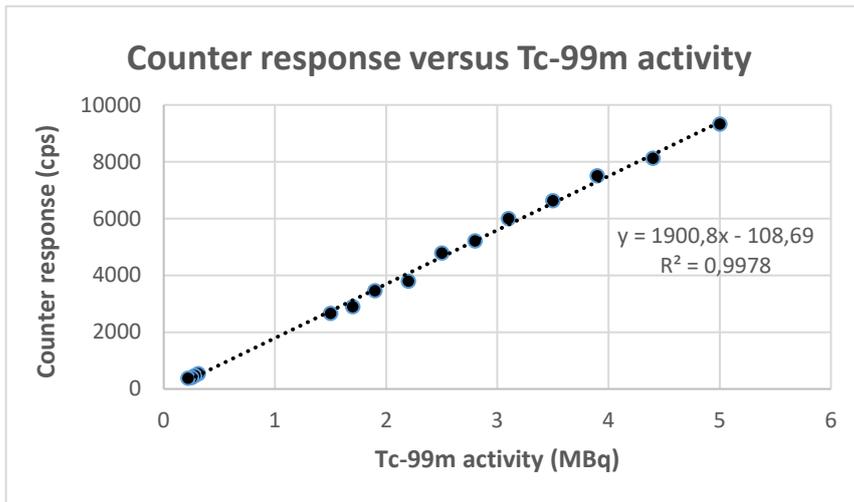
Figure 3.2 Response of the counter for Tc-99m activity
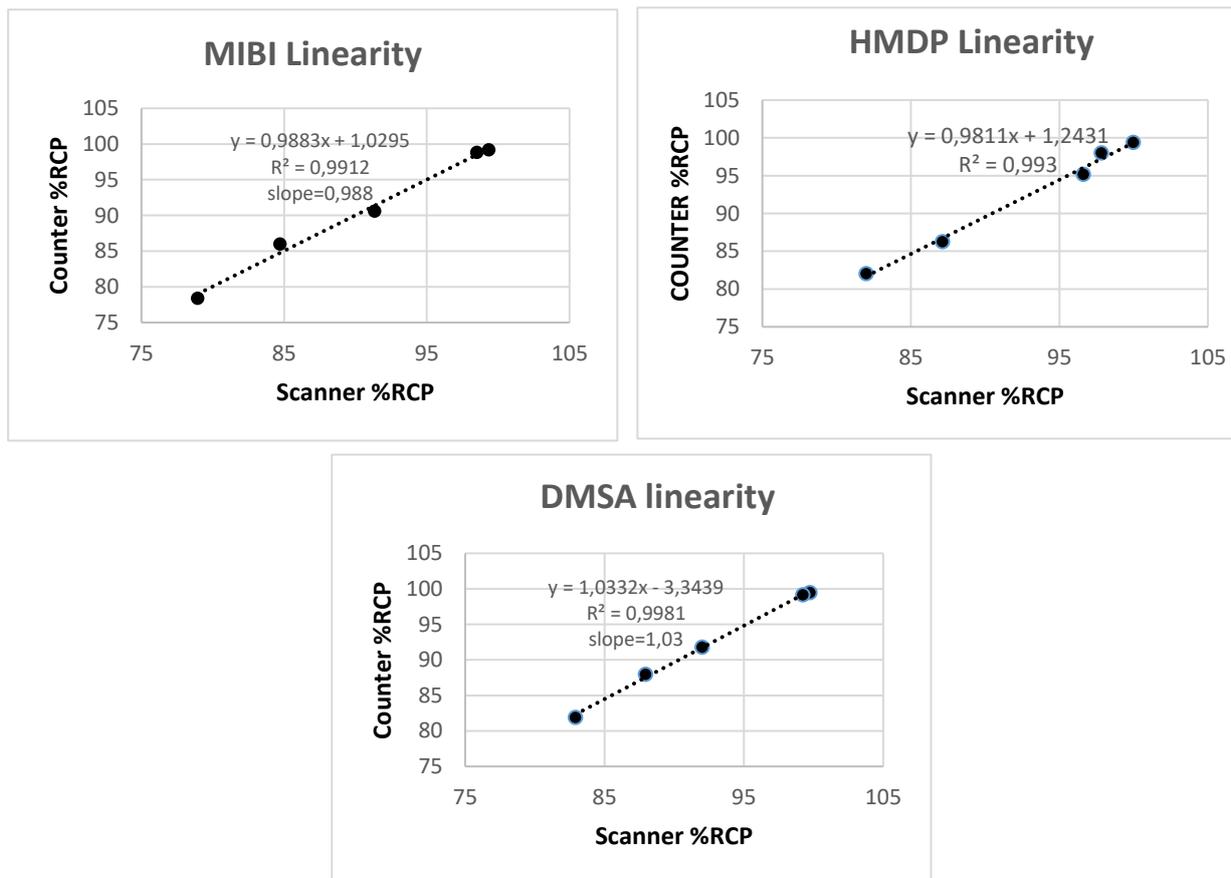
Figure 3.3 Linearity of the proposed method for the 3 products, counter response

compared with RCP values determined with the scanner

41

5. Robustness

Results from 5 replicates of a spiked MIBI sample with one part counted with slight deviation in counter position compared very well with those obtained with the counter in the normal position.

Table 3.4 Robustness of the counter

Change	%RCP Mean ±RSD counter at normal position for both parts	%RCP Mean ±RSD counter tilted 5° for the strip section containing the impurity	%RCP Mean ±RSD counter tilted 5° for the strip section with the bound compound
MIBI (n=5)	91.5±1.0	91.8±2.6 (P=2.9*)	91.9±2.3 (P=2.4*)

* P values for ANOVA of the respective RCP compared to RCP obtained with the counter in the normal position

6. Limit of detection and limit of quantitation

The results from twenty counts of the blank sample (n=20) gave 0.6 counts per second as a mean value of the blank with standard deviation of 0.1, for a LOD value of 0.9 counts per second. The calculated LOQ was 1.6 counts per second. Based on the linearity curve this represents approximately 0.06 MBq.

Discussion

1. The importance of method validation

Recommended analytical methods are supplied with package inserts from radiopharmaceutical kits and by pharmacopoeial monographs. In radiopharmacies with limited funding, it may be difficult to follow the recommended analytical methods due to lack of adequate equipment and limited availability of consumables. Other disadvantages of many of the recommended methods include the time required to complete the tests, which delays administration of the short-lived products to patients, and use of relatively large volumes of solvents when solid phase extraction (SPE) or HPLC are used (Seetharaman et al 2004). Thus, there is a clear need for practical, simple, faster (Hammes et al. 2004; Mihon et al. 2016) and low-cost methods. Any alternative methods should be validated prior to use (Leonardi et al. 2012).

The current study describes the validation of a cost-effective quantification method for instant thin layer radiochromatography strips using a counting system (RadEye B20) available at YGH, cross-validated with a routine method used at the GMP compliant radiopharmacy at UMCG.

In the current work, the validation parameters prescribed by the ICH (The United States Pharmacopeial Convention <1225> 2008), i.e. linearity, precision, intermediate precision, accuracy, assay range, method robustness, limit of detection and limit of quantitation were all included for each of 3 SPECT radiopharmaceuticals commonly used at YGH (HMDP, DMSA, MIBI).

2. Standards and Sampling

In validation of analytical methods, reference standards are generally used for comparison. Certified reference standards for ^{99m}Tc impurities do not exist in the radiopharmaceutical industry due to the short half-life and short shelf life of the radiolabeled complexes as well as absence of a stable isotope of technetium. In our study, samples containing varying concentrations of ^{99m}Tc-RP were obtained by spiking with adequate quantities of sodium pertechnetate and measured with a validated method for comparison with the proposed alternative counting method. Seetharaman et al. used a similar approach to determine linearity of their method (Seetharaman et al. 2006).

43

3. Accuracy

Accuracy can be described as being as near as possible to the expected value. For the experiments in this study, we used a similar approach to Mambilima, comparing differences between values from standard to proposed methods (Mambilima 2016). We calculated the difference between RCP mean value of the standard method and the method of study for the three radiopharmaceuticals at each RCP level after spiking with pertechnetate. All differences in RCP were smaller than 1.5%, confirming the method of study met the accuracy criteria. Different approaches have been used in the literature. Mihon et al. used the percentage recovery within a predetermined specified range to demonstrate the accuracy of the method he proposed (Mihon et al. 2016), while Leonardi et al. in 2012 used the RSD value of the mean percentage recovery to validate the accuracy of their method (Leonardi et al. 2012). As we used a TLC method here, the recovery parameter is not relevant, because all used sample material will remain present during the analytical procedure, which is not the case for methods using chromatography columns like HLPC.

In this study HMDP, DMSA and MIBI accuracy analysis met the acceptance criteria at the five RCP levels tested. Thus, for the three radiopharmaceuticals the comparison of respective results obtained from the two counting systems is good. This indicates that the proposed counting method using the RadEye B20 counter is demonstrated to be as good as the Veenstra VCS-203 TLC scanner.

4. Precision

Precision can be defined as the closeness of measurements to each other. In contrast with accuracy, no relation is investigated between the result and the expected result, or true value. The validation of precision in the current work was twofold: firstly, we evaluated repeatability with five replicates of a spiked sample for each product. The RSD values of the impurities were much lower than acceptance value 15%. It should be noted that for RCP measurements (i.e. the active pharmaceutical ingredient or radiopharmaceutical compound) such a high RSD value is not acceptable. The proposed method using the RadEye B20 counter is regarded as precise. Secondly, we did intermediate precision tests by the evaluation of the method using different operators, and analysis at different time points, and found no significant differences. Seetharaman used a similar approach to compare a standard ITLC method

previously developed by Chen et al. (Chen et al. 1993) to a simpler method developed using a solid phase extraction cartridge (Seetharaman et al. 2006). Even though our survey meter is a relatively simple piece of equipment, these results encourage us to advocate the use of the RadEye B20 counter, especially in a low-cost setting.

5. Linearity and range

The range of radiochemical purity between approximately 80% and 100% used in the study was chosen in order to include RCP values recommended by manufactures for patient use (95% to 100%) and to be in accordance with ICH and pharmacopeia recommended minimum of five concentrations in the range from 80% to 120 % for finished products or assays of drug substances (The United States Pharmacopeial Convention <1225> 2008). Our samples contained approximately 2 to 3 MBq/5 µl.

To prove linearity, the analytical method should exhibit proportionality to the concentration of the analyte at the chosen range; r^2 should be determined as well as the equation and slope of the regression line. We studied the response of the RadEye B20 counter to Tc-99m radioactivity. Linearity was demonstrated between 0.2 and 5 MBq. As the maximal amount of counts expected for QC strips is much lower than the maximum of the range of the RadEye B20 counter, which can count up to 500 kcps according to its specifications, we expect no dead time problems using our method.

6. Robustness

Parameters such as mobile phase composition, and ambient temperature influencing the stationary and mobile phase flow are typically used to evaluate robustness, especially for HPLC methods (Mihon et al. 2016). In this work, we evaluated the suitability of a counting system, which is independent of chemical separation. Careless positioning of the survey meter might lead to differences in counting geometry between the two parts of a chromatography strip. Based on the possibility of slight differences in counting position of the counter, the robustness of the counting system was checked by comparing counts with a correctly positioned counter against a slightly tilted counter. No significant differences in count rates were demonstrated, so the method could withstand at least a small variation in positioning the counter.

7. Limit of Detection and limit of quantitation

Some authors argue that limits of detection and of quantitation are not required for RCP determination with thin layer radiochromatography. Todde et al. indicate that LOQ as usually included in validation studies, may not be relevant where the RCP is determined as a ratio between areas or counts, and no absolute quantitative measurement is required to be performed (Todde et al. 2014). Seetharaman et al. (2006) state that determination of LOD and LOQ can be omitted as only products with RCP values higher than 90% are used clinically. It is however important to know if the counting device is able to distinguish the lowest expected or clinically meaningful impurity activity from background counts.

Tc-99m products are usually required to have a RCP of 90 or 95%, i.e. not more than 5% of impurities may be present. Exact quantification of impurities larger than 2 % may not be mandatory, as it will not affect the acceptance of the product. Calculated from our linearity measurements, 2% of our 5 MBq samples would give a count rate of approximately 80 cps, or 50 times the LOQ of the survey meter. Looking at the calibration curve, uncertainties are present in the lower range of radioactivity. We think that small impurities will rather be overestimated than underestimated. Thus, the survey meter can be used, but care should be taken not to use very low amounts of Tc-99m, as impurities might then be below the LOQ.

8. Specificity

The specificity of chromatographic methods depends on the separation of different chemical entities, e.g. the active pharmaceutical ingredient and any impurities. For HPLC, this means that the peaks of the different chemical entities should be clearly separated. In our evaluation of the method of quantification of the distribution of radioactivity, the chemical separation was done with thin layer chromatography, and the same strip was then used for both counting methods. We therefore did not include specificty in our validation. For the same reason we also did not test for impurities other than pertechnetate.

9. Summary

Guidelines for analytical method validation as presented by international regulatory bodies and national authorities (e.g. ICH, FDA) and described in available literature, usually do not provide much information for radioanalytical methods such as radio-TLC (ICH Q2R1, EMEA 2006). A notable exception is the Guide published by the EDQM in 2018 which does address validation of analytical methods for radiopharmaceuticals. Using internationally recommended parameters, we validated our proposed method by comparison with a validated method routinely used in a GMP unit.

10. Limitations

Limitations of this study include the non-availability of reference standards for Tc-99m labelled compounds since there are no stable isotopes of technetium (Todde et al. 2014). The range over which the linearity curve for the survey meter was determined, includes typical activities for sample drops used for RCP analysis of Tc-99m radiopharmaceuticals with iTLC, but should be extended at the lower end to unequivocally include activities expected in impurities representing 0.5% of the sample. Furthermore, the linearity measurement with the survey meter for Tc-99m should be extended to include higher activities if radiopharmaceuticals with higher radioactivity concentrations are to be analysed.

In validation studies, sample preparation should be done very carefully, and effects like possible adhesion of impurities to containers used in preparing samples should be considered in the design of the tests. Furthermore, spiking samples with pertechnetate may result in additional labeling of the Tc-99m-radiopharmaceutical, which may cause an additional uncertainty in the outcome of the experiments.

Conclusion

Compared to the standard method, our alternative method was linear, accurate, specific in the range of product concentrations internationally recommended for patients, precise, and robust. The value of the successful validation of the proposed method is twofold: Firstly, it has increased the awareness of the importance of validation among staff in our unit, and secondly, a cost-effective method is now available and can be used in any other low-income Nuclear Medicine units.

Declarations for publication

Authors' contributions

FPE performed the experiments, analysed the data and drafted the manuscript; HHB helped with design of the experiments, with drafting of the manuscript and provided critical feedback on the manuscript, SMR helped with general design of the study, provided critical feedback on the manuscript and assisted with revision of the manuscript. DAZ helped with interpretation of the results and revised the manuscript. All the authors read and approved the final manuscript.

Conflict of interest

All authors declare that they have no competing interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgements

References Chapter 3

Ajay S, Rohit S. Validation of Analytical Procedures: A comparison of ICH vs. Pharmacopoeia (USP) vs. FDA. International Research Journal of Pharmacy 2012;3:39-42

Amin KC, Patel S, Oke A, Saha GB. An Alternative Method for Determining the Radiochemical Purity of ^{99m}Tc-Tetrofosmin. Journal of Nuclear Medicine Technology 2011;39:51-54

Boersma HH, Stolk LM, Kenis H, Deckers NM, Vanderheyden JL, Hofstra L, Heidendal GA, Reutelingsperger CP The ApoCorrect assay: a novel, rapid method to determine the biological functionality of radiolabeled and fluorescent Annexin A5. Analytical Biochemistry 2004;327(1):126-34

Chen F, Decristoforo C, Rohrbacher B, Riccabona G. A simple two-strip method to determine the radiochemical purity of technetium-99m mercaptoacetyl triglycine. European Journal of Nuclear Medicine 1993;20:334-338

Decristoforo C and Zolle I. Quality Control Methods of 99mTc Pharmaceuticals. In Zolle I, (ed.). Technetium-99m Pharmaceuticals; Preparation and Quality Control in Nuclear Medicine. Berlin Heidelberg. New York: Springer; 2007. pp. 123-143

Dondi M, Kashyap R, Paez D, Pascual T, Zaknun J, Mut F, et al. Trends in Nuclear Medicine in developing countries. Journal of Nuclear Medicine 2011;52:16S-23S

Elsinga P, Todde S, Penuelas I, Meyer G, Farstad B, Faivre-Chauvet A, et al. Guidance on current good radiopharmacy practice (cGRPP) for the small-scale preparation of radiopharmaceuticals. European Journal of Nuclear Medicine and Molecular Imaging 2010;37:1049-1062

European Directorate for the Quality of Medicines & HealthCare: Guide for the elaboration of monographs on Radiopharmaceutical Preparations. 2018. https://www.edqm.eu/sites/default/files/guide_guide_for_the_elaboration_of_monographs_on_radio-pharmaceutical_preparations_october_2018.pdf [Accessed 15 September 2019]

Faria DP, Buchpiguel CA and Marques FLN. Alternative chromatographic system for the quality control of lipophilic technetium-99m radiopharmaceuticals such as $[^{99m}Tc(MIBI)_6]^+$. Brazilian Journal of Medical and Biological Research 2015;48:902-907

Hammes R, Joas LA, Kirschling TE, Ledford JR, Knox TL, Nybo MR, et al. A better method of quality control for ^{99m}Tc- Tetrosfosmin. Journal of Nuclear Medicine Technology 2004;32:72-78

IAEA. Operational Guidance on Hospital Radiopharmacy, A safe and Effective Approach. Vienna: International Atomic Energy Agency; 2008. pp. 28-30

IAEA. Good practice for introducing radiopharmaceuticals for clinical use. IAEA-Tecdoc-1782. Vienna: International Atomic Energy Agency; 2016;ISSN 1011-4289;1782

ICH. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology. EMEA, 2006

Krause S. Good analytical method validation practice. Deriving acceptance criteria for the AMV protocol: part II. 31-46 http://www.ivtnetwork.com/default/file/Good%20Analytical%20part%20IIpdf [Accessed 10 October 2016]

Krause S. Good Analytical Method Validation Practice, Part I: Setting-Up for Compliance and Efficiency. Journal of Validation Technology 2002;9:23-32

Leonardi N, Casale G, Nicolini J, Zubata P, Salgueiro M and Zubillaga M. Validation of a paper chromatographic methodology as an alternative for determination of the radiochemical purity of $Na^{18}F$. Journal of Nuclear Medicine Technology 2012;40:271-274

Loveless V. Quality control of compounded radiopharmaceuticals, continuing education for Nuclear pharmacist and Nuclear Medicine Professionals. University of New Mexico Health Sciences Center, College of Pharmacy, 2009; 15 (XV), Lesson 3, p 9-12

Luebke AL, Wilary DM, Mahoney DW, Hung JC. Evaluation of an alternative radiochemical purity testing method for technetium-99m sestamibi. Journal of Nuclear Medicine Technology 2000;28(4):259-263

Mambilima N. Validation of radiochemical purity analysis methods used in two tertiary public hospitals in South Africa. Thesis submitted in partial fulfilment of the requirements for the degree of Master of Science in Nuclear Medicine in the Faculty of Medicine and Health Sciences at Stellenbosch University 2016

Mihon M., Tuta C., Manea C., Ion A., Lavric V. Validation of the HPLC method for determination of identity and radiochemical purity of [^{18}F]Na U.P.B. Scientific Bulletin 2016;Series B,78 (1)

Millar AM, Beattie LA, Craig F and O'Brien LM. An evaluation of GMCP-SA as a replacement for ITLC-SG when measuring the levels of radiochemical impurities in 99m Tc-radiopharmaceuticals by thin-layer chromatography. Journal of Labelled Compounds and Radiopharmaceuticals 2009;52:538-542

OSTEOCIS 3mg, Kit for radiopharmaceutical preparation. Summary of Product Characteristics, IBA Molecular 2016

RENOCIS, Kit for preparation of technetium [^{99m}Tc] - succimer injection. Summary of Product Characteristics, CIS bio international 2016

Saha GB, Fundamentals of Nuclear Pharmacy, 6th edition. New York: Springer; 2010. pp. 153-164

Seetharaman S, Ballinger J, and Sosabowski MH. Simplified method for determining the Radiochemical Purity of ^{99m}Tc-MAG3. Journal of Nuclear Medicine Technology 2006;34:179-183

STAMICIS, Kit for radiopharmaceutical preparation. Summary of Product Characteristics, IBA Molecular 2016

The United States Pharmacopeial Convention. Validation of compendial procedures (general information 1225). The United States Pharmacopeia and National Formulary (UPS37-NF32). Rockville: The United States Pharmacopeia; 2008

Todde S, Kolenc Peitl P, Elsinga P, Koziorowski J, Ferrari V, Ocak E, et al. Guidance on validation and quantification of processes and operations involving radiopharmaceuticals.

European Journal of Nuclear Medicine and Molecular Imaging Radiopharmacy and Chemistry 2017;2:8

Todde S, Windhorst AD, Behe M, Bormans G, Decristoforo C, Faivre-Chauvet A, et al. EANM guideline for preparation of an investigational medicinal product dossier (IMPD). European Journal of Nuclear Medicine and Molecular Imaging 2014;41:2175-2185

Vincenti LP, Samuel A and Zarf F. Establishing radiopharmaceutical standards at a Nuclear Medicine unit in Malta. International Journal of Radiology and Imaging Technology 2016;2:012

Chapter 4

Implementation of air quality monitoring

in a low-income radiopharmacy unit

Fany P Ekoume[1, 3], Hendrikus H Boersma[2], Sietske M Rubow[3]

[1]Yaoundé General Hospital, Cameroon; [2] University Medical Centre Groningen, The Netherlands;

[3]Stellenbosch University, Tygerberg, South Africa

Abstract

Background: Environmental control is of the utmost importance to protect radiopharmaceuticals against microbial contamination. The Department of Nuclear Medicine at Yaoundé General Hospital in Cameroon has introduced a quality control program including passive air sampling. This paper evaluates the trends of air quality monitored by settle plate exposure over the year 2017.

Sampling was performed by positioning opened tryptic soy agar plates for 2 to 4 hours at six predetermined locations where radiopharmaceuticals are prepared and dispensed, either during work, or while work stations were not used ("rest") After exposure the plates were incubated at 30 to 35 °C for 72 hours and the number of colony forming units per hour was determined. Based on the GMP grade of the sampling location and the corresponding limits recommended by the European Commission, the percentage of settle plate results exceeding the limit were recorded and microorganisms identified. Interventions aimed at improving cleanliness of the radiopharmacy

environment were introduced after 6 months. Post intervention sampling evaluated the effect of the interventions using Fisher's two-sided exact test (P<0.05).

Results: At each site, 77 samples at rest and 40 samples during work were collected before intervention, versus 76 samples at rest and 33 samples during work after the intervention. The reduction in percentage of plates exceeding the action limit were as follows: In the closed cabinet at rest: 55%; during work: 42%. In the LAF at rest: 14%; during work: 11%. In the open bench area at rest 45% and during work 45%. On the lab floor, the decrease was 6% at rest and 10% during work. The microorganisms identified were *Staphylococcus epidermidis*, other gram positive bacteria, and *Candida* species. Significant improvements were found at all sample locations with all p ≤0.05.

Conclusion: Passive air sampling was implemented and it was confirmed that subsequent simple interventions led to a marked improvement in the air quality.

Keywords: Quality control, radiopharmacy, microbiological monitoring, passive air sampling

Background

Radiopharmaceutical injections are, like any other injectable product, in need of special protection, as potential microbiological contamination could harm the patient. Therefore, the environment in which sterile injections are prepared should be controlled, clean and free of microorganisms. Monitoring results help to alert the staff about the performance of the equipment, procedures, and the operators' techniques and skills, and it can guide effective preventive actions. The real value of a microbiological monitoring program lies in its ability to confirm consistent, high quality environmental conditions at all times.

To assess the level of microbial contaminants or other harmful substances and control the microbiological status of working environments for aseptic processing, a range of sampling methods are available (The United States Pharmacopeial Convention <1116> 2013). These include particle counting and methods monitoring growth of microorganisms.

Three different microbial contamination risk levels are defined in the literature (The United States Pharmacopeial Convention <797> 2012). If products are compounded from sterile commercial drugs using sterile devices and where procedures involve only a few basic steps in a closed system with simple aseptic transfer and manipulation, the procedure is regarded as low risk. The medium risk level involves areas in which multiple sterile commercial products are prepared, complex aseptic manipulations are done and procedures cover a long period of time. Quality assurance procedures include all steps required at low risk level, as well as more challenging tests like media fill tests. When products are prepared from non-sterile ingredients, compounded using non-sterile devices prior to terminal sterilization, or products are exposed to conditions worse than class A air quality for longer than an hour, the procedures are regarded as high risk. Radiopharmacies working with technetium-99m generators and commercial kits work with closed systems which carries a relatively low risk, but as multiple doses are dispensed from the kits over the span of several hours, the work should probably be categorized as medium risk. According to the IAEA, these procedures should therefore be performed in a Grade A clean air environment (e.g. a vertical laminar air flow unit or isolator), and the room air should also meet class C or D requirements and these environments should be subject to regular microbiological monitoring (IAEA 2008 (a)). Such monitoring procedures include air sampling.

Specific locations for air sampling are determined considering the proximity of the exposed products to the environment. Using pre-defined sampling sites allows comparison of the level of contamination of an area or site over time (Sutton S 2015). Microbiological sampling sites are best selected considering human activity during production operations, as contamination is strongly related to location, movement and behaviour of personnel within a critical zone. Areas where monitoring is critical are those related to airborne contamination, entry points where materials and equipment move from areas of lower to higher classification, and areas around doors and airlocks.

The logical step following results of environmental monitoring which exceed recommended limits, is the introduction of corrective and preventive actions (CAPA). Personnel flow and behaviour, operations, and materials are factors that could be analysed to try to pinpoint the root source of contaminants (WHO November 2012).

The work reported in this paper concerns a radiopharmacy unit that is not GMP compliant and that is only able to perform passive air sampling. Nevertheless, the unit strives to comply with Good Radiopharmaceutical Practice (GRP) recommendations, and has recently implemented environmental monitoring by passive air sampling. This paper describes the trends of air quality monitored by settle plate exposure over the year 2017 at Yaoundé General Hospital (YGH) in Cameroon, and the effect of relatively simple, low-cost corrective actions.

Methods

Passive air sampling was performed in the YGH Radiopharmacy by positioning opened tryptic soy agar plates (TSA, diameter 90 mm) for 2-4 hours at locations where radiopharmaceuticals are prepared and dispensed. The sampling was carried out both when there was no work activity in the area ("at rest" or "resting") and during work activity ("during work" or "working") in predetermined areas in a closed cabinet (CC) (2 sites), laminar air flow cabinet (LAF) (2 sites), on the open bench (OB) (1 site) and the floor at the radiopharmacy entrance (F) (1 site). After exposure the plates were incubated at 30 to 35 °C for 72 hours. The observed number of colony forming units (CFU) were corrected to reflect a standardised 4-hour period. Where possible, plates were sent to the microbiological unit for identification of microorganisms. The total number of measurements for each sampling site during the 6-month interval was determined. Based on the recommended GMP grade of the sampling location and the corresponding recommended limits, the percentage of settle plate results exceeding the limit was recorded.

Several interventions aimed at improving cleanliness of the Radiopharmacy environment were introduced after 6 months. Operator training was scheduled for once a month. Wall and window cleaning on a regular basis were introduced. All syringes were removed from cardboard boxes and placed in plastic containers prior to transfer into the room. Cotton swabs were replaced with gauze, sterile gloves were introduced for any operation performed in the radiopharmacy rooms and all the lab coats used in the radiopharmacy were sterilised on a regular basis. Easy to clean plastic shoes were introduced for each operator at the entrance of the radiopharmacy. For any cleaning operation in the radiopharmacy, sterile water is now used rather than tap water. At the end of each week, the CC and LAF are sterilised by drops of 10% formol on gauze and remain exposed for the whole weekend.

After the introduction of the changes, samples were collected for six months to evaluate the effect of the change. The number of plates exceeding the limit recommended by the European Commission GMP Guidelines (2008) for the corresponding class (at rest and during work before and after intervention) was recorded and the percentage of reduction in exceeding plates calculated. To assess the statistical differences between data before and after intervention, Fisher's two-sided exact test was performed

using STATA Statistics version 15.1 (copyright 2017 StataCorp, Texas 778). P values of less than 0.05 were considered statistically significant.

Results

A total number of 1356 plates were collected; 702 before the intervention and 654 plates after the intervention. 77 samples at rest and 40 samples during work were collected at each site before intervention, versus 76 samples at rest and 33 samples during work after the intervention. The results from all sampling sites exhibit a clear improvement both at rest and during work with data of the reduction in exceeding plates ranging from 6% to 60%. The lowest percentage of reduction was found on the floor (6% at rest and 10% during work) while the highest reduction was recorded in the closed cabinet (between 49% and 60%). All the results are represented in tables 4.1 to 4.3 below.

In total, only 9% of settle plates before intervention were not contaminated, compared to 53% after invention. Significant improvements were found at all sample locations with all p ≤0.05.

Table 4.1 Settle plate results in all sites during work (W) and at rest (R)

Location	Site	Recom-mended Class	Limit (CFU/4h)	Plates exceeding limit before intervention		Plates exceeding limit after intervention		Reduction in exceeding plates	
				Rest	Work	Rest	Work	Rest	Work
Closed cabinet	site 1	A	<1	69 (89%)	33 (82%)	29 (38%)	11 (33%)	51%	49%
	site 2	A	<1	71 (92%)	34 (85%)	25 (32%)	15 (45%)	60%	40%
LAF	site 1	A	<1	15 (19%)	10 (25%)	6 (7%)	4 (12%)	12%	13%
	site 2	A	<1	20 (25%)	13 (32%)	7 (9%)	7 (21%)	16%	11%
Open bench	site 1	C	50	46 (59%)	24 (60%)	11 (14%)	5 (15%)	45%	45%
Floor	site 1	D	100	38 (49%)	22 (55%)	33 (43%)	15 (45%)	6%	10%

The number of plates exceeding the relevant limit is shown for each site, followed by the percentage in brackets.

Table 4.2 Fisher's two-sided exact test of data from plate exposure before versus after corrective action

Area	Rest	Work
Closed cabinet	F=0.14	F=0.01
LAF	F=0.40	F=0.02
Open bench	F=33.42	F=3.60
Floor	F=12.82	F=4.74

P was always <0.05

Table 4.3 Microbial trends in the closed cabinet in 2017

	January – June (before intervention)				July - December (after intervention)			
	Staphylo-coccus	Other gram + bacteria	*Candida* species	none	*Staphylo-coccus*	Other gram + bacteria	*Candida* species	none
N	85	99	0	11	33	44	12	58
%N	72%	84%	0%	9%	30%	40%	11%	53%
Total	117	117	117	177	109	109	109	109

N: number of plates contaminated with different microbial species

Discussion

Classification and maintenance of an environment depend on a number of factors including the premises, equipment characteristics, operating staff's clothing and behaviour, and cleaning procedures (Todde et al. 2017; Kastango and Bradshaw 2004, IAEA 2008 (b)). According to Todde et al. (2017) the qualification of classified environments in radiopharmacies can be difficult, due to lack of instrumentation and skills. The trends of microbial contaminants in the current study revealed the presence of viable microorganisms at all sampling sites. The general overview of all sites exhibits higher concentration of viable microorganisms during work than when there was no work performed. An exception was the slightly reversed tendency in the closed cabinet where there were more viable microbes at rest than during work before intervention. This pattern persists after intervention. The most likely explanation of the situation is the position of the cabinet close to the entrance of the radiopharmacy in an area of high human traffic, and construction work around the building. The LAF which is further removed from the entrance in a more sheltered position is less exposed to traffic and dust, which is reflected in lower microbial contamination values. In addition, the LAF is used less frequently and by more experienced staff members than the closed cabinet. That the least reduction in exceeding plates was found in the floor samples is not surprising as it is difficult to maintain a clean environment close to an entrance door.

Although target limits for passive air sampling are recommended (Elnour et al. 2018), there are discrepancies regarding sampling procedures including nutrient media, incubation condition (Gordon et al. 2014), and sampling sites. The reasons for such diverse methods could arise from the fact that critical sampling points depend essentially on the setting and the locations posing microbiological risk to the product (The United States Pharmacopeial Convention <1116> 2013). Authors refer to different environments, thus methods and limits may differ. Diverse nutrient media are used. Elnour et al. in 2018 used blood agar nutrient media, Napoli et al. (2012 (a) and (b)) used TSA for active air sampling and maltose salt agar for passive air sampling for their experiment. The medium should be selected according to target or expected microorganisms to obtain optimal growth. Naik and coworkers estimated microbial air contamination and monitored the quality of air and Tshokey et al. did comparisons of two methods

of air sampling plus assessment of air quality in an operation theatre using blood agar plates (Naik S et al. 2018, Tshokey T et al. 2016). We used TSA nutrient for our passive air sampling because it is easily available, and low-cost. All-purpose TSA containing enzymatic digests of casein and soybean meal is a non-selective nutritious medium for a wide range of organisms, providing enough nutrients to allow a variety of microorganisms to grow. It is in fact recommended for microbiological environmental monitoring in pharmaceutical facilities by the USP (The United States Pharmacopeial Convention <1116> 2013). Air sampling is commonly done both during work when contamination is expected to be higher, as well as when there is no activity in facilities (Napoli et al. 2012 (a), Caggiano et al. 2014).

Extensive interventions, including reduction of activities involving traffic around the environment, training on personnel hygiene and behaviour in the laboratory, and introduction of sterile coats and radiopharmacy dedicated shoes that can easily be cleaned, helped to improve the situation, with significant differences in all sample locations. All these interventions were easy to implement and did not involve major expenses. A continuous assessment program is currently in place to monitor and evaluate the contaminant level at each sampling site on a regular basis.

Microbiological trending is quite critical for facilitation of process improvement. It is a supporting tool for several aspects including illustration of the level of compliance, the efficacy of sanitization, and the gowning process. Moreover, it helps in understanding which microorganisms pose a risk and proactively preventing future environmental problems (The United States Pharmacopeial Convention <1116 > 2013; Elsinga et al. 2010). According to Sandle, microorganisms primarily encountered in cleanrooms are gram-positive bacteria associated with human skin (Sandle 2011). We indeed found mostly gram-positive bacteria including *Staphylococcus* in our study. There was a remarkable reduction of *Staphylococcus epidermidis* and other gram-positive bacilli after the intervention. The reduction of the number of bacterial colonies and increase in number of sterile samples illustrate the effectiveness of the implementation of our interventions to improve cleanliness. The presence of *Candida* species after implementation of interventions may be due to presence of construction workers moving in and out of the Radiopharmacy room during the second half of 2017.

In GMP-compliant facilities, the risk in areas in which radiopharmaceuticals are normally prepared is primarily related to human errors. In a non-compliant facility, which is not well-designed, lacks clean air supply, and with poor maintenance of cabinets and filters, products are exposed to other risks arising from the environment in the radiopharmacy unit. While our results show a marked reduction in contamination risk, the internationally recommended levels of clean air have not been achieved and more effort is still needed, especially regarding facility design.

Conclusion

Passive air sampling was implemented and it was confirmed that interventions led to an improvement in the air quality in the Yaoundé General Hospital radiopharmacy unit. Continued efforts should be made for the sustainability of the quality of products prepared. Other low-income radiopharmacy units could use similar methods to evaluate and improve air quality in their unit.

Declarations for publication

Authors' contributions

FE performed the experiment, analysed the data and drafted the manuscript; HHB helped with design of the experiments, with drafting of the manuscript and provided critical feedback on the manuscript, SMR helped with general design of the study, provided critical feedback on the manuscript, and critically revised the manuscript. All the authors read and approved the final manuscript.

Competing interests

All authors declare that they have no competing interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgements

References Chapter 4

Caggiano G, Napoli C, Coretti C, Lovero G, Scarafile G, De Diglio O and Montagna MT. Mold contamination in a controlled hospital environment: a 3-year surveillance in southern Italy. BMC Infectious Diseases 2014;14:595

Elnour A, Abdelfattah M, Negm S, and Kassim T. Microbiological surveillance of air quality: A comparative study using active and passive methods in operative theater. International Journal of Pharmaceutical and Phytopharmacological research 2018;(8)1:33-38

Elsinga P, Todde S, Penuelas I, Meyer G, Farstad B, Faivre-Chauvet A, et al. Guidance on current good radiopharmacy practice (cGRPP) for the small-scale preparation of radiopharmaceuticals. European Journal of Nuclear Medicine and Molecular Imaging 2010;37:1049-1062

European Commission: EudraLex The Rules Governing Medicinal Products in the European Union Volume 4: EU Guidelines to Good Manufacturing Practice Medicinal Products for Human and Veterinary Use. Annex 1 Manufacture of Sterile Medicinal Products. 2008. https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2008_11_25_gmp-an1_en.pdf [Accessed 6 August 2019]

Gordon O, Berchtold M, Staerk A, and Roesti D. Comparison of different incubations for microbiological environmental monitoring. Journal of Pharmaceutical Science and Technology 2014;68:394-406

IAEA, Technetium-99m Radiopharmaceuticals: Manufacture of kits. Technical reports series No. 466. Vienna: International Atomic Energy Agency; 2008. pp. 26-49

Kastango ES, Bradshaw BD. USP chapter 797: Establishing a practice standard for compounding sterile preparations in pharmacy. American Journal of Health-System Pharmacy 2004;61:1928-38

Naik S, Ghogare H and Bhalchandra M. Estimation of microbial air contamination of operation theaters by settle plate method at tertiary care hospital, Aurangabad, India. International Journal of Current Microbiology and Applied Sciences 2018;7(1):1059-1061

Napoli C. Prevention of healthcare associated infections: Which sampling method should be used to evaluate air bio-contamination in operating rooms? Epidemiology 2012;2:2

Napoli C, Marcotrigiano V, Montagna M. Air sampling procedures to evaluate microbial contamination: a comparison between active and passive methods in operating theatres. BioMed Central Public Health 2012;12:594 (a)

Napoli C, Tafuri S, Montenegro L, Cassano M, Notamicola A, Lattarulo S, Montagna M, Moretti B. Air sampling method to evaluate microbial contamination in operating theatres: results of comparative study in an orthopaedics department. Journal of Hospital Infection 2012;80:128-132 (b)

Sandle T. A review of cleanroom microflora: Types, Trends, and Patterns. Journal of Pharmaceutical Sciences and Technology 2011;65(4):392-403

Sutton S. Critical Components of Effective Environmental Monitoring. Pharmacy Purchasing and Product. March 2015. www.pppmag.com [Accessed on 11 May 2018]

The United States Pharmacopeial Convention. Pharmaceutical compounding – sterile preparations (general information 797). The United States Pharmacopeia and National Formulary (USP35-NF30). Rockville: The United States Pharmacopeia; 2012

The United States Pharmacopeial Convention. Microbiological control and monitoring of aseptic processing environments (general information 1116). The United States Pharmacopeia 36. Rockville: The United States Pharmacopeia; 2013

Tshokey T, Somaratne P, Agampodi S. Comparison of two air sampling methods to monitor operating room air quality and assessment of air quality in two operating rooms with different ventilation systems. International Journal of Infection Control 2016;12(3):1-8

Todde S, Kolenc Peitl P, Elsinga P, Koziorowski J, Ferrari V, Ocak E, et al. Guidance on validation and quantification of processes and operations involving radiopharmaceuticals. EJNMMI Radiopharmacy and Chemistry 2017;2:8

World Health Organization. Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities Points to consider for Manufacturers of human vaccines November 2012 Vaccine Quality and Regulations (VQR), Quality, Safety, and Standards (QSS) Essential Medicines and Health Products (EMP) Department, World Health Organization (WHO), Geneva, Switzerland, pp. 1-37

Chapter 5

A comparative study of passive air sampling in different radiopharmacies

Ekoume FP (1, 3), Boersma HH (2), Rubow SM (3)

(1) Yaoundé General Hospital, Cameroon; (2) University Medical Centre Groningen, The Netherlands; (3) Stellenbosch University, South Africa

Note: For publication in a journal, the three radiopharmacies described in this chapter were named as follows: Yaoundé General Hospital - radiophamacy I, Tygerberg Hospital - radiopharmacy II, and University Medical Centre Groniningen - radiopharmacy III.

Abstract

Passive microbiological air sampling is a relatively simple and affordable method for evaluation of air quality. In this paper, air sampling results from two African radiopharmacies (designated I and II) with different economic backgrounds were evaluated.

In the two African radiopharmacies, settle plates were exposed at predetermined locations in comparable working areas and then incubated. The number of colonies per hour of exposure were determined. Based on recommended limits for the respective areas, results from the two units were compared and data from routine settle plate counts from a Good Manufacturing Practice-compliant European facility (radiopharmacy III) were used as standard.

Radiopharmacy I, in a developing country, had the highest incidence of results exceeding recommended limits. Results from air sampling at radiopharmacy I differ significantly from those from radiopharmacy II at the comparable sampling sites. The results reflect the facility design and standard of operation.

For radiopharmacies unable to afford the systems required to provide a clean room, it is difficult to reach the recommended levels of air quality. A validated class II LAF cabinet which is well maintained and used correctly, can play an important role to provide microbiologically clean air in such radiopharmacies.

Keywords: Quality control, radiopharmacy, microbiological monitoring, passive air sampling

Introduction

Healthcare facilities can harbour many microorganisms, including environmental opportunistic and airborne pathogens. For this and many other reasons, a consistent quality assurance program, involving a combination of validated processes and well-established tests, is a key factor in pharmaceutical production. Regulatory bodies and pharmacopoeias stipulate comprehensive standards (Food and Drug Administration 2004, EDQM 2018, The United States Pharmacopeial Convention <797> 2012), in order to ensure patient safety. The sterility of radiopharmaceuticals depends on the quality of air of the unit in which they are prepared and dispensed (Elsinga 2010). Due to the risk of product contamination and the negative impact thereof on patient safety, special focus is placed on control of the environment. Continuous environmental monitoring methods and well-designed processes are practical aspects to consider for mitigation of the risk of contaminating products.

Air quality monitoring must ideally comprise different techniques implemented continuously for a constant overview of microbial levels throughout the whole facility. Recommended monitoring techniques include particle monitoring, active air sampling, surface contact plates or swabs, operator fingerprints and settle plates or exposure plates (Beaney 2016, The United States Pharmacopeial Convention <825> 2019). According to GRP guidelines it is best to monitor both viable and non-viable particles. Constant monitoring helps pinpointing high risk areas which assists in addressing the problems in a targeted manner. Information gathered by regular monitoring will help to respond timeously if trends approaching limits are observed, enabling preventative measures.

Studies in literature describe different procedures for air monitoring. The choice of the optimal method could depend on the availability of equipment or consumables, difficulty of procedures or possible ways to address the relevant issue on site. An example is passive air sampling using settle plate exposure in identified sites. Diverse media are used (tryptic soy agar (TSA), blood agar, Sabouraud dextrose agar), and incubation temperatures and periods vary. Some authors sample while the area is not used and others both when the sites are used and when they are unused (Gordon et al. 2014, Naik et al. 2018, Tshokey et al. 2016, Napoli et al. 2012).

Guidelines for radiopharmacy practice in many countries prescribe complex facilities, especially air handling units, and extensive quality assurance and documentation requirements. In developing countries, these guidelines are currently not always met, due to several factors, including the high cost of facilities and equipment. In numerous countries in Africa, enforcement of the international guidelines would lead to closure of radiopharmacies, and consequently, loss of Nuclear Medicine services, as they depend on radiopharmacies to provide the required diagnostic and therapeutic products.

Good Manufacturing Practice (GMP) and Good Radiopharmacy Practice (GRP) recommendations highlight that, in order to prevent microbial contamination of products, Tc-99m radiopharmaceuticals should be radiolabelled and dispensed in a laminar air flow cabinet (LAFC) situated in a grade C clean room, or an isolator in a grade D clean room. Radiopharmaceuticals are prepared and dispensed under different circumstances in the three radiopharmacies included in this study. Radiopharmacy III in Netherlands is a well-equipped GMP compliant unit. Radiopharmacy II is relatively well-equipped and has a well maintained dedicated shielded LAFC, but located in a room that does not meet clean room standards, while radiopharmacy I has a closed cabinet that has not been subjected to regular maintenance and is located in an unclassified room.

Implementing good practice with limited funding remains a challenge faced by many sub-Saharan radiopharmacies. The question arises if we can produce safe radiopharmaceuticals even when the facility does not meet regular requirements for structure and equipment. How does a sub-optimal facility impact on air quality in critical areas? If the level of air quality does not meet the recommended standards, are structural improvements always essential?

This work studies outcomes of passive air sampling in the radiopharmacies of two large teaching hospitals in Cameroon and South Africa with economic status regarded respectively as lower middle income and upper middle-income countries. The results from a GMP-compliant radiopharmacy in a large teaching hospital in the Netherlands, a high-income country (World Bank 2019), were used as standard.

Methods

The passive air sampling method in the two African radiopharmacies entailed exposure of tryptic soy agar plates (TSA, diameter 90mm) for 2 to 4 hours at locations where radiopharmaceuticals are prepared and dispensed. The plates were incubated at 30 to 35°C for 72 hours and colony forming units were counted. The observed number of colony forming units (CFU) were corrected to reflect a standardised 4-hour period. Routine sampling at radiopharmacy III was performed during the work session in the relevant areas, while at radiopharmacies I and II, samples were collected both during work (working) and when the LAF cabinet or closed cabinet was not used (resting). The colony counts were evaluated according to limits described in the EU classification for the relevant areas in radiopharmacies (IAEA, 2008, European Commission 2008). Depending on the type of operation performed, and whether samples were collected at rest or during work and based on the recommended limit for microbes present in sampling sites, the number of plates exceeding the limit were recorded.

Data from radiopharmacy I and radiopharmacy II were compared using Fisher's two-sided exact test. P values of less than 0.05 were considered statistically significant. Data of routinely collected samples from radiopharmacy III served as a standard of air quality monitoring.

Results

At radiopharmacy I, a total of 654 samples was included in the study, 456 samples collected at rest and 198 during work. At radiopharmacy II, 875 samples were collected during the period of study (560 samples during work and 315 samples at rest) in all sampling sites. At radiopharmacy III, 1825 samples were collected during work.

The percentage of plates exceeding the recommended limits in the three centres are represented as follows:

Table 5.1 presents the percentage of the settle plates (including resting and working) exceeding the relevant recommended limit during work and at rest in each sampling area. Figure 5.1 shows differences between working and resting status. Summaries of the statistical analyses describing the differences

between corresponding areas at radiopharmacies I and II are provided in Addendum table A.9. The difference was significant in the cabinets where radiopharmaceuticals are prepared and on the open bench. In both radiopharmacies, there was no significant difference between the results obtained during work and resting (Addendum table A.10).

Table 5.1 Percentage of total number of settle plates exceeding relevant colony count limits

	Limits (CFU/4h)	I	II	III
Area where RP are prepared	< 1	37%	6%	3%
Other class A area	< 1	12%	7%	2%
Class C area	50	15%	8%	0%
Class D area	100	44%	-	0%
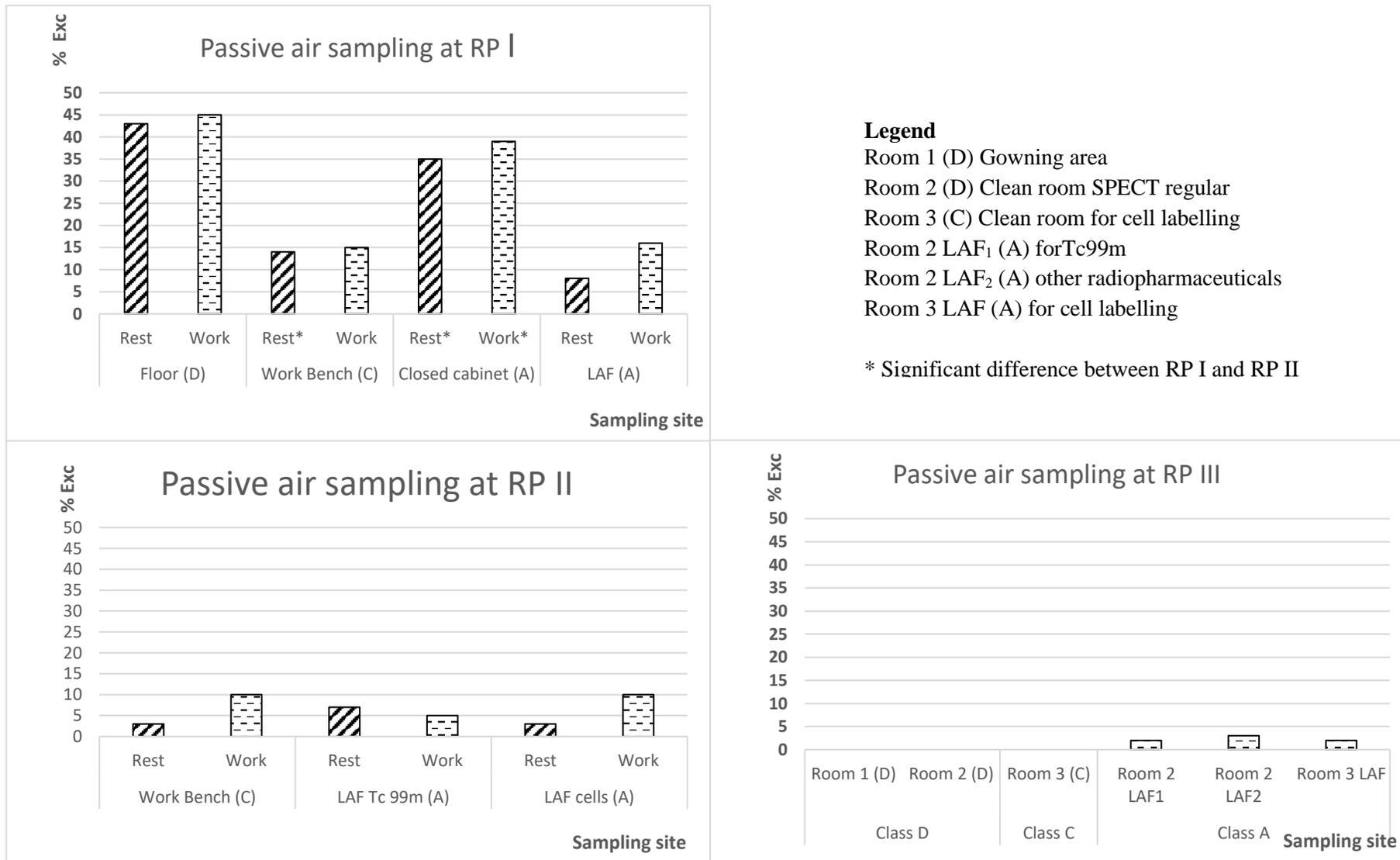
Figure 5.1 Passive air sampling at the 3 radiopharmacies (RP)

Discussion

The current study is part of quality assurance programme monitoring aspects recommended in literature (European Commission 2008) to improve microbial safety in two African radiopharmacies. Radiopharmacy I started implementing passive air sampling in its radiopharmacy unit during the year 2017, and after a baseline study implemented corrective actions to improve the quality of air (Ekoume 2018). The values shown in this study are all after the improvements.

The highest percentage of colony counts exceeding the relevant limit were seen in radiopharmacy I. Of the different areas monitored in radiopharmacy I, the LAF had the lowest number of contaminated settle plates. This LAF is located in the quietest area in radiopharmacy rooms with the least human traffic and activities. Radiopharmacy II recorded less contamination in all areas. As expected from a GMP-compliant facility, radiopharmacy III had the lowest number of colony counts exceeding limits at all sites.

The work in radiopharmacies I and II could not be directly compared with the results from radiopharmacy III due to differences in the incubation temperatures and times. Radiopharmacy I and II incubated the TSA for 72 hours at 30 to 35 °C, while at radiopharmacy III, the plates were incubated for 48 hours at 32°C (for bacterial growth), followed by 5 days at room temperature to allow fungal growth. As neither of the two African radiopharmacies have rooms with clean air supply, many viable organisms tended to be present, which could result in a fast overgrowth per plate. Despite the less than ideal circumstances of the 3-day incubation at the higher temperature, radiopharmacy I did detect *Candida* species on several settle plates, both in earlier work (Ekoume 2018), and during the current study, indicating that the method used was sensitive enough to detect at least some species of fungi.

Results from the current work highlight existing deficiencies and the need for improvement in design and work methods, especially in radiopharmacy I. Recommendations for a new facility were submitted and accepted by the hospital management and a maintenance engineer for the unit was recruited to be trained for maintenance of all the equipment in the unit.

In earlier work, radiopharmacy 1 showed that a number of simple and affordable techniques can lead to a marked improvement in the air quality in the area where radiopharmaceuticals are prepared and dispensed (Ekoume 2018). Despite these measures, there is still a high incidence of viable microorganisms in areas that should, according to international recommendations, meet class A air standards. In radiopharmacy II, the LAF dedicated to Tc-99m radiopharmaceuticals fares much better. It is worthwhile considering the factors that may contribute to this difference. Although the room in radiopharmacy II is not designed as a clean room and does not reach clean room status, the dedicated LAF is regularly monitored, cleaned and maintained. Staff members are specifically trained to implement correct working procedures in the LAF. All items used in the LAF are disinfected before placement in the LAF, and staff wear sterile gowns and sterile, powder-free gloves. A second staff member is present in the radiopharmacy to assist with documentation and quality control of the products, thus minimising the number of times the operator may have to interrupt and resume tasks inside the cabinet.

Neither of the African radiopharmacies could achieve a level of air quality equivalent to that of the GMP-compliant facility, which has a suite of clean rooms with a dedicated ventilation system. Thus there remains some risk of microbial contamination of products.

Despite the fast growth of radiopharmacy, there are no published studies describing air quality monitoring in radiopharmacies in Africa. Using a simple and affordable method, the current study successfully identified the trends of microbial contamination in the two African radiopharmacies included in the study. This study highlights the value of air quality monitoring in the African context. Simple measures like additional staff training, improved hygiene measures and reduction of movement in the radiopharmacy rooms can contribute to reduce the presence of microbes in the radiopharmacy. However, these steps will not reduce the risk of microbial contamination of products to a GMP-compliant level.

The best solution is evidently to follow international recommendations, i.e. invest in a clean room facility which should then be appropriately used, monitored and maintained. This high-cost solution

may not be achievable in developing countries with limited funds to support nuclear medicine and radiopharmacy facilities.

The current work shows that an intermediate option can bring the environment for radiopharmaceutical preparation and dispensing almost to the required standard. Working in a validated, well-maintained class II LAF cabinet can contribute largely to provide microbiologically clean air. It is important that staff is well-trained, uses good cleaning protocols and suitable garments and adheres strictly to working protocols in the cabinet.

Conclusion

The current study successfully investigated the microbiological air quality in two African radiopharmacies. It describes an affordable and useful monitoring method that can easily be used by other radiopharmacies with limited resources.

In order to provide the optimal environment with the lowest possible microbial risk for intravenous radiopharmaceuticals, laminar air flow cabinets or closed cabinets in clean rooms are recommended. When clean rooms are not available, it is difficult to reach the recommended levels of air quality. This work shows that correct use of a validated, well-maintained class II LAF cabinet can contribute largely to provide microbiologically clean air and can thus play an important role in facilities that cannot afford the systems required to provide a clean room.

Declarations for publication

Author contributions

FE performed the experiments, analysed the data and drafted the manuscript; HHB helped with design of the experiments, with drafting of the manuscript and provided critical feedback on the manuscript, SMR helped with the general design of the study, provided critical feedback on the manuscript, and revised the manuscript. All the authors read and approved the final manuscript.

Competing interests

All authors declare that they have no competing interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgements

References Chapter 5

Beaney AM. Quality assurance of aseptic preparation services: Standards. 5th edition. London: Pharmaceutical Press; 2016. pp. 80-92

European Commission: EudraLex - The Rules Governing Medicinal Products in the European Union Volume 4: EU Guidelines to Good Manufacturing Practice Medicinal Products for Human and Veterinary Use. Annex 1 Manufacture of Sterile Medicinal Products. 2008. https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2008_11_25_gmp-an1_en.pdf [Accessed 6 August 2019]

European Directorate for the Quality of Medicines (EDQM) & HealthCare: Guide for the elaboration of monographs on Radiopharmaceutical Preparations. 2018. https://www.edqm.eu/sites/default/files/guide_guide_for_the_elaboration_of_ monographs_on_radio-pharmaceutical_preparations_october_2018.pdf[Accessed 15 September 2019]

Ekoume F, Rubow S, Boersma H. Implementation of Environmental Monitoring in a Low Income Radiopharmacy Unit. World Journal of Nuclear Medicine 2018;17(4):345

Elsinga P, Todde S, Penuelas I, Meyer G, Farstad B, Faivre-Chauvet A, et al. Guidance on current good radiopharmacy practice (cGRPP) for the small-scale preparation of radiopharmaceuticals. European Journal of Nuclear Medicine and Molecular Imaging 2010;37:1049-1062

IAEA. Operational Guidance on Hospital Radiopharmacy, A safe and Effective Approach. Vienna: International Atomic Energy Agency; 2008. pp. 28-30

Naik S, Ghogare H and Bhalchandra M. Estimation of microbial air contamination of operation theaters by settle plate method at tertiary care hospital, Aurangabad, India. International Journal of Current Microbiology and Applied Sciences 2018;7(1):1059-1061

Napoli C., Marcotrigiano V. and Montagna M. Air sampling procedures to evaluate microbial contamination: a comparison between active and passive methods in operating theatres. BMC Public Health 2012;12:594

Food and Drug Administration. Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice. Rockville: FDA; 2004

The United States Pharmacopeial Convention. Radiopharmaceuticals Preparation, compounding, dispensing, and repackaging (general information 825). Rockville: The United States Pharmacopeia; 2019

The United States Pharmacopeial Convention. Pharmaceutical compounding – sterile preparations (general information 797). The United States Pharmacopeia and National Formulary (USP35-NF30). Rockville: The United States Pharmacopeia; 2012

Tshokey T, Somaratne P, Agampodi S. Comparison of two air sampling methods to monitor operating room air quality and assessment of air quality in two operating rooms with different ventilation systems. Int J Infect Control. 2016;12(3):1-8

World Bank. World Bank Country and Lending Groups. https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups. [Accessed 16 August 2019]

Chapter 6

Evaluation of aspects of practice in two African radiopharmacies

Fany P Ekoume[1, 3], Hendrikus H Boersma[2], Sietske M Rubow[3]

[1]Yaoundé General Hospital, Cameroon; [2] University Medical Centre Groningen, The Netherlands;

[3]Stellenbosch University, Tygerberg, South Africa

Abstract

An affordable approach is applied to evaluate the level of microbial safety for radiopharmaceuticals, used in two African Nuclear Medicine units. Media fill testing (MFT) and fingertip testing (FT) was implemented at radiopharmacy I to evaluate staff members' ability to maintain sterility during manipulation of radiopharmaceuticals. The rate of contaminated products as determined by sterility testing at radiopharmacy I and II was 1.8% and 0.38% respectively during the period of study. The presence of contaminated samples and a comparative study with routine data from a GMP-compliant European radiopharmacy III recording 0% product contamination, indicate that more effort is needed in both centres. Radiopharmacy I also had a higher rate of contaminated fingertip plates than radiopharmacy III. MFT and FT at radiopharmacy I identified problems with implementing aseptic technique, understanding of procedures, and the ability of workers to apply learned skills in the unit. The skills of a newly recruited staff member were improved through dedicated corrective actions.

Keywords: Quality control, radiopharmacy, media fill test, fingertip test, sterility test.

Introduction

With the worldwide expansion of radiopharmacy through the creation of new PET and SPECT units, it is important to ensure that good practice is well established in each unit, including small scale production facilities (Elsinga 2010). Quality control tests (QC) and quality assurance (QA) programs, including validation of methods, are important tools recommended to ensure the quality of products. They provide evidence of the suitability of the procedure and help to provide safe final products (Ajay et al. 2012). Microbial safety of radiopharmaceuticals remains a relevant topic drawing the attention of scientific communities and leading to updating pharmacopoeias (The United States Pharmacopeial Convention <825> 2019, IAEA 2016). This aspect of quality assurance involves a range of monitoring parameters including sterility testing, microbial environmental monitoring (surface testing, air sampling), and process and aseptic technique validation using media fill tests. Periodic sterility testing of radiopharmaceuticals, and validation and revalidation of each step of the entire procedure by media fill and fingertip testing, are recommended for optimal microbiological safety in radiopharmacy (The United States Pharmacopeial Convention <825> 2019, Elsinga et al. 2010, Todde et al. 2017, Lange et al. 2015).

Sterility testing of radiopharmaceuticals is conducted to ensure that a batch of products is free of viable microbes (bacteria, yeasts, moulds or any other microorganisms). However, satisfactory results of tests indicate only that the examined sample has no contaminating organisms under the conditions of the test. This test can be done by inoculating the product directly into appropriate culture media, followed by incubation at suitable temperatures. Observation and interpretation of results should be done by a well-trained operator at regular intervals during the duration of the incubation. Products comply with the requirements for sterility if no turbidity develops in the media or if there is no evidence of growth in the media. If any growth is found in the media, identification of the microbe and the possible origin of the microbe should follow in order to start appropriate corrective action (The United States Pharmacopeial Convention <71> 2012). Aspects affecting the sterility of pharmaceutical products include facility design, environmental monitoring, personnel training and monitoring, and aseptic technique.

Aseptic technique validation should be compulsory for any operator involved in preparation and dispensing of sterile radiopharmaceuticals. Media fill testing with culture media is used to validate pharmaceutical processes. It is adapted to reflect the manipulations carried out by the operators, simulating the most challenging and stressful conditions in their duties (The United States Pharmacopeial Convention <825> 2019, Sigward et al. 2012, Urbano 2013). Other aspects like cleaning and disinfecting the workstation, hand hygiene, garbing as described in the standard operation procedures (SOPs), and aseptic technique should also be included in staff qualification.

As most radiopharmaceuticals are administered intravenously, particular attention should be paid to the safety of products prepared and dispensed in radiopharmacies that are relatively poorly equipped and funded. In this context, we evaluated aspects of Good Radiopharmacy Practice (GRP) implemented in a basic radiopharmacy in Cameroon (named radiopharmacy I hereafter) and a relatively well-established radiopharmacy (II) in South Africa. This work evaluates sterility of radiopharmaceuticals prepared at radiopharmacies I and II, and the aseptic status of radiopharmaceutical preparation methods and staff competence at radiopharmacy I by media fill with fingertip testing. Results of routine tests from a GMP compliant radiopharmacy (III) in the Netherlands are provided for comparison.

Methods

1. Sterility testing

Samples were obtained from routinely prepared Tc-99m radiopharmaceutical (RP) vials containing at least 2 ml product after dispensing of the last patient dose. The vials were set aside for sterility samples and the Tc-99m was allowed to decay (60-90 hours). Samples were selected to ensure that all different kits used in the facility were represented in the sterility test series. A microbiology laboratory operator inoculated tryptic soy broth (TSB) with the decayed RP. Inoculated broth was incubated firstly between 20 and 25 °C for 7 days then at 30 to 35 °C for 7 days and visually monitored for any signs indicating microbial growth.

The rate of contamination was calculated as the number of radiopharmaceuticals contaminated divided by the total number of radiopharmaceuticals tested for sterility at the relevant hospital.

2. Media fill and fingertip testing

Aseptic process qualification by media fill testing (MFT) or process simulation was done by 4 workers involved in the radiopharmaceutical preparation at radiopharmacy I. The test included the facility's existing procedures for cleaning and disinfection, garbing and aseptic technique. TSB was prepared and filled as shown in figure 6.1, simulating all the steps currently used for daily radiopharmaceutical preparation and dispensing. The protocol was conducted in 6 replicates for operators' initial qualification and repeated in case of failure until successfully completed. Thereafter each operator had to repeat the test approximately every 6 months as routine evaluation or requalification. Media fill testing was performed immediately after a work period to introduce a fatigue factor. The media were incubated and identification of contaminants followed when necessary. Quality control of the media included growth promotion tests. All the growth media used were provided by qualified microbiologists from the microbiology laboratory in the same hospital.

TSA (Tryptic Soy Agar) plates were used for gloved fingertip testing of the sterile gloves after completion of each media fill. Immediately after the MFT, without disinfecting the gloves, each finger of both hands was printed on the agar plate in such away to have the five fingertips from right hand in

the same plate and those from the left hand in the second plate. At the end of each routine test a total of 5 vials (all shown in figure 6.1) and 2 plates were ready for incubation. For each initial validation 30 vials and 12 settle plates were collected for incubation. Plates were incubated at 25-30 °C for 3 days and vials for 7 days at 20 to 25° C, then at 30 to 35 ° C for additional 7 days.

The rate of vials contaminated during media filling (number of contaminated vials divided by total number of vials filled), the rate of plates contaminated during the fingertip operation (number of contaminated plates divided by total number of plates used) and the rate of media fill failure (number of tests failed divided by the total number of media fill tests performed) were calculated.

New staff members who failed the MFT were retrained (theoretical and practical training). For completeness, retraining included not only aseptic technique, but also general radiopharmacy skills like knowledge of equipment and materials, radiation safety and waste management.
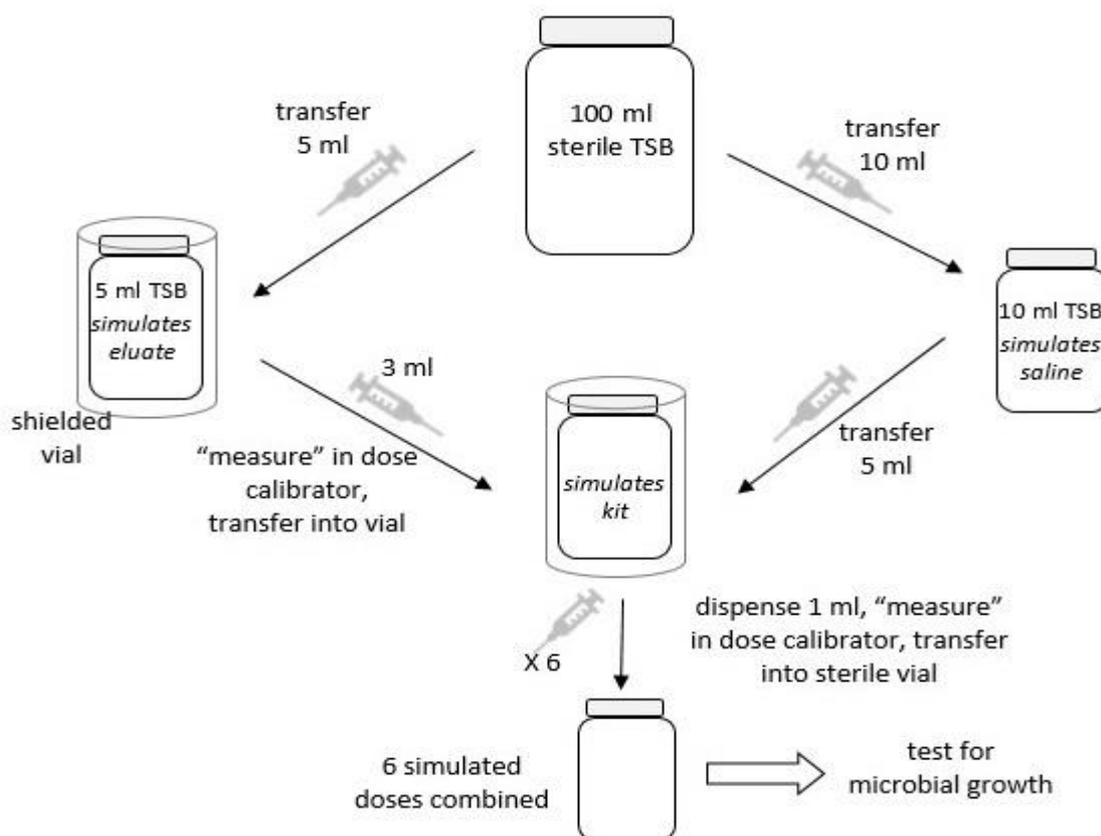
Figure 6.1 Simulation of radiopharmaceutical preparation

Results

The results of sterility testing of radiopharmaceuticals routinely produced at radiopharmacy I are summarized in the table 6.1, those for radiopharmacy II in table 6.2. At radiopharmacy III, samples from 103 SPECT radiopharmaceuticals were evaluated for sterility between 2018 and 2019 and none of them presented any growth.

Table 6.1 Sterility test results at Radiopharmacy I over an 18-month period

Radiopharmaceuticals	No of samples tested	No of samples showing microbial growth
Pertechnetate (eluate)	81	2
HMDP	16	0
MIBI	3	0
DTPA	7	0
DMSA	1	0
Total (5 different radiopharmaceuticals)	108	2 (1.8%)

Table 6.2 Sterility test results at radiopharmacy II over a 4 year period

Radiopharmaceuticals	No of samples tested	No of samples showing microbial growth
MDP	34	0
MIBI	26	0
MAA	28	0
Nanocolloid	12	0
MAG3	32	0
Tin colloid	19	0
DTPA	35	0
HMPAO	20	0
DMSA	20	0
RBC	7	0
BRIDA	8	0
Eluate	16	1
Total (12 different products)	275	1 (0.38%)

Table 6.3 Radiopharmacy I operators' media fill and fingertip test results

(Jan 2017 to Jan 2019)

Operator	No of tests	MFT vial contamination rate	FT contamination rate
A	3 validation	0% of 115	8% of 46
	5 routine		
B	4 validation	0% of 140	14% of 56
	4 routine		
C	4 validation	0% of 140	10% of 56
	4 routine		
D	5 validation	1% of 175	30% of 70
	5 routine		

Four operators were involved in the production of radiopharmaceuticals at radiopharmacy I.

Table 6.4 Radiopharmacy III media fill and fingertip test results

Operator	No of tests	MFT contamination rate	FT contamination rate
E	1 validation	0% of 80 vials	28% of 32 plates
	10 routine		
F	13 routine	0% of 65	7% of 26
G	12 routine	0% of 60	1% of 24
H	1 validation	0% of 70	17% of 28
	9 routine		
I	4 routine	0% of 25	12% of 8
J	1 validation	0% of 70	21% 0f 28
	8 routine		
K	1 validation	0% of 30	0% of 12
L	2 validation	0% of 60	21% of 12

Eight operators currently producing SPECT radiopharmaceuticals were included in the current study at Radiopharmacy III.

The operators at radiopharmacy I failed 12 tests from a total of 34 media fill tests performed (mostly due to failed fingertip tests) for an overall failure rate of 35%, although only 2 of the 570 filled vials (0.3%) were contaminated. At radiopharmacy III, 4 cases of MFT failure were found in a total of 61 tests done, all due to fingertip test failure. A failure rate of 6% was recorded with 0% of vials contaminated during filling processes.

Discussion

Quality Control in radiopharmacy has unique challenges due to the short half-life of many radionuclides, which does not allow lengthy test procedures like sterility testing prior to use of the products. Working in a clean environment and following defined operating procedures like aseptic transfer techniques endorsed by retrospective sterility tests on radiopharmaceuticals is essential to ensure their sterility. The TSB medium that was used in the current work is a multipurpose medium allowing the growth of a wide range of aerobe and fungal microorganisms.

Sterility data from two different African radiopharmacies was evaluated in this work, and compared with results from a large GMP-compliant European unit. Radiopharmacy I serves the Nuclear Medicine unit of a teaching hospital in an African country classified as lower middle-income by the World Bank (World Bank data 2019). Radiopharmacy I does not have clean room facilities, and due to financial limitations, the cabinet in which Tc-99m tracers are prepared and dispensed is not maintained to function as a class A clean environment. Radiopharmacy II in a large hospital in an upper middle-income country in Africa also does not have a clean room, but the shielded laminar air flow cabinet is well-maintained to provide a class A clean air environment for Tc-99m generator elution, kit preparation and dispensing of patient doses. Radiopharmacy III meets GMP criteria, with the necessary suite of clean rooms, sterile attire and quality management system. This radiopharmacy serves a large teaching hospital in a European high-income country.

For all three centres only preparation and dispensing of products in closed or nearly closed systems are included in this study. Normally, the risk of contaminating vials is very low in such situations, as there is no exposure of the ingredients or products to microbial risk. The risk is however increased, because radiopharmaceutical kits are usually prepared to supply multiple individual doses, and the pertechnetate vial is penetrated by needles more than twice during elution and preparation of several kits for the day. Thus, Tc-99m radiopharmaceuticals should be classified as medium-risk according to the USP criteria (The United States Pharmacopeial Convention <797> 2008, Gurajala et al. 2015). The risk level is highest in Radiopharmacy I, where the work station cannot be maintained at ISO 5 level or class A.

Sterility is a critical parameter for the safety of radiopharmaceuticals for human use (Boschi A and Duatti A. 2019, Statuto et al. 2016) and sterility testing is therefore essential. The existing literature suggests contamination rates between 0.86% (Weatherman et al. 2013, radiopharmaceuticals) and 5.2% (Trissel et al. 2005, compounded pharmaceutical products). Values obtained in our study from both middle-income centres are lower than the rate described by Trissel et al. in 2005. It should however be noted that Trissel et al. describe competency evaluation for medium-risk-level compounding procedures, which are more complex than most procedures involving preparation and dispensing of Tc-99m radiopharmaceuticals. The higher contamination rate at radiopharmacy I compared to radiopharmacy II can be ascribed to the difference in status of the facility and equipment.

The contaminated product in both units originated from Tc-99m generator eluate. Due to the relatively long shelf-life of Tc-99m generators and the use of eluates to prepare radiopharmaceuticals for many patients each day, this is a cause for concern. Microbial contamination of radionuclide generators could theoretically occur in the generator, which is eluted with sterile saline solution one or more times per day over a period of approximately two weeks. The generator eluates provide the pertechnetate for preparation several different kits in the course of a day, as well as pertechnetate doses for thyroid imaging. If contamination occurred in the generator, kits prepared from pertechnetate from that generator in the following days would also be contaminated. At both our study sites, no contaminated kits were identified. If there is a high risk of such contamination, a simple filtration step using a 0.22 µm filter could be implemented. Furthermore, according to Sorensen et al. (1977) and Allwood and McCarthy (1980), Tc-99m generators are not a favourable environment for microbial life. Another possibility is contamination during use of a vial of eluate. With multiple septal penetrations of technetium eluate vials during a workday, the possibility of microbial contamination remains a risk, even when good aseptic technique is used (Snowdon G, 2000). At radiopharmacy II, not all radiopharmaceutical vials are subjected to sterility testing, thus we do not know if the contaminated eluate did lead to contamination of kits prepared on the same day. In the more optimal situation of radiopharmacy III, no microbial contamination of technetium generator eluates was observed.

One of the aims of the MFT is to indicate if sterility problems are due to operator technique rather than to environmental factors. All four operators at radiopharmacy I involved in the preparation and dispensing of radiopharmaceuticals were new to the MFT technique and repeated the validation exercise more than twice. Thereafter they seemed to become familiar with the process by successfully completing the validation test. Three workers completed all the tests without contaminating vials during the filling process. A newly appointed staff member contaminated 1 % of the MFT vials and also had the highest rate of contaminated fingertips.

Observation of the newly recruited operator at radiopharmacy I during the test revealed inadvertent touching of vial septa and omitting disinfection of the vial prior to puncture. This case illustrates the importance of strict adherence to decontamination procedures. Fingertip failure at radiopharmacy I is most likely due to incorrect cleaning of the work station, including the use of tap water before disinfection with ethanol. The cleaning procedure has been altered, and suitable disinfecting procedures have been implemented.

The MFT operators' failure rate were 35% at Radiopharmacy I and 6% at radiopharmacy III, and the contamination rates during vial filling process were 0.3% and 0% respectively. The number of plates contaminated from fingertip operation was higher than the number of vials contaminated from MFT in both centres. There seems to be no association between the operator's experience and the fingertip results. Sigward and co-workers found no association between staff members' years of experience and media fill results (Sigward et al. 2012). The fact that even experienced staff sometimes fail media fill or fingertip tests, supports the need for improving guidelines for good pharmacy practice.

The contaminating microorganism found in the MFT vial at radiopharmacy I was *Staphylococcus epidermidis,* which was also identified in the eluate vials during the sterility testing in this study. This matches several reports of contamination of sterile products with microorganisms associated with human skin. The contamination of sterility test samples and media fill vials in radiopharmacy I took place during a period in which students observed work in the radiopharmacy, i.e. while there was movement of people in the radiopharmacy.

92

For better control of microbial safety of radiopharmaceuticals, the QA programme in both these African radiopharmacies includes environmental monitoring. Results of passive air sampling in radiopharmacies I and II during similar time periods as the sterility tests reported here, are the subject of another publication (see chapter 5). However, no air quality results were available for the dates of the failed sterility test samples. At both radiopharmacies only a fraction of radiopharmaceutical products are submitted for sterility testing, and air quality monitoring was not done every day. Thus it is not possible to directly link the sterility failure to specific air quality results. The frequency of both air quality monitoring and sampling for sterility testing should be increased if the relationship between air quality and sterility failures were to be investigated, especially in radiopharmacies operating without clean room facilities.

The findings of the current work revealed similar contamination levels to those described in literature (Weatherman 2013). Technetium-99m radiopharmaceuticals are prepared as multi-dose vials, but the contents are used within 6 to 8 hours. The risk of micro-organisms multiplying in radiopharmaceutical vials is relatively low compared to other pharmaceuticals with longer shelf-lives. The possibility of a patient developing an infection after administration of a contaminated radiopharmaceutical is small but definitely present. Both radiopharmacies should therefore strive to improve their quality management in order to reach a 0% microbial contamination rate.

Conclusion

This paper describes successful evaluation of sterility testing of radiopharmaceutical compounds, prepared in two African radiopharmacies. An important finding of the study was that, in general, there were only a very small number of positive sterility tests. The percentage of microbially contaminated eluate in radiopharmacy I was higher than that for radiopharmacy II. Media fill tests helped radiopharmacy I to identify serious deviations from aseptic technique which were corrected by retraining staff. Both sterility testing and aseptic technique validation are simple and affordable aspects of GRP

that were introduced in radiopharmacy I in response to a self-audit. Implementation of similar quality assurance methods is highly recommended to any other radiopharmacy working with similar limitations.

Declarations for publication

Author contributions

FPE performed the experiments, analysed the data and drafted the manuscript. HHB helped with the design of the experiments and with drafting of the manuscript. He also provided critical feedback on the manuscript. SMR helped with the general design of the study, provided critical feedback on the manuscript, and revised the manuscript. All the authors read and approved the final manuscript.

Conflict of interest

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no financial support for this work that could have influenced its outcome.

Acknowledgements

References Chapter 6

Ajay S, Rohit S. Validation of Analytical Procedures: A comparison of ICH vs. Pharmacopoeia (USP) vs. FDA. International Research Journal of Pharmacy 2012;3:39-42

Allwood MC and McCarthy TM. Microbial and pyrogenic contamination of molybdenum 99/technetium 99m radionuclide generators. International Journal of Pharmaceutics 1980;4:185-193

Boschi A, Duatti A. Radiopharmacy/Radiochemistry for Conventional Single-Photon Emitting and Therapeutic Radiopharmaceuticals. In Volterrani D et al., editors. Nuclear Medicine Textbook. Cham: Springer; 2019. pp. 1009-1018

Elsinga P, Todde S, Penuelas I, Meyer G, Farstad B, Faivre-Chauvet A, et al. Guidance on current good radiopharmacy practice (cGRPP) for the small-scale preparation of radiopharmaceuticals. European Journal of Nuclear Medicine and Molecular Imaging 2010;37:1049-1062

Gurajala R, Shrikanthan S and Amin K. USP 797 Intrusive media fill challenge for compounding radiopharmaceutical. Journal of Nuclear Medicine 2015;56(supplement 3):1160

IAEA. Good practice for introducing radiopharmaceuticals for clinical use. IAEA-Tecdoc-1782. Vienna: International Atomic Energy Agency; 2016; p. 1782

Lange R, Ter Heine R, Decristoforo C, Penuelas I, Elsinga P, van der Westerlaken M et al. Untangling the web of European regulations for the preparation of unlicensed radiopharmaceuticals: a concise overview and practical guidance for a risk-based approach. Nuclear Medicine Communications 2015:36(5):414-421

Sigward E, Fourgeaud M, Vazquez R, Guerrault-Moro MN, Brossard D and Crauste-Manciet S. Aseptic simulation test challenged with microorganisms for validation of pharmacy operators. American Journal of Health System Pharmacy 2012;69:1218-1224

Snowdon G. Is your technetium generator eluate sterile? Journal of Nuclear Medicine Technology 2000;28:94-95

Sorensen K, Kristensen K, and Fransden P. Microbial contamination of radionuclide generators. European Journal of Nuclear Medicine 1977;2:105-107

Statuto M, Galli E, Bertagna F, Migliorati E, Zanella I, Di Lorenzo D et al. The strange case of the [^{13}N]NH$_3$: validation of the production process for human use. Nuclear Medicine Communications 2016;37:412-421

The United States Pharmacopeial Convention. Pharmaceutical compounding sterile preparations (general information 797). Rockville: The United States Pharmacopeia; 2008

The United States Pharmacopeial Convention. Radiopharmaceuticals Preparation, compounding, dispensing, and repackaging (general information 825). Rockville: The United States Pharmacopeia; 2019

The United States Pharmacopeial Convention. Sterility test (general information 71). Rockville: The United States Pharmacopeia; 2012. pp. 69-74

Todde S, Kolenc Peitl P, Elsinga P, Koziorowski J, Ferrari V, Ocak E, et al. Guidance on validation and quantification of processes and operations involving radiopharmaceuticals. European Journal of Nuclear Medicine and Molecular Imaging Radiopharmacy and Chemistry 2017;2:8

Trissel LA, Gentempo JA, Anderson RW, Lajeunesse JD. Using a medium-fill simulation to evaluate the microbial contamination rate for USP medium-risk-level compounding. American Journal of Health System Pharmacy 2005;62:285-8

Urbano N, Modoni S, Schillaci O. Media fill test for validation of autologous leukocytes separation and labelling by ^{99m}Tc-HmPAO. Nuclear Medicine and Biology 2013;40:104-108

Weatherman KD, Augustine S, Christoff J. and Galbraith W. Establishing benchmark rates of microbial and bacterial endotoxin contamination for radiopharmaceuticals compounded in commercial Nuclear Pharmacy settings. International Journal of Pharmaceutical Compounding 2013;17(2):169-174

World Bank. World Bank Country and Lending Groups. https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups. [Accessed 16 August 2019]

Chapter 7

Discussion and Conclusion

Recommendations and rules from national and international regulatory bodies for SPECT radiopharmaceuticals are sometimes not fully adhered to due to limited funding allocated to healthcare in developing countries. To address regulatory requirements, an optimized quality assurance programme can assist in ensuring the efficacy and safety of pharmaceutical products.

The aim of this study was to investigate the conditions to ensure safe and efficacy of products. Different aspects that influence the quality of radiopharmaceuticals are addressed in this dissertation in five chapters. Two radiopharmacies in teaching hospitals in Africa (Yaoundé General Hospital and Tygerberg Hospital) are used as examples, with a GMP-compliant radiopharmacy in the Netherlands serving as a model of a facility that meets requirements of international guidelines. The identification of important factors was done by self-audits in the radiopharmacies. The first topic addressed was the identified need for validation of methods at YGH, resulting in validation of a radioactivity quantification method for RCP determination. Further chapters address the microbial safety of radiopharmaceuticals at YGH and TBH, including air quality monitoring, sterility testing of products at YGH and TBH, and the evaluation of aseptic work skills of staff at YGH.

The IAEA's QUANUM tool was adapted by incorporating elements of an earlier IAEA questionnaire for radiopharmacy to specifically address GRP. This modified version of the audit instrument was very useful to establish the status of radiopharmacy practice in the radiopharmacies included in this dissertation. The findings of the self-audits are reflected by the results of microbiological studies described in later chapters of the dissertation.

Based on the outcome of the initial quality audit at YGH, relatively simple, affordable corrective actions were implemented to improve the standard of radiopharmacy practice. These included staff training,

introduction of annual performance reviews, installation of a transfer hatch, rearrangement of room lay-out, and improved record keeping. The re-evaluation of the radiopharmacy at YGH demonstrated clear improvement in the quality of practice. Additional, more complex or higher cost improvements were recommended to hospital management.

Radiochemical purity (RCP) is a key factor determining the biodistribution and therefore efficacy of radiopharmaceuticals. To evaluate the reliability of the method for radiochemical purity determination at YGH, an experimental validation of the method for quantification of the distribution of radioactivity on radiochromatograms was conducted using the method of the GMP-accredited UMCG radiopharmacy as comparator. The value of this study was two-fold: Firstly, it demonstrated validation of a method in radiopharmacy addressing all relevant parameters internationally recommended for validation of analytical methods. Secondly, the use of a simple alternative device for counting the radioactivity on thin layer strips was validated and can be implemented in daily work at YGH. This method, employing a small contamination monitor, may also be useful for other radiopharmacies.

The use of settle plates to monitor air quality in radiopharmacies can be implemented without the need for specialised air samplers or particle counters. The method demonstrated that viable organisms were often present in the areas where radiopharmaceuticals are prepared in the two African radiopharmacies studied in this work. In addition, YGH introduced several corrective actions, aiming improvement of the air quality, and their beneficial effect was clearly demonstrated. Nevertheless, the air quality in the closed cabinet at YGH was still far from the recommended class A clean air level. Despite the lack of a clean room, the results at TBH demonstrate the value of correct use of a well-maintained type II biosafety laminar air flow cabinet to provide the best possible environment for preparation of Tc-99m radiopharmaceuticals.

Having evaluated the air quality in the radiopharmacies, the next question addressed the impact of air quality and staff competency on the sterility of radiopharmaceuticals. Although the microbiological contamination rates of radiopharmaceuticals in both African radiopharmacies is low, both should strive to improve their performance to prevent possible serious consequences for patients. Media fill testing is a valuable tool by which radiopharmacies should evaluate methods and the competency of staff.

Summary of limitations

A weakness of the current study is the fact that the work is based only in two African radiopharmacy units, which limits the extent to which the conclusions and recommendations can be generalised. Unfortunately, including radiopharmacies from more African countries in the study was not feasible. Furthermore, the audits included in the work were self-assessments performed by staff members from the respective radiopharmacies who may have interpreted the audit questions differently. For complete adherence to international standards, external audits are recommended. For a true comparison of different practices, a single audit team should evaluate facilities. However, the aim here was not to perform a quantitative comparison of the three radiopharmacies, but to demonstrate the level of adherence to GRP.

Limitations of the validation study are described in chapter 3. An important aspect that should be taken into account in the validation of any method to measure radioactive samples, is to include the entire range of possible activities to be measured.

Due to limited availability of radiopharmaceuticals and the fact that our sterility samples were taken from the volume remaining in radiopharmaceutical vials after patient doses were dispensed, our sterility sample numbers were relatively small. Larger sample sizes for sterility tests and media fill tests (e.g. collected over a longer time period) would allow a more reliable statistical comparison of the different radiopharmacies, and possibly permit better analysis of relationships between the different aspects of microbial monitoring addressed in this work.

Future directions

Further work can support the findings of this study. Surface sampling at critical locations in the radiopharmacies would help to provide more complete monitoring data for microbial safety of radiopharmaceuticals. It would be useful to investigate if passive air sampling according to

pharmacopoeial methods (using different incubation temperatures and longer incubation) would be feasible at least in LAF cabinets which demonstrated low settle plate contamination rates in the current study. Collecting more data on both sterility of radiopharmaceuticals and air quality would allow better statistical analysis. Lastly, based on the useful outcomes from the work described in this dissertation, another attempt can be made to obtain permission to expand the study by collecting data at more African radiopharmacies, to reflect a broader range of circumstances.

Recommendations for future improvement and development at YGH:

- Training of a medical physicist for the nuclear medicine department, who would be, amongst others, responsible for regular QC of equipment;

- Training of an engineer to service and as far as possible repair radiopharmacy and nuclear medicine equipment;

- Designing the new radiopharmacy to have all rooms (e.g. change room, radiopharmaceutical preparation room, QC room, etc.), adjacent to each other, with a layout that allows unidirectional movement of staff and products;

- Emphasize again the earlier recommendations regarding a clean room facility for the radiopharmacy.

Recommendations for future improvement and development of TBH radiopharmacy:

- Implement broth fill and fingertip tests to evaluate competency of staff preparing radiopharmaceuticals in laminar flow cabinets;

- Address shortcomings found during the self-audit.

The results obtained in this doctorate will contribute significantly to enhancing the quality of work and implementation of quality assurance standards in radiopharmacies in low-income countries:

- The adapted QUANUM tool described in the current work should be useful for audits by African radiopharmacies.

- Other radiopharmacies working under comparable conditions can use the methods described in this study as an example of implementing good practice for radiopharmacies.

- Investment in establishing and maintaining well-designed clean room facilities and suitable equipment for quality control monitoring remains the best option for ensuring safety and efficacy of radiopharmaceuticals. Where this is financially not feasible, a good quality management system, a correctly used and well-maintained good LAF, appropriate staff training and evaluation of their competence as well as strict adherence to aseptic technique can to a large extent overcome the disadvantages of a sub-optimal facility.

Conclusion

Requirements for radiopharmacy design and procedures described in international guidelines cannot all be met by radiopharmacies in many African countries, including Cameroon and South Africa. Factors affecting the safety and efficacy of radiopharmaceuticals at TBH and YGH are described in this work. The sub-optimal facility and lack of equipment maintenance negatively impact the air quality at YGH, but simple structural and procedural improvements lowered the level of microbes detected by passive air sampling. The better facility and well-maintained equipment at TBH demonstrated better air quality in the radiopharmacy, despite the lack of a classified pharmaceutical clean room.

The results obtained in this study indicate that implementation of a good quality management plan can make a major contribution to safety and efficacy of radiopharmaceuticals even if the facility and equipment cannot meet internationally recommended standards.

The scientific value of the study lies in its originality as no published work is available until now describing implementing radiopharmacy practice guidelines in the African context. In addition, this

study provides useful information to hospital radiopharmacy facilities in many African countries and the rest of the world which do not have access to all recommended equipment for their daily operations.

Addenda

Addendum to Chapters 1 and 2

Table A.1 Status of SPECT radiopharmacy at the three hospitals

Components	Status YGH	Status TBH	Status UMCG
Staff and training	Responsible person: MSc Radiopharmacy 1 Nuclear Medicine technician with six months training in a well-equipped unit 1 Scientist with a Bachelor's degree in Biology with 4 months training in a well-equipped unit 1 Scientist with a Bachelor's degree in Biology and European course in radiopharmacy level 1	Responsible pharmacist: PhD, registered specialist in Radiopharmacy 7 Nuclear Medicine radiographers (technologists) take turns to work 1 week in the radiopharmacy (National diploma or degree course includes Radiopharmacy theory and practice	3 Qualified Persons (Pharmacist/ PhD, 2 Clinical Pharmacists), 4 Radiochemists (PhD, 2 Clinical Radiochemists), 2 GMP-officers (1 BSc), 15 technicians for Radiopharmacy production (7 BSc in chemistry)

	1 Chief nurse with 3 months training in a well-established unit in Europe		
Facility (rooms)	2 rooms, not cleanroom status	Separate rooms for receipt and unpacking, radiopharmaceutical preparation, QC, cell labelling Not cleanroom status	One room for receipt, quarantine storage with fridge and freezer as well as unpacking. 2 Cleanroom facilities: one for Shortliving radiopharmaceuticals (PET) and one for longer living radiopharmaceuticals (SPECT, Therapy and ^{89}Zr-PET) Separate QC room.
Equipment	1 glove box (not clean air) 1 fume hood 1 laminar air flow cabinet 1 shielded dispensing area 1 dose calibrator	Shielded LAF cabinet for preparation and dispensing of radiopharmaceuticals, includes generator safe with lift mechanism Biohazard class II LAF for cell labelling 2 dose calibrators Chromatography scanner	SPECT facility: 3 LAF-hoods and one Hotcell Grade A/B for aseptic filling of ^{89}Zr-antibodies. QC facility UPLC, HPLC, endotoxin testing, sterility testing, chromatogram scanner, pH-meter, and gamma counter.

107

	3 contamination monitors	Well counter 2 Centrifuges Several contamination monitors etc.	
Radionuclides	^{99m}Tc, ^{131}I	^{99m}Tc, ^{123}I, ^{131}I, ^{111}In, ^{51}Cr	^{99m}Tc, ^{123}I, ^{131}I, ^{125}I, ^{111}In, ^{81m}Kr, etc.
Radiopharma-ceuticals	MIBI (methoxyisobutylisonitrile), DTPA (diethylene triamine penta acetic acid), HMDP (hydroxy methylene diphosphonate), nanocolloid (colloidal rhenium sulphide), DMSA (dimercaptosuccinic acid)	^{99m}Tc: MIBI, DTPA, MAG3, DMSA, MDP, nanocolloid, tin colloid, HMPAO, MAA, Technegas, Pyrophosphate, etc. RBC and Leucocytes ^{123}I: NaI, MIBG ^{111}In: platelets	^{99m}Tc: Pertechnetate, MAG3, tetrofosmin, DTPA, DMSA, HDP, nanocolloid, tin colloid, HMPAO, MAA, mebrofenin, HMPAO, HSA, RBC, ^{99m}Tc-labelled Leucocytes Krypton generator ^{123}I: NaI, MIBG, SAP (Serum Amyloid Protein). ^{131}I: NaI, HSA, Hippuran ^{125}I: Iothalamate

108

Details of self-audits at the three radiopharmacies discussed in this study

The questions in the tables below were published by the IAEA in the Operational Guidance on Hospital Radiopharmacy: A Safe and Effective Approach in 2008. This publication also provides background information on the requirements for hospital radiopharmacies working at different operational levels. The questions address those requirements.

In the following tables the answers Yes or No are presented in the format Y/Y, with the first answer indicating the initial audit status, and the second answer relating to the follow-up audit.

Questions numbers starting with "1." are relevant for operational level 1, and "2." for operational level 2. Class A indicates aspects that are required and that should be addressed urgently, class B standards should be reached although they are not compulsory.

The level of conformance (LoC) was graded as follows:

0   the component was absent in the unit

1   the component was planned

2   the component was partially implemented

3   the component was largely implemented

4   the component was fully implemented

Items scoring 0, 1, or 2 are considered non-compliant.

Items scoring 3 and 4 are considered compliant.

Items marked ''n/a'' are not applicable.

Table A.2 Staffing scoring

Question	Component	Class	Y or N Before/ After	YGH Initial score	YGH Follow-up score	Y or N	TBH Score	Y or N	UMCG score
1.1	Is there a professional responsible for the radiopharmacy? Provide details.	A	Y/Y	4	4	Y	4	Y	4
1.2	Is the radiopharmacy unit operated under the direction of a person with appropriate training as defined by local or national regulations?	A	Y/Y	4	4	Y	4	Y	4
1.3	Are there written staff training manuals for all grades of staff?	B	N/Y	0	3	N	0	Y	4
2.1a	Calibration of equipment- please provide details and training records	A	N/N	0	0	Y	3	Y	4
2.1b	Working practices in the radiopharmacy - please provide details and training records	A	Y/Y	3	3	Y	3	Y	3

2.1c	Preparation of individual doses - please provide details and training records	A	Y/Y	4	4	Y	3	Y	4
2.1d	Quality control and analytical techniques - please provide details and training records	A	N/Y	1	3	Y	3	Y	4
2.1e	Dose release - please provide details and training details	A	Y/Y	4	4	N	2	Y	4
2.1f	Record keeping - please provide details and training records	A	Y/Y	3	3	Y	3	Y	3
2.1g	Cleaning - please provide details and training records	A	Y/Y	3	3	N	2	Y	4
2.2	Is there a system for formal approvals of all documentations including radiopharmaceutical (RP) preparation, QC and formal release to patient?	B	N/Y	3	3	Y	4	Y	4
2.3	What training is provided to staff performing final checks on all products prepared before release for patient use?	A	N/Y	0	3	Y	3	Y	3

2.4	Are there training records for all staff performing cell labelling, e.g. RBC, WBC?	**B**	n/a			N	1	Y	4
2.5	Is there an annual performance review to check the competencies of radiopharmacy staff?	**B**	N/Y	0	3	N	1	Y	4
	Total			29	40		36		55
	Maximum possible score			52	52		56		56
	Score (%)			56%	77%		64%		93%
	Conforming			8	12		9		14
	Non-conforming			5	1		5		0
	Conforming factors (%)			61.5%	92.3%		64.3%		100.0%

112

Table A.3 Facility scoring

Question	Component	Class	Y or N Before/ After	YGH Initial score	YGH Follow-up score	Y or N	TBH Score	Y or N	UMCG score
1.4	Does the unit have appropriately finished rooms (including adequate lighting, appropriate finishes to walls, floors, ceilings and ventilation) and a shielded dispensing station?	A	N/N	0	0	Y	3	Y	4
1.5	Is there a shielded dispensing station available?	A	Y/Y	3	3	Y	4	Y	4
1.6a	For operational level 1b is there a shielded dispensing station and/or a fume hood available? [Is there a fume cupboard with suitable filters for volatile radioactive materials such as ^{131}I solutions?]	A	Y/Y	3	3	Y	4	Y	4

1.6b	[If only radioiodine capsules are handled is the package opened in a well-ventilated area?]	**A**	N/Y	0	3	Y	4	Y	4
1.7	Is there a validated (annual check on air-flow, safety and challenge testing) fume hood with suitable filters for handling radioiodine solutions?	**A**	N/N	0	0	Y	3	Y	4
1.8	Are there records and logs kept for all equipment irrespective of whether maintenance and calibration is performed 'in-house' or by external contractors?	**B**	Y/Y	3	3	Y	3	Y	4
2.6	For operational level 2: Are there regular checks on validated Class II type B microbiological safety cabinets located in a dedicated room?	**A**	N/Y	0	3	Y	4	Y	4
2.7	Are manometer readings of pressure differentials across HEPA filters recorded daily?	**B**	N/Y	0	3	N	0	Y	4

114

2.8	Are there periodic records of air velocity determination for LAF cabinets or isolators?	B	N/Y	0	3	Y	3	Y	4
2.9	Is challenge testing of the HEPA filters in LAFs and isolators carried out annually?	B	N/N	0	0	Y	4	Y	4
2.10	For negative pressure isolators: Before preparation takes place, are gloves or gauntlets visually inspected and integrity tests carried out and recorded?	B	n/a			n/a		Y	4
2.11	Is there a system and record of planned preventative maintenance for all equipment in the radiopharmacy including the refrigerator?	B	N/N	0	0	Y	3	Y	2
2.12	When clean rooms are used, are the over-pressures gauges monitored and recorded daily?	B	N/Y	0	3	N	2	Y	4

Total	9	24	37	50
Maximum possible score	48	48	48	52
Score (%)	18.8%	50.0%	77.0%	96.1%
Conforming	3	8	10	12
Non-conforming	9	4	2	1
Conforming factors (%)	25.0%	66.7%	83.3%	92.3%

116

Table A.4 Purchase of materials scoring

Question	Component	Class	Y or N Before/ After	YGH Initial score	YGH Follow-up score	Y or N	TBH Score	Y or N	UMCG score
1.9	Are there suitable protocols and trained staff for the purchase of approved or Marketing Authorized radiopharmaceuticals?	A	N/Y	0	3	Y	3	Y	4
1.10	Are all goods received checked and recorded against the order for correctness of delivery?	B	N/Y	0	3	Y	3	Y	4
1.11	Are records kept for batch numbers and quantities received?	B	N/Y	0	4	Y	4	Y	4
1.12	Are visual inspections and label checks carried out prior to acceptance?	B	Y/Y	3	4	Y	3	Y	4
2.13	Do all products, kits and generators have product approval, marketing authorisation, or bear a product licence number?	A	Y/Y	4	4	Y	4	Y	4

2.14	How many unlicensed or unapproved products are used each year and is there a record of them?	A	n/a			Y	4	Y	4
2.15	For all unlicensed kits, radiopharmaceuticals or radio-chemicals are the prescribers or responsible medical doctors made aware of his/her responsibilities?	A	n/a			Y	4	Y	4
2.16	Do the suppliers or reagents and unapproved products provide a "Certificate of Analysis"?	B	Y/Y	4	4	Y	3	Y	4
	Total			11	22		28		32
	Maximum possible score			24	24		32		32
	Score (%)			45.8%	98.4%		87.5%		100.0%
	Conforming			3	6		8		8
	Non-conforming			3	0		0		0
	Conforming factors (%)			50.0%	100.0%		100.0%		100%

Table A.5 Dispensing protocols scoring

Question	Component	Class	Y or N Before/After	YGH Initial score	YGH Follow-up score	Y or N	TBH Score	Y or N	UMCG score
1.13	Are there specific written radiopharmacy procedures for dispensing operations undertaken in the radiopharmacy?	B	N/Y	2	4	Y	4	Y	4
1.14	Under operational level 1a: Are there written procedures for the aseptic dispensing and labelling of unit doses of ready-to-use radiopharmaceuticals?	B	n/a			Y	4	Y	4
1.15	Is there a system for labels which assesses quality, number produced and number applied to dispensed doses?	A	Y/Y	3	4	n/a		Y	4
1.16	For operational level 1b: Do the written procedures contain clear safety and monitoring instruction for dispensing radioiodine solutions or capsules?	A	N/Y	0	4	N	2	Y	3

No.	Question								
1.17	Under operational level 1b are there written procedures for calibration assay, preparation and dispensing of individual patient radionuclide therapy?	A	N/Y	0	3	Y	4	Y	3
1.18	Can the audit and documentation for each RP batch be traced from the prescription to the actual administration of individual patient doses?	A	Y/Y	3	4	Y	4	Y	4
	Total			8	19		18		22
	Maximum possible score			20	20		20		24
	Score (%)			40.0%	95.0%		90.0%		91.6%
	Conforming			2	5		4		6
	Non-conforming			3	0		1		0
	Conforming factors (%)			40.0%	100.0%		80.0%		100.0%

Table A.6 Preparation protocols scoring

Question	Component	Class	Y or N Before/ After	YGH Initial score	YGH Follow-up score	Y or N	TBH Score	Y or N	UMCG score
2.17	Are there written and approved procedures for the use of generators and reconstitution of each radiopharmaceutical kit used?	A	Y/Y	4	4	Y	4	Y	4
2.18	Are SOPs independently reviewed and approved at specified intervals?	B	N/Y	1	3	Y	3	Y	4
2.19	Is the preparation of ^{99m}Tc radiopharmaceuticals from kits and generators carried out in a LAF cabinet?	A	Y/Y	4	4	Y	4	Y	4
2.20	Are there set criteria before release for preparation for patients use? Is this undertaken by the same operator or a different individual?	B	N/Y	1	3	Y	3	Y	4
2.21	Can each individual patient dose be traced to a specific generator and kit batch number?	A	N/Y	1	3	Y	4	Y	3

2.22	Under operational level 2b: Do the written procedures for any autologous preparation, e.g. red and white blood cells, include a clear instructions on safety, cleaning and decontamination?	**A**	n/a			Y	4	Y	4
2.23	Are there written procedures for the preparation and dispensing of approved kit formulations of radio-labelled biological e.g. monoclonal antibodies, peptides?	**A**	n/a			n/a		Y	4
	Total		11	17		22		28
	Maximum possible score		20	20		24		28
	Score (%)		50.0%	85.0%		92.0%		100.0%
	Conforming		2	5		6		7
	Non-conforming		3	0		0		0
	Conforming factors (%)		40.0%	100.0%		100.0%		100.0%

Table A.7 QA/QC scoring

Question	Component	Class	Y or N Before/ After	YGH Initial score	YGH Follow-up score	Y or N	TBH Score	Y or N	UMCG score
1.19	Are daily QC checks performed on radionuclide calibrators?	A	Y/Y	4	4	Y	4	Y	4
1.20	What quality checks are undertaken on a supplier before purchase?	B	Y/Y	4	4	Y	3	Y	4
1.21	Are periodic quality checks on radiopharmaceuticals (RP) performed?	B	N/Y	1	4	Y	4	Y	4
1.22	Is there a written procedure for dealing with product/s failing to meet the required standard?	B	N/Y	0	4	N	0	Y	4
1.23	Is there a record of complaint/s and any associated follow-up and investigation?	B	N/Y	0	4	Y	4	Y	4
1.24	Are there written procedures and records for regular contamination surveys of the radiopharmacy unit?	A	N/Y	0	4	Y	3	Y	4

2.24	**For operational level 2 are there records for the following:**	**B**							
2.24a	Purchase of radioactive products and ingredients	**B**	Y/Y	4	4	Y	4	Y	4
2.24b	Generator elution, yield, [^{99}Mo] molybdenum breakthrough and aluminium ion breakthrough	**B**	Y/Y	4	4	Y	4	Y	3
2.24c	Product preparation, QC and release	**B**	N/Y	0	4	Y	3	Y	4
2.24d	Environmental and microbiological monitoring	**B**	N/Y	0	3	Y	3	Y	4
2.24e	Aseptic process, aseptic operator validation and trend analysis	**B**	N/Y	0	4	N	0	Y	4
2.24f	Laboratory cleaning and maintenance	**B**	N/Y	0	4	N	0	Y	3
2.24g	Equipment and plant calibration and maintenance	**B**	N/N	0	0	Y	3	Y	3
2.24h	Radioactive contamination monitoring and radioactive waste disposal	**B**	Y/Y	3	4	Y	3	Y	4

2.24i	Product defects and SOPs non-conformance, i.e. when a procedure is performed in a manner other than that described in the relevant SOP	B	N/Y	0	3	Y	4	Y	4
2.24j	Independent inspection and audit	B	N/Y	0	3	N	1	Y	4
2.25	In line with the IAEA "Operational guidance on Hospital Radiopharmacy" document, are there records of routine microbiological monitoring of the preparation area in the radiopharmacy?	A	N/Y	0	3	Y	3	Y	4
2.26	Are there calibration and linearity checks of the dose calibrator response over the complete range of activities measured at least annually?	A	Y/Y	3	4	Y	4	Y	4
2.27	Is there set programme for checking the quality of radiopharmaceuticals (RP)?	B	N/Y	0	4	Y	4	Y	4
2.28	Considering patient safety, are certain simple checks performed on prepared radiopharmaceutical, e.g. mini-chromatography?	A	N/Y	0	3	Y	4	Y	4

2.29	For operational level 2 is a [⁹⁹Mo] Molybdenum breakthrough measurement performed on the first eluate from each [⁹⁹ᵐTc] Technetium generator and repeated when the generator is moved?	A	Y/Y	4	4	Y	4	Y	4
2.30	Is aluminium ion breakthrough checked on the first eluate from a [⁹⁹ᵐTc] Technetium generator?	A	N/N	0	0	Y	4	N	0
2.31	Are changes in the source of any kits, diluents or vehicle used, needles, syringes, swabs and sterile containers used within radiopharmacy recorded?	B	N/Y	0	3	N	1	Y	4
2.32	On first use of a new batch or first new delivery of RP kits is radiochemical purity performed?	B	N/Y	0	4	Y	4	Y	4
2.33	Laboratory cleaning and maintenance	A	n/a			Y	4	Y	3
2.34	Is there regular pH testing of RP carried out?	B	N/Y	0	3	Y	3	Y	3

2.35	Prior to release for patients is each individual radioactivity dose checked?	**A**	Y/Y	3	4	Y	4	Y	4
2.36	Is there a record of the formal approval/release by an authorized person before a product is administered to a patient?	**A**	Y/Y	3	4	N	0	Y	4
2.37	Are there written procedures for the recall of defective products?	**A**	N/Y	0	4	n/a		Y	4
2.38	Is there a record of complaints and any associated follow-up and investigation?	**B**	Y/Y	3	4	Y	4	Y	4
2.39	Is there a system of recorded self-inspection and reports evaluation?	**B**	N/Y	0	3	N	0	Y	4
2.40	Is there a system for external audit or peer review process?	**B**	N/N	0	0	N	1	Y	4
	Total			36	104		87		117
	Maximum possible score			124	124		124		128
	Score (%)			29.0%	83.9%		70.0%		91.4%

127

Conforming	10	28	23	31
Non-conforming	21	3	8	1
Conforming factors (%)	32.3%	90.3%	74.0%	96.9%

Table A.8 Waste scoring

| Question	Component	Class	Y or N Before/ After	YGH Initial score	YGH Follow-up score	Y or N	TBH Score	Y or N	UMCG score
1.25		**A**	Y/Y	4	4	Y	4	Y	4
1.26		**A**	N/Y	0	4	N	0	Y	4
1.27		**A**	Y/Y	4	4	Y	4	Y	4
	Total			8	12		8		12
	Maximum possible score			12	12		12		12
	Score (%)			66.7%	100.0%		67.0%		100.0%
	Conforming			2	32		2		3
	Non-conforming			1	0		1		0
	Conforming factors (%)			66.7%	100.0%		66.7%		100.0%
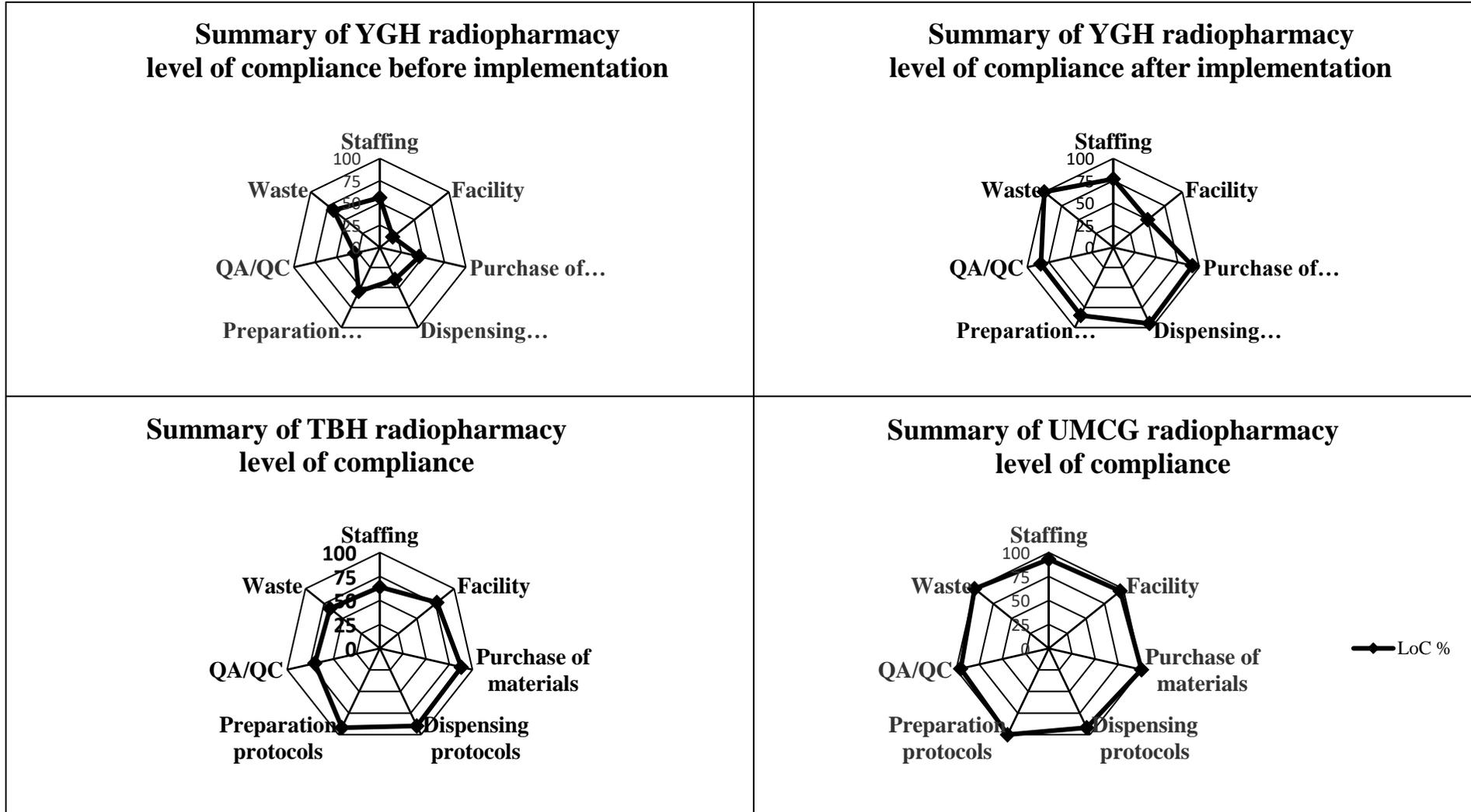
Figure A.1 Summary of radiopharmacies' level of compliance. For radiopharmacy at YGH, the compliance is shown for the initial audit

(before implementation) and for the audit after corrective actions were implemented (after implementation)

Addendum to Chapter 5

Table A.9 Fisher's exact test of data from plate exposure

at Radiopharmacy I versus Radiopharmacy II

Area	Rest	Work
Where RP are prepared (Class A)	$P < 0.01$*	$P < 0.001$*
Other Class A LAF	$P > 0.05$	$P > 0.05$
Class C	$P < 0.05$*	$P > 0.05$

* Difference between Radiopharmacy I and Radiopharmacy II is statistically significant

Table A.10 Fisher's exact test of data from plate exposure

rest versus work at Radiopharmacies I and II

Area	Radiopharmacy I	Radiopharmacy II
Where RP are prepared (Class A)	$P > 0.05$	$P > 0.05$
Other Class A LAF	$P > 0.05$	$P > 0.05$
Class C	$P > 0.05$	$P > 0.05$
Class D	$P > 0.05$	n/a