DETERMINATION OF THE QUALITY OF TECHNETIUM 99m ELUATE BEING USED AT THE UNIVERSITY TEACHING HOSPITALs IN LUSAKA, ZAMBIA

By

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DECLARATION

I, **ZULU JENIPHER** hereby declare that this dissertation herein presented for the degree of master of clinical pharmacy has not been formerly submitted in every detail or in part at this or any other university.

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ABSTRACT

Good quality of Technetium 99m eluate (^{99m}Tc) as a radionuclide used in diagnostic imaging procedures is important for proper diagnosis, to avoid patients' unnecessary exposure to radiation and to reduce the number of repeat nuclear imaging. However, presence of impurities in ^{99m}Tc eluate, may produce radiolytic effects, bio-distribution and/or inadequate localization in organs of interest, resulting in poor images. The impurities may interfere with diagnostic interpretation of the images by masking and mimicking disease. The main objective of this study was to determine the quality of ^{99m}Tc eluate, while specifically we determined the radionuclidic and radiochemical, chemical impurity, pH and physical characteristics of ^{99m}Tc eluate.

A cross sectional study, was conducted at the University Teaching Hospitals (UTHs – Adult Hospital) and National Institute of scientific and industrial research (NISIR) in Lusaka from September 2016 to March 2017. Six ^{99}Mo/^{99}Tc generators that were available during the study period were sampled from each generator collected at two time points (96 hours and 120 hours from the time manufacture date). The radionuclidic purity, radiochemical purity, chemical purity, pH and physical characteristics of the of the ^{99m}Tc eluate obtained from the Molybedenum-99/Technetium-99 (^{99}Mo/^{99}Tc) generators were determined and results were compared with the British Pharmacopeia standards and those set by the manufacturers. GraphPad Prism version 5 was used to analyse the data with the p value of < 0.05 considered statistically significant.

The median for radionuclidic purity at 96 hours was 0.003 (0.002 – 0.005) and at 120 hours was 0.003 (0.00 – 0.015) p value=0.39. The median pH at 96 hours was 5.94 (5.79 – 6.05) and at 120 hours was 5.96 (5.82 – 6.09); p = 0.06. For radiochemical test at 96 hours it was 98.3 (97.5 – 98.9) percent and at 120 hours was 98.8 (98.4 – 99.3) percent p = 0.06.

All the parameters that were analysed were within the acceptable ranges and hence met the British Pharmacopeia standards

Keywords: Nuclear medicine, Technetium 99m eluate, radionuclidic purity, radiochemical purity, ^{99m}Tc generators, University Teaching Hospital, Zambia,

DEDICATION

I devote my work to my Lord Jesus Christ through whom I find strength every day and also to my husband Dr. Ernest Chipasha and my five sons; Ernest Junior, Clement, Dalitso, Mwila and Theophilus for their support and love during this piece of work despite the less time I spent with them.

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LIST OF ACRONYMS

DTPA	Calcium Trisodium Pentetate Tin Dichloride 2 - Hydrate 4 - Aminobenzoic Acid
IAEA	International Atomic Energy Agency
GMP	Good Manufacturing Processes
^{99}Tc	Technetium-99
^{99m}Tc	Technetium -99m
MAA	Micro albumin aggregated
PH	potential of Hydrogen
UTH	University Teaching Hospital
KeV	kilo electron volts
MBT	Molybdenum Breakthrough
NISIR	National Institute of Scientific and Industrial Research
Rf	Reference front value
P	Probability value

DEFINITION OF KEY TERMS IN THIS STUDY

Elution: a method of "washing off" an adsorbed substance from a solid adsorbing matter (such as ion-exchange resin) with a liquid

Eluate: Product of the elution process

Isomer: nuclides having the same atomic and mass numbers but differing in energy and spin of the nuclei. For example, ^{99m}Tc and ^{99}Tc are isomers

Isotonic: a solution that has the same salt (sodium chloride) concentration as the cells and blood (medicine net.com).

Isotope: nuclides having the same atomic number (same number of protons in the nucleus but different number of neutrons) for example C-12 and C-14

Labelling: a process of tagging a molecule with a radionuclide

Metastable (m): an excited state of a nuclide that decays to another excited state or the ground state with a measurable half-life

^{99}Mo/^{99m}Tc generator: a device in which a short-lived daughter is separated chemically and periodically from a long-lived parent adsorbed on adsorbent material. For example, ^{99m}Tc is separated from ^{99}Mo from the ^{99}Mo/^{99m}Tc generator by eluting with saline

Molybdenum 99: is made from molybdenum trioxide which is a naturally occurring compound from which ^{99m}Tc is made.

Nuclear medicine: is a medical specialty and branch of Medicine that uses of radioactive substances in treatment, diagnosis and research.

Nuclear Pharmacy - as the specialty of pharmacy, nuclear pharmacy is dedicated to the procurement, storage, preparation or compounding, dispensing and patient counselling, as well as assuring the acceptable quality of radiopharmaceuticals.

Pharmaceutical: a molecule that is used to tag a radio nuclide during the labelling process

Quality: is a property that products should have to satisfy requirements specified by international standards

Quality Assurance: Is a sum total of organised arrangements made by object of ensuring that products will be of quality required by their intended use or verification of the quality of products to safety that the predefined standard specification of the products can be accepted or refused according to these verifications

Radionuclide: is an atom that has excess nuclear energy making it unstable.

Radiopharmaceutical: is a radioactive drug that can be administered safely to humans for diagnostic and therapeutic purposes. An example of radiopharmaceutical is ^{99m}Tc-DTPA

Radionuclidic purity: is the fraction of the total radioactivity in the form of the stated radionuclide. Any extraneous radioactivity such as ^{99}Mo in ^{99m}Tc radiopharmaceuticals is an impurity. An example of radionuclidic impurity is ^{99}Mo in levels above 0.015MBq.

Radiochemical purity: the fraction of the total radioactivity in the desired chemical form. If 99mTc-Macroaggregated albumin is 90% pure, then 90% of the radioactivity is in the ^{99m}Tc-MAA form

Relative Front Value: this is the ratio of distance travelled on the medium (stationary Phase) by a given radiochemical species to the distance travelled by a mobile phase's solvent front

Technetium 99 m: is a short lived metastable nuclear isomer (half-life -6hours) used in nuclear medicine produced from molybdenum 99

Technetium 99: is an isotope of technetium which decays with a half-life of 211,000 years

CHAPTER ONE

INTRODUCTION

1.1 Background

Technetium 99m (^{99m}Tc) is the radionuclide used today intravenously in 80 to 85% of the procedures in nuclear medicine worldwide (Richards and Tucker, 1982). ^{99m}Tc is preferred due to its favourable characteristics (Richards and Tucker, 1982 and Peter 2005) which include short half-life of six hours, availability and is less expensive among others. It is eluted from a Molybedenum 99/Technetium 99m (^{99}Mo/^{99m}Tc) generator from what is termed as parent–daughter relationship using isotonic saline as shown below.

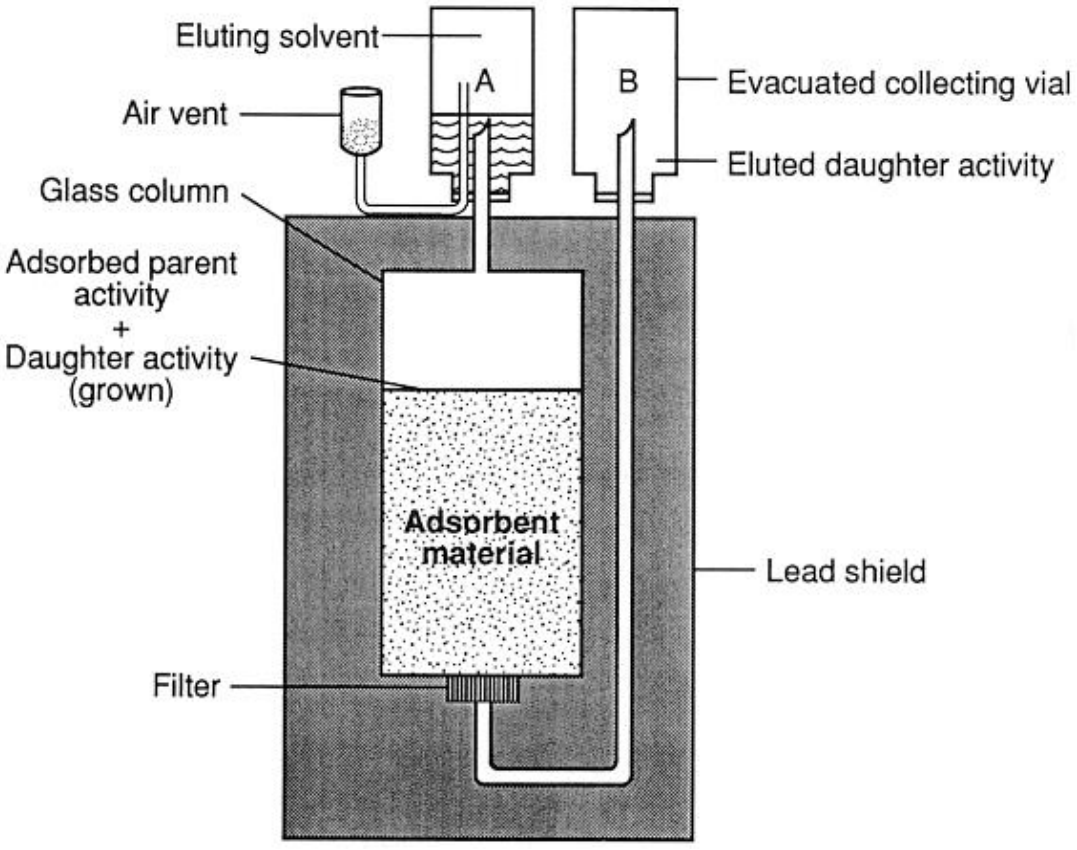

Figure 1: Cross Section of a ^{99}Mo/^{99m}Tc generator (as adapted from Fundamentals of Nuclear Pharmacy by G.B. Saha 2010)

As can be seen in the diagram above, the normal saline from the eluting solvent A is collected as the eluted daughter in the collecting vial B. The above process, called milking, can

accidentally collect other unwanted substances in the final product (^{99m}Tc eluate). The quality of images generated after this product is administered to the patient can affected resulting in erroneous diagnosis of different ailments.

Some studies have revealed presence of contaminants in the ^{99m}Tc eluate. In Brazil, a study was conducted to determine ^{99}Mo contamination in a nuclear medicine patient submitted to a diagnostic procedure with ^{99m}Tc. An in-vivo measurement technique was developed to verify the accuracy of internal contamination and results indicated the presence ^{99}Mo as a contaminant. A similar study in Iran set out to determine ^{99}Mo contamination in ^{99m}Tc eluate obtained from ^{99}Mo/^{99m}Tc generators. Their results showed that certain methods of producing generators promote ^{99}Mo contamination (Momennezhad, Sadeghi and Zakavi., 2010). In Croatia a study was undertaken to determine radionuclidic purity of ^{99m}Tc eluate for use in nuclear medicine. Though ^{99}Mo could not be detected in all the ^{99m}Tc eluates as the main radio contaminant, results revealed the presence of three long gamma emitters ^{103}Rubidium, ^{106}Rubidium and ^{131}Iodine (Vucina 1989). In an effort to reduce ^{99}Mo contamination, a study in Malaysia devised a new way of producing ^{99}Mo/^{99m}Tc generators and their results showed that alumina dry type of producing ^{99}Mo/^{99m}Tc generators reduces ^{99}Mo contamination (Ibrahim *et al*, 2012). In a study that was done in Germany (by Hammermaier, Reich and Bogl) the researcher set out to determine the quality of ^{99}Mo/^{99m}Tc generators, seven different ^{99}Mo/^{99m}Tc generators all loaded with fission molybdenum were sampled. Their specific objectives were to test the ^{99m}Tc eluate for its radionuclidic and radiochemical purity, checking the pH value and test to detect soluble aluminium in the eluate. Gamma spectrometry and chromatography were used to analyse the ^{99m}Tc eluate. The results were significant as all the eluates showed high and sufficient radionuclidic purity. The elution yields were between 85 and 122% meeting the required standards. All eluates had pH values between 5.0 and 6.5, and aluminium content below 1microgram/ml. The generators were therefore found to have good performance and generally proved to be a good source of ^{99m}Tc pertechnetate and hence were of good quality as no contaminants were found (Hammermaier, Reich and Bogl., 1996).

As can be seen from some of the studies above, there is a possibility of contaminants in the ^{99m}Tc eluate. Most of the studies on the subject are from developed countries with little or no information locally and regionally so far. Presence of contaminants may cause unnecessary radiation exposure, can also affect radiolabelling and localization thereby affecting image quality which may result in delays in diagnosis as repeat imaging will definitely be required (Andrew 2000).

1.2 Problem Statement

Improper compounding techniques while preparing ^{99m}Tc eluate may lead to presence of undesired impurities and contaminants (Ponto, 2004; Yoshihara 1996 and Ioveless 2009). Presence of impurities in ^{99m}Tc eluate may produce radiolytic effects, biodistribution and/or inadequate localization in organs of interest, resulting in poor images (Uccelli and Bosch, 2013; Emran 2013; Lopez and Monroy, 2013, Ponto, 2004 and Guedes-silva 2014), this may lead to repeat nuclear imaging. The impurities may interfere with diagnostic interpretation of the images by masking and mimicking disease diagnosis. Therefore, the quality of ^{99m}Tc eluate is important before it's used in patients (Theobald 1994; Pauwels et al 1977 and Gad 2008).

This research, therefore, intended to determine the quality of ^{99m}Tc eluate being used at UTH with the hope of improving, diagnosis, management and possibly reduce repeat imaging.

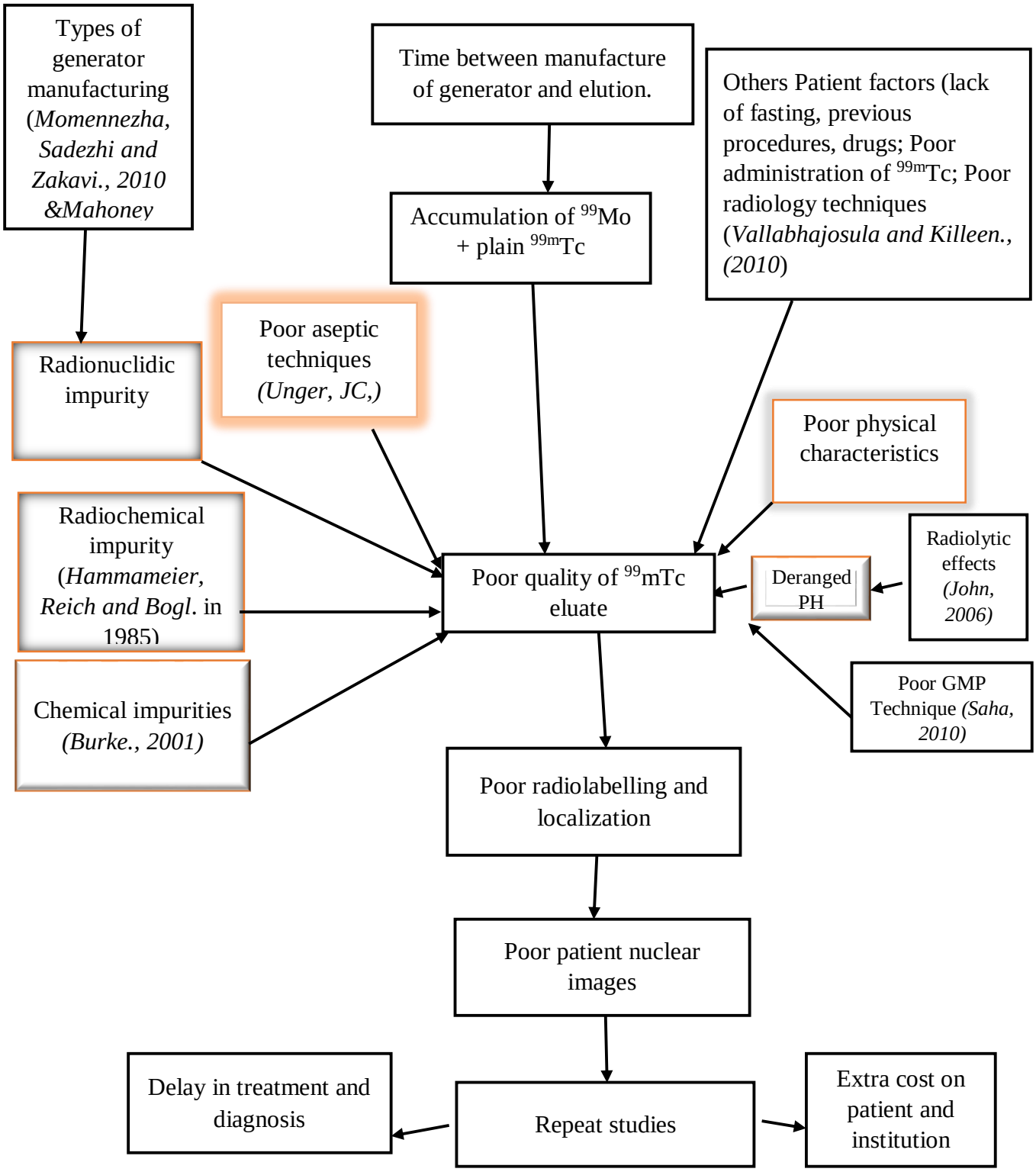

Figure 2: Conceptual Framework

Figure above showing different factors that affect the quality of ^{99m}Tc eluate and outcomes of the poor quality of ^{99m}Tc eluate.

1.3. Significance of the Study

The presence of contaminants in the ^{99m}Tc eluate causes unnecessary radiation exposure, affects radiolabelling and localization, affect image quality and can cause organ damage (Vallabhajosula *et al*., 2010). Contamination with molybdenum alone introduces a dose coefficient of 50 times higher than that of ^{99m}Tc eluate, this high dose is associated with high energies photons emitted by ^{99}Mo leading to poor images and cell damage in the human body. The other complications include delay in diagnosis and extra expense on both the patient and the institution. Literature search showed no statistics available on the quality of ^{99m}Tc eluate being used in developing countries, Zambia inclusive as most of the studies that we came across on the subject were from developed countries.

In Zambia, it is not clear why there are some repeat imaging (about 10%) at UTH Nuclear Medicine due to poor imaging. Literature search showed that a study such as this had not been done in Zambia particularly at the UTH and thus warranted the need for this study. Hence checking the quality of ^{99m}Tc eluate being used in Zambia will enhance timely accurate diagnostic interpretation of images and early diseases treatment. The finding can also serve as a foundation for future research and will also add to literature.

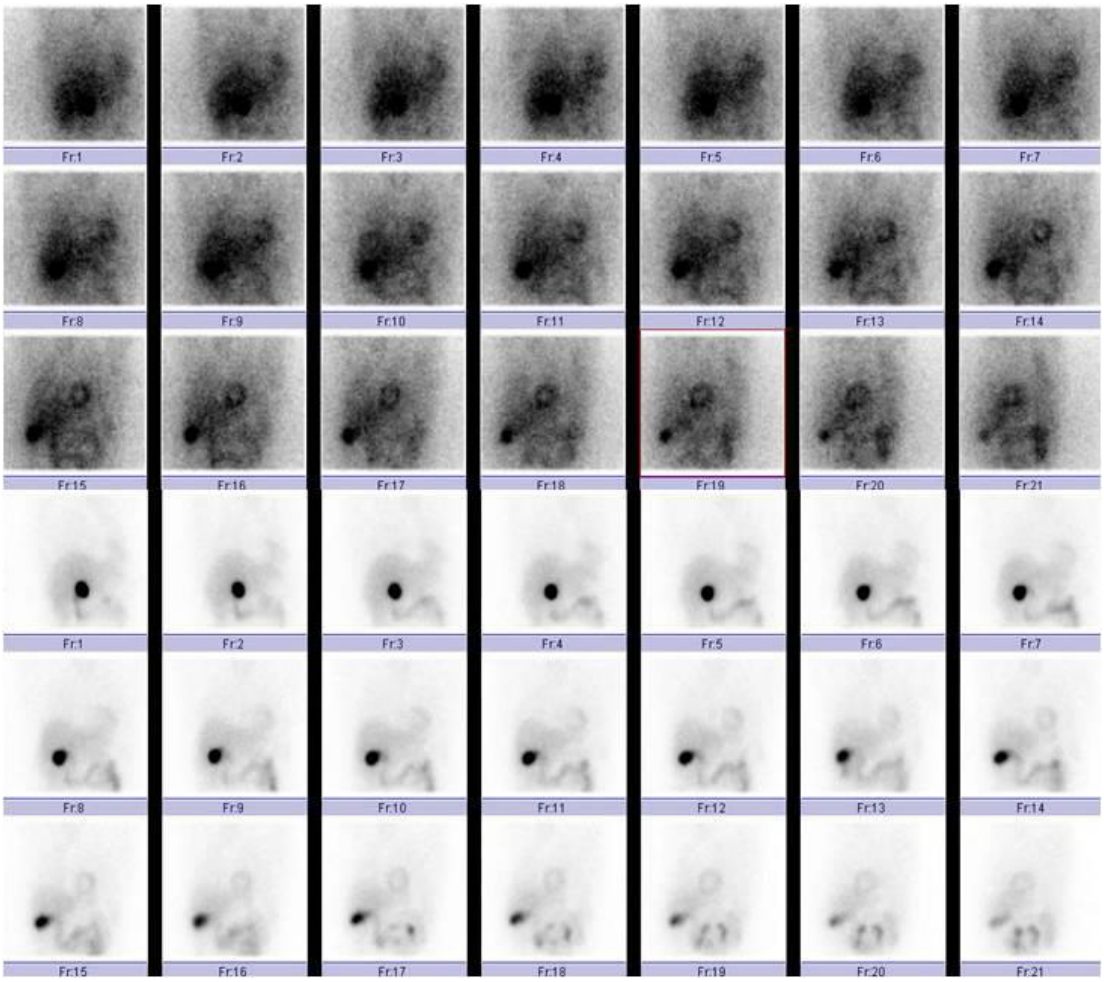

Figure 3: below shows poor image quality as a result of ^{99}Mo contamination - Momennazhad *et al* 2010

Projections from myocardial perfusion scans: the upper three rows show increased background activity and poor image quality as a result of ^{99}Mo contamination compared to the normal state (lower three rows-Momennazhad, Sadezhi and Zakavi., 2010).

1.4 Research question

What is the quality of ^{99m}Tc eluate being used at the University Teaching Hospital in Zambia?

1.5 General objective

To determine the quality of ^{99m}Tc eluate obtained from ^{99}Mo/^{99m}Tc generators.

1.6 Specific Objectives

1.6.1 To determine the radionuclidic impurity in the eluate

1.6.2 To define the radiochemical impurity in the eluate.

1.6.3 To determine the chemical and physical characteristics of the eluate.

1.6.4 To measure the pH range of the eluate.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview

The main purpose for this study was to determine contaminants in ^{99m}Tc eluate obtained from ^{99}Mo/^{99m}Tc generators. The findings from literature review showed that literature on the quality of ^{99m}Tc eluate locally or regionally was not available at the time of our search as most researches that was so far come across were from developed countries whose institutions and human capital are well established as elaborated below. It was again found that there was not much literature available on our topic at the time of our search.

2.2 Determination of ^{99}Mo Contamination in a Nuclear Medicine Patient

In 2005, researchers in Brazil conducted a study to determine ^{99}Mo contamination in a nuclear medicine patient submitted to a diagnostic procedure with ^{99m}Tc. They acknowledged that ^{99m}Tc is a radionuclide which is widely used for diagnostic imaging in nuclear medicine. Elutions for ^{99m}Tc from ^{99}Mo/^{99m}Tc generators were supplied by the Nuclear energy Research institute (IPEN). This process of elution was usually carried out in nuclear pharmacy laboratory located in hospitals and clinics. Depending on the quality of the generator and manner of processing during the elusion process, ^{99}Mo can be extracted from the column of the generator, becoming a radionuclidic impurity of the ^{99m}Tc eluate. ^{99}Mo emits high-energy photons, beta particles and its presence degrades the quality of the image and increases the radiation dose delivered to the patient. An *in-vivo* measurement technique was developed to verify the accuracy of internal contamination by ^{99}Mo in nuclear medicine patients. Direct measurements were made in a volunteer who underwent myocardial scintigraphy with ^{99m}Tc sestamibi. The results indicated the presence of internal contamination of the patient with ^{99}Mo. The activity was tracked for several days, and an assessment of the radiation dose from the contamination with ^{99}Mo was made and one of its effects was poor images which led to repeats.

2.3 Determination of ^{99}Mo Contamination in ^{99m}Tc Elute obtained from ^{99m}Tc/^{99}Mo Generator

In 2010 Momennezhad and others carried out a research in Iran in order to determine ^{99}Mo contamination in ^{99m}Tc elute obtained from six ^{99}Mo/^{99m}Tc generators over a period of six months. Generators in Iran were supplied by the Iran Atomic Energy Agency and by private companies from foreign countries. They measured ^{99}Mo contamination in ^{99m}Tc eluate from different generators in a period of one year. The radionuclide impurity of the ^{99m}Tc elute were studied on two types of radionuclide generators that is those produced in Iran and those that were imported from other countries. *In-vitro* measurements were performed using dose calibrator and direct measurements were made, using a standard canister at the time of milking of the generators and also in subsequent hours of milking. A mean of ^{99m}Tc impurity was found in generators A and B to be 0.00932 ± 0.0043 and 0.0170 ± 0.0127. The results showed that the ^{99}Mo contamination in ^{99m}Tc was lesser than the maximum accepted activity remit of 0.015. They concluded that the difference in ^{99}Mo levels in generators A and B may had reflected the different methods of production of generators, as well as the quality control procedures during compounding. The authors concluded that the mean of ^{99}Mo contamination in generators produced in Iran Atomic Energy Organisation was lesser than generators imported from foreign origin.

2.4 Quality Control of ^{99}Mo/^{99m}Tc Generators

In a study that was done in Germany by Hammermaier and others in 1985 on quality control of ^{99}Mo/^{99m}Tc generators, seven different ^{99}Mo/^{99m}Tc generators all loaded with fission molybdenum were sampled over a period of seven months. The main objective of their study was to test the ^{99m}Tc eluates for their radionuclidic and radiochemical purity (determining elution efficiency), measuring the pH value and tests to detect soluble aluminium in the eluates were also performed. Gamma spectrometry and thin layer chromatography were used to analyse the ^{99m}Tc eluate. The results were significant as all the eluates showed high and sufficient radionuclidic purity. The elution yields were between 85 and 122% meeting the required standards. All eluates had pH values between 5.0 and 6.5 (normal range is 4 – 8), and aluminium content below 1microgram/ml. The generators were therefore found to have good performance and generally proved to be a good source of ^{99m}Tc pertechnetate.

2.5 Radionuclidic Purity of ^{99m}Tc eluate for use in Nuclear Medicine

A Croatian researcher studied radionuclidic purity of ^{99m}Tc eluate for use in nuclear medicine. The available ^{99}Mo/^{99m}Tc based fission generators were studied using gamma spectrometry. The main radio contaminant that was being looked at was the presence of ^{99}Mo in the eluates. In all eluates, three long living gamma emitters ^{103}Rubidium, ^{106}Rubidium and ^{131}Iodine were found. These radioisotopes were fission products. They were formed from the fission of ^{235}Uranium to ^{99}Mo during the separation and purification process. It was however found that the ^{99}Mo was within the accepted level therefore, the ^{99m}Tc eluates fulfilled the criteria for nuclear medical applications (Vucina 1989).

2.6 Minimizing ^{99}Mo contamination in ^{99m}Tc pertechnetate from the elution of ^{99}Mo/^{99m}Tc generators

The Malaysian study by Ibrahim et al looked at reduction of ^{99}Mo content in the ^{99m}Tc eluate. The authors devised a new way of producing ^{99}Mo generator as a dry type alumina chromatographic column generator other than the commonest way of producing ^{99}Mo generators using the fission method. It was discovered that this method significantly reduced the ^{99}Mo contamination. The study was conducted on five generators only.

2.7 Influence of the Generator in-Growth Time on the Final Radiochemical Purity and Stability of Radiopharmaceuticals

At Legnaro laboratories of the Italian National Institute for Nuclear Physics, a feasibility study which started in 2011 on impact of different/isomeric ratios on the preparation of different ^{99m}Tc-labelled pharmaceutical kits revealed that both the ratio and the specific activity were basically different in the final accelerator-produced ^{99m}Tc generators. The aim of this study was to evaluate the possible impact on the preparation of different ^{99m}Tc-labelled pharmaceutical kits and the radiochemical purity of the ^{99m}Tc eluate. A set of measurements of the ^{99m}Tc eluate eluted from a standard ^{99}Mo/^{99m}Tc ^{99} generators, was done, and results showed that both the radiochemical purity and stability of these radiopharmaceuticals were not affected and conformed to the standards (ucceli and Bosch., 2013).

CHAPTER THREE

METHODOLOGY

3.1 Overview

The previous chapter highlighted a number of studies that were done by a number of people mainly from Europe in line with the research topic. In this chapter, the following the following will be discussed; research design, research site, study population, sampling procedure and sample size, methods and materials, data collection tools and techniques, data analysis and ethical considerations.

3.2 Study Design

It was a laboratory based cross-sectional study.

3.3 Study site

There were two study sites namely the hot lab located within the Nuclear Medicine Unit at the University Teaching Hospital and the National Institute for Scientific and Industrial Research(NISIR). NISIR was one of the sites because it has some equipment that was supposed to be used during the research which UTHs does not have. NISIR is 7km approximately off Great East Road airport round about.

3.3.1 Hot Lab (I)

The research was done within the University Teaching Hospital as this is the only tertiary referral hospital which provides Nuclear Medicine services for all the people in Zambia. At this lab, the following tests were done; to determine the radiochemical purity, chemical purity and to check for physical characteristics of the eluate.

3.3.2 NISIR (II)

The research was also conducted at National Institute for Scientific and Industrial Research (NISIR). The following test was done at NISIR, which was to determine the pH range of the eluate since the hot laboratory at UTH does not have pH meter or Paper. NISIR was chosen

because it has a specific laboratory that handles radioactive substances and has the equipment that was needed for the research which UTH does not have.

3.4 Study Population

The study population included all the $^{99}Mo/^{99m}Tc$ generators that were received at UTH during the data collection period of the research which was between September 2016 and February 2017.

3.5 Sample size Justification

The consumption rate for $^{99}Mo/^{99m}Tc$ generators in 2015, 2016 and thus far 2017 has been one per month. ^{99m}Tc eluate is a radioisotope meaning it is a radioactive substance to which unnecessary exposure should never be promoted. The study period was from September 2016 to March 2017, hence all the six generators that came during the study period were considered as the study sample.

3.6 Methods and Materials

The method employed was experimental and observational since it involved performing certain procedures in the laboratories and then looking at the characteristics of the ^{99m}Tc eluate and comparing them to the standards in the reference books. The sampled $^{99}Mo/^{99m}Tc$ generators were given code numbers. The $^{99}Mo/^{99m}Tc$ generators were coded generator one to six, then two samples from each $^{99}Mo/^{99m}Tc$ generator were collected twice, 96 hours and 120 hours from the time of manufacture. Orders for $^{99}Mo/^{99m}Tc$ generators are made once a week (every Wednesday by 12:00hours), then the company manufactures the $^{99}Mo/^{99m}Tc$ generators once every week (every Thursday) and are dispatched to the buyers every Friday. As an institution, we have no control on the UTH agents who clears UTH items at the airport and so the $^{99}Mo/^{99m}Tc$ generators are only cleared on Monday and delivered on the same day. Since the $^{99}Mo/^{99m}Tc$ generators contains radioactive substances that are in constant reaction, the time for the first and second elution from when the samples A and B were collected were recorded from as 96 and 120 hours respectively.

3.6.1 Radionuclidic Test

Radionuclidic purity is defined as the ratio of the stated radionuclide activity to the total radioactivity normally given as a percentage (Kowalsky, 2004 and Welch 1977). The materials used included ^{99}mTc eluate, a dose calibrator (brand name is capintec found at 7 Vreeland, Florham park, New Jersey 07932 USA). The process involved a step by step measure of ^{99}Mo impurity, the procedure is part of an in built mechanism in the dose calibrator (Capintec-CRC®-15Rradioisotope dose Calibrator Owner's manual chapter 8 pages 4 – 8). However, other impurities that are gamma emitters are not detected on a dose calibrator hence the reason to use gamma spectrometer at NISIR although unfortunately, this could not be done due to lack of nitrogen liquid at NISIR.

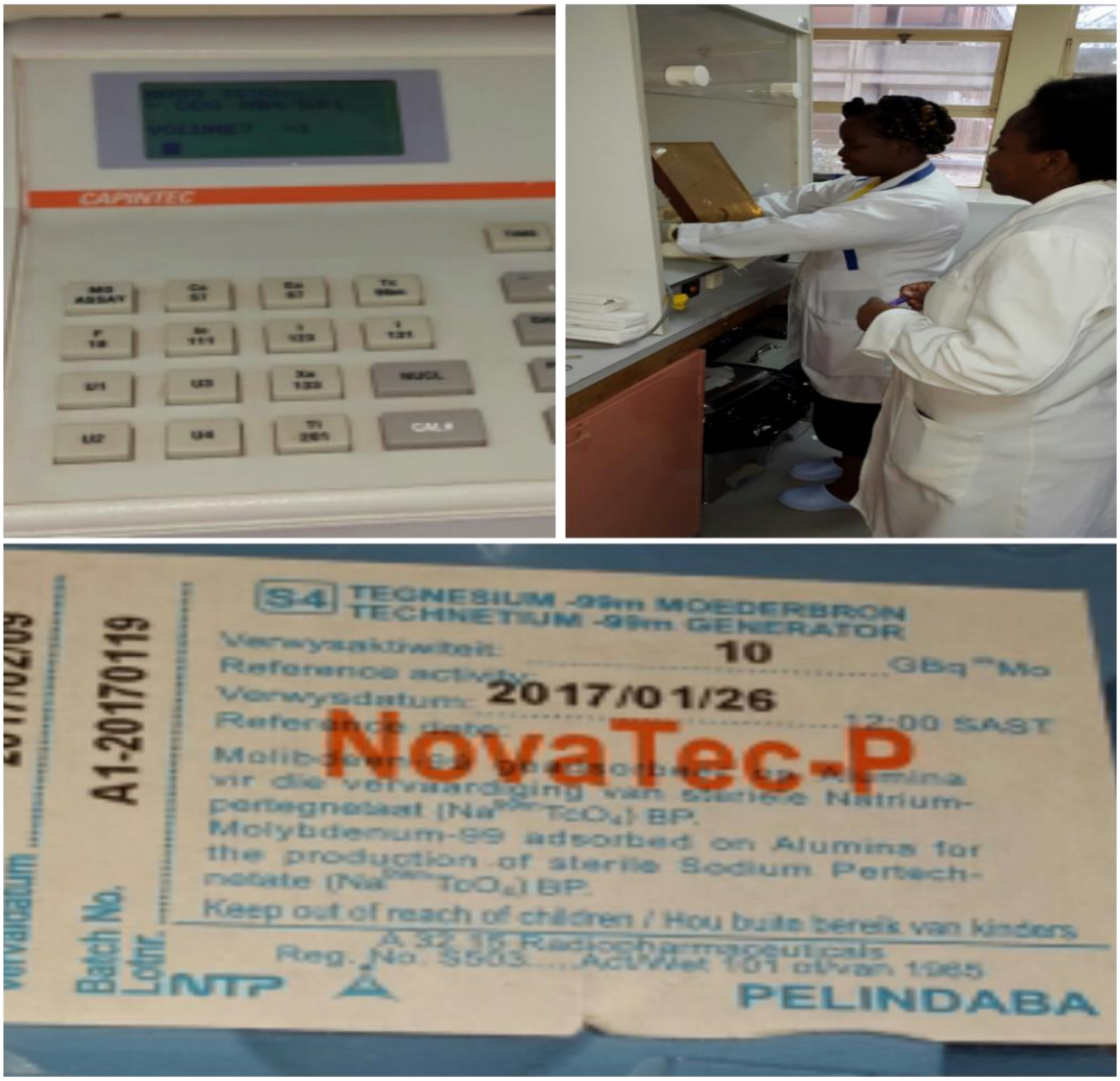

Figure 5: Student performing radionuclidic purity test under observation as explained above

3.6.2 Radio Chemical Test

Radio chemical purity is the proportion of the total radioactivity existing in the desired chemical form in a radioactive pharmaceutical (Mahoney 1998). The expected impurities with regards to ^{99m}Tc eluate are free technetium ^{99m}Tc pertechnetate, hydrolysed-reduced ^{99m}Tc (which is insoluble) and bound ^{99m}Tc. These impurities were determined using thin layer chromatography which consisted of a stationary phase and a mobile (solvent) phase. The materials required included ^{99m}Tc eluate and whatman 31ET paper for chromatography, acetone solution as a solvent and a dose calibrator. The whatman 31ET comes as an A4 sheet of paper. The strips were cut in sizes of 0.9 cm x 8.5 cm with the origin at 1 cm from the bottom and solvent front is 6.5 cm from the origin.

A drop (0.05ml) was applied to the strip of whatman 31ET paper at the origin and placed in a vial containing acetone as the solvent. The components of a mixture exhibited different capabilities of solubility depending on their chemical properties. Each radiochemical species travelled a characteristic distance and this helps to calculate the relative front value. Then strips were cut according to the reference value and used to determine the number of counts on a dose calibrator, then calculations were performed to determine percentage bound and percentage of radiochemical impurities. This procedure was done according to the instruction by the manufacturer's leaflet of Whatman 31ET paper (found at Biodex Medical Systems; 20 Ramsey road Shirley New York, USA) and yield reference was compared to the standards from the British Pharmacopeia which should be should be between 80% - 100% (BP 2016). These methods are usually validated alternative cheaper methods.

$$\text{Reference Value} = \frac{\text{distance travelled on medium by a radiopharmaceutical}}{\text{Distance traversed by the mobile phase}}$$

$$\%^{99m}\text{Tc} = \frac{\text{number of counts from lower cut strip}}{\text{X 100}}$$

Figure 4: Student performing the radiochemical purity test as explained above

3.6.3 Chemical Impurity

Chemical Purity is the proportion of total mass present in the stated chemical form (Simon *et al*, 2012 and Thomson 2002). This refers to the amount of undesirable chemical species (which are non-radioactive) present in the radiopharmaceutical. In elution of ^{99m}Tc, aluminium is always the possible chemical contaminant since the parent radionuclide ^{99}Mo is embedded onto aluminium column hence may at times be milked into the eluate in more the than the limited quantities. The materials that were required included ^{99m}Tc eluate from the sampled ^{99}Mo/^{99m}Tc generators, ammonium salt of aurin tricarboxylic acid indicator and the test The procedure to detect the presence of aluminium was done by applying a drop of ^{99m}Tc eluate and a drop of a standard solution containing approximately 10mcg/ml of aluminium to a calorimeter test paper impregnated with ammonium salt of aurin tricarboxylic acid indicator. The two spots were compared in pink colour formation (aluminium forms a light pink colour when it reacts with the mentioned acid indicator). The intensity of the pink colour is proportionate to the amount of the aluminium present. This procedure was done according to the leaflet instruction by the manufacturer (Biodex Medical Systems found at 20 Ramsey road Shirley New York, USA) of the aluminium kit. This method is validated alternative cheaper method of checking for aluminium content. Other more expensive sophisticated methods exists.

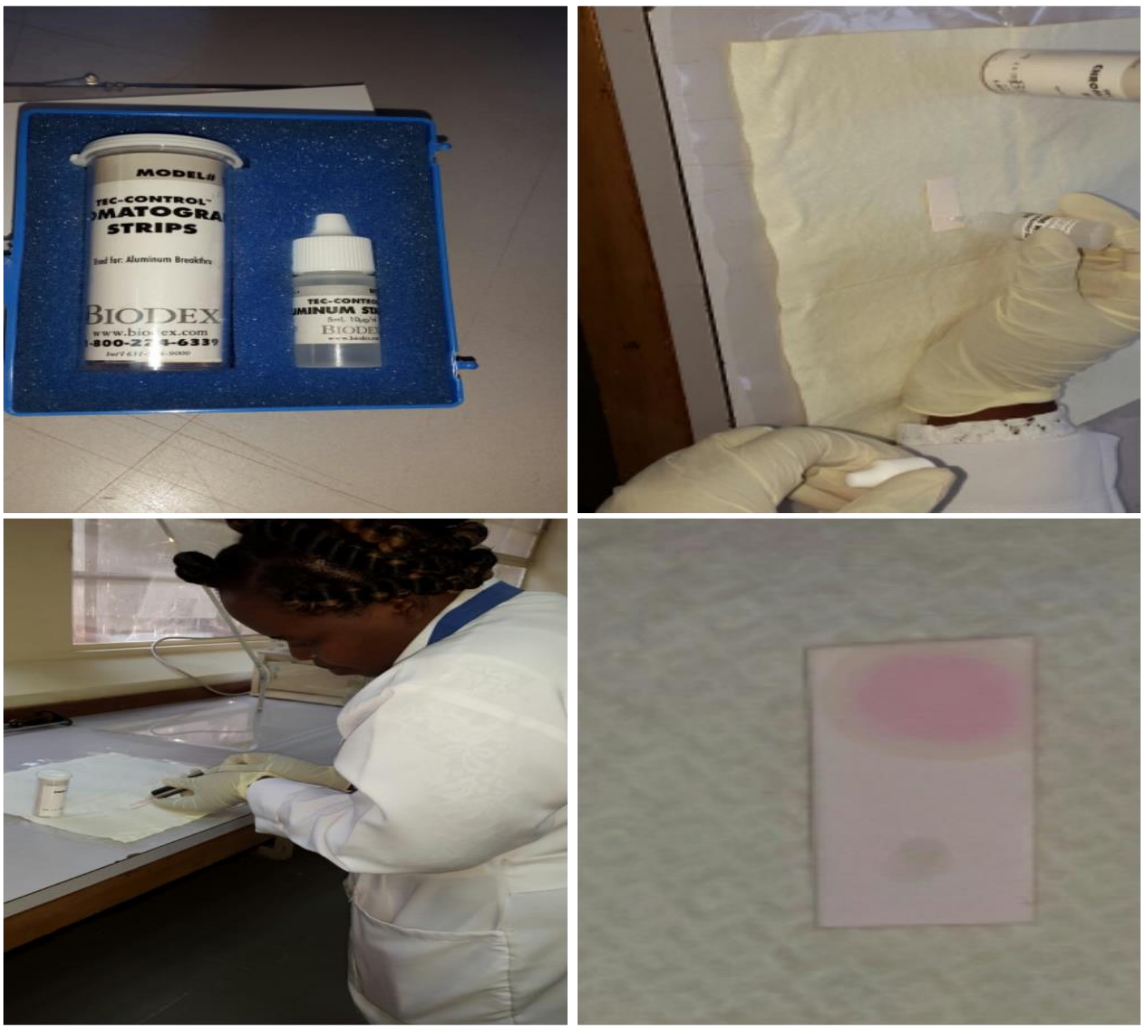

Figure 6: Student performing chemical purity test as explained above

3.6.4 Physical Check

A radiopharmaceutical's physical appearance is important. A true solution of a ^{99m}Tc eluate should be clear, colourless with no particulate matter. The materials involved were ^{99m}Tc eluate and a clear vial. The physical check involved visual inspection of the colour of the ^{99m}Tc eluate using the naked eyes against a white background by the student with verification from the supervisor.

3.6.5 pH of Eluate

In order to ensure stability and integrity, radiopharmaceuticals should be formulated at an appropriate pH. Ideally, the pH of a parenteral radiopharmaceutical should be that of the blood (7.4), however due to high buffer capacity of the blood it is acceptable for the pH to range between; 4 to 8 (BP 2016). The materials required were; a pH meter (manufacturers are found at 197 Fabrick, St Strildom Park, Johannesburg-South Africa), standard buffers and ^{99m}Tc eluate. The pH meter has a probe is the sensitive part which is dipped in the solution to be measured and the reading reflects on the screen, it is always kept in a holding solution (buffer solution). Rinsing the probe in between use of different solutions is very cardinal. The pH meter probe was thoroughly cleansed and placed in a vials containing ^{99m}Tc eluate, then the readings were observed and recorded.

Figure 7: Student conducting a pH check

3.7 Data collection tools and techniques

The qualified nuclear pharmacist at UTH hot laboratory was observing and helping in performing various tests using the guide from standard reference books. Similarly, the senior chemist at NISIR laboratory was observing and helping in performing various tests that were done at NISIR. Checklist and tables were used to collect raw data. The data that was collected was primary data in nature and collection was done by observing the test results according to the specific objectives. Secondary data was also used from official reference materials for comparison purposes with the analysed data from the samples.

3.8 Data Analysis

Baseline information of the generators were checked and compared with the standards. To compare same generator at 96 and 120 hours, Paired T-test was used after checking whether the data was normally distributed or not using D'Agostino-Pearson omnibus test. This method was used on three specific objectives namely; to determine the radionuclidic impurity in the eluate, to determine the radiochemical (i.e. unwanted salts of pertechnetate) impurity in the eluate and to determine the pH range of the eluate. Statistical Product for Social Solutions was used to analyse the physical and chemical tests of the eluate, specifically frequencies via descriptive statistics was used. All statistical analyses were done using GRAPHPAD Prism version 6.01 (Graph Pad Software Inc., La Jolla, CA, USA). For all statistical tests a p value of <0.05 was considered statistically significant.

3.9 Ethical Considerations

Permission to conduct the research was sought from the University of Zambia Biomedical Research Ethics Committee (UNZA-BREC), UTH management and NISIR. This Research involved no human subjects as it was purely done in the laboratories, hence it had very minimal risks. Caution was made by wearing protective clothing to minimize radiations. Standards operating procedures were followed to ensure safety of environment. The actual Company name was concealed (Labelled as Company X) to ensure confidentiality.

CHAPTER FOUR

PRESENTATION OF RESULTS

4.1 Overview

In this chapter, the results have been presented in form of figures and tables starting from the background information of the ^{99m}Tc Eluate and including all the test results that were carried out.

Table 4.2: Background Information of the ^{99}Mo/^{99m}Tc Generators

Table 4.2 below showing the background information of the six generators that were collected and analysed between January 2017 and March 2017. The six generators were sourced from the same company and were manufactured between August 2016 and February 2017. Packaging and appearance were intact on delivery and the batch numbers were as indicated in Table 4.2 for each generator. The instructions on the labels were clear and easy to understand. The generator sizes were of short expiry and were either 10 or 18 GBq, the size ordered was dependent on the number of patients booked.

Generator Code No.	Supplied By	Name of Product	Man. Date	Batch No.	Generator	Exp. Date
1	Company X	TECHNETIUM-99m GENERATOR (NOVOTECH)	26/08/16	A2 - 20160306	18GBq	2016/09/27
2	Company X	TECHNETIUM-99m GENERATOR (NOVOTECH)	07/10/16	A4 - 20161006	10GBq	2016/10/27
3	Company X	TECHNETIUM-99m GENERATOR (NOVOTECH)	18/11/16	A4 - 20161117	10GBq	2016/12/08
4	Company X	TECHNETIUM-99m GENERATOR (NOVOTECH)	16/12/16	A2- 20161214	18GBq	2017/01/05
5	Company X	TECHNETIUM-99m GENERATOR (NOVOTECH)	20/01/17	A1- 20170119	10GBq	2017/02/09
6	Company X	TECHNETIUM-99m GENERATOR (NOVOTECH)	03/02/17	A2- 20170213	10GBq	22/02/17

Table 4.3: Compiled Report for all Results for ^{99m}Tc Eluate tests

Table below showing all the results for the tests done to assess quality control of the ^{99m}Tc Eluate being used at the University Teaching Hospital

TESTS	SPECIFICATIONS (BP 2016)	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Appearance	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
Chemical purity (Aluminium Content)	DIP PINK=+ve LIGHT PINK/COLOURLESS=-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
pH of the Eluate	4 – 8	5.96	5.98	6.03	6.07	6.11	6.13	5.92	5.93	5.76	5.79	5.81	5.83
Radiochemical purity	➢ <95%	99.5	99.7	99.4	99.6	99.7	99.8	99.5	99.7	99.6	99.8	99.8	99.5
Radionuclidic purity- MBT	< 0.15mCi	0.003	0.000	0.001	0.003	0.005	0.006	0.005	0.042	0.002	0.003	0.003	0.000

Table 4.4: Physical and Chemical Characteristics of ^{99m}Tc Eluate

Table below showing the physical and chemical characteristics of generators from which the samples were collected. The letters A and B represent samples collected from the eluates of generators 120 hours from time of manufacture. British Pharmacopeia (BP) standard specification requires that physical tests of ^{99m}Tc should be colourless and particle free while the chemical test of ^{99m}Tc in terms of aluminium content test should be light pink or colourless denoted by the negative sign (-ve). The test results in the Table 4.2 show that both the physical and chemical characteristics of ^{99m}Tc elutes were within the recommended BP standard reference specification because upon testing the samples were found to be particle free and colourless.

Generator (99-mTc) Sample		**Reference (Specifications-BP 2016) & Test Results**	
		Physical (Appearance)	**Chemical purity (Aluminium Content)**
		Clear and colourless	Dip Pink = +Ve or Light Pink/Colourless = -Ve
1	**A**	Clear and colourless	-ve
	B	Clear and colourless	-ve
2	**A**	Clear and colourless	-ve
	B	Clear and colourless	-ve
3	**A**	Clear and colourless	-ve
	B	Clear and colourless	-ve
4	**A**	Clear and colourless	-ve
	B	Clear and colourless	-ve
5	**A**	Clear and colourless	-ve
	B	Clear and colourless	-ve
6	**A**	Clear and colourless	-ve
	B	Clear and colourless	-ve

TABLE 4.5: SHOWING ANALYSED CHEMICAL TEST RESULTS

As seen in table 4.5 below, the frequency (12) is standing for the samples from the 6 generators that were collected twice (at 96 and 120hours from time of manufacture). All the 12 samples were negative meaning they did not contain aluminium hence yielded a 100% cumulative and valid percentage.

Chemical Test

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid Negative	12	100.0	100.0	100.0

TABLE 4.6: SHOWING ANALYSED PHYSICAL TEST RESULTS

Similarly, as can be seen in table 4.5 below, the frequency number (12) is standing for the samples from the 6 generators that were collected twice (at 96 and 120hours from time of manufacture). All the 12 samples were clear and colourless meaning they complied with the standards and hence yielded a 100% cumulative and valid percentage.

Physical Test

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid clear and colourless		12	100.0	100.0	100.0

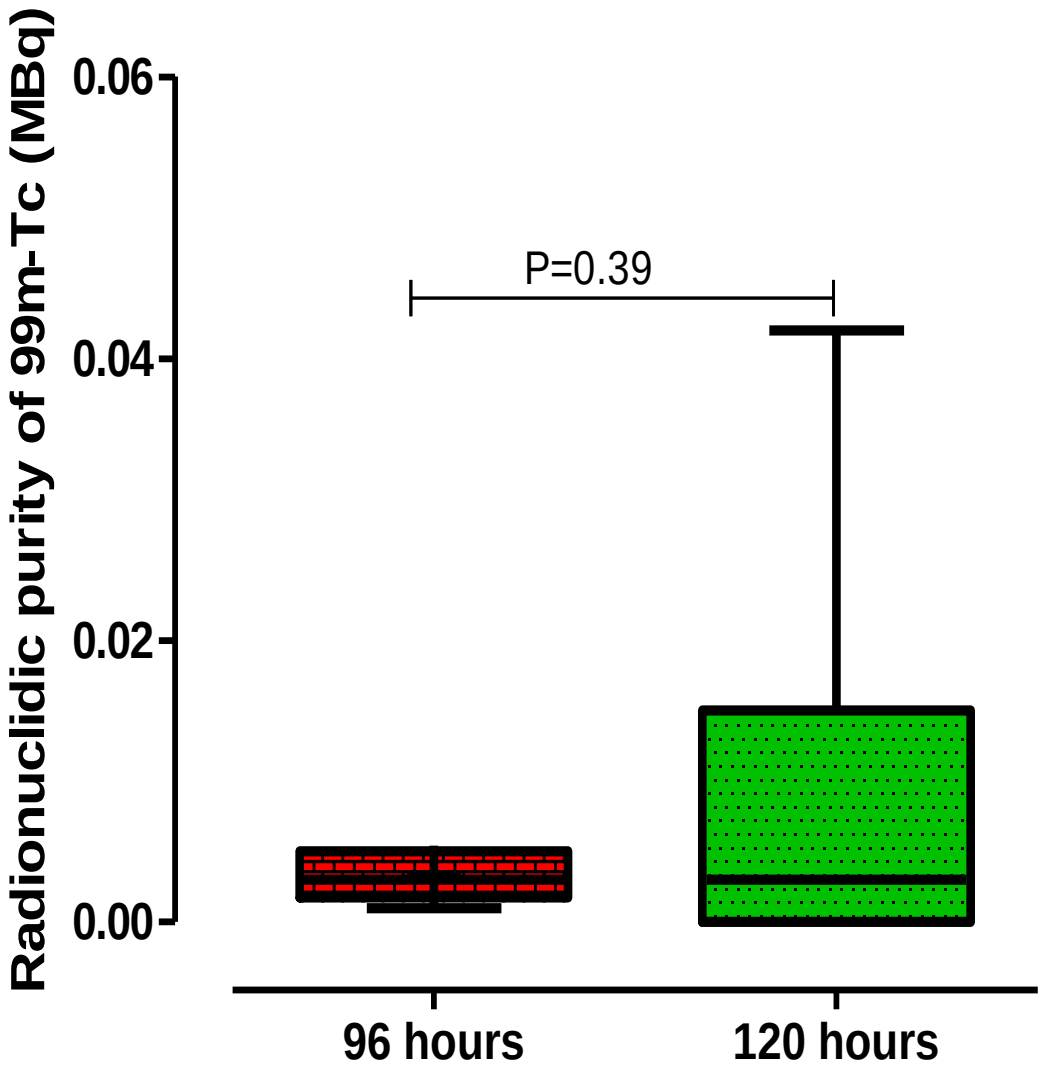

Figure 8: Levels Of Molybedenum In ^{99m}Tc Eluate. Minimum level should be 0.55MBq

The figure 4.1 above is showing ^{99}Mo levels of samples for ^{99m}Tc eluate that were collected at 96 hours from the time of manufacture from the six generators were compared to ^{99}Mo levels of samples for ^{99m}Tc eluate that was collected 120 hours from the time of manufacture from the six generators. The median for radionuclidic purity (^{99}Mo content) at 96 hours was 0.003 with interquartile ranges of 0.002 to 0.005 and 0.003 at 120 hours with interquartile ranges of 0.00 to 0.015; p value=0.39. The medians were comparable p = 0.39. There was 100% conformity to the standards for physical and chemical characteristics for all samples. The samples were within the BP standard reference range of 0.015 µCi.

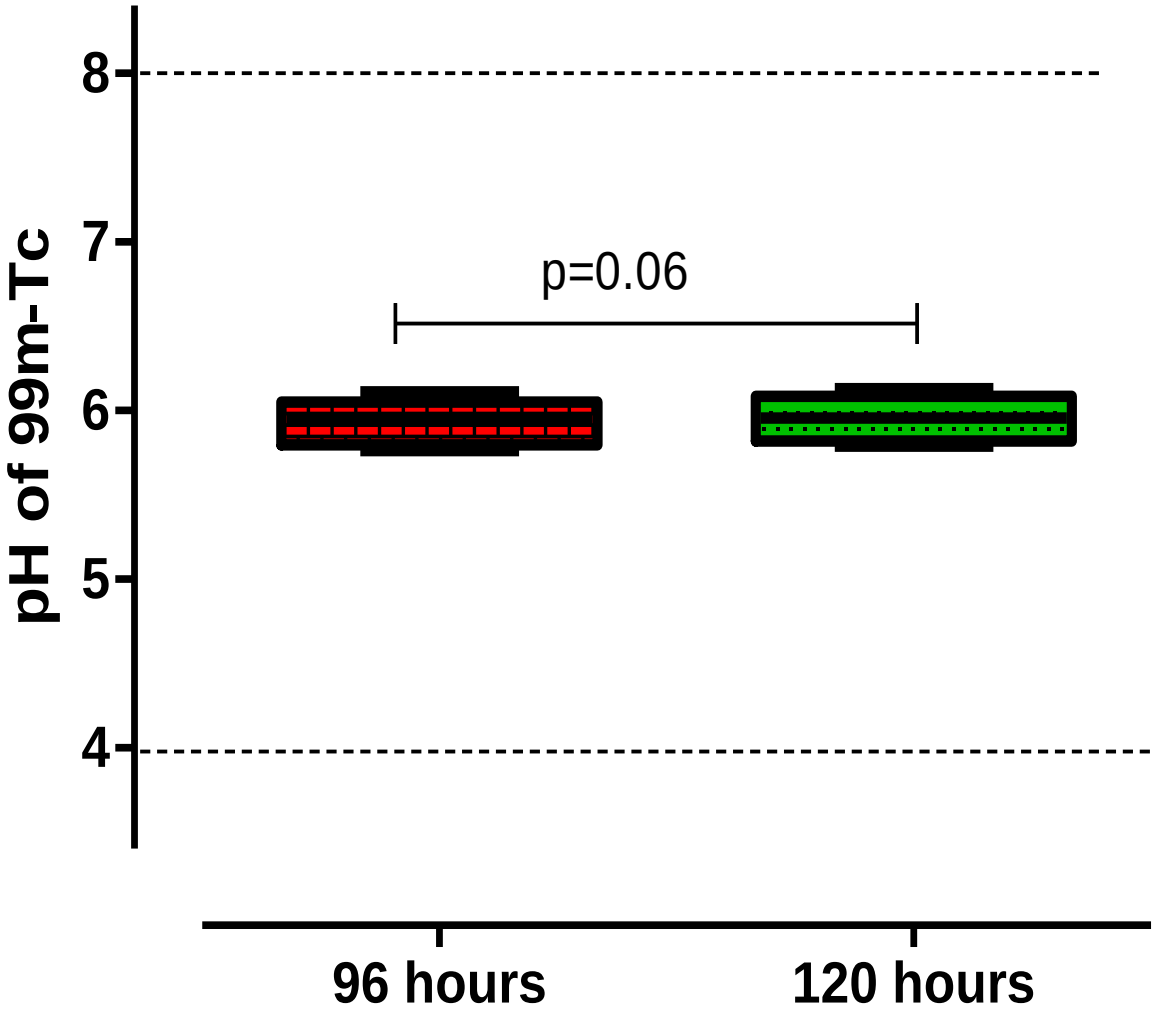

Figure 9: Showing pH Levels Of ^{99m}Tc Eluate. Normal range is 4 to 8 as indicated by the dotted lines

The figure 4.2 above is showing pH of ^{99m}Tc eluate. The pH of samples for ^{99m}Tc eluate that was collected at 96 hours from the time of manufacture from the six generators was compared to the pH of the samples for ^{99m}Tc eluate that was collected 120 hours from the time of manufacture from the six generators. The pH at 96 hours and 120 hours from the time of manufacture were comparable; The median for pH at 96 hours was 5.94 and the interquartile was 5.79 to 6.05 and 5.96 at 120 hours with interquartile range of 5.82 to 6.09; p = 0.06. All the samples were within the British Pharmacopeia (BP) standard reference range (pH 4 to 8).

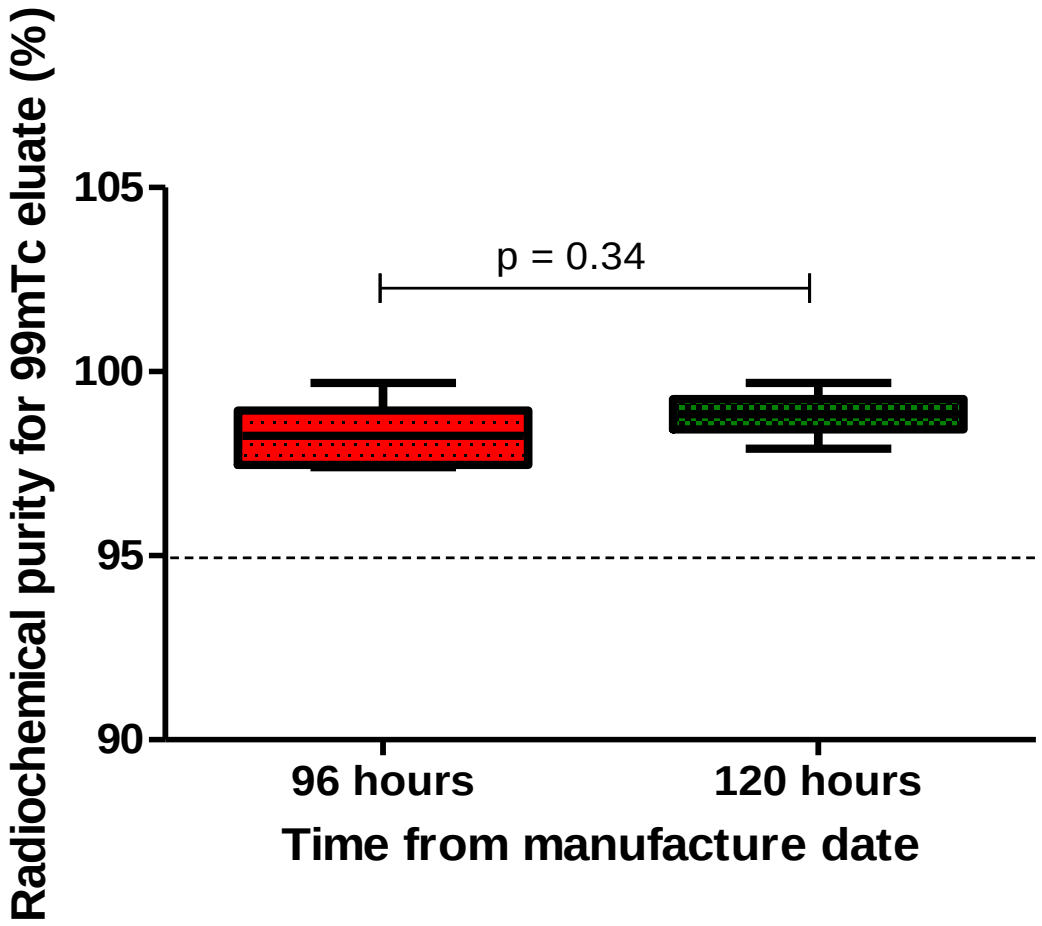

Figure 10: Levels of ^{99m}Tc pertechnetate in ^{99m}Tc Eluate. Elution yields should be above 95%.

Figure 4.3 above is showing levels of ^{99m}Tc pertechnetate. The box whisker at 96 hours represents ^{99m}Tc pertechnetate levels of samples for ^{99m}Tc eluate that was collected at 96 hours from the time of manufacture from the six generators while the box whisker at 120 hours represents ^{99m}Tc metastable pertechnetate levels the samples for ^{99m}Tc eluate that was collected 120 hours from the time of manufacture from the six generators. Unpaired t-tests was used to assess differences between the two groups. The ^{99m}Tc pertechnetate at 96 hours and 120 hours from the time of manufacture were comparable; The median for radiochemical test (elution yield) at 96 hours was 98.25% and the interquartile was 97.48% to 98.95; and 98.85% at 120 hours with interquartile range of 98.43% to 99.25%; p = 0.06. The samples were above the minimum requirement (95%) according to the BP standard reference range hence proved to of good quality.

CHAPTER FIVE

DISCUSSIONS

5.1 Overview

In the pharmaceutical manufacturing industry, inclusive of radiopharmaceutical manufacturing, strict observance to GMP principles is essential to guarantee that any medicine made are safe and effective (Gad, 2008; IAEA Doc 649 1992 & IAEA hospital guidelines 2008). Quality control in nuclear medicine is one of the areas that should be practised to ensure that the radiopharmaceuticals being given to patients is efficacious to produce intended results which are quality medical images.

5.2 Radionuclidic Purity

In our study the mean levels for ^{99}Mo content at 96 and 120 hours was 0.00317 +/- 0.0016 and 0.00918 +/- 0.0163. This was in conformity with the requirements of the BP standards which states that the level of ^{99}Mo should be above 0.015% hence the radionuclidic purity gave satisfying results. This was similar to the figures seen in the study done by Momennezhad and others which were 0.00932 +/-0.0043 and 0.017 +/- 0.0127 for generators 1and 2; and Ibrahim and others whose ^{99}Molevels ranged from 0.0038 +/- 0.0126 and 0.00913 +/- 0.0123 respectively. Although Vucina's study in Croatia recorded normal ^{99}Mo levels, other long gamma emitters were discovered in the ^{99}mTc Eluate.

5.3 Radiochemical Purity

^{99m}Tc eluate is acquired by elution of a ^{99}Mo/^{99m}Tc generator in Nuclear Medicine. ^{99}Mo whose half-life is 66 hours deteriorates by beta rays to 87% ^{99m}Tc and ^{99}Tc 13%. Radiochemical purity is then determined by determining the % yield of the required salt which is the sodium pertechnetate ^{99m}Tc. In this study, the radiochemical purity was found to range from 99.5% to 99.7% therefore it conformed to the requirements of the British Pharmacopeia and hence proved to be a good source of sodium pertechnetate ^{99m}Tc. This % yield was much more compared to that realised by Ibrahim et al which was only above 80%.

5.4 pH Measurements

It was eminent in the study done by Reich and others that the pH range of the eluates were between 5 and 6.5 while our study revealed similar pH levels of 5.8 to 6.1. Although they were in conformity with the requirements in the BP standard, distance from UTH to NISIR might have played a role regarding the amount present at the time of measurement. Every second that passes for a radioisotope contributes to its decay and loss of hydrogen ions which eventually affects pH readings. It took 45 minutes to reach NISIR for pH measure.

5.5 Chemical Purity

There are different ways of measuring the presence of aluminium in the ^{99m}Tc eluate. In our study, the presence of aluminium was measured in a different way compared to how Hammermaier and others measured it where all the samples had the aluminium content which were below the minimum 1mcg/ml and Ibrahim and others presented it as less than 5parts per million (<5ppm). In our case, we used the aluminium kit which was done by smearing a drop of ^{99m}Tc eluate and a drop of a standard solution containing approximately10mcg/ml of aluminium onto a calorimeter test paper impregnated with ammonium salt of aurin tricarboxylic acid indicator. The two spots were then compared in terms of the intensity of the pink colour (aluminium forms a light pink colour when it reacts with the ammonium salt of aurin tricarboxylic acid in the indicator). The intensity of the pink colour is proportionate to the amount of the aluminium present (negative=light pink/clear, positive=deep pink). All the samples indicated a clear colour meaning no trace of aluminium was present).

5.6 Physical Test

Regarding physical studies, our findings indicated that all the samples complied with the expected BP standards. These results were similar to the findings from the study by Ibrahim and others.

5.7 Challenges

The distance from UTH to NISIR is quiet long (about 45 minutes' drive), any minute for radioisotopes counts in terms of degradation of the isotope. Isotope degradation promotes loss of hydrogen ions and hence capable of affecting the pH of the sodium pertechnetate ^{99m}Tc. Despite this limitation, the pH levels were however within the expected range according to the British Pharmacopeia.

5.8 Limitations

Apart from checking for ^{99}Mo, our study intended to check for long gamma emitters like Tc, Rb, I, Sn and In from sodium pertechnetate ^{99m}Tc eluate using the gamma spectrometer at NISIR. Unfortunately, the spectrometer was not functional at NISIR during the time of study hence that could not be done.

5.9 Conclusion

There were no radionuclidic impurities, there were no radiochemical impurities, and there was no chemical impurity. The chemical and physical characteristics were according to the expected standards. Therefore the quality of ^{99m}Tc eluate being used at the University Teaching Hospital in Lusaka Zambia seems to be of acceptable quality.

5.10 Recommendations

1. All generators should be consistently checked for quality even though no impurities were found since other studies have reported presence of impurities.
2. Capacity to do quality control on labelled pharmaceutical should be built to check for other possible impurities since quality control is currently possible on the primary eluate only.

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Appendix I: Checklist

Background

Source of Generator (Company)	
Generator Batch Number	
Expiry Date	
Generator Code number	

Physical Studies

Presence of Particles	
Colour	
Labelling	

Chemical Studies

Radiochemical Purity	
Radionuclidic purity	
Chemical Purity	
pH of Eluate	

Appendix II: Table Showing Compiled Results for All the Tests

No	Generator	Elution Time (hrs)	Tc (mCi)	Mo (µCi)	Rb	I	Sn	In	Al	pH (normal range :4-8)	Std for Mo
1	A	0									0.15
1	B	6									0.15
2	A	0									0.15
2	B	6									0.15
3	A	0									0.15
3	B	6									0.15
4	A	0									0.15
4	B	6									0.15
5	A	0									0.15
5	B	6									0.15
6	A	0									0.15
6	B	6									0.15