Chapter One
Introduction

1.1 About nuclear medicine.

Nuclear medicine is defined as: [the branch of medicine that uses radiation and nuclear properties of radionuclides to provide diagnostic information about the functioning of a person's specific organs (diagnosis) and to treat diseased organs or tumors (therapy). It occupies a unique position in the allied health sciences because of its strong dependence on quantitative or mathematical results (Sam A. K, 2008).

The science and clinical practice of nuclear medicine involve the administration of trace amounts of compound labeled with radioactivity (radionuclides)that are used to provide diagnostic information in a wide range of disease states (Simon R. Cherry et al , 2003).

The first nuclear medicine used on a human was in 1956. In the future, nuclear medicine may be known as molecular medicine. As our understanding of biological processes in the cells of living organisms expands, specific problems can be developed to allow visualization, characterization and quantification of biological processes at the cellular and sub-cellular levels .Nuclear medicine is an ideal specialty to adapt to the new discipline of molecular medicine, because of its emphasis on function, and its utilization of imaging agents that are specific for a particular disease process (Kieran Maher et al, 2006).

1.2 The Radiation and Isotopes Center of Khartoum.

Till recently, RICK has been the only center specialized in nuclear medicine in Sudan. It has two departments: one is a radiotherapy department, and the other is a nuclear medicine department for in -vivo and in- vitro studies, using radionuclides.

The idea of establishing this center was initiated by the undersecretary of the Ministry of Health in Sudan, who wrote an application to the WHO calling for assistance. The experts commission sent by the WHO in 1958 to assess the situation recommended the establishment of the center (Abdul Gadir E.M. 2005).

In cooperation with the IAEA, the government of Sudan established the center in 1962.The Ministry of Health bore the task of training the workers. Work was started in 1966, two years before the official inauguration in December 1968. The nucleus of nuclear medicine section was a small laboratory established at Khartoum Hospital in 1964, the first time that nuclear medicine was introduced in Sudan (Abdul Gadir E. M. 2005).

1.3 Problem of the study

The culture of quality control of radiopharmaceuticals in RICK has never taken root as part and parcel of nuclear medicine practice (Sam A. K, Derar A. A. 2009).Absence of quality control program affects the quality of studies and the radiation protection of the patients, co-patients and the department employees.

1.4 Importance of the study

This study is an attempt to help introduce the culture of quality control of in-house prepared radiopharmaceuticals in RICK, through conducting many Q.C. tests, whose results may constitute a base for future Q.C. procedures not only in RICK but in other nuclear medicine centers in Sudan.

1.5 Objectives of the study

1.5.1 General objective

The general objective of this study is to evaluate the 99mTc- labeled radiopharmaceuticals quality control procedures in the Radiation and Isotope Center of Khartoum.

1.5.2 Specific objectives

- To measure the radiation exposure rates in different areas in the nuclear medicine department of RICK.
- To determine the amount of ^{99}Mo breakthrough in different generator eluates.
- To physically inspect the appearance of $^{99m}TcO_4$ solutions for color, particles and turbidity changes.

- To test $^{99m}TcO_4$ eluates for pyrogenicity via rabbit test.
- To measure the remaining (unused) $^{99m}TcO_4$ activities after different generators elutions.
- To calculate the percentage of unused activities to the total activity of each eluate.
- To calculate the amount and percentage of prepared and unprepared activities of the remaining activities.
- To measure the external radiation exposure emanating from a new generator package.
- To test the generator package surface for radiation contamination (wipe test).
- To measure the radiation doses received by some body organs during elution, preparation and injection of radiopharmaceuticals.
- To measure the radiation dose emanating from the patient during imaging.
- To provide purposely selected information to be utilized for futuristic research and knowledge in nuclear medicine field.

1.6 place and duration of the study.

The study was conducted at the Radiation and Isotope Center of Khartoum during the period 2010 – 2016

1.7 Ethical considerations.

Approval, and all ethical and safety aspects were strictly considered throughout the study conducting. The three rabbits involved in the tests were subject to veterinary test and approval in advance.

1.8 Limitations of the study

In addition to the unavailability of most necessary facilities in RICK, the frequent changes of the ^{99m}Tc generator (three times), forced the researcher to

annul the recorded data many times. That was why the research took longer time than scheduled.

1.9 The study outlines

The study was encompassed in five chapters, as follows:

Chapter one included: an introduction, chapter two encompassed the literature review; chapter three included research materials and methods, chapter four showed the results and chapter five included the discussion, conclusion and recommendations, in addition to the references and appendices.

Chapter Two

Literature Review

2.1Theoretical Background

2.1.1. Nuclear pharmacy

2.1.1.1 Introduction

We generally come across two terms in the literature: nuclear pharmacy and radio pharmacy. There is no difference between the two terms, and they can be used interchangeably. The use of one term or the other is a matter of individual choice (Gopal, B. Saha 1998).

In a nuclear pharmacy radiopharmaceuticals are prepared, stored and dispensed primarily for human use, just as regular drugs are in a pharmacy (Gopal, B. Saha 1998).

Radiopharmacy has become firmly established as a specialist branch of the pharmaceutical service and as an integral part of the multidisciplinary team providing a nuclear medicine service (P.F.Sharp et al. 1989).

The nuclear pharmacy is staffed with trained personnel such as radiopharmacists and radiochemists, that is, chemists or pharmacists with special training in radiopharmaceutical chemistry. In the nuclear radiopharmacy the remedy for any adverse reaction in humans due to the administration of radiopharmaceuticals is sought and found. The nuclear pharmacists can provide education and consultation to the patient and health care personnel in this field (Paul E. Christian et al. 2004).

2.1.1.2 Design of a nuclear pharmacy

Several problems should be kept in mind when designing a nuclear pharmacy unit. Protection of personnel from radiation hazard, avoidance of contamination of work area and radiation- detection instruments, clean air circulation in the dispensing area and disposal of radioactive waste are the most commonly encountered problems. The design should take into account daily operational protocols, proper utilization of available space and provisions for future growth (P.F Sharp et al, 1989). A nuclear pharmacy should be located within or near the nuclear medicine department. The nuclear pharmacy area can be as small as 12x12 ft (4 m x4 m) room, depending on the volume of

the operation. For a larger operation, the unit may consist of several rooms. Ideally, it should have enough space for accommodating offices, accounting room and a health physics laboratory on one side of a corridor, and a hot level laboratory, a compounding room, a store room, and a dispensing area on the other side (see fig. 2.1). The whole area should have minimal access to the public and the patients in order to avoid radiation hazard. Many institutions have a designated area for the storage and disposal of radioactive waste from all departments, and the nuclear pharmacy can share this facility for its own storage and waste disposal (Gopal B. Saha, 1998).

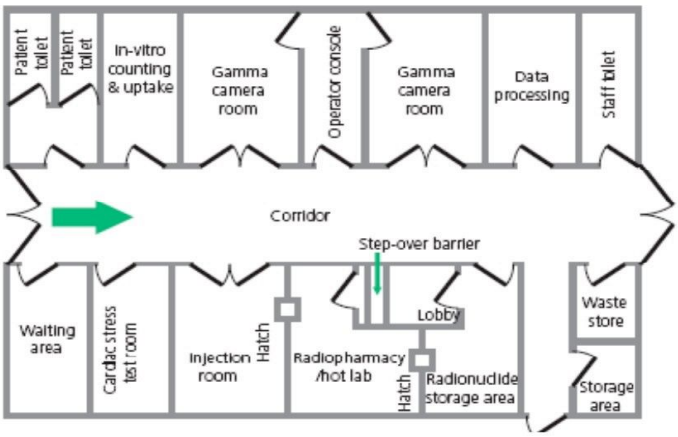


Fig (2.1) A nuclear medicine department design including the location of radio-pharmacy room.

The laboratory area where compounding and dispensing are done should be equipped with work benches made of stainless steel or wood covered with laminated plastic. The floor should be made of removable tiles or should be covered with rubber matting; in the event of spillage the contaminated tiles or rubber matting can be readily replaced with new ones. In each laboratory there should be an appropriate number of stainless steel sinks deep enough to prevent splashing. Each laboratory should be equipped with exhaust fume hoods fitted with filters to absorb gaseous and particulate radioactive materials, particularly radioiodine and radioxenon. A laminar flow hood should be

installed in the dispensing area for a sterile environment. The storage area should be well built with thick concrete walls, and the walls of the storage safes should be lined with lead for radiation shielding. A safety shower should be installed for use in the case of major body contamination.(Gopal B. Saha, 1998).

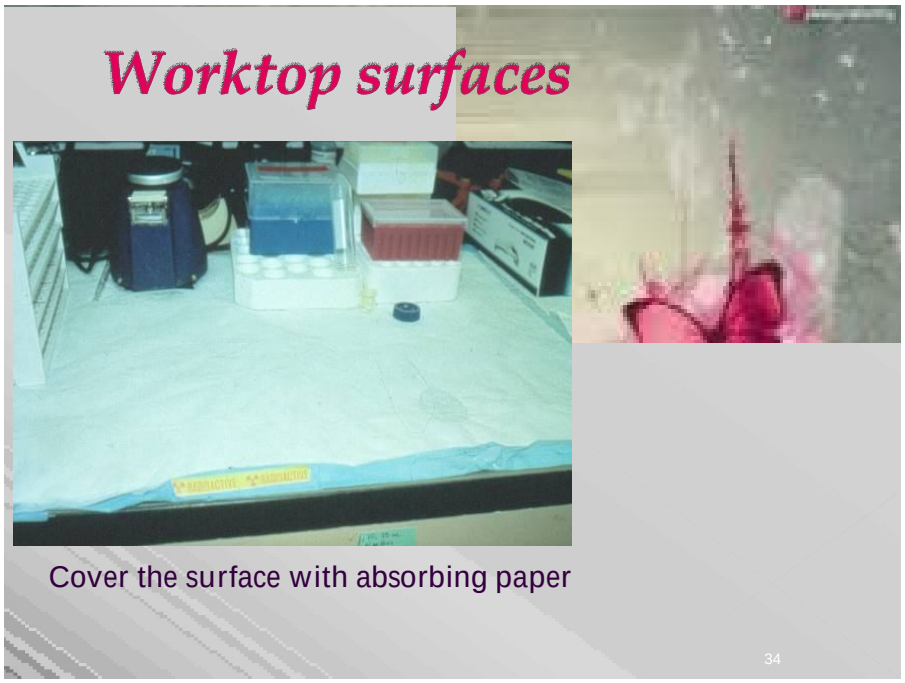


Fig.(2.2) shows the lab worktop surface covered with absorbing materials.

Various pieces of equipment are essential for a good operation of nuclear pharmacy. Examples are: (1) a dose calibrator capable of measuring a wide range of various types and levels of radioactivity , (2) chromatography equipment ,(3) radiation survey meters, (4) an area monitor , (5) a PH meter, (6) a light microscope for particle size determination,(7) a NaI (TI) or Ge (Li) detector coupled to a multichannel analyzer to identify contaminants in radiopharmaceuticals, (8) lead- lined refrigerators and freezers to store cold kits and radiopharmaceuticals under refrigeration , (9) a hot water bath, (10) a dry heat oven , and (11) a well-type NaI (TI) counter equipped with an automatic sample changer for counting many samples. In addition, lead barrier shields are essential for handling radioactive materials behind them. A sufficient number of lead containers of various designs to accommodate vials and syringes containing radioactivity are very important for transporting radioactive materials. Lead-lined or leaded gloves, aprons, and eyeglasses are essential pieces of radiation safety equipment in any nuclear pharmacy operation. An autoclave is needed to sterilize certain materials in nuclear

pharmacy, and a freeze-dryer would serve to lyophilize materials, if needed. An incubator is useful for incubation in sterility testing of radiopharmaceuticals. Certain basic equipment such as a balance, centrifuge, pipettes, and a calculator should be available in nuclear pharmacy. Record keeping of nuclear pharmacy data facilities should also be available.(Gobal B. Saha, 1998).

Table 2.1 shows unavailable nuclear pharmacy equipment in RICK.

Equipment / facility
Chromatography equipment
PH meter
Light microscope
Lead – lined refrigerator
Well-type NaI(Tl) counter with sample changer
Dry heat oven
Lead gloves
Lead aprons
Eyeglasses
Autoclaves
Centrifuge
Tongs

Table (2.2) Available materials and facilities in RICK, which are used for QC procedures.

Material / Facility	Field of use
Gamma cameras	Imaging and QC tests.
Radionuclide generator	source of Tc^{99m}
Pharmaceuticals kits	radionuclide studies
Dose calibrator	measurement of radioactive Materials doses.
Refrigerator	keeping of kits
Survey meter	measurement of radioactive Contamination.
Normal saline	Generator elution.
Lead shields	Radiation protection.
Syringes ,butterfly , gloves, face masks, cotton gauzes..etc.	Different usages.
waste containers	for keeping radioactive and Non-radioactive waste.

2.1.1.3 Operation of a nuclear pharmacy

The daily operation of a nuclear pharmacy involves the following steps : (1) receiving of radioactive materials, (2) preparation of radiopharmaceuticals , (3) quality control tests of radiopharmaceuticals , (4) storage , (5) dispensing , (6) radioactive waste disposal ,and (7) infectious waste disposal .

Table 2.3 daily nuclear pharmacy steps done/ not done in RICK:

Step	Done	Not done
Receiving of radioactive materials	√	
Preparation of radiopharmaceuticals	√	
Radiopharmaceuticals Q.C tests		√
Storage of radioactive substances	√	
Dispensing of radiopharmaceuticals	√	
Radioactive waste disposal	√	
Infectious waste disposal		√

According to the standards and regulations of NM worldwide practices, including those adopted by the IAEA, and national regulations such as those promulgated by the USNRC, the radioactivity of any radiopharmaceutical that contains a photon –emitting radionuclide must be measured by a dose calibrator prior to administration to patients or for human research purposes. Obviously, the administration of the prescribed amount of activity to the patient requires proper operation of the dose calibrator (Zeinaliet et al, 2008).

This requires that, before the day's operation is begun, the nuclear pharmacist must ensure that all equipment in the nuclear pharmacy, such as the dose calibrator, survey meter, and NaI (Tl) detector are in good operating condition. If a malfunction is noted in any instrument , it must be remedied before any measurement is made .All personnel in the nuclear pharmacy must wear laboratory coats and gloves while handling radioactive materials .A pair of long tongs should be used in the handling of a high activity , preferably behind a lead barrier shield (Gobal B. Saha, 1998).

Monitoring of packages is required (according to the US Code of Federal Regulation - 10 CFR 20) if the packages are labeled as containing radioactive materials. Briefly, the packages should be monitored within three hours if delivered during normal hours, or within three hours from the beginning of the next working day if delivered after working hours. The survey must be done on the surface of the package and at one meter using a GM survey meter, and the readings should not exceed the limits of 200 mR / hr and 10 mR/ hr respectively .The wipe test of the package surface must be done according to 10 CFR 20, and the limit for the test is 0.003µCi (6600 dpm or 111Bq) per 300 cm^2. (Gopal B. Saha,1998).

^{99m}Tc – radiopharmaceuticals are prepared daily for nuclear medicine studies. After the daily elution of the Moly generator, the activity should be assayed in the dose calibrator and tests for molybdenum breakthrough, PH, and radiochemical purity by chromatography should be performed. The ^{99m}Tc vial should be identified with a label containing the information as the total activity, concentration, time of calibration … etc. This information should be recorded on a generator control sheet. (Gopal B. Saha, 1998).

2.1.2 Radionuclides

Radionuclides are unstable nuclei having a neutron excess or deficit which transform spontaneously (decay) until they become stable nuclei, with the emission of any combination of alpha, beta, and gamma radiation (Donald R. Bernier et al, 1997).

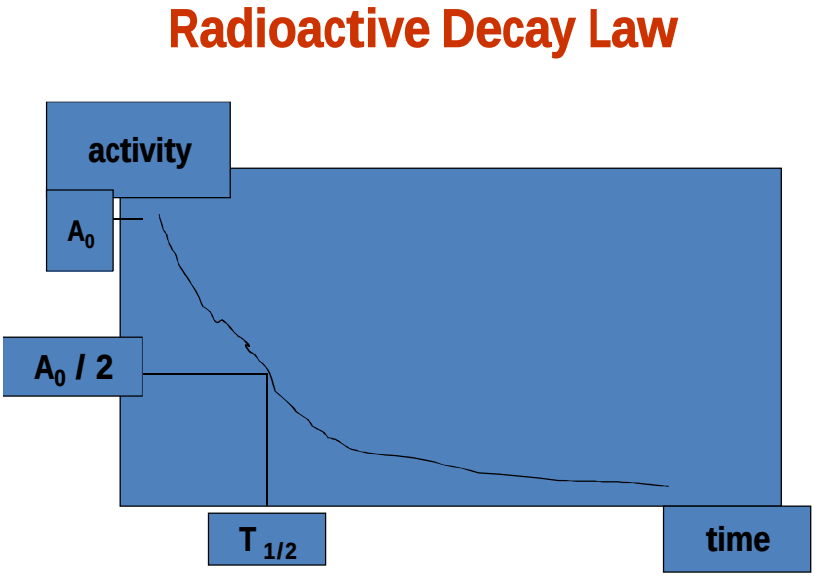


Fig. 2.3 shows the radioactive decay law.

Naturally occurring radionuclides such as uranium, radon, carbon-14 and potassium-40 contribute to our background radiation exposure, whether external to the body or internal. (Donald R. Bernier et al,1997).

These naturally occurring radionuclides are not used in medical imaging. Only artificial radionuclides with certain properties are used. Irene Curie and Federic Joliot were the persons who paved the way to the use of radionuclide tracers in biomedical research and clinical medicine (William R. Hendee 2002).

Radionuclides used in nuclear medicine are all synthetic and fall into three broad groups: generator-produced, reactor- produced and cyclotron-produced (Sam A. K 2008).

Radionuclides are produced using nuclear reactions. Nuclear reaction is a term usually taken to mean a process in which the atomic nucleus is changed through reactions with elementary particles or other atomic nuclei. (Sam A. K., 2008)

A shorthand notation for the nuclear reaction is:

T (P, E) R. The nuclides outside the bracket are the target and product respectively. Inside the bracket are the incident and the emitted particles respectively. (Sam A. K., 2008).

Any configuration of protons and neutrons forming an atom is called a nuclide. (Paul E. Christian et al, 2004).

Most nuclear reactions pass through an intermediate stage, the transition nucleus or the compound nucleus, losing its excitation energy by emission of gamma quanta or particles.(Sam A. K., 2008)

Nearly 3000 nuclides are known, of which 2700 are radioactive, and the rest are stable.(Paul E. Christian et al , 2004).

2.1.2.1 Technetium$^{99m:}$

2.1.2.1.1 Historical Background

Technetium is the forty third elements in the periodic table. It was not conclusively isolated until 1936, when Perrier and Serge showed that radioactivity obtained by the irradiation of molybdenum with deuterons in Berkeley cyclotron belonged to isotope of the "missing" element ekamanganese. Only in 1947 was it named technetium, from the Greek word technetos, meaning artificial. It was introduced into nuclear medicine in 1957 with the development of the ^{99}Mo – ^{99m}Tc generator at the Brookhaven National Laboratory. The first clinical use of technetium, in 1961 at the University of Chicago, heralded a new era of nuclear medicine. The first commercial ^{99}Mo – ^{99m}Tc generator was made available in 1965 (IlseZolle, 2007).

Today, 22 isotopes and isomers of this element, with mass numbers ranging from 90 – 110, are known. The half – lives of these isotopes range from less than one second to 4.2 x10^6 years .^{99m}Tc is the most significant of the metastable isotopes, emitting gamma rays to decay with a half – life of 6 h to ^{99}Tc which has a half- life of 2.1x 10^5 years. It is one of two elements with Z less than 83 that have no stable isotopes; the other element is promethium (Z less than 61) (P.E.Sharp et al 1989; Pual E. Christian et al 2004; Sam A.K; 2008).

Tc^{99m}has gained importance as a radionuclide for nuclear medicine imaging because of its favorable bio-distribution, excretion characteristics and

relatively short physical half-life, coupled with its 140 Kev gamma emission. It is easily combined with a large range of pharmaceuticals suitable for specific organ imaging. In addition, it may be used alone as pertechnetate (99mTc O_4) to study a number of organs whose cells either accumulate it or excrete it .As a result, ^{99m}Tc is the most widely used of the radionuclides in nuclear medicine, either as the radioactive label for a range of pharmaceuticals, or as pertechnetate to image the brain, thyroid, salivary glands or gastric mucosa. The usefulness of pertechnetate for this purpose is based on its bio-distribution in the body. The importance achieved by ^{99m}Tc today is far beyond what might have been expected for an element with no stable isotopes. It plays a great role in the temporary nuclear medicine diagnostic investigations .It constitutes almost 80% of the radiopharmaceuticals which are used in this vital branch of medicine (P.E. Sharp et al 1989; Sam A.K.; Derar A.A.2009). It is better to justify this importance by mentioning the unique properties of this element in details:-

2.1.2.1.2 Properties of technetium – 99m:-

It has suitable physical half- life of six hours, which is similar to the time from preparation to injection. If the half – life is too short, much more activity must be prepared than is actually injected. It's half – life is sufficiently long so that needed medical information can be obtained, yet short enough to minimize radiation dose to the patient.(Ramesh Chandra, 2001; Penelope Allisy et al. 2008).

- It emits mono energetic gamma rays of 140 Kev , high enough to well penetrate the tissues and low enough for efficient detection .This is because it is close to optimum for a NaI (Tl) crystal (150-200 Kev) of the gamma camera.
- It decays to a radionuclide of a very long half- life i.e^{99}Tc ($2.1X10^5$).
- It has a high specific activity (high activity per unit volume).
- It is easily and firmly attached to a number of pharmaceuticals without affecting the metabolism thereof (Penelope Allisy et al 2008).
- Readily available at the hospital site in a generator system.
- It has a reasonable cost.
- It is available in a sterile, pyrogens -free, and carrier - Free State from ^{99}Mo – ^{99m}Tc generators (Gopal B. Saha, 1998).

2.1.2.1.3 Chemistry of technetium -99m:

Technetium is a silvery – grey metal that tarnishes slowly in moist air, and it belongs to group V11B (Mn, Tc and Re). It can exist in eight oxidation states, namely, 1- to 7+, and the common oxidation states of these are+7, +5 and +4. When it is obtained from a generator in normal saline (0.9% Nacl) as pertechnetate ion (^{99m}Tc O_4), technetium is in the +7 valence state, which is the most stable of all valence states of technetium in aqueous solutions. Technetium forms both soluble and insoluble compounds. With the exception of pertechnetate and ^{99m}Tc – sulfur colloid, all other radiopharmaceuticals containing technetium are in an oxidation state lower than seven. Therefore, technetium needs to be reduced to a lower oxidation state before it can be utilized in a variety of useful ligands such as DTPA.(Gobal B. Saha; David H. et al 2ooo; Paul Christian et al 2004; Sam A.K.2008).

The reduction process is very important since the pertechnetate ion is a mild oxidizing agent and, chemically, a rather non-reactive species which does not label any compound by direct addition (17, 20). Various reducing agents that have been used are stannous chloride (Sncl2. $2H_2$ o), stannous citrate, stannous tartrate, concentrated Hcl, sodium borohydride (Na BH_4), dithionite and ferrous sulfate. Among these, stannous chloride is the most commonly used reducing agent in most preparations of ^{99m}Tc – labeled compounds (Gobal B. Saha 1998; Paul E. Christian 2004).Another method of reduction of ^{99m}Tc from +7 oxidation state to a lower oxidation state involves the electrolysis of a mixture of sodium pertechnetate and the compound to be labeled using an anode of zirconium.

The amount of ^{99m}Tc atoms in the ^{99m}Tc eluate is very small, and therefore only a minimal amount of Sn^{2+} is required for reduction of such a small quantity of ^{99m}Tc. However, enough $Sn^{2+}$is added to ensure complete reduction. The ratio of Sn^{2+} ions to ^{99m}Tc atoms may be as large as10^6 (Gopal B. Saha 1998).

The reduced ^{99m}Tc species are chemically reactive and combine with a wide variety of chelating agents. A schematic reaction would be:

Reduced ^{99m}Tc + chelating agent $\longrightarrow$ ^{99m}Tc – chelate.

In a typical preparation of ^{99m}Tc – radiopharmaceuticals in the kit, the quantity of free pertechnetate usually remains within the acceptable limit. However, the presence of oxygen in the vial, particularly before the oxidation of ^{99m}Tc, can

cause oxidation of the stannous ion to stannic ion whereby the amount of stannous ion available for reduction of Tc^{7+} decreases. This results in an increase in free $^{99m}TcO_4$ in ^{99m}Tc- radiopharmaceuticals. Further, the high activity of ^{99m}Tc in the presence of oxygen can cause radiolysis of water or other products in the sample, producing hydroxyl (OH.), alkoxy (RO.) and proxy (RO_2.) free radicals. These species interact with ^{99m}Tc – chelates producing free $^{99m}TcO_4$ in the sample .However, limits of ^{99mTc} activity suggested for adding to the commercial kits are sufficiently low such that the radiolytic affects are normally negligible. The above effects can be mitigated by using sufficient quantity of stannous ion and by avoiding oxygen, air, or any oxidizing agent in the vial throughout its shelf life. In some kits such as MDP kits, antioxidants (e.g. ascorbic acid and gentisic acid) are added to prevent oxidation (Gopal B. Saha 1998).

After the reduction of ^{99m}Tc, it may undergo hydrolysis when it is reduced in an aqueous medium in the absence of any complexion agent. In this case, reduced ^{99m}Tc reacts with water to form various hydrolyzed species, depending on the PH, duration of hydrolysis and presence of other agent's .The analysis of chemical reactions shows that hydrolyzed technetium is a compound of ^{99m}Tc O_4complexed with other ingredients (e.g. .SnO, MoO_3 or Al). This hydrolysis competes with the chelation process of the desired compound and thus reduces the yield of ^{99m}Tc- chelate. The hydrolyzed species can also interfere with the diagnostic test in question if they are present in large quantities in the radiopharmaceutical (Roy P. Parker et al 1984; Gopal B. Saha 1998).

The hydrolytic reaction may also occur to some extent even in the presence of commixing agent, and the presence of hydrolyzed^{99m}Tc species in a radiopharmaceutical preparation may be missed unless the analytical procedures are well chosen (Roy P. Parker et al 1984).

The use of stannous chloride has a disadvantage in that the Sn^{2+} ion also readily undergoes hydrolysis in aqueous solution at PH 6 to 7 , and forms insoluble colloids . These colloids bind to reduced ^{99m}Tc and thus compromise the labeling yield. For this reason, an acid is added to prevent the hydrolysis of Sn^{2+} before the reduction of technetium if the preparation is made using basic ingredients rather than a kit (Gopal B. Saha 1998). These two disadvantages, namely the hydrolysis of reduced ^{99m}Tc and Sn^{2+}, can be circumvented by adding enough chelating agents. The latter will bind to reduced ^{99m}Tc and Sn^{2+}

, and thus prevent their hydrolysis . The ratio of the chelating agent to Sn^{2+} should be large enough to ensure complete binding. Binding between the chelating agent and reduced ^{99m}Tc or Sn^{2+} is highly dependent on the affinity constant of the chelating agent. If it is a weak chelating agent (e.g. phosphate compounds), then hydrolyzed species in the ^{99m}Tc – labeled preparation tend to be relatively high. However, if the chelating agent has a high- affinity constant (e.g. DTPA), then the amount of hydrolyzed species will be minimal (Gopal B. Saha 1998).

At any rate, in a preparation of a ^{99m}Tc – labeled compound, three ^{99m}Tc species may be present:-

1. “free" ^{99m}Tcas $^{99m}TcO_4$that has not been reduced by Sn^{2+}.
2. "Hydrolyzed" ^{99m}Tc, such as $^{99m}TcO_2$ that did not react with the chelating agent; this includes reduced ^{99m}Tc bound to hydrolyzed Sn^{2+} ($Sn(OH)_2$).
3. “Bound “99mTc – chelate, which is the desired compound formed by binding of reduced ^{99m}Tc to the chelating agent (Goal B. Saha1998).

In most routine preparations, the major fraction of radioactivity is in the bound form. The free and hydrolyzed fractions are undesirable and must be removed or reduced to a minimum level so that they do not interferer significantly with the diagnostic test in question (Gopal B. Saha 1998) .

Table 2.4 shows the oxidation states of ^{99m}Tc in ^{99m}Tc- radiopharmaceuticals.

Oxidation state	^{99m}Tc – radiopharmaceuticals.
Tc^{7+}	$^{99m}TcO_4$ and technetium hepato- Sulfide($^{99m}Tc_2S_7$)
Tc^{5+}	Tc^{99m} – citrate , ^{99m}Tc gluconate and ^{99m}Tc –gluceptate prepared by $Sncl_2$ reduction in aqueous solution , Diaminodithiol (DADT), EDTA and ^{99m}Tc- HMPAO.
Tc^{4+}	Tc O2. XH_2 O and HEDP, Depending on solution PH.
Tc^{3+}	^{99m}Tc complexes like EDTA , DMSA And HIDA when prepared in acid solution.
Tc^{1+}	^{99m}Tc – sestamibi and tertbutyl , Methyl , cyclohexile and phenyl isocyanide complexes

N.B: no useful $Tc^{6+,}$ $Tc^{2+}$and Tc^{0} complexes have been developed for clinical use, so they have been omitted (Gopal B. Saha 1998).

2.1.3 Radionuclide Generators

2.1.3.1 Introduction

The generator concept was developed in the early years of the twentieth century in order to provide a supply of radon gas from 226Ra.(Roy P. Parker et al 1984).

The first commercial radionuclide generator was produced in the United States in early 1960's, at the Brookhaven National Laboratories. Since then, a number of different types of generators have been developed for various purposes in nuclear medicine. (M.M Khalid 2011).

Generators are parent-daughter systems involving a long–lived parent radionuclide that decays to a short half- life daughter. The parent and it's daughter nuclides are not isotopes; therefore, chemical separation is possible.(Gopal B. Saha 2010,M.M Khalid 2011). The importance of radionuclide generators lies in the fact that they are easily transportable and serve as sources of short-lived radionuclides in situations of no cyclotron or reactor facilities. For hospitals not located close to a radionuclide production facility , the only practical way to obtain short –lived radionuclides is by use of a generator .(Adlan A.A.2007).

Radionuclide generator consists of a glass or plastic column fitted at the bottom with a fretted disc. The column could be made of ceramic with ^{99}Mo adsorbed onto its top surface .The column is filled with adsorbent material such as ion exchange resin or alumina on which the parent radionuclide is adsorbed. Daughter radionuclides are generated by the decay of the parent radionuclide until either a transient or secular equilibrium is reached in several half- lives .After that the daughter appears to decay with the half- life of the parent. Because of the differences in chemical properties, the daughter activity is eluted with appropriate solvent, leaving the parent on the column. After the elution, the daughter activity builds up again and can be eluted repeatedly (Gobal B.1998; Kieran et al 2006; M.M. Khalid 2011). An ideal radionuclide generator should be simple and sturdy for transportation. The generator eluate should be free of parent nuclide and the adsorbent material.

Several radionuclide generators are available for ready supply of short – lived radionuclides (see table 2.4 below).

Table 2.5 shows the main radionuclide generator systems.

Parent	physical $T_{1/2}$	daughter	physical $T_{1/2}$	decay product
^{68}Ge	280 d	^{68}Ga	68 min	^{68}Zn
^{81}Rb	4.5 d	^{81}Kr	13.5 s	^{81}Kr
^{99}Mo	67 h	^{99m}Tc	6 h	^{99}Tc
^{113}Sn	117 d	^{113}In	100 d	^{113}In
^{132}Te	78 h	^{132}I	2.3 h	^{132}Xe
^{82}Sr	25 d	^{82}Rb	75 s	^{82}Kr

Of these generator systems, the ^{99}Mo / ^{99m}Tc generator is the workhorse of nuclear pharmacy in nuclear medicine (Gopal B.1998)

2.1.3.2 ^{99}Mo – ^{99m}Tc generator

This generator has been the most commonly used radionuclide generator in nuclear medicine practice worldwide since its first commercial introduction in 1965. Originally, the device was named (radioisotope generator) and the old term is still often used even though the term (isotope) for designation of nuclear species has been replaced with the term (radionuclide). It has several characteristics and attractive properties, which include the following:

- Cost effective and simple to use.
- Sterile and pyrogen - free.
- High radionuclidic and radiochemical purity.
- Used to produce many ^{99m}Tc- labeled radiopharmaceuticals frequently used in nuclear medicine departments.
- Ideal half – life of the daughter nuclide (6 h), and optimum energy (140 Kev - 90% abundance).
- Mo is produced by the (n, f) fission reaction instead of the (n,γ) reaction (to have a carrier- free ^{99}Mo). It has a half- life of 66h and decays by beta negative emission(87%) to metastable state ^{99m}Tc and in (13%) to ground state ^{99}Tc,

while ^{99m}Tc has a half- life of 6 h and decays to ^{99}Tc by isomeric transition with the emission of 140 Kev gamma photons . (Ervin B. Podgorsak 2010, M.M. Khalid 2011).

The ^{99}Mo – ^{99m}Tc generator is constructed with alumina ($Al_2 O_3$) loaded in a plastic or glass column. In a typical ^{99}Mo-^{99m}Tc generator, the column is filled with alumina, ^{99}Mo is part of the molecule sodium molybdate, and the eluting solution is oxidant – free physiologic saline (0.9% sodium chloride solution) . ^{99}Mo used in these generator is produced either by irradiation of ^{98}Mo with neutron or by fission of ^{235}U in a reactor. The latter is carrier – free and, therefore, has very high specific activity. The ^{99}Mo activity is adsorbed on alumina in the chemical form Mo O_4^{-2}(molybdate) ,and in various amounts . The amount of alumina used is about 5-10 grams. Currently, all generators use fission- produced ^{99}Mo. The fission – produced^{99}Mois carrier – free and therefore has very high specific activity (Gopal B. Saha 2001; Ramesh Chandra 2004).

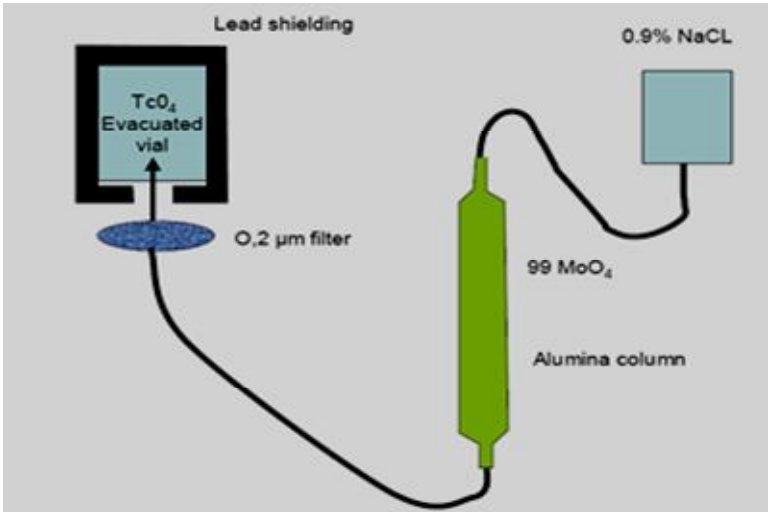


Fig. (2.4) shows the main components of ^{99}Mo/ ^{99m}Tc generator

^{99}Mo- ^{99m}Tc generator is used in 90% of radionuclide imaging as it fulfills most of the desired criteria (Penelope Allisy et al 2008).

There is a choice of many different generator systems, all based on the aluminum oxide column eluted by a saline solution. Both pressure and vacuum systems are employed. In some systems the column is kept wet and in others

the bed is kept dry between elutions. The wet column generator contains a reservoir of normal saline that is connected to the alumina column. After elution, of this generator, saline remains on the column, leading to the formation of water radiolysis products, which are reducing agents? This causes reduction of the ^{99m}Tc and deceased yields of ^{99M}Tc O_4, since the reduced ^{99m}Tcspecies do not elute from the column (Paul E. Christian 2004; Sam A.K 2008)

The dry column generator system was developed to alleviate poor elution yields of $^{99m}TcO_4$, by removing saline from the column after elution. This decreases the amount of radiolysis products formed. The dry column generator employs a 5-20 ml saline charge, which is applied to an exterior part of the generator. An evacuated vial draws saline through the generator to remove ^{99m}Tc O_4, followed by air to dry the column. Leaving the air on the column promotes oxidation of any reduced ^{99m}Tc species back to the +7 valance state of $^{99m}TcO_4$ which can then be reduced.(Paul E. Christian 2004; Ramesh Chandra 2004; M.M. Khalid 2011).

Dry column generators are preferable due to the repeated withdrawal of saline from the column after routine generator usage by an evacuated tube , which prevents the formation of hydrogen peroxide (H_2 O_2) and perhydroxyl free radical (HO_2), which , if present in the ^{99m}Tc eluate , can interfere with the ^{99m}Tc labeling procedures because they can act as oxidants.(M.M. Khalid 2011).

The elution and operating principle of ^{99}Mo-^{99m}Tc generator is fairly straightforward. ^{99m}Tc is removed from the column in the form of sodium pertechnetate (Na99mTcO4), by drawing a solution of sodium chloride (NaCl, 0.9% w/v) eluant through the column.

This process is known as eluting the generator, and the resulting solution as the eluate .The vial containing the eluant is first inverted onto a needle on top of the generator. Another evacuated vial is inverted onto the other needle. Vacuum in the vial on the other needle draws the eluant through the column and elutes the daughter nuclide, leaving the parent nuclide on the column. It is thought that ^{99}Mo remains on the column because the 2^- charge on the ^{99}Mo O_4 ion binds with a greater affinity to the alumina than the $^{99m}TcO_4$ion (with only a single negative charge (Gopal B.Saha1998, P.F Sharp et al 1989)

After adsorption of ^{99}Mo on alumina, ^{99m}Tc grows by the decay of ^{99}Mo until its maximum activity is reached after approximately four half- lives.

Table (2.6) Radioactive decay correction coefficients for inserted^{99}Mo activity.

Hours	Coefficient	Days	Coefficient
1	0.990	1	0.780
3	0.969	2	0.609
6	0.940	3	0.475
9	0.911	4	0.370
12	0.883	5	0.289
15	0.856	6	0.225
18	0.830	7	0.176
21	0.805	8	0.137
24	0.780	9	0.107
		10	0.083
		11	0.065
		12	0.051
		13	0.040
		14	0.031

Table (2.7) the coefficient $(1- e^{-0.115})$ following the time of elution in hours

Time since the last elution in hours	$(1- e^{-0.115})$
1	0.100
3	0.273
6	0.470
9	0.617
12	0.720
15	0.797
18	0.851
21	0.892
24	0.922
30	0.959
50	0.995

Sodium pertechnetate is obtained from the ^{99}Mo / ^{99m}Tc generator as a salt which dissociates in water into two ions, sodium and pertechnetate:

$Na^{99m}TcO_4 \longrightarrow Na^+ + {}^{99m}TcO^-_4$ (Sam, 2008).

The positively charged sodium cautions are present to balance the negatively charged pertechnetate anions, and any other positively charged ion can serve the same role. Following the i.v injection, the positively charged ion does not follow the same biological pathway as pertechnetate ion (James H. Thrall and Harvey A. 2001).

In any generator, if the half – life of the parent is longer than the half- life of the daughter radionuclide, an important phenomenon occurs which is the basis of the generators presently used in nuclear medicine .Under this condition (i.e. the half – life of parent is longer than that of the daughter), and in due course, an equilibrium is established between the parent and daughter radionuclides. In the state of equilibrium the ratio of the amounts (number of radio nuclei present) of the two radionuclides becomes constant .The two radionuclides also maintain a constant ratio (this ratio is in general close to unity) with time , even though the half-lives of the two radionuclides are quite different . In effect, the daughter radioactivity decays with an apparent half – life of the parent rather than its own half-life. (Ramesh Chandra 2001; Sam A.A. 2008).

The state of equilibrium is sometimes classified in two categories, transient and secular. When the half- life of the parent is not long in comparison with the daughter half- life, (parent half – life is at least 10-50 times greater than that of the daughter half-life), the equilibrium is transient. When the half- life of parent is much longer (100- 1000 times greater) than the half-life of the daughter, the equilibrium is secular. (Ramesh Chandra 2001; Sam A.A. 2008).

Once equilibrium has been achieved between the parent and daughter, it can be disturbed only by chemical separation (i.e. milking) of the two radionuclides. . Fresh supply of daughter radionuclide can be obtained by milking the generator after each four daughter half – lives, although 50% of the maximum supply could be obtained after one daughter half-life. (Ramesh Chandra 2004).

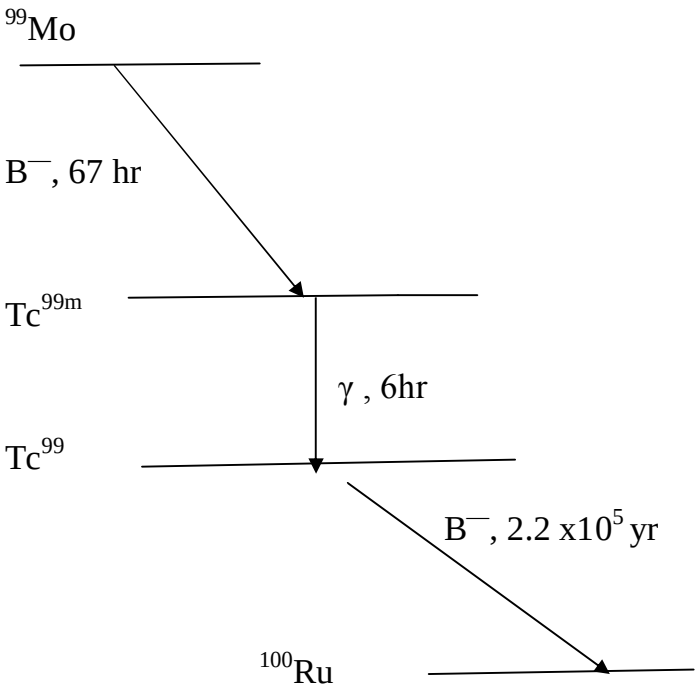


Fig. (2.5) Decay scheme for 99Mo

After the elution process is finished , the activity in the vial is measured , the concentration calculated (concentration =activity / volume of eluant) and the Na $^{99m}Tc\ O_4$ is used, after mixed with the appropriate pharmaceutical, for radionuclide imaging of different organs , according to the dose allocated for each organ .

For any generator system to be of practical use in nuclear medicine, the milking process should be convenient and rapid. The eluate must be sterile, apyrogenicity and it's PH in a range of 4.5 – 7.5. Other factors should also be considered, namely the efficiency, parent breakthrough, radiation shielding and specific concentration. Efficiency = the activity eluted per total activity of daughter x 100%. It is also called generator yield (70 – 90%). (Ramesh Chandra 2004).

2.1.4 Radiopharmaceuticals

A radiopharmaceutical is defined as a radioactive compound which, when administered for purposes of diagnosis or therapy, elicits no physiological response from the patient (Sam 2008).

It is also defined as: a chemical compound tagged with a radionuclide and prepared in a form suitable for human use. (Ramesh Chandra 2001).

A third definition states that the radiopharmaceutical is a radioactive compound used for the diagnosis and therapeutic treatment of human diseases.(Gopal B. Saha 1998).

A more detailed definition states that radiopharmaceuticals are medical products designed for the use in the investigation or treatment of human diseases, which contain a radionuclide as an integral part of the main ingredients, and a stable compound which called pharmaceutical agent to produce a pharmacological response in the patient (Abdul Wahid B. 2001).

All radiopharmaceuticals are legend drugs and are subject to all regulations that apply to other drugs .Any material administered to humans must be sterile and non-pyrogenic. In designing a radiopharmaceutical, a suitable pharmaceutical is chosen on the basis of its preferential localization.(M.M. Khalid 2011).

Radiopharmaceuticals differ both in their physical and chemical form as well as in the radionuclide involved. The radiopharmaceutical must distribute itself in the body in such a manner that the aim of the test is fulfilled and an accurate understanding of its biological behavior is essential. Good choice of the radionuclide will, in the case of diagnostic procedures, minimize the radiation dose to the staff and patient and maximize the information gained.(Roy P. Parker et al 1984).

Although the term radiopharmaceutical is most commonly used, other terms such as radiotracer, radio-diagnostic agent and tracer are also used.(Gopal B. Saha 1998).

Since a radiopharmaceutical consists of a radionuclide and a biochemical, two considerations apply in designing or developing a radiopharmaceutical; one relating to the radionuclide and the other relating to the biochemical. The choice of a radionuclide for imaging purpose is chiefly dictated by the necessity of minimizing the radiation dose to the patient and the detection characteristics of present-day nuclear medicine instrumentation (Ramesh Chandra 2001).

In short, the radionuclide should have the following desirable properties:

-A physical half-life of a few hours, similar to the time from preparation to the injection. If the half –life is too short, much more activity must be prepared than is actually injected.

-Decaying to a stable daughter or at least one with a very long half-life.

-Emission of gamma rays (which produce the image) but no alpha or beta particles nor very low energy photons (which have a short range in tissue and deposit unnecessary dose in the patient).Decay by isomeric transition or electron capture is therefore preferred.

-Emission of gamma rays of energy range 50-300 Kev, and ideally about 150 Kev, which is high enough to exit the patient but low enough to be easily collimated and easily detected.

-Ideally, emission of mono-energetic gamma rays, so that scatter can be eliminated by discrimination with a pulse height analyzer. Other non-penetrating radiations, e.g. alpha and beta particles are undesirable due to their high linear energy transfer (LET), which is the fraction of energy deposited per cm of travel. This results in almost quantitative absorption in the body. The few particles that manage to escape the patient's body can't penetrate the crystal to interact. Consequently, alphas and betas are not imageable, and because of their high LET, they confer a very significant radiation dose to the patient.(Sam A.K. 2008).

-Easily and firmly attached to the pharmaceutical at room temperature but has no effect on its metabolism.

-Readily available at the hospital site.

-Of high specific activity i.e. high activity per unit volume.

Both the radionuclide and the biochemical should:

-localize largely and quickly in the target (tissue of diagnostic interest).

-be eliminated from the body with an effective half-life similar to the duration of examination, to reduce the dose to the patient. Ideally, the effective half-life equals approximately 1.5 times the duration of diagnostic procedure.(Sam A.K. 2008).

-Of low toxicity.

-form a stable product both in vivo and in-vitro.

-be readily available and inexpensive per patient dose (Penelope et al. 2008).

-be taken by the desired organ (or part of the organ) in such a way as to differentiate substantially between the normal condition and the pathological condition (Ramesh Chandra, 2001).

The target to non-target ratio should be as large as possible. The target uptake rate is also a key characteristic of an ideal radiopharmaceutical because it influences the period after which imaging acquisition is done. For example, $^{99m}TcO_4$is preferable to ^{123}I-NaI because the thyroid imaging procedure can be performed after 20 min of dose administration, while with ^{123}I-NaI it takes 4-6 h to launch the imaging session.(NM Khalid,2011)

It must be emphasized, however, that no single radiopharmaceutical actually has all the above mentioned properties (P.F. Sharp, 1989).

The definition of an ideal radiopharmaceutical in nuclear medicine procedures varies according to its use (Ramesh Chandra, 2001).

The usefulness of a radiopharmaceutical is dictated by the characteristics of the two components thereof. A pharmaceutical is first chosen according to its preferential localization in a given organ or its participation in the physiologic function of the organ .Then a suitable radionuclide is tagged onto the chosen pharmaceutical, such that after administration of the radiopharmaceutical, radiations emitted from it are detected by a radiation detector (Gobal B. Saha 1998).To help in the selection of a suitable biochemical, a wealth of information has been acquired in the field of pharmacology. A number of physiochemical variants determine or affect the distribution and localization of drugs in tissues .Three important determinants in this regard are : route of administration ,blood flow to the organ or tissues and excretion by the tissues .

Radiopharmaceuticals ,with few exceptions , are nearly always administered intravenously ,primarily because this is the fastest way to introduce a drug into the circulatory system to the body .Blood flow , which can be severely affected in diseases , essentially determines the fraction of the administered dose that will be delivered to a particular organ or tissue during the first transit .Since blood serves as a carrier for the drug , another property , binding to plasma proteins , plays an important role in the localization of the drug or chemical in a given tissue .Extraction of a drug or chemical from circulation and localization in tissue may occur in four ways : simple diffusion , filtration through small pores in the membranes , active transport and Phagocytosis (Ramesh Chandra,2001).

Radiopharmaceuticals are used in a variety of chemical forms as follow:

a- Incorporated into inorganic or organic molecules i.e. carrier + radionuclide. the carrier provides for the affinity to a special body tissue. The radionuclide is intended for the purpose of detection only (diagnostics) or interaction with the tissue (therapeutics) .These range from small inorganic species like ^{201}LI – TICL, ^{131}I–NaI, ^{111}In- Incl, $Na_3{}^{32}Po_4$, to large proteins such as ^{99m}Tc-HSA and^{125}I- serum albumin.

b- Pure elements e.g.^{133}Xe and ^{81m}Kr for pulmonary inhalation imaging.

c- Ionic form e.g.^{131}I.

The design of these compounds is based solely upon physiological function of the target organ. Unlike radiographic procedures, which depend almost on tissue density differences, external imaging of radiopharmaceuticals is essentially independent of the density of the target organ .Every organ acts differently from a chemical point of view. For instance, the thyroid takes up iodine; the brain consumes quantities of glucose…etc .With this knowledge, radiopharmaceuticals are able to attach various radionuclides to biologically active substances. Once a radioactive form of one of these substances enters the body, it is incorporated into the normal biological process and excreted in the normal way. The distribution of the radiopharmaceutical within the body is determined by the physiochemical properties of the drug, the stability of the label, the purity of the radiopharmaceutical preparation, the pathophysiological state of the patient and the presence or absence of interfering drugs.

Radiopharmaceutical products are available in four different categories, namely:-

Ready–to-use radiopharmaceuticals, instant kits for preparation of ^{99m}Tc products kits requiring heating and products requiring significant manipulation.

Examples of each category:

-Prepared products:

^{123}I-capsules, ^{131}I- hippuran, ^{67}Ga-citrate,^{201}Tl- chloride, ^{133}Xe-gas, ^{99m}Tc pertechnetate.

-Instant ^{99m}Tc- kits:

Disofenin (diisopropyl-IDA –DISIDA).

DTPA, GH, HDP, MDP, Mebrofenin, MIAA, MAA, PYP.

-^{99m}Tc-kits requiring heating:-

MEG3, Sestamibi, Sulfur colloid, tebroxime.

-Products requiring significant manipulation:-

Cr- RBC's ,^{99m}Tc-RBC'S , ^{99m}Tc-WBC's, ^{111}In-platelets, ^{133}Xe in saline , ^{123}I-MIBG .

Distinction between radiopharmaceutical and radiochemical is important to recognize .There are many similarities between them .From the standpoint of both chemistry and radionuclidic purity ,there is usually minimal or no difference .The ^{201}TI-chloride sold as a radiochemical most likely came from the same cyclotron run as the radiopharmaceutical grade ^{201}TI-chloride used for diagnostic imaging .Radiopharmaceuticals ,however ,have undergone a very lengthy and expensive regulatory process as well as expensive physiochemical testing (PH, isotonicity, particle-size determination , and chemical parameters) , and biological testing to insure that the final product is sterile, pyrogen-free , safe for human use and efficacious. This includes animal studies prior to release the product for sale.

Radiochemical typically don't undergo this vigorous testing and neither their sterility nor apyrogenicity is guaranteed .Radiochemical usage is usually limited to chemical and biological research; in addition, the tracers used in RIA procedures are usually radiochemical grade.

Radiopharmaceuticals are designed for diagnostic imaging and therapy procedures, but may also serve as tracers in research projects (Sam, 2008).

2.1.4.1 Properties of an ideal radiopharmaceutical in details:-

2.1.4.1.1 Type of emission:-

The radionuclide should be a pure gamma ray emitter .This is because in diagnosis the objective is to image a biological structure. For images to be formed, the radiation must be penetrating enough to pass through the subject and into the detector. Therefore, the best radionuclide for diagnostic procedure is that which emits pure gamma ray, decaying either by electron capture or isomeric transition.

Other non- penetrating kinds of radiation, e.g. alpha and beta particles are undesirable for two reasons: due to their high LET, which results in almost quantitative absorption in the body. The few particles that manage to escape the patient's body can't penetrate the crystal to interact. Alphas and betas are, therefore, not image able. In addition, they confer a very significant radiation dose to the patient.

2.1.4.1.2 Imaging energy:-

From an imaging standpoint, the ideal imaging energy range is 100-250 Kev. Currently available imaging equipment has been tailored to function best in this range. Image quality is suboptimal above or below it. Despite this limitation, there are certain radioisotopes that are commonly used in diagnostic studies whose gamma ray energies are below 100 Kev or above 250 Kev. For example, on the low end, ^{201}TI and 133Xe emit photons with energies of about 70-80 Kev, while on the high end, 67Ga and 131I emit photons of 300 Kev and 364.5 Kev respectively. Commonly used radioisotopes considered ideal from an energy standpoint include 99mTc, 111In, and 123I.

2.1.4.1.3 Effective half-life:-

Ideally a radiopharmaceutical's effective half- life equals approximately 1.5 times the duration of the diagnostic procedure.

The effective half –life of a radiopharmaceutical in a biological system is dependent on both the physical half-life and the biological clearance (biological half-life) (James H. Thrall, 2001).

Physical half –life of a radionuclide is the time taken for its activity to decay to half of its original value. It is a fixed characteristic of the radionuclide; cannot be predicted for a given radionuclide in any way and is unaffected by any agency such as heat, pressure, electricity or chemical reactions. It can range from fractions of a second (useless in imaging) to millennia in the case of ^{99}Tc (also useless in imaging). If the pharmaceutical alone is administered, it is gradually eliminated from the tissues, organs and whole body by the usual metabolic process of turnover and excretion. Such a process can be regarded as having a biological half-life. If the radiopharmaceutical is administered to a person, the radioactivity in specific tissues, an organ or the whole body decreases because of the simultaneous effects of radioactive decay and metabolic turnover and excretion. The radiopharmaceutical can be regarded as having an effective half-life .The effective half-life is shorter than either the

biological half-life (Tbio) or physical half-life (T_{ph}).The relation between them is:

$1/T_{eff}=1/T_{bio}+1/T_{ph}$. $T_{eff}=T_{ph} \times T_{bio}/ T_{Ph} +T_{bio}$. (Penelope Allisy et al, 2008)

A radiopharmaceutical that is administered to a patient should remain in the target tissue long enough to do its job properly , but no longer than necessary , so that radiation exposure is minimized. This approximation provides a good compromise between our desire to minimize radiation dose to the patient and maximize the dose to be injected in a way that the counting statistics are good and image quality is optimal.

In the special case where the biological half-life of a particular compound is very long compared to the physical half-life e.g.^{99m}Tc-sulfur colloid in the liver, $1/ T_{bio}$ will be a very small number, and the effective half-life therefore equals the physical half-life. The classical example of an ideal effective half-life is that of ^{99m}Tc- MDP (6 hours). Since bone imaging is a four-hour procedure, the ratio of effective half- life to duration of the test is 1.5: 1, considered ideal. On the other hand, 99mTc-sulfer colloid has an effective half-life of 6 hour in the liver, but the procedure takes only one hour. This 6:1 ratio does not mean that a liver scan is a bad procedure to perform , rather that the compound has a residence time in the liver that is longer than desirable , resulting in an increase in the radiation dose to the target organ .

2.1.4.1.4 Target: non-target ratio:

The radiopharmaceutical should have a high target: non-target ratio i.e. the concentration within the organ or tissue under examination should be higher than the concentration at other organs or tissues. If the ratio is not high enough (5 : 1 minimum for planner imaging , and about 2: 1 for SPECT imaging), a non- diagnostic scan can result , making it difficult or impossible to distinguish pathology from background . For example, when performing a thyroid scan, ideally, the entire radioactivity will be in the thyroid and nowhere else in the neck region. While liver uptake of the radioiodine would be undesirable dosimetrically, it would have no impact on the actual imaging process since it is not in the field of view. For some procedures, e.g. bone imaging, there are two targets: non-target ratios that must be considered. It is important to see bones against soft tissue so the bone: soft tissue ratio must be acceptably high. It is also important to be able to identify a metastatic lesion on bone tissue so the tumor: bone ratio must also be high. These ratios are multiplicative; if the

tumor: bone ratio is 5: 1, and the bone: soft tissue is also 5:1, then the tumor: soft tissue is 25: 1. The result of a very low target: non-target ratio may be a non-diagnostic scan, resulting in an unnecessary radiation dose, a delay in the diagnosis and the necessity of repeating the procedure.

2.1.4.1.5 Chemical reactivity:

Ideally, radioisotope for diagnostic imaging must have a higher ability to readily bind to wide variety of compounds under physiological conditions .Special consideration must be given to availability 0f substrates for radiolabelling reactions. Not every compound can be labeled with every isotope and, in fact, labeling is often quite selective. Compounds which demonstrate acceptable biodistribution often become useless when a radio-metal is added or the molecule is iodinated. Even minimal changes in the molecular structure are often enough to completely change the biodistribution. (Sam, 2008)

2.1.4.1.6 Cost and availability:

Radiopharmaceuticals must be stable both in pre- and post- reconstitution. If a particular compound performs well for a particular procedure, but is only available at on hospital nationwide, its use will be extremely limited. In addition, in the current economic climate, use of radiopharmaceuticals costing hundreds of dollars is limited; especially if less expensive alternates are available (Sam, 2008)).

2.1.4.1.7 Preparation and quality control:

Preparation of the drug should be simple and requires relatively little manipulation on the part of the preparer. In addition, no complicated equipment or time consuming steps should be involved. If the radiopharmaceuticals are manufactured in- house, it is essential that quality control be performed in every batch of drug prepared to insure that each individual preparation will produce a high quality image while minimizing the radiation dose to the patient. Altered biodistribution caused by improperly prepared drug can destroy image quality and have a significant impact on the internal radiation dose to the patient (Sam, 2008).

2.1.4.1.8 Uses of radiopharmaceuticals:

^{99m}Tc imaging agents are considered class of radiopharmaceuticals due to the excellent nuclear decay characteristics of ^{99m}Tc. Most of the first generation of ^{99m}Tc radiopharmaceuticals are simple volume radiotracers indicating the space and the fluid flow in certain compartment. Another more important group can localize in specific tissues depending on their biological state. These components are capable of detecting pathological alterations in tissues. Examples of 99mTc radiopharmaceuticals used in RICK:-

2.1.4.1.8.1 Liver and spleen imaging agents:-

The liver is an organ of reticuloendothelial system and it consists two main cell types, the parenchymal (polyclonal) cells perform the metabolic functions and account for 85% of the cell population. The second type is the reticuloendothelial (Kupffer) cells which are also present in the spleen and bone marrow. These cells phagocytose foreign particles of colloidal dimensions .Radioactive colloids are rapidly removed from the circulation by these cells. Any lesion that displaces normal liver tissue will appear as photopic site, as it does not take up the radio-colloid. Liver imaging radiopharmaceuticals include:-

a- ^{99m}Tc- sulfur colloids (99mTc –SC):-

These are considered the most popular 99mTc liver / spleen imaging agents. ^{99m}Tc-SC is formed by acid – catalyzed conversation of soluble thiosulphate ($S_2 O_3^{-2)}$ to an insoluble ^{99m}Tc-hepatosulfide (Tc_2 S7), which co-precipitates with colloid sulfur. Sulfur colloid kits are formulated in liquid forms, containing thiosulphate, Hcl acid and gelatin as stabilizers of the colloidal particles which are formed during preparation .The mixture is heated at 100^0 F for 5 – 10 min., depending on the manufacturer. Alternatively, it may be heated in a microwave oven for 12- 25 seconds, depending on the particular oven and the power level selected. Buffer is added to the reaction mixture to raise the PH to 5.5. The ^{99m}Tc-SC is then cooled prior to quality control testing and injection. The colloidal particles size ranges from 0.1 to 2 microns. The SC particles are phagocytized by the reticuloendothelial cells with an extraction efficiency of 95%.

Blood clearance time is 2-3 minutes. Particles size is an important determinant of their distribution. Particles between 0.3-1.0 microns are predominantly phagocytized by the Kupffer cells of the liver. Small particles (< 0.3 micron)

distribute primarily to the bone marrow. Particles larger than one micron distribute mostly to the spleen, while very large particles will be deposited in the lungs. Uptake of the tracer reflects distribution of hepatic perfusion and functioning reticuloendothelial cells in the liver (Kupffer cells) and spleen.

Although Kupffer cells make up less than 10% of the liver cell mass, they are distributed uniformly throughout the liver. In the normal situation, about 85% of the total activity is trapped into the liver, 10% in spleen and 5% in bone marrow.

B-^{99m}Tc –phytate (inositol hexaphosphate):-

This compound is also known as myo-inositol hexaphosphate and myo-inositol 1, 2, 3, 4, 5, 6- hexakis-phosphate. ^{99m}Tc-phytate has been proposed as an alternative agent to ^{99m}Tc-SC for liver /spleen imaging for determining liver size and shape , and for the investigation of a malignancy, infection trauma ,and cirrhosis; because ^{99m}Tc- SC suffers from the drawback of a rather complicated and time consuming preparation .

^{99m}Tc-phytate is prepared from instant freeze dried kit contains an active ingredient inositol-hexaphosphate and stannous chloride as reducing agent by two main methods:

i. The in vivo method which involves reconstitution of the kit with pertechnetate to form soluble ^{99m}Tc-phytate complex, which after i.v administration forms insoluble ^{99m}Tc-calcium phytate colloid suitable for liver/spleen imaging.
ii. The invitro preparation of the colloid involves reconstitution of the sterile, pyrogen-free, lyophilized ingredients of the kit with sodium pertechnetate and addition of calcium chloride solution to produce ^{99m}Tc- calcium phytate colloid which is then administered by i.v injection.
iii. ^{99m}Tc-Tin colloid :-

Above PH 4.0 stannous chloride forms insoluble hydrated stannous oxide. Under regulated reaction conditions 99mTc-tin oxide particles can be formed which are trapped to the liver.

Table (2.8) Imaging protocol of the liver scan using ^{99m}Tc –Sulfur colloid .

Radiopharmaceutical	^{99m}Tc-SC.
Activity administered	5 – 10 mCi
Patient preparation	No preparation is needed, but there should be no retained radiographic contrast agent in the abdomen
Time after injection	10 – 15 min.
Counts	2000 Cs / cm^2.
Projections	AP, RAO, LAO, LPO, RL and PA.
Camera and collimator	Large field of view camera ,low Energy, parallel whole collimator.

Liver and spleen scintigraphy should be performed before administration of any iodinated or barium containing contrast agents. Such agents , if retained in the body (particularly barium in the colon), can result in artifactual defects in the liver or spleen (Paul E Christian, 2004).

When injecting the colloid, it is important that blood does not clot in the syringe, as this will trap the colloid and form small radioactive emboli in the lungs with hot spots on the scan. The position of the costal margin should be marked together with any mass in the right upper quadrant which may be related to the liver (P.F Sharp et al 1989).

2.1.4.1.8.2 Lung imaging agents:-

i. ^{99m}Tc- MAA :-

This can be prepared from an instant freeze dried kit containing MAA particles with size ranging from 10 -90 µm, with the majority between 10 and 40µm as compared to 7µm of an average capillary size. Thus, MAA greater than 10µm are trapped allowing for lung visualization through capillary blockage. The particles are performed by heating a stirred solution of album containing sodium acetate and tin chloride in the appropriate PH. Speed of stirring must be carefully controlled in order to obtain the suitable aggregates.

Table (2.9) Imaging protocol of perfusion lung scan:

Radiopharmaceutical	^{99m}Tc-MAA, ^{99m}Tc-HAM.
Activity administered	2mCi (80 MBq)
Collimator	Low energy, general purpose.
Patient preparation	Patient flat or semi recumbent , for slow I.v injection during which Patient breathes deeply.
Images acquired	AP, PA, RPO, LPO, RL and LL.
Views counts	100 s exposure per view.

In case of patient with severe pulmonary hypertension, the MAA should be injected slowly. MAA is contra- indicated in patients with right to left cardiac shunt, where it will enter the system circulation and may result in micro emboli in the brain and kidneys (P.F Sharp et al 1989).

ii. Radioactive xenon: This is used, after being dissolved in saline solution, to demonstrate the distribution of blood flow. It is given intravenously, and because the gas is relatively insoluble, it comes out of solution as it reaches the air contained in the alveoli. Its distribution can be measured during breath holding and corresponds to pulmonary capillary blood flow. Regions of the lung that are collapsed or consolidated, as in pneumonia, appear to have no blood flow because the alveoli contain no air. Other agents are used in ventilation lung study, and include ^{99m}Tc – DTPA aerosol, ^{133}Xe and ^{81m}Kr.

2.1.4.1.8.3 Renal imaging agents:-

Renal imaging of radiopharmaceuticals provides the general evaluation of the integrity of the renal perfusion and urodynamics and quantification of functional renal mass. Most of 99mTc –radiopharmaceuticals used for renal scanning fall under four distinct groups of compounds, namely polyalcohols (e.g. insulin, mannitol, sorbitol,etc) , hydroxy acids(e.g. DTPA,GH), mercapto- acid derivatives (e.g. DMSA, MAG-3 ..etc.) and polypeptides (e.g. gelatin caseidin).

1. ^{99m}Tc-DMSA :-

This commonly known as ^{99m}Tc –succiner, and is used in the scintigraphic evaluation of renal parenchymal disorders. In fact, it is currently the first choice radiopharmaceutical for quantitative or visual determination of functioning renal mass. ^{99m}Tc-DMSA kit contains a sterile, pyrogen-free freeze –dried mixture of 1.0 mg dimercaptosuccinic acid, 0.42 mg stannous chloride dihydrate (0.38 mg $SnCl_2$-$2H_2O$), and 0.7o mg onohydrochloric acid are used for PH adjustment. Usually after 10 min incubation, after mixed with saline, the reconstituted solution is ready for i.v injection. The preparation of 99mTc-DMSA requires special attention. Four different complexes can be formed, depending on the PH, concentration of the reactants and the incubation time. These complexes are differing in biological characteristics. The complex more suitable for renal imaging is formed at PH 2.5 .The succiner component of DMSA consists of more than 90% mesoisomer, and less than 10% d, l isomer. Aseptic procedures normally employed in making additions and withdrawal from sterile, pyrogen – free containers, should be used during addition of sodium pertechnetate solutions and during the withdrawal of doses for patient administration. The suggested dose range to the average patient (70 Kg) for renal parenchymal imaging is 74 –222 MBq (2-6 mCi) of ^{99m}Tc-DMSA. About 0.5% of the injected activity is cleared from the plasma and retained in renal cortex 60 min after administration. Approximately 6% of the activity is excreted in the urine within two hours; about 20 % of the activity is concentrated in each kidney at six hours. Acceptable images may be obtained 1 – 2 hours post injection. Delayed images may be taken up to 24 hours for patients with advanced real failure.

2- ^{99m}Tc –GH:

^{99m}Tc- GH (commonly called Gluceptate) is a polyhydroxy monocarboxylic acid, available as sodium or calcium salt. Approximately 50% - 70% of ^{99m}Tc –GH is bound to plasma proteins after administration. It is cleared from the

target organ (kidney) by glumerular filtration (about 8o %) and active tubular secretion (about 20%) into the urine. It is eliminated from the body through renal system excretion, about 35- 40 % over one hour, 45 – 50% over two hours and about 70 % over 24 hours.

3- ^{99m}Tc – DTPA:

It is commonly called 99mTc –Pentetate. DTPA is a water soluble compound that is rapidly eliminated from the body, and available in instant freeze dried kits. ^{99m}Tc DTPA is prepared by adding about 2- 8 ml oxidant free 99mTco4 to the vial. The mixture is then swirled and let stand for required period of time. ^{99m}Tc-DTPA is a very stable compound; hence the problem of re-oxidation and hydrolysis are less frequently encountered with this radiopharmaceutical. It is generally clear, colorless and free from visible, foreign, particulate matter. Two ITL-chromatographic systems are required to obtain radiographic purity of ^{99m}Tc-DTPA:

System A : acetone/ whatman 3mm,

Origin : reduced –hydrolyzed 99mTc and 99mTc-pentetate,

R_fo.8 – 1.0: free ^{99m}Tc- pertechnetate;

System B: Saline (0.9 % Na cl) ITLC – SG,

Origin : reduced - hydrolized^{99m}Tc (RHTC),

R_f0.8 – 1.0: free 99mTc – pertechnetate and 99mTc-pentetate. The minimum acceptable radiochemical purity is 90%. ^{99m}Tc – pentetate is currently used for brain imaging, dynamic renal function studies, lung ventilation imaging and alveolar – capillary membrane permeability studies following conversion of aerosol, and liquid phase gastric emptying half – time determination. Its biodistribution depends on route of investigation of administration and study being performed. Following i.v administration, it is rapidly cleared from plasma into urine with a very little plasma protein binding (3- 5 %). Renal activity peaks 3-5 min post injection via glumerular filtration only.

Following i.v administration, ^{99m}Tc-DTPA is exclusively eliminated by kidneys through 100% glumeriolar filtration without any tubular re-absorption. Renal retention is very little, about 3-4% at one hour and < 1% at 24 hour. Its biological half-life is about 1- 2 hours; about 50% of the injected dose is excreted over 1.5 hour and about 95% of the remaining is excreted over 24 hours.

4-^{99m}Tc MAG- 3:-

This new renal agent presents biological characteristics close to those of 131I – Hippuran. Both compounds are excreted by the kidneys, mainly by tubular secretion. It is available in kits. After addition of oxidant free pertechnetate to the vial contents, it should immediately be placed into boiling water bath for 10 minutes before administration. It has rapid blood clearance and renal excretion via tubular secretion and glumerular filtration.

2.1.4.1.8.4 Bone Imaging agents:-

The most relevant bone imaging agents can be classified into three different chemical structures:

A. Inorganic phosphates that have phosphorous –oxygen bonds, e.g. pyrophosphate and polyphosphates.
B. Organic phosphonates that have strong phosphorous – carbon bonds, e.g. MDP, medronic acid, HMDP, oxidronic acid and HEDP (1- hydroxyl-ethylidene-1, 1 – diphosphonate) – Etidronic acid.
C. Imidodiphosphonates :-

^{99m}Tc –labeled diphosphonates are used for diagnostic bone scintigraphy. Application is especially recommended in case of primary bone tumor and bone metastases of other tumors (e.g. prostate, breast and lung cancers).
The required dose for an adult patient (70 Kg) is 370 – 470 MBq at the time of application. The labeling should be carried out within 3 – 6 GBq pertechnetate activity. In case of children, the activity to be administered is to be determined with Webster's formula:

$$A_{\text{children}}\,(\text{MBq}) = \frac{(N+1) \times A_{\text{adult}}}{N+7}$$

Where N is the child age in years.
After i.v administration, 99mTc – MDP, ^{99m}Tc –HMDP, ^{99mTc} –HEDP and others, leaves the blood and concentrates in the skeleton and, to a negligible extent, to soft tissues.
A much smaller quantity of injected 99mTc-Diphosphonates binds to the blood plasma proteins, which results in a very slight body background. The fraction unbound to the skeleton washes out from the body in the urine.

In patients, approximately half of the injected activity binds to the skeleton .In case of the healthy; this value does not exceed 31%. However, in bone metastases, almost 40% of the total dose accumulates in metastases. Therefore, metastases conspicuously show up against the background on the image of the skeleton. The same applies to the fractures, inflammations osteoporosis and hyperparathyroidism. Maximum bone uptake occurs 1-2 hours after injection and remains unchanged for 72 hours.

Washout from the body proceeds with urine. Maximum activity appears in the kidneys 20 min after injection. At normal kidney function, about 60% of the total injected activity shows up within 6 hours in urine.

2.1.4.1.8.5 Hepatobiliary imaging agents:-

Hepatobiliary scintigraphy is an instrumental imaging tool in the diagnosis and evaluation of hepatobiliary diseases, such as acute and chronic cholecystitis, biliary obstruction and bile leaks for tailoring better approach to the management and treatment of the patient.

Acute cholecystitis is referred to as sudden inflammation of the gallbladder that causes severe abdominal pain. In 90% of cases, acute cholecystitis is caused by gall stones in the gallbladder which obstruct the cystic duct. Severe illness, alcohol abuse and, rarely, tumors of the gallbladder may also cause cholecystitis. An ideal radiopharmaceutical for hepatobiliary imaging should be rapidly extracted from the blood by the hepatocytes, pass rapidly through the hepatocytes and concentrate in the bile. There should be little or no re-absorption from the intestinal tract, and minimal excretion in the urine. The proposed radiopharmaceuticals include ^{99m}Tc-IDA, which is the most important one, and has multiple derivatives. These derivatives are organic anions, predominantly bind to albumin in the circulation and are carried to the liver. Protein binding helps to decrease renal excretion. IDA compounds are taken up by hepatocytes and are not conjugated prior to their excretion (they are excreted unchanged).

Table (2.10) cholescintigraphy protocol.

Radiopharmaceutical	^{99m}Tc- IDA ,di-ethyl IDA or di-isopropyl IDA.
Activity administered	75 MBq (2 mCi). [Up To 300 MBq if patient is Jaundiced] .
Patient preparation	fasting for at least 6 h
Collimator used	low energy, general purpose
Images acquired	Anterior abdomen (liver At top of FOV) at 5, 20, 40 And 60 min, lateral/oblique and delayed images up to 3h in some cases.

2.1.4.1.8.6 Brain imaging agents:-

The principle of brain imaging is governed by a mechanism called the Blood Brain Barrier (BBB) , which excludes many substances from entering the brain from the blood . The BBB is selective : some substances such as water, glucose, sodium chloride …etc , enter the brain readily , whereas compounds such as sodium nitrate, sodium iodide , sucrose, bile pigments and many commonly used radiopharmaceuticals do so with difficulty or not at all . The breakdown of BBB, as in the case of tumors or other diseases, results in the penetration of the latter compounds into the brain.

Based on the principle of BBB, radiopharmaceuticals for brain imaging can be broadly grouped into two categories: diffusible and non-diffusible. Diffusible tracers are typically lipophilic and readily cross the BBB. Examples are 99mTc-HMPAO, ^{99m}Tc-ECD and 18F–FDG. Non-diffusible tracers are hydrophilic and cannot cross the BBB except in abnormal tissues where the BBB is broken. Examples are ^{99m}TcO4 and ^{99m}Tc-DTPA.

In earlier years, in the absence of diffusible radiopharmaceuticals, non-diffusible tracers were the agents of choice for brain imaging. Now these agents are no longer used for brain imaging (Gopal B. Saha 1998).

Table (2.11) Radiopharmaceuticals of brain imaging.

(Diffusible agents)

characteristics	^{123}I-IMP	^{99m}Tc-HMPAO	^{99m}Tc-ECD	^{99m}Tc-FDG	^{18}F-fluoro-dopa
$T_{1/2}$ (physical)	13.2 h	6 h	6 h	110min	110 min
Photon energy (Kev)	159	140	140	511	511
Usual dose (mCi)	3 – 5	10 - 20	10 - 20	5 – 10	5 – 10
Usual time for imaging (h)	0 – 3	0.3 - 2	0.5 - 1	0.6	0.3-0.5

NB : 1 mCi = 37 MBq

$^{99m}TcO_4$, ^{99m}Tc –DTPA, and ^{99m}Tc-glucoheptonate are the three principals radiopharmaceuticals used for brain imaging. When injected intravenously, each will demonstrate areas of increased vascularity and pass through a damaged BBB. $^{99m}TcO_4$ is the least expensive but has the disadvantage of accumulating in the normal choroid plexus and the thyroid and salivary gland. Because of this, oral potassium per chlorate, 500 mg, is administered 30 -45 min before injection. Image quality using ^{99m}Tc-DTPA and ^{99m}Tc – glucoheptonate is better than that obtained with 99mTcO4 due to their rapid plasma clearance and their lack of uptake by the choroid plexus and salivary glands. Moreover, false negative brain scan may be obtained with ^{99m}TcO4 when a bone study has been performed in the recent past.
N.B characteristics of ^{99m}Tc- DTPA and ^{99m}Tc –glucoheptonate have already been mentioned within the part of renal imaging agents (P.F Sharp et al 1989).

2.1.4.1.8.4 Thyroid imaging agents:

Radionuclide imaging plays an important role in the investigation of patients with thyroid disorders, especially those with solitary nodules. It serves to confirm the presence of a nodule within the thyroid identifies the function characteristics of the nodule and may demonstrate the presence of multiple nodules. A variety of radiopharmaceuticals has been and is currently employed for this purpose. $^{99m}TcO_4$ is the most readily available radionuclide employed for thyroid imaging. Pertechnetate ions are trapped by the thyroid in the same

manner as iodine through an active transport mechanism, but are not organified (P.F. Sharp et al 1989). Thyroid imaging agents include:-

i. Sodium iodide ^{123}I :

This agent has excellent physical properties as an imaging agent. It decays by electron capture with photon energy of 159 Kev and a half –life of 13 hours,(see table 2.9). The gamma emission of ^{123}I allows excellent imaging with low background activity. The major disadvantages of this agent are high cost, because it is produced by cyclotron, and problems with availability and delivery. Despite these restrictions, ^{123}I is the iodine of choice for thyroid imaging (Al Najar B, 1996).

^{123}I is supplied in a capsule or a solution for oral administration. It may contain other chemical forms of radioactivity, and these should not exceed 5% of the total radioactivity, according to the USP XXV111 (Gobal B. Saha, 1998).

^{123}I is preferred to $^{99m}Tc\ O_4$ in cases of thyroid ectopic tissues, because it shows greater uptake in the thyroid compared with salivary glands. It is also preferred incase of retro-sternal goiter due to much lower background activity in the rest of the mediastinum area. It is also used for whole body imaging for detection of distant metastasis of thyroid cancer (Yousef, 2010).

ii. ^{99m}Tc – pertechnetate ($^{99m}Tc\ O_4$) :-

Thyroid imaging is most often performed through the use of 99mTc O_4. (Becker et al 1996). It is an ideal choice for thyroid imaging because of its six-hour half –life, 140 Kev gamma emission and ready availability either from molybdenum generator or when purchased in bulk.(Atkins HL, Richard P). The short physical half –life (six hours) and isomeric transition mode of decay of 99mTc result in a more favorable patient radiation absorbed dose than 131I, and permit routine use of several milli-curies of 99mTcO4for diagnostic studies. Atypical scanning dose of ^{131}I (100 µCi) results in an absorbed dose to the adult thyroid of 20 rad and a one rad whole body dose , whereas a 4 mCi dose of $^{99m}TcO_4$ yields an absorbed dose of less than one rad to the thyroid and 0.1 rad absorbed dose to the whole body . (Paul E.Christian et al, 2004).

The most common artifact of $^{99m}TcO_4$, when injected intravenously for thyroid studies, is produced by activity secreted by the salivary glands and swallowed by the patient. This usually presents a linear area of esophageal activity in the midline of the image. If this complicates interpretation or causes confusion

with enlarged pyramidal lobe, repeat imaging should be performed using oblique images or after cleaning the esophagus by drinking water (Fred A. Mettleret al, 2006).

In case of congenital organification defect of thyroid, radioiodine scan usually shows no activity in the thyroid, and is not possible to distinguish this from an absent thyroid. In this case 99mTc O_4 is used as it clearly shows the presence of thyroid because the trapping mechanism of the gland is intact (Yousef, 2010).

iii. ^{131}Iodine :-

^{131}Iodine decays by beta emission and has a half – life of 8 days. The principle gamma emission of 364 Kev is considerably higher than the ideal for imaging with gamma cameras .The major disadvantages are its long physical half- life and high beta emission which cause relatively high radiation dose to the thyroid(about one rad / µCi),although the whole body dose is acceptable. The high radiation dose makes ^{131}I undesirable for routine thyroid imaging (Yousef, 2010).

There is no justification for using ^{131}I for diagnostic imaging of the thyroid gland itself. Whole body 131I imaging is used in patients with known thyroid carcinoma to identify residual thyroid bed activity and distal metastasis (Paul E. Christian, 2004).

Recently, however, it has been suggested that whole body 131I is less sensitive for the detection or exclusion of metastatic thyroid carcinoma than the post-recombinant TSH serum Thyroglobulin level (Robins RJ et al, 2002).

^{131}I is used to treat hyperthyroidism (Graves' disease, multi-nodular toxic goiter and toxic solitary nodule or adenoma), and to ablate normal thyroid tissue or treat thyroid carcinoma with local or distal metastases. The beta particle emission during 131I decay results in a profound radio biologic effect (Paul E. Christian, 2004).

iv ^{125}I :

^{125}I is not used for thyroid imaging because of its low energy (27.4 and 35 Kev) photons which are too weak to penetrate the neck tissue and to yield considerable radiation counts for a scan of good quality. It is used mainly for RIA studies as 125I – radio iodinated serum albumin (RISA), which is prepared under certain chemical, PH, temperature and medical conditions.

Table (2.12) Radio pharmaceuticals used in thyroid imaging

R.Ph.	Usual dose	Administration Route	Physical $T_{1/2}$	Imaging time(hr)
$^{99m}TcO_4$	4 mCi	I.V	6 h	0.3 – 1.0
^{123}I	200-600µCi	oral and I.V	13 h	1-2
^{131}I	4 -6 µCi	Oral	8.04	4 – 24

Table (2.13) Imaging protocol of thyroid in RICK:

Patient preparation	Discontinuation of medications that interfere with thyroid uptake.
Radiopharmaceutical	$^{99m}TcO_4$.
Activity administered	5 mCi (adult dose).
Time of imaging	20 min. post injection
Patient position	Supine, neck extended, head Immobilized.
View	Ap , with marker to the SSN
Total counts	300 Kc's
Collimator	LEGP.
Matrix size	256 x 256
Window size	20 %.

2.1.4.2 Quality control tests of radiopharmaceuticals:

2.1.4.2.1 Introduction:

Quality control in radiopharmaceutical preparation is important to protect the patient and to ensure that the radiopharmaceutical localizes in the intended area. As with any parenteral substance, radiopharmaceuticals must be sterile and free of pyrogens and chemical, radiochemical, or radionuclidic impurities. Throughout the preparation process, Q.C of radiopharmaceuticals is primarily performed on ^{99m}Tc – radiopharmaceuticals. The first step in Q.C. is to ensure that the radionuclide is pure (Fred A. Mettler 2006).

Quality control tests must be performed after the final step of preparation of the radiopharmaceutical. The quality control tests associated with the kits formulation and production of radionuclides from primary sources are performed by manufacturers, while those associated with the final step of the preparation of the radiopharmaceutical are performed in the radiopharmacy. When a labeled compound is prepared for use in human body or in animal tests, extremely high standards of purity have to be maintained. The QC of radiopharmaceuticals before use when prepared in-house is of paramount importance as it serves to assure that the person preparing the radiopharmaceuticals has not made some blunder in carrying out the instructions of preparing the generators and the kits . The QC also permits the preparer to assure that the used kit gives the tagging .In addition , it provides information about whether the preparation can be successful in situations in which following the exact instructions was not feasible or was not what the preparer deemed best for the current situation (Sam,2008 , Sam and Derar 2009).

Some QC requirements, like toxicity, are the responsibility of the manufacturer. Toxicity is the negative pharmacologic response of the tissue to the administered drug. Radiopharmaceuticals do not evoke a pharmacologic response because only a minute amount of the tracer is used. However, the manufacturer is required to assure that the non- radioactive component of the radiopharmaceutical is not toxic at the milligram level. This is because toxicity arises from the pharmaceutical part of the radiopharmaceutical, not from the radionuclide part (Sam, 2008, James H. Thrall, 2001).

Like all other drugs for human administration, radiopharmaceuticals should undergo strict and routine quality testing procedures in addition to their own specific tests for radionuclidic and radiochemical purity (N.M Khalid, 2011).

The Q.C tests can be simply classified as physiochemical and biological tests. Physiochemical tests include physical appearance, particle size, PH, and chemical, radionuclidic, and radiochemical purity, in addition to radionuclide concentration and determination of isotonicity. Biologic tests establish sterility, apyrogenicity, toxicity of the material and biological distribution. Tests such as sterility, apyrogenicity and toxicity, isotonicity and PH, and absence of foreign particulate matter represent common criteria apply to all inject able pharmaceutical products. Some of these tests can be measured directly on products prior to use. However, one of the characteristics of many diagnostic radiopharmaceuticals is the inclusion of short – lived radionuclides. These radionuclides often have half –lives measured in hours, minutes or even seconds, making meaningful testing of the end products impractical. Most diagnostic radiopharmaceuticals ^{99m}Tc with a half – life of six hours. In these circumstances, great emphasis must be placed on adoption of an effective program of QA to minimize the risk and maximize the benefits. There are two divergent opinions as to whether all or some of these tests are to be performed daily or only as an aid to trouble – shooting. The authors advocate that these tests should be performed daily prior to administration of radiopharmaceuticals (Sam, 2008, Azuwuikeet al 1994).

Physical characteristics should be observed for any radiopharmaceutical for the first and frequent use. Color alterations should be identified for both true solution and colloidal preparations .True solutions should also be checked for turbidity and presence of any particulate matter; in colloidal preparations, determination of particle size is of most interest. Radiochemical purity is defined as the proportion of the stated radionuclide that is present in the stated chemical form. Image quality (as a function of the radiopharmaceutical biological distribution) and the radiation absorbed dose are directly related to the radiochemical purity. Radiochemical impurities are produced from decomposition due to the action of solvent, change in temperature or PH, light, presence of oxidation or reducing agents and radiolysis. The stability of a compound is time - dependent and; the longer the time of exposure to light is, the higher the probability of decomposition will be. Sodium ascorbate, ascorbic acid and sodium sulfite are normally used for maintaining a radiopharmaceutical stability (N.M Khalid2011).

Quality control is the most important factor to consider for the patient care in general. Quality of the radiopharmaceutical should be of an acceptable standard before it is administered to patients. Impure radiopharmaceutical may create unwanted problems and could lead to false diagnosis. Professional results are dependent upon using good quality radiopharmaceutical in the purest acceptable form .Impurities will change the imaging statistics due to the different distribution characteristics of labeled technetium , free pertechnetate and colloid (David Henderson et al,2000).

Chemical purity must be carried out routinely although analytical tests to check radionuclidic, radiochemical and results are obtained retrospectively. This also applies to the routine sterility and pyrogen testing which must be carried out on parenteral radiopharmaceuticals. This is achieved by the following:

- All procedures being documented and strictly observed.
- The keeping of accurate and up-to-date records.
- Routine monitoring of the production environment with respect to microbiological, particulate and radiological examinations.
- Planned preventive maintenance on equipment and instruments routinely used within the department, including calibration check (P.F.Sharp, 1989).

Good housekeeping and clean dress are essential to good radio-pharmacy practice .Housekeeping is a prime responsibility of the radio-pharmacist. The responsibility for the approval and release of the final products lies with the radio-pharmacist and not the QC pharmacist .The role of radio-pharmacist in the QC should be clearly defined and as much of QC work as possible be left to QC personnel (Buck A. Rhodes, 1977).

Types of impurities expected in ^{99m}Tc – radiopharmaceuticals can be classified into three categories as follows:

1- Radionuclidic impurities e.g.^{99}Mo.
2- Chemical impurities e.g. Al^{+3} ion.
3- Radiochemical impurities : labeling of ^{99m}Tc to different ligands results in presence of the following radiochemical impurities as by – products in the final preparation :-

a- Free pertechnetate (^{99m}Tc O_4), due either to the presence of insufficient reducing agent (Sn^{2+}), in the vial or prior oxidation of the added Sn^{2+} to Sn^{4+} by air in vial.
b- Hydrolyzed reduced ^{99m}Tcspecies ((RHTc) , such as :

- ^{99m}Tc – tin colloid :

  $Sn^{2+} + H_2O \longrightarrow$ tin hydroxide.

  Tin hydroxide + Tc $\longrightarrow$ colloid.

  Hydrated Tc – oxide ($TcO(OH_2).H_2O$).

  $Tc(VII) + Sn^{2+} \longrightarrow Tc(IV) + Sn^{3+}$.

  $Tc(IV) + H_2O \longrightarrow TcO_2$ (insoluble in water).

  In addition, ^{99m}Tc tartrate can be formed as a radiochemical impurity in the preparation of ^{99m}Tc- MAG-3, and also stereo chemical impurities are expected in ^{99m}Tc-HMPAO (Sam, 2008).

  Free pertechnetate could accumulate in organs such as the thyroid, stomach and salivary glands. While imaging other organs in these regions, it could influence the underlying structures and mask the observation. Free pertechnetate also creates an unwanted radiation burden on other organs.

  Similarly, the colloids (small particles) could accumulate in the liver and spleen and create an unwanted radiation burden and mask imaging of other organs in these areas (David Henderson et al 2000).

  The preparation is said to be radionuclidically pure if it is free from radionuclides of other elements and if the activity lies in a single chemical form (C. Keller, 1988).

  Here are examples of Q.C tests:

2.1.4.2.2 Physical appearance test:

Prior to iv injection, the radiopharmaceutical is visually examined to assure that it is either particulate or a true solution. Particulates include colloids, macro-aggregated albumin, micro-spheres and blood cells. True solutions include all other liquid radiopharmaceuticals. The test is done for observing any change in color, turbidity or presence of particles in non- particulate (true) radiopharmaceutical solutions. The physical appearance is important both on receipt and subsequently. One should be familiar with the color and state of a radiopharmaceutical. Any deviation from the original color and clarity should be viewed with concern because it may reflect changes in the

radiopharmaceutical that would alter its biologic behavior, and it should not be administered to humans (Gopal B. Saha, 2010).

2.1.4.2.2 Particle size test:-

Colloidal or aggregate preparations should be checked for size, e.g. in sulfur colloid preparations, the particle size (0.1 – 1 µm) may vary considerably from batch to batch , and can be checked by means of a microscope .

In MAA preparations particle size varies between 10 and 100 µm. Preparations of particles larger than 150µm should be discarded because of the possibility of pulmonary arterial blockade by these particles (Gobal B. Saha, 2010).

Particle size control is essential in applications in which radiopharmaceuticals in the form of particulate suspensions. These applications include lung scanning, liver/ spleen scanning and lymph node detection. For example, a lung scanning agent containing particles which are too small (less than 0.3 µm) can show liver uptake. The presence of large particles (greater than 500 nm) in lymphatic agents can result in poor clearance from the injection site, resulting in poor visualization of the lymph.

Standard particle size measurement technique , including microscopy , laser – light scattering , Coulter counter and filtration , can all be applied to measure this parameter .The particle size of colloids is checked with an ultra-microscope and should be 1-100 nm (Sam, 2008) .

2.1.4.2.3 Molybdenum breakthrough test:

This is one of the so-called radionuclidic purity tests; the other is Aluminum breakthrough test. Radionuclidic purity is defined as: the proportion of the stated radionuclide that is present in the stated chemical form (18 N.M Khalid, 2011).

^{99}Mo activity is likely to be eluted in trace quantities along with ^{99m}Tc activity. This is called the ^{99}Mo or Moly breakthrough. The presence of ^{99}Mo gives unnecessary radiation dose to the patient. According to the Nuclear Regulatory Commission (NRC) regulations, the acceptable limit for ^{99}Mo breakthrough is 0.15 µCi (5.5 KBq) per millicurie (37 MBq) of ^{99m}Tc at the time of injection. As a rule, if the ratio is less than 0.038 at time of elution, the material will be suitable for injection for at least 12 hours .^{99}Mo breakthrough is determined by the detection of high energy photons (740 Kev – 780 Kev)of ^{99}Mo in a dose

calibrator after stopping the 140 Kev photons of ^{99m}Tc in a lead container (6mm thick). The above mentioned limit is obtained by dividing the activity (µCi) of ^{99}Mo by the activity (mCi) of ^{99m}Tc (Gobal B Saha 2001 , Sam A. K ,2008) .It is well known that 4 mm of lead shielding reduces the ^{99m}Tc radiation by a factor of 5x 10^4 , whereas the ^{99}Mo radiation is reduced by a factor of 2 (Fred A. Mettler 2006 ,Sam A. K ,2008).

The test procedure can be done as follows:-

a- Place the empty lead shield in the dose calibrator and zero the calibrator.
b- Place the eluate in the lead shield and record the activity reading using the ^{99}Mo assay dial setting.
c- Remove the lead and record the activity at the ^{99m}Tc setting.
d- Determine the activity ratio by dividing the ^{99}Mo activity by the ^{99m}Tc activity, which should be less than 0.15 µCi^{99}Mo per mCi^{99m}Tc, and less than 5 µCi per dose given to the patient, as mentioned before. Deviation of ^{99}Mo activity may be due to the lead container not fulfilling the measurement requirements, so the lead shielding used must be of homogeneous quality and contains no holes in the casting. The presence of holes will allow part of the ^{99m}Tc activity to contribute to the ^{99}Mo measurements and results in high ^{99}Mo values .For any patient injected with larger amounts of ^{99}Mo, testing of the generator eluate for the presence of this radionuclide impurity is mandatory (Sam, 2008).

2.1.4.2.4 Aluminum ion breakthrough test:

Contamination of eluates with alumina from column chromatographic generators has been a source of considerable concern. Labeling procedures can be adversely affected by small quantities of aluminum compounds, and this contamination should be monitored often (Buck A. Rhodes, 1977).

Soluble "aluminum ion breakthrough" can, under certain conditions, appear in the eluate. While not harmful to the patient, it interferes with preparation of ^{99m}Tc- RBC labeling, ^{99mTc}-bone agents and ^{99m}Tc-sulfer colloid and thus causes degradation in image quality due to poor incorporation of ^{99m}Tc into the carrier. Very large colloid particles (sulfur colloids) , which may be produced during preparation of ^{99m}Tc – SC , due to high levels of aluminum ions in the eluate , may result in increased lung uptake at the expense of liver uptake. It is therefore, mandatory to check every eluate of ^{99m}Tc generator for aluminum ion impurity and to prove that the level of this chemical impurity does not

exceed 10µg Al^{3+}per ml of eluate which is recommended as an acceptable limit (James H. Thrall, 2001, Sam, 2008).

The test kit that measures Al^{3+} ion is a special colorimetric test paper strip containing a chemical sensitive to the presence of aluminum ion at the microgram level. The test is performed by placing a drop of the eluate on one end of the test paper, a drop of a standard solution of Al^{3+}, with concentration of 10 ppm, is placed on the other end of the test strip. If the color at the center of the drop of eluate is less red than that of the standard solution, the eluate has passed the aluminum breakthrough test, i.e. the eluate contains less than 10 µg/ ml of Al^{3+}, which is the limit set out by the USP, and lies within the BP and EP limit of 20µg/ml. The test may be carried out on the remains of the first eluate from each generator (Sam 2008, P.F.Sharp1989).

2.1.4.2.5 Radiochemical purity test:

The European Pharmacopoeia(EP) , The British Pharmacopoeia (BP) and the United States Pharmacopoeia (USP) defined the radionuclidic purity as : (the ratio of the radioactivity of the radionuclide concerned to the total radioactivity of the source) .It was also defined as : the proportion of the stated radionuclide that is present in the stated chemical form (P.F.Sharp ,1989, N.M Khalid, 2011) .

The USP minimum acceptable radiochemical purity level is 90% for ^{99m}Tc – MAA, ^{99m}Tc –DMSA, ^{99m}Tc – DTPA, ^{99m}Tc- MDP, ^{99m}Tc –MAG3…..etc. Radiochemical impurities arise from decomposition due to the action of solvent, change in temperature or PH, exposure to light, presence of oxidizing or reducing agents and radiolysis. It is very important to control the levels of radionuclidic impurities in the radiopharmaceuticals strictly, as their presence will result in poor- quality images due to poor localization in the organ of interest and the high background from surrounding tissues. The impurities change the imaging statistics due to the different distribution characteristics of labeled technetium, free pertechnetate and colloid. It could also potentially increase the radiation dose to the patient. Image quality (as a function of the radiopharmaceutical biological distribution), and the radiation absorbed dose are directly related to the radiochemical purity. Free and hydrolyzed ^{99m}Tc forms in many ^{99m}Tc – labeled preparations, secondary HMPAO complex in ^{99m}Tc- HMPAO preparations and free ^{131}I – iodine in the ^{131}I – iodine labeled proteins are good example of the radiochemical impurities. The effect of

impurities depends on their concentration and type of clinical investigation (Sam, 2008, N.M. Khalid, 2011).

The substance is said to be radio-chemically pure if it is uncontaminated with other substances containing the same radionuclide, or with inactive substances (C. Keller,1988).

Several analytical methods are used to determine the radiochemical purity of a given radiopharmaceutical, including:-

Thin Layer Chromatography (TLC): which was developed by Hye in 1967, is considered the most commonly used method for the determination of radiochemical purity in nuclear medicine. The main principle of a TLC system is that a mobile phase (solvent) migrates along a stationary phase (adsorbent) by the action of the capillary forces. Depending on the distribution of components between the stationary and mobile phases, a radiopharmaceutical sample spotted onto an adsorbent will migrate with different velocities, and thus impurities are separated. In ITLC, each component in a given sample is identified by an Rf value, which is defined as (the ratio of the distance traveled by the sample component to the distance the solvent front has advanced from the original point of starting the chromatography test in the stationary phase) .The main principles of separation are adsorption (electrostatic forces), partition (solubility) and ion exchange (charge) , and the movement of the mobile phase may take either ascending or descending modes. When the solvent front moves to the desired distance, the strip is removed from the testing container, dried and measured in an appropriate radiation detector; histograms are obtained for the activity of all sample components (N.M. Khalid, 2011).

The standard TLC materials include: standard TLC plates which are available as glass plates, plastic or aluminum foils covered with the stationary phase. A wide range of stationary phases are commercially available, including silica gel, reversed – phase silica, aluminum oxide, synthetic resins and cellulose. The main advantage of standard TLC materials is that they have the ability to provide relatively high resolution test , while the relatively long developing time of the mobile phase (mainly longer than 30 min) through the high - particle –size adsorbent material (20μm) , is considered its main disadvantage.

Saline, water, acetone Methyl Ethyl Ketone (MEK), ethanol acetic acid chloroform and acetonitrile represent the most common group of mobile

phases used as the mobile partner in most TLC procedures (N.M. Khalid , 2011).

In the Instant Thin Layer Chromatography (ITLC) , the plates are made of fiber glass sheets integrated with an adsorbent , usually silica gel , and can be cut to any size , developing an economic chromatographic solution . Due to the fine mesh material , the migration properties are increased many fold compared to the standard TLC materials , reducing the chromatographic time to less than 5 min without affecting the separation of radiochemical impurities . Because of these advantages, ITLC materials are the most frequently used for the stationary phase in nuclear medicine since they fulfill the need for a rapid and accurate method for testing the radiochemical purity of a radiopharmaceutical sample. Silica stationary phases have been produced for ITLC as silica gel (ITLC – SG) and silica acid (ITLC –SA). ITLC – SG is the most widely used adsorbent for routine radiochemical purity determination. Papers (whatman no. 1 and whatman 3MM) were commonly used in the early days of chromatography, although they are still used and recommended for many chromatographic procedures. The main disadvantage of paper chromatography is the poor resolution it provides; however, whatman 3MM is the material of choice for partition chromatography procedures. The third type is the aluminum oxide (aluminum – coated) plates which are commonly used for separation of some radiopharmaceuticals (e.g. sesta MIBI). The forth material is cellulose which can interact with water and serve as a stationary phase for separation of polar substances by paper chromatography; also, it can be used in powder form as an adsorbent for TLC (N.M. Khalid, 2011).

The stationary and mobile phases are used such that one determines the level of hydrolyzed / reduced (HR – Tc) impurities, while the other determines free $^{99m}Tc\ O_4$ (Tc). By calculating the ratio of activity associated with the impurity to the activity of the total strip, the amount of impurities present may be quantified, and the radiochemical purity may be calculated using the equation:

Radiochemical purity ratio = 100 – (%Tc + % HR – Tc) (Sam, 2008).

Three Tc species may co-exist in any ^{99m}Tc – labeled preparation, as radiochemical impurities, namely: free pertechnetate, hydrolyzed reduced and bound 99mTc. Both free and hydrolyzed reduced ^{99m}Tc can give artifacts on scans, which may mislead diagnosis or make assessing scans difficult. It is recommended that every radiopharmaceutical is checked for HRTC, which is a radio-colloid taken up in the liver and spleen. Its response in the drug causes

degradation in image quality due to poor incorporation of ^{99m}Tc into the carrier (Sam, 2008).

Paper chromatography or ITLC is routinely used to estimate the amounts of these three components and hence the labeling efficiency .ITLC is an adsorption chromatography in which sample components are separated , based on the interaction between a thin layer of adsorbent (250mm) and a selected solvent .It is a simple and inexpensive technique commonly used to determine the number of components in a mixture , identify the identity of the compounds and judge the purity of a synthesized compound or to monitor the extent of progress of a chemical reaction . It also permits the optimization of the solvent system for a given separation problem .In comparison with column chromatography, it only requires small quantities of the compound and is much faster as well. In principle, a minute amount of radiopharmaceutical solution is spotted on a paper (Whatman filter strip) or an ITLC plate coated with adsorbent such as silica gel (SG), poly silisic acid (SA), or alumina (Al_2O_3) and then dipped into an appropriate solvent contained in a developing chamber. The different components in the sample will distribute themselves between stationary phase (adsorbent) and the mobile phase (solvent), depending on their distribution coefficient. Electrostatic forces of the stationary phase tend to retard different components , while the mobile phase carries them along .This effect ,combined with the solvent polarity and the solubilities of the different components in the solvent , cause the individual components to move at different speeds and appear as spots at different distances along the stationary phase . The purity of solvents used is important to ensure reliability of test results. Contaminants, such as water or other chemicals, can alter the system and give inaccurate results .Solvents can evaporate, absorb moisture from the air or become contaminant with other solvents. Usually 85% methanol, acetone methyl ketone (MEK) , or saline are the most commonly used solvents .Each component is characterized by an Rf value in a given solvent / media system ,which is defined as the ratio of the distance traveled by the component to the distance the solvent front has advanced from the point of application . These values are established with known standard and used for identification of individual components in a given radiopharmaceutical. It is obvious that the separation patterns of the radiochemical species obtained are either at the origin (Rf = 0) or at the solvent front (Rf = 1) (Sam, 2008, Abdel Salam, 2005).

In a typical chromatography, 5 to 7 cm long and one cm wide ITLC or whatman no. one or three strips are used for analysis of ^{99m}Tc radiopharmaceuticals. A small sample volume is spotted at the origin of the chromatographic strip, and it is then placed vertically in a developing chamber (usually 20 ml vial fitted with a screw cap) containing the appropriate solvent. The solvent front moves to a desired distance through capillary action carrying the deposited substances along with it at different rates. As the solvent moves past the spot, equilibrium is established for each component of the mixture between the molecules of that component which are adsorbed on the solid and the molecules which are in solution. The components will differ in solubility and in the strength of their adsorption to the adsorbent, solvent system, amount of material spotted and temperature. Therefore , some components will be carried further up the plate than others .In addition , an impure sample will often develop as two or more spots , while a pure sample will show only one spot . When the solvent has reached the top of the plate, the plate (strip) is removed from the developing chamber, dried and the separated components of the mixture are visualized. If the compounds are colored, visualization is straightforward. Otherwise, the separated spots are visualized with UV light or by placing the plate in iodine vapor. The components then appear as a series of spots at different locations up the plate. The different components in a given sample can be identified from their ratio of fronts (RF) as follows:

$$Rf = \frac{\text{Distance from origin to center of substance spot}}{\text{Distance from origin to solvent front.}}$$

Rf is a characteristic for any given compound on the same stationary phase using the same mobile phase for development of the plates .The most effective way to identify a compound is to spot known substances next to unknown substances on the same plate. The Rf values range from 0 to 1.0 .Rf value of 0.5 means that the spot corresponding to the substance travels exactly as far as the solvent travels along the plate .If a component migrates with the solvent front , the Rf is 1 , while the Rf for the component remaining at the origin is (0) . Rf values are established with known components and may vary under different experimental conditions (Sam, 2008, Abdel Salam, 2005).

For insoluble ^{99m}Tc- radiopharmaceuticals (e.g.-MAA, Tc-MIAA), only one solvent chromatography is necessary to test for the presence of free

pertechnetate. HRTc cannot be measured in these products, as there is no simple system can effectively separate colloidal HRTc from an insoluble product (Abdel Salam, 2005).

Performing the ITLC analysis consists of a number of steps: 1- Preparation of the developing container .2- Making the ITLC plate 3- Activation of the ITLC plate 4- Spotting the sample on the ITLC plate 5- Development of the ITLC plate 6- Drying the plate 7-Vizualization of the substance spots and 8- Measurement of the Rf values and data analysis. These steps can be discussed in details as follows:

Preparation of the developing container:

The container can be a specially designed chamber, a jar with a lid, or a beaker with a watch glass on top. Pour the mobile phase (solvent) into the developing tank to a depth of 0.5 cm.

Marking the ITLC plate:

Obtain a silica gel TLC plate that is approximately 2 cm wide and 5 cm long. Mark the ITLC plate as follows; using a pencil (pencil must be used rather than pen because inks are moved by many developing agents):

a- Lightly draw a straight line parallel to the short dimension of the plate, about 1cm from one end of the plate. Don't scratch the silica gel or make a trough with the pencil.
b- Lightly make two small marks perpendicular to this line to divide the line into thirds. These subdivided lines will serve as a guide for placing the substance spots and as a point from which to measure Rf values.
c- Lightly draw a second line parallel to the first line and about 1cm from the other end of the plate. The solvent front will be allowed to rise to this second line when developing the plate. Handle the plates carefully in order to avoid disturbing the coating of adsorbent or get them dirty.

Activation of the ITLC plate:

Place the marked ITLC plate in oven at $50\text{-}60^0$ for 15 – 20 min. to activate it. Activation involves driving off water molecules that bind to the polar sites on the plate.

Spotting the ITLC plate:

The sample to be analyzed is added to the plate in a process called (spotting). The spotter can be a glass capillary or 1 µL microcap. The thin end of the spotter is dipped into the analyzed solution and the solution will then rise up in the capillary by capillary forces. Touch the plate briefly at the start line and allow the solvent to evaporate and spot at the same place again to get a concentrated small spot (if the solvent is water evaporation will be slow; it may be hastened by placing the plate in the oven for 5 minutes). Avoid spotting too much material; because this will deteriorate the quality of the separation considerably (tailing) .The spots should be far enough away from the edges and from each other as well. If possible, spot the compound or mixture together with the starting materials.

Development of the ITLC plate:

An ITLC plate can be developed in a beaker or closed jar. Place a small amount of solvent (mobile phase) in the developing container to a depth of 0.4 – 0.5 cm. Using tweezers, pick up the ITLC plate at the top, which is the end opposite where the subdivided pencil line is drawn. Place it carefully in the developing container so that it stands somewhat , but not excessively , tilted, i.e. the bottom of the plate should be somewhat away from the wall of the bottle, while the top of the plate rests against the wall of the bottle . It is important not to allow the ITLC plate to tilt too much in the developing tank. If the plate is excessively tilted, solvent will not advance uniformly along the plate and development will not take place properly. Similarly, if the bottom of the plate is against the wall of the tank, solvent will advance more rapidly up the edges of the plate than in the middle, causing the substances to be pushed toward the center of the plate as they move up. Leave the plate in the tank until solvent has advanced to the top pencil line on the plate. Development normally requires at least 30 min. When the solvent front has advanced to the top pencil line, use the tweezers to withdraw the plate from the tank .Non- polar solvents will force non- polar compounds to the top of the plate, because the compounds dissolve well and do not interact with the polar stationary phase.

Drying the ITLC plate:

Place the plate flat on a clean dry surface and allow the solvent to completely evaporate. If the solvent is not highly volatile, this can be facilitated by placing the plate on a flat surface in an oven at a temperature of 50 - 60^0c (higher

temperature will melt the plastic substrate material). When the plate is completely dry, it is ready for visualization.

Visualization of the developed ITLC tank:

If the substances being separated are colored, the spots can be seen without any further effort. Using a pencil, draw a boundary around each spot that matches the shape of the spot. Many substances are colorless and don't show up on the white silica gel unless steps are taken to make them visible. These include the use of iodination, ninhydrin technique, ultraviolet lamp….. etc.

Measurement of the Rf and data analysis:

The components visible as separated spots, are identified by comparing the distances they have traveled with those of the known reference materials. The distance between the two horizontal pencil lines is the distance of solvent advance. Measure the distance of the start line to the solvent front (=d). Then measure the distance of center of spot to the start line (=a). Divide the distance the individual spot moved by the distance the solvent moved. The resulting ratio is called Rf (ratio of the fronts of retardation factor).

There are some problems encountered in ITLC analysis, these include:

a- Over-large spots (smear): sample spots made by using ITLC capillaries should be no larger than 1 – 2 mm in diameter, because component spots in the developed plate will usually be larger than the size of the initial spot. If the initial spot is larger than 2 mm in diameter, then components with similar Rf values may not be resolved properly because their spots will be so large that they will overlap considerably and may appear to be one large spot. Small initial spots, on the other hand, maximize the potential of complete separation of components.

b- Uneven advance of solvent front: A common problem in ITLC is uneven advance of solvent along the plate. Instead of a straight line, the solvent front may appear to bow either up or down in the center. Uneven advance of solvent leads to uneven advance of substance spots, and inaccurate Rf values. A frequent cause of uneven solvent advance is the use of non-flat bottomed developing tank. Glass bottles usually have bottoms that curve upward from the edges to the center. When placing the ITLC plate on such curved surface, the shape of the solvent line may mirror the shape of the container bottom. It is therefore important to use flat-bottomed developing tanks in ITLC. A bowed solvent front may also result from placing too little developing solvent in the

tank; improper cut of the plate so that the sides are not exactly perpendicular to the bottom edge; and from the excessive tilting of the plate in the chamber. Care in choosing and using a developing chamber is the best defense against curved solvent fronts. Water is seldom used as a developing solvent because it has a tendency to produce a dramatically curved front. This may be due to its high surface tension.

c- Streaking: Sometimes a substance will move along ITLC plate as a long streak, rather than as a single discrete spot. This is the result of spotting the plate with highly concentrated substance, more than the moving solvent can handle. The solvent moves as much substance as it can, but a substantial amount of substance is left behind. The substance is dragged along by the solvent leaving a trail of substance that may sometimes span the entire distance between the start line and the solvent front. Streaking can be eliminated by systematically diluting the spotting solution until development and visualization show the substances moving as single spots, rather than elongated streaks.

d- Distorted solvent front: this may be due to evaporation of solvent in the tank, strip touching the side of chromatography tank, and tank moving after the strip has been placed therein.

e- No separation: this is caused by: incorrect solvent used, incorrect ITLC medium used and incorrect sample (Sam, 2008, David Henderson et al, 2000).

In order to reduce any error chance in chromatography, here are some helpful hints:-

- Perform analysis in duplicate.
- Minimize sample spot on the chromatographic strip (use 25-27 gauge needle).
- Use gloves when handling strip and when sampling the radiopharmaceutical.
- Always use a pencil in marking the origin and solvent front, because if a pen is used, the inks will be separated up the strip.
- Use correct and fresh solvent.
- Use good mixing of solvent.

In conclusion, chromatography is the preferred technique for measuring radiochemical purity because: it is simple to use, quick and easy to perform, inexpensive and readily available, consistent and its results are comparable with Gold Standard Methods. ITLC-SG is superior to paper chromatography (TLC) because the solvent moves faster, separation is quicker, and the separation appearance is distinct due to electrostatic charge interaction with

solvent. It is extremely useful to perform in cases where unexpected imaging occurs. In these cases, if chromatography confirms that the radiopharmaceutical is in the correct form, you will then have to look at other causes for imaging abnormalities. If, however, the analysis proves that the radiopharmaceutical is not correctly labeled i.e. large percentage of free pertechnetate is present; the reconstitution labeling method employed should be checked. Also, vials from the sample batch should be examined visually to check that there is in fact reagent in the vial or that the product has not expired …… etc. (David Henderson et al, 2000).

2.1.4.2.6 Sterility test:

Sterility refers to absence of any viable bacteria or micro-organisms that could develop into something living. All preparations for human administration must be sterilized by suitable methods that depend on the nature of the product, the solvent and various additions. Assessment of sterility is most commonly performed by culturing samples with special growth media. There are four methods of sterilization used in pharmaceutical practice, namely sterilization by moist heat, by dry heat, by membrane filtration and by radiation (Sam, 2008).

The presence of micro-organisms (bacteria, fungi ….etc) should be examined for all pharmaceuticals intended for human administration and is defined by sterility test. Living organisms can also produce metabolic bi-products (endo-toxins) that can undesirably affect the radiopharmaceutical preparation , so special testing procedures should be applied (pyrogenicity and toxicity tests) (N.M. Khalid ,2011).

The manufacturer's instructions must always be carefully followed when assembling and eluting the generator to ensure that the eluate is sterile. It is not possible to carry out a test for sterility prior to administration, but regular tests should be carried out to maintain surveillance of procedures and techniques (Roy P. Parker et al 1984).

The objective of the sterility test is to ensure that sterilization process mainly by autoclaving for long-lived radiopharmaceuticals, and membrane filtration for short-lived ones, are conducted properly. A proper sterility test involves the incubation of the radiopharmaceutical sample for 14 days. The second elution (taken as early as possible after the first elution), and final elution from each generator are used as test samples .Each week, a different prepared dose is sent

as test sample, so that over a period of time the range of injections commonly made in the department have all been covered .There are two methods of sterility testing:

Colony culture and radiorespirometry:

a- Colony culture: here the radiopharmaceutical sample is incubated in Fluid thioglycollate medium (30-35^0 c) for growth of aerobic and non-aerobic bacteria, or in soybean case-in medium (20-25 ^{0}c) for fungi, molds, aerobic and facultative non-aerobic bacteria. The test medium is observed for 7-14 days, and the presence of bacteria is determined by bacterial growth. The result of this test is only available after the radiopharmaceutical has been administered; therefore it is not adequate for testing the radiopharmaceutical sterility.

b- Radiorespirometry: here the radiopharmaceutical sample is incubated in a culture medium containing ^{14}C-glucose or ^{14}C –acetate, at 37^{0}c for 3-24 hr. If bacteria are present in the sample, they metabolize the medium to 14CO2, which is measured in a liquid scintillation counter .This is a faster technique for radiopharmaceutical sterility testing.

As mentioned above, the sterility test requires 14 days, so ^{99m}Tc – labeled compounds and other short – lived radiopharmaceuticals could be released prior to the completion of the test (Sam, 2008).

2.1.4.2.7 Pyrogenicity:

Pyrogens are either polysaccharides or proteins produced by the metabolism of micro-organisms. They are mainly soluble and heat stable, represented primarily as bacterial endotoxins. Pyrogens can be present even in sterile preparations, i.e. it is quite possible for a particular solution to be sterile but still be highly pyrogenic when injected into patients. While they become asterile from bacterial, fungal and yeast growth, pyrogenicity arises from certain metabolic by-products of these micro-organisms. Therefore , every product designed for parenteral use must be sterile and pyrogen- free .Pyrogens are non- volatile , water-soluble substances that , when injected into the body , they can produce symptoms of fever , chills , malaise , joint pain , sweating , headache, leucopenia, flushing and dilation of the pupils. Pyrogenic reactions can develop in patients within 30 minutes to 2 h after administration, but usually subside in 10 to 12 hours after onset. These reactins are rarely fatal, however, when injected intrathecally, are estimated to be 1000 times as potent as when injected intravenously. They are usually 0.5-1µm in size, and cannot

be destroyed by normal methods of sterilization and are not filterable. For these reasons, control of pyrogens must be achieved by using only pyrogen-free reagents and glassware and maintaining very high pharmaceutical standards throughout the preparation .The preparation must be carried out in the appropriate clean or aseptic facilities. According to the EP test for pyrogen, all single dose parenteral injections of 15 ml or greater must be pyrogen – free. ^{99m}Tc radiopharmaceuticals do not yet have to comply with the EP test for pyrogen, as they are administered in doses of 1-2 ml. Some radiopharmaceutical preparations, however, must comply with this test, and problems again arise due to short shelf-life (Sam, 2008,).

Substances derived from the cell walls of bacteria, known as endotoxins, are the prime examples of pyrogens, but various chemicals also can add pyrogens to the radiopharmaceutical solution. In order to prevent pyrogen contamination of the radiopharmaceutical, all glassware and equipment should be heated at 200^0c for 2hr. Pyrogenicity testing was developed from the rabbit test to a more sophisticated and rapid method called the Limulus Amebocyte Lysate (LAL) method .The test is used for the detection of endotoxins – type of pyrogens. The principal of the test is based on the gelation of clot table proteins of the Lysate of Amebocyte from the blood of the horseshoe crab, limulus Polyphemus, when it is incubated with a sample solution .The reaction between endotoxins and LAL was first described by Levin and Bang (1964). The application of Limulus test as a quality control measure for parenteral products was produced by Cooper and co-worker beginning in (1970). LAL, which is isolated from the horseshoe crab (limulus), reacts with gram-negative bacteria endotoxins in nano-gram, or greater, concentration. LAL is commercially available in lyophilized form in a kit, and should be stored at 5^0C. A sample of 0.1 ml of buffered radiopharmaceutical (PH 6 - 8) under test is usually added to 0.1 ml of LAL, at 37^0 C, and observed for 15- 60 minutes for positive gel formation, which indicates pyrogens presence. The thicker the gel, the greater the concentration of pyrogens in the sample .Gram – negative endotoxins is known as the most important source of pyrogen contamination. LAL test is a rapid, simple, relatively inexpensive and very sensitive pyrogenicity test (five times sensitive than rabbit test). The test, however, requires meticulous handling, because minute contamination may lead to a false result .Sensitivity of LAL is defined as the lowest concentration of endotoxins that will produce a firm gel after one hour of incubation at 37^0c, and that will remain intact when inverted carefully. LAL is sensitive to heat,

especially in liquid form, so it should be dispensed just prior to testing and refroze immediately. LAL test, however, shall not replace the rabbit test, but to be used when the rabbit test cannot be easily applied. This is because LAL test can only detect the presence of endotoxins. Pyrogens may be present which are not of endotoxins origin, and these would only be detected by the rabbit test (P.F. Sharp, 1989, Sam 2008,).

In the rabbit test (the second type of pyrogens testing), the sample of radiopharmaceutical is injected in three mature rabbits on mg/Kg basis, so that the animal dose approximates the human dose. The test sample is injected into the ear's vein of each rabbit. The rectal temperatures are then taken at 1, 2, and3 hours, and the data recorded before and after the injection. If there is a rise in temperature that is less than 0.6^0c for each rabbit or less than 1.4^0c for all three rabbits, the sample is assumed to be pyrogens-free. The increase in temperature can, however, be caused by radioactivity of the preparation rather than indicating the presence of pyrogens. Hence, the preparation must be allowed to decay before the test (Fred A. Mettler, 2006, Sam A.K. 2008).

2.1.4.2.8 Radioactive package monitoring:

Packages containing radioactive materials e.g. the generator package, should be monitored for external exposure within three hours if delivered during normal hours, or within three hours from the beginning of the next working day if delivered after working hours. Survey must be done on the package surface, and at one meter, using a GM survey meter. The readings should not exceed the limits of 200 mR /hr at the surface, and 10mR /hr at one meter distance. The exposure reading in mR /hr at one meter from the package surface is called Transport Index (TI), and it must be indicated. The label RADIOACTIVE must be shown on the package. The maximum TI value is 10 (10 mR/hr). A warning label must identify the contents and amounts of radionuclides in Becquerels, as well as the shipping document inside (Gopal B. Saha 1998).

2.1.4.2.9 The wipe test:

The wipe test indicates no removable contamination in excess of 6600 dpm/ 300 cm^2. The test is done by swabbing 300 cm^2 areas on the surface of the package containing the radioactive material, using absorbent paper, and counting the swab in a NaI (TI) scintillation counter. For reading the swab,

gamma camera could be used to read the swab in one minute (Gopal B. Saha 1998).

Fig. 2.6 The contamination monitor (left) and survey meter (right) in RICK.

2.1.4.2.10 PH and Isotonicity

The PH of the final compounded radiopharmaceutical preparation should be checked to ensure that its value is within the acceptable range as defined by the USP, package insert or literature. The PH of a radiopharmaceutical normally varies between 2 and 9, because of the high buffer capacity of the blood. The PH is checked with universal PH paper.

Isotonic solution is defined as solution having the same osmotic pressure as a body fluids, blood or saliva, depending on the intended use. Control of Isotonicity requires an osmometer to allow for fast determination on small volumes.

The Isotonicity of inject able drug products should be equal to that of a o.9% NaCl solution , and the PH should preferably approximate that of blood (PH 7.4) . While iv injection of hypertonic or hypotonic solutions, or those with very low or very high PH, is not recommended, it is possible to inject compounds with these characteristics if done slowly (Sam, 2008).

2.2 Previous Studies:

Seibert and co-workers (1923), demonstrated the micro- biological origin of pyrogens and the necessity of high quality water as the vehicle for injectables, and promoted the use of the rabbit- fever response test for their detection.

Pauwels and Feitsma (1977), aimed to evaluate a number of radio-chromatographic methods and to establish some daily practice guidelines for radiochemical quality control of ^{99m}TC- labeled radiopharmaceuticals, recognizing the fact that each nuclear medicine facility should be able to perform simple radiochemical quality tests on currently used radiopharmaceuticals.

Browns S. Baker MH (1986), after following the survival of bacteria in non-radioactive and radioactive kits and generator eluate over a period of five days, reported that the response of bacteria in the non- radioactive materials ranged through death, survival and growth. The response in the radioactive materials was either death or survival (i.e. no growth). They suggested an improved test method.

Brandau (1994) revised guidelines for radiation protection in radiopharmaceuticals Q.C. For the first time the revision of the "Guideline for radiation protection in medicine "defines extensive Q.C procedures for radiopharmaceuticals. The principles of ^{99}Mo – ^{99m}Tc generator, the preparation of ^{99m}Tc – radiopharmaceuticals and the origin of the most frequent radiochemical impurities were illustrated in the study. The study added a base for the determination of the radiochemical purity of the most important radiopharmaceuticals in clinical nuclear medicine.

Ponto (1998), carried out an extensive 12 years of experience study on ^{99m}Tc radiopharmaceutical preparation problems. The objective was to investigate chemical reactions involved in preparing these radiopharmaceuticals occasionally result in products of substandard purity levels. The result obtained was that fifty (0.2%) of 20,972 samples had substandard radiochemical purity; none was administered to a patient. Thirty three (66%) of that fifty substandard sample were obtained from the first elution of a new generator and/ or the elapse of more than 12 hours from the preparation time.

Decristoforo C. Siller R. Chen F. and Riccabona (2000), conducted a retrospective study about radiochemical purity of routinely prepared

radiopharmaceuticals on a number of 2090 samples. They reported mean radiochemical purity of 96.9 %. They related this low percentage to the substandard preparations .They stressed the need for quality control in the preparation of radiopharmaceuticals, and they provided original values of radiochemical purity on routinely prepared ^{99m}Tc radiopharmaceuticals.

Decristoforo et al. (2000) conducted a retrospective study on radiochemical purity of routinely prepared ^{99m}Tc radiopharmaceuticals. A number of 2090 samples out of 7000 preparations of 20 different ^{99m}Tc radiopharmaceuticals were tested, using standard methods over a period of more than seven years. Seventy four (3.54%) preparations failed to meet radiochemical purity limits. The researchers ascribed that failure to laboratory – related conditions.

Snowdon G.M (2000) assessed the sterility of multi-dose ^{99m}Tc generator eluate vials at the end of a working day. He randomly collected 10 vials over a period of 10 weeks after their activity reached background, and sent them to an independent micro-biology laboratory for sterility testing. He reported that the testing confirmed the validity of their departmental protocol for radiopharmaceutical preparation. Thus, he reported that the sterility testing had become part of their Q.C program.

The researcher found variations in the efficiency of showing the radiochemical impurities among the systems he used. He attributed the increment of impurities shown by some systems to factors like hand touching, the way of cutting the emulsion layer, and bad preparation and mixing of the eluate with the pharmaceuticals vials. The study showed that the impurity levels of ^{99m}Tc-MDP in some systems were far below the levels recommended by both the USP and manufacturer.

Derar A. M. (2006), conducted a study on radiochemical purity of ^{99m}TC – MDP and ^{99m}Tc –DTPA using four ITLC systems in RICK.

He reported that the (levels of impurities of the radiopharmaceuticals were not exactly determined, and they were subject to contamination with micro-organisms, due to low level of cleanliness at the work area). Through observation he noticed that the level of cleaning in the hot laboratory and the injection room is not adequate. He also observed that the doses were injected by a nurse without supervision by a physician or a technologist. As for the generators, he noticed that they are not usually checked for leakage, and the

eluate is not usually checked for molybdenum breakthrough, and other Q.C tests on the eluate and radiopharmaceuticals are not performed.

Adlan A.A. (2007) evaluated the Q.C tests in RICK. He noticed that (the Q.C tests were not normally performed in the department)

Ali M. (2008) measured and evaluated the radiation dose received by RICK staff members using TLD chips. Results obtained by the staff were as follows: technologist: 6.75 mSv / h, physicist: 7.89 mSv /h, radio pharmacist: 6.1 mSv /h and nurse: 8.1mSv/h. The researcher concluded that all these doses agreed with the international recommendations.

Sam A. K, and Derar A.A (2009) conducted a study on the determination of the radiochemical purity of the dynamic renal imaging agent (^{99m}Tc –DTPA – Amersham Company), using four different instant layer chromatographic systems in RICK. The study agreed with Adlan that (culture of Q.C) of radiopharmaceuticals has never taken root as part and parcel of nuclear medicine practice in RICK). The study came out with a result that the purity levels (96.7 – 99.9 %) were higher than the levels proposed by USP (90%) and the manufacturer (95 %).

Thuwayba et al (2014) conducted a study in RICK, in which they studied and measured – among other things – the radiation doses received by the nuclear medicine staff (in µSv/ h) as follows:

Doses received during generator elution: hands (86), eyes (4) and whole body (52). Doses received during injection of bone dose which was (20 mCi): hands (210), eyes (57) and whole body (34). Doses received during injection of thyroid dose (5mCi): hands (9.5), eyes (3.5) whole body (4.2).

Maida Y. Mohamed (2016) conducted a study on the doses received at the injection room of RICK and found that the doses were within the accepted international levels. The author was faced with difficulty in calculating the annual dose owing to the absence of personal monitoring devices.

Osama Ibrahim Mohamed (2016) performed a study on the performance of the dose calibrator in RICK, including the measurement of background, accuracy, linearity and geometry. The study showed that the dose calibrator was in a good condition, and was reliable in measuring those aspects for the time being.

Chapter Three

Materials and methods

3.1 Materials

3.1.1 Gamma cameras:

The nuclear medicine department of RICK had three gamma cameras at the time of doing the experiments of this research , two of them were out of order .The following table shows the description and status of each one :

Table (3.1) shows available gamma camera machines and their working status in RICK.

Gamma camera	Current status
ZLC 370, single head, Phillips	out of order
Phillips , dual head	out of order
Nucline^{Tm} SPIRIT, Medisco , dual Head , with all accessories	Functioning

3.1.2 Radionuclide generator:

The generator used in RICK at the time of study was Mo99/ Tc^{99m} generator of the following specifications:

Type: ECZacibasi / Monrol, Turkey.

Activity: 15 GBq.

Registration no. : 223 / 48

Package: Type A package – UN 2915.

3.1.3 Pharmaceuticals kits:

These are the chemical agents which are added to the Tc^{99m} to form the radiopharmaceuticals injected to patients for different studies. They are kept in a refrigerator and stored in the injection room.

3.1.4 Dose calibrator: Type: REF: VIK – 202 .Venester Instruments VOC- 404, Netherland. SN. : 21401- 5051 – 05.

3.1.5 Contamination monitor:

The specifications of contamination monitor used in the tests included :

Type: CoMo 170 contamination monitor.

Manufacturer: NUKLEAR – Medizintechnick Dresden GmbH.

Detector size: 170

Dimensions: 280x125x135mm (L, W, H).

Nuclides: 25 nuclides.

Result display: in CPS or Bq / $cm^{2.}$

3.1.6 Survey meter: Type: Inovision 451p Universal Survey Meter.

Meter model: 451p

Probe model: NIA

Calibration source: Cs – 137.

Accuracy: Within 10% of reading between 10% and 100% of full scale indication on any range, exclusive of energy response.

Radiation detection: Beta above 1 Mev, gamma and X-rays above 25Kev.

Detector and Chamber: 300 cc volume pressurized air ionization chamber to 6 atmospheres.

Serial number: 389.

Calibration factor: 1.25

Calibration date: 20.4.2014.

3.1.7 Normal saline: Normal saline 0.9% NaCl.

3.1.8 Lead blocks: Lead blocks: these are very heavy and thick blocks, each block of 6 cm thick, used as shield in the hot lab for the generator and radioactive sources

3.1.9 Lead shields: these include vials and syringes shields of different sizes and thicknesses.

3.1.10 Thermometer: 11Thermometer.www.holdenmedical.com , modern digital thermometer, used to measure the rabbits temperature in pyrogenicity test.

3.1.11 Waste containers: These are bins classified as bins for radioactive waste put behind lead shields, and bins for non- radioactive waste.

In addition to syringes, butterfly, gloves, face masks, cotton, gauzes..etc.

Table (3.2) Summary of available materials and facilities in RICK and their field of use in QC procedures.

Material / Facility	Field of use
Gamma cameras	Imaging and QC tests.
Radionuclide generator	source of Tc^{99m}
Pharmaceuticals kits	radionuclide studies
Dose calibrator	measurement of radioactive Materials doses.
Refrigerator	keeping of kits
Survey meter	measurement of radioactive Contamination.
Normal saline	Generator elution.
Lead shields	Radiation protection.
Syringes ,butterfly , gloves, Face masks, cotton gauzes..etc.	Different usages.
waste containers	for keeping radioactive and Non-radioactive waste.

Table (3.3) Q.C materials and facilities not available in RICK, in spite of their importance.

Material / facility	field of use
ITLC solvents	R.Ph purity test.
ITLC stationary phase	R.Ph purity test.
ITLC reading equipment	R.Ph purity test.
Lycate kits	LAL (pyrogenicity) test.
Lead canisters	Mo^{99} breakthrough test.
Al^{3+}	Al breakthrough test.
Test paper strip	Al breakthrough test.
PH meter	reading of solutions PH.
Personnel monitoring devices	Radiation protection.
Light microscope	determination of particle size
Multichannel analyzer	differentiation of radionuclides
Hot water bath	some NM studies
Dry heat oven	Q.C procedures

3.1.12 RICK employees:

Table (3.4) the number of RICK nuclear medicine department

employees at the time of study experiments (2015).

Employees	Number
Specialists	5
Technologists	8
Radio-pharmacists	3
Nurse	1
Worker/ cleaner	1
Receptionists	2

3.1.13 Animals:

The animals used in pyrogenicity test included the use of three mature rabbits of different colors, to avoid confusion.

3.1.14 Inclusion criteria.

These included all the criteria mentioned in the specific objectives.

3.1.15 Exclusion criteria.

These were excluded because of the lack of tests materials and facilities, and they included the following:

- Aluminum ion breakthrough test
- LAL test for pyrogenicity.
- Sterility test.
- Isotonicity and PH tests.
- Radionuclide concentration level test.
- Thin layer chromatography test.

3.2 Methods:

3.2.1 Methods of data collection:

Data were collected from relevant text books, magazine, internet sites, and previous studies personal communications, in addition to observation, tests and experiments.

3.2.2 Methods of data analysis:

Data were analyzed using (SPSS) statistical program, including measurements, equations, percentages, means, which were represented in terms of tables, graphs and diagrams.

3.2.3 Techniques of different tests:

3.2.3.1 Observation tests:

In these tests, application of radiation safety rules was directly observed by the researcher during the generator elution, radiopharmaceuticals preparation and injection and during patient imaging. Notes about the application of pre-set rules were immediately recorded.

3.2.3.2 Personal dose measurement:

The radiation doses received by the hands, eyes and whole body of working persons during the generator elution, radiopharmaceuticals injection and patients imaging were measured, using radiation dose meter.

3.2.3.3 Wipe test:

This was used to detect and measure the radiation contamination on a new generator package surface using contamination monitor. A package area of 300 cm^2 was swabbed by an absorbent paper, which was inserted into a NaI (Tl) scintillation counter, and the level of contamination was recorded.

3.2.3.4 Pyrogenicity test:

This was performed to detect the presence of pyrogens in the Tc^{99m} eluate. Three mature rabbits were used whose temperatures were measured before and after the eluate injection, using modern digital thermometer which was inserted into the rabbit rectum. The temperatures were recorded and compared to the acceptable international level of temperature rise after the eluate injection.

3.2.3.5 Package external radiation monitoring:

This was done to measure the external radiation emanating from a new generator. The radiation was measured three hours after the generator delivery, using GM survey meter, and the readings were registered and analyzed.

3.2.3.6 Measurement of room's doses:

Here, a radiation survey meter was used to measure the radiation dose inside the hot lab, the injection room, and the imaging room, the waiting area of injected patients, the reception and the toilets. The doses were recorded and compared to the internationally accepted limits.

3.2.3.7 Mo^{99} breakthrough test:

This test is usually performed to assess the amount of Mo^{99} in the Tc^{99m} eluate. Mo^{99} energy (740-780 Kev) is higher than Tc^{99m} energy (140 Kev). The eluate was vial, inserted in a 6-mm thick lead shield, was placed into the dose calibrator, using the Mo^{99} assay dial setting, and the activity reading (Mo^{99} activity) was recorded. The shield was removed and the activity at the Tc^{99m} setting was recorded. The ^{99}Mo activity was divided by the Tc^{99m} activity and the ratio was obtained, and compared to the internationally permissible level (should be less than 0.15 µCi Mo^{99} per one mCi Tc^{99m}).

Chapter four

Results

4.1 Results obtained through observation

Table (4.1). (A) Application of radiation safety rules during generator elution and handling of ^{99m}Tc –radiopharmaceuticals.

No	Rule	A	P	N
1	Wear lab coat		√	
2	Wear disposable gloves.	√		
3	Wear face mask.		√	
4	Apply time, distance and shield principles.	√		
5	Change gloves at regular times.			√
6	Use of vial shields.	√		
7	Use of syringe shields.	√		
8	Wear personal radiation monitors.			√
9	Where possible, wear finger exposure monitor.			√
10	Measure total elution activity.	√		
11	Measure each patient's dose before injection.		√	
12	Label vials or syringes containing radioactive Materials.			√
13	Cover work surfaces with absorbent materials.			√
14	Work behind an enclosed shielded area.	√		
15	Place the generator behind a lead shield.	√		
16	Use of remote handling tool in carrying out radioisotopes			√
17	Do not eat, drink, smoke or apply cosmetics.	√		

A = fully applied, P = partially applied, N = not applied.

Table (4.1) (B) Contribution percent of the application of each rule to the total rules application (17 rules).

Rules	frequency
Applied rules	8
Partially applied rules	3
Not applied rules	6

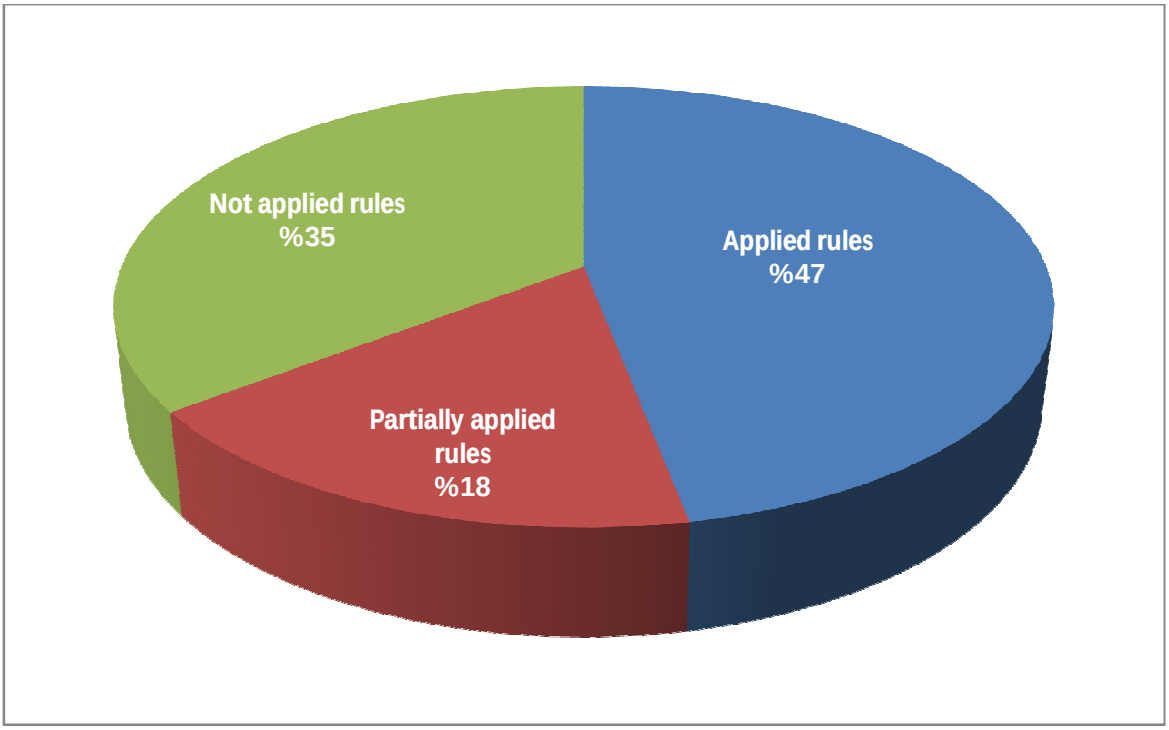


Fig (4.1) shows percentages of applied, partially applied and unapplied rules to the total rules.

Table (4.2) (A) Application of radiation safety rules during preparation of radiopharmaceuticals in RICK.

No.	Rule	A	P	N
1	Wear lab coat.		√	
2	Wear disposable gloves and face masks.		√	
3	Wear personal monitoring devices at all times in the radiation areas.			√
4	Where possible, wear finger exposure monitor.			√
5	Use spill trays in preparation area.			√
6	Keep radioactive solutions in shielded labeled containers		√	
7	Label syringed dose with R. Ph. name, dose and time.			√
8	Always change the needle immediately after drawing up dose from the vial.			√
9	Use long forceps or tongs when lifting vials of radioactive materials.			√

A = fully applied, P = partially applied, N = not applied

Table (4.2) (B) Contribution percent of the application of each rule to the total rules application (9 rules).

Rules	Frequency
Applied rules	0.00
Partially applied	3
Not applied	6

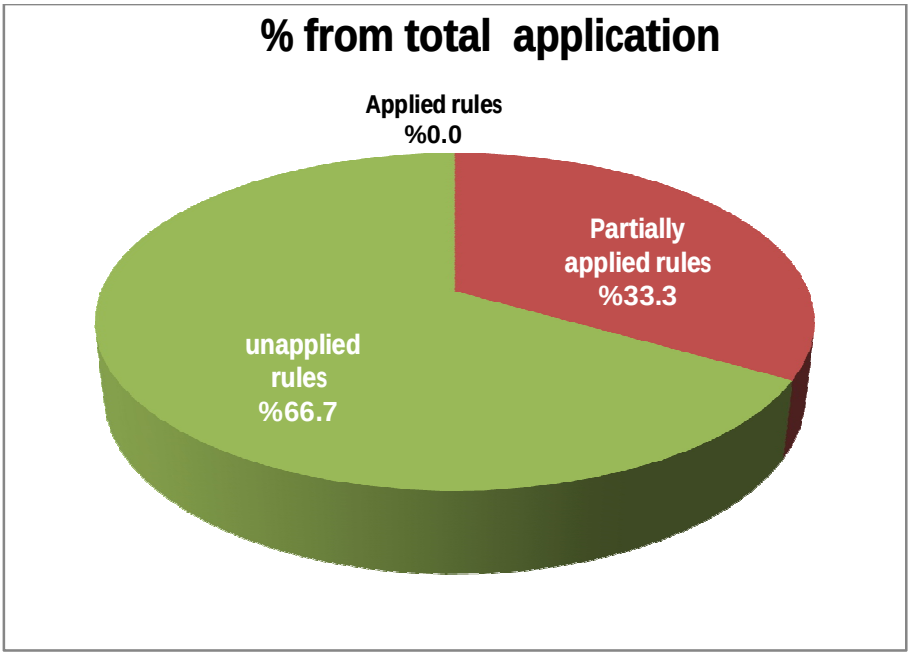


Fig. (4.2) shows percentages of applied, partially applied and unapplied rules to the total rules.

Table (4.3) (A) application of safety rules before and during radiopharmaceuticals injection.

No	Rule	A	P	N
1	Wear lab coat.		√	
2	Wear disposable gloves and mask.		√	
3	Always swab vials with fresh alcohol swab immediately before use.			√
4	Prepare R.Ph. under aseptic conditions.			√
5	Wipe test and monitor all work areas for radioactive contamination.			√
6	Monitor hands/ gloves for contamination after each Procedure.		√	
7	Dispose of radioactive waste only in designated, labeled and properly shielded containers	√		
8	Dispose of all syringe needles in a separate container.			√
9	Keep all decontamination materials together inconvenient location.			√
10	Always apply the time, distance and shielding principles.	√		
11	Change gloves at regular times.			√
12	Wear personal monitoring devices.			√
1 3	Where possible, wear finger exposure monitor			√
14	Check each patient dose in a dose Calibrator before injection.		√	
15	Check the real R.Ph. for the real patient.	√		
16	Use disposable sterile syringe for each patient.	√		
17	Do not allow the needle tip to touch any other surface before injection.	√		
	R .Ph's are administered only by N.M specialist			√
19	Physically check Tc^{99m} O_4 solution before injection to patients.			√
20	Ask females for pregnancy or breast feeding before injection.	√		
21	Swab injection site.	√		
22	Apply time, distance and shielding rules during injection.	√		
23	Put radioactive swabs after injection in a lead container.			√

A = fully applied, P = partially applied, N = not applied.

Table (4.3) (B) Contribution percent of the application of each rule to the total rules (23 rules).

Rules	Frequency
Applied rules	8
Partially applied	5
Not applied	10

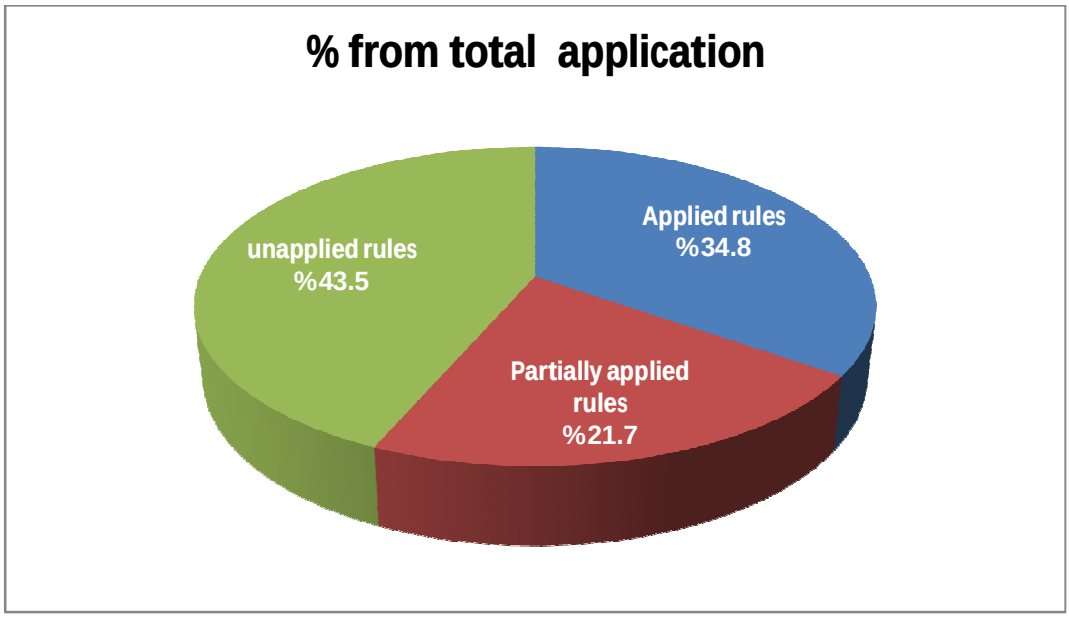


Fig. (4.3) shows percentages of applied, partially applied and unapplied rules to the total rules.

Table (4.4) (A) Application of radiation safety rules during patient imaging.

No.	Rule	A	P	N
1	Wear lab coat.		√	
2	Wear disposable glove and mask.			√
3	Wear personal monitoring device.			√
4	Where possible, wear finger exposure monitor.			√
5	Apply time , distance and shielding rules	√		
6	image patients within the prescribed imaging protocol.	√		

Table (4.4) (B) Contribution percent of the application of each rule to the total rules application (6rules).

Rules	Number	% from total application
Applied	2	33.3
Partially applied	1	16.7
Not applied	3	50

Table (4.5) Application of daily operations of nuclear pharmacy in RICK.

No	Operation step	A	P	N
1	Receiving of radioactive materials.	√		
2	Preparation and dispensing of radiopharmaceuticals.	√		
3	Storage of radiopharmaceuticals.	√		
4	Q.C tests of radiopharmaceuticals.			√
5	Check radiation detectors and meters operation status.			√
6	Radioactive waste disposal			√
7	Infectious waste disposal			√

4.2 Results obtained through tests :

Table (4.6) Personal radiation doses received by some organs during the first elution of generator in RICK.

Organs	received dose(μSv/h)	permissible limit(mSv/yr)	Remarks
Hands	86	750	Inside block shield.
Eyes	4	50	Behind block shield.
Whole body	52	50	Behind block shield.

Table (4.7) shows the personal dose received by some organs during radiopharmaceutical injection for bone (dose – 20 mCi) and thyroid (dose 5 mCi) imaging.

Organ	Dose(μSv/ h) (Bone inj.)	Dose(μS/h) (Thyroid inj.)	Remarks
Hands	68	9.5	Syringe shielded.
Eyes	47	3.5	Syringe shielded.
Whole body	44	4.2	abdominal area, syringe shielded.

N.B: permissible dose is 50 mSv / yr. (NCRP publication No 43).

Table (4.8) shows the wipe test for radiation contamination of generator package surface.

Generator	Test result	Max. permissible limit
First generator	939dpm / 300cm^2	6600 dpm / 300/cm^2
Second generator	1100 dpm / 300cm^2	6600 dpm / 300 cm^2

Table (4.9) shows the external radiation exposure from new generator packages.

Generator no.	exposure at surface (mR / h)	exposure at one meter(mR/h)	maximum permissible value (mR / h)
One	89	4	200 at surface.
Two	96	5	10 at one meter.

Table (4.10) (A) the remaining (unused) activities after each generator elution.

Elution no.	Total activity (mCi)	Remaining activity (mCi)	%
First	1730	1730	100
Second	1256	675	53
Third	960	438.7	46
Fourth	757	424.76	56
Fifth	590	285.38	48
Sixth	430	172	40
seventh	340	73.7	22
Eighth	267	79.9	30
Ninth	214	12.1	56
Tenth	166	24.5	15
Eleventh	130.7	66	50
Twelfth	96	7.89	8
Thirteenth	72	9.48	13
Total	7008.7	3999.41	57

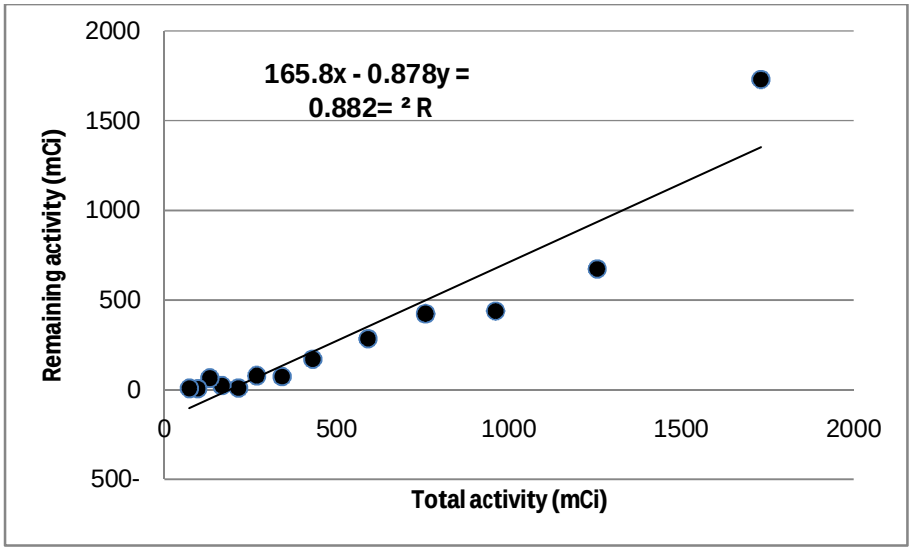


Figure (4.4) scatter plot diagram shows the rate of remaining activity per the total activity which is 0.878mCi/mCi

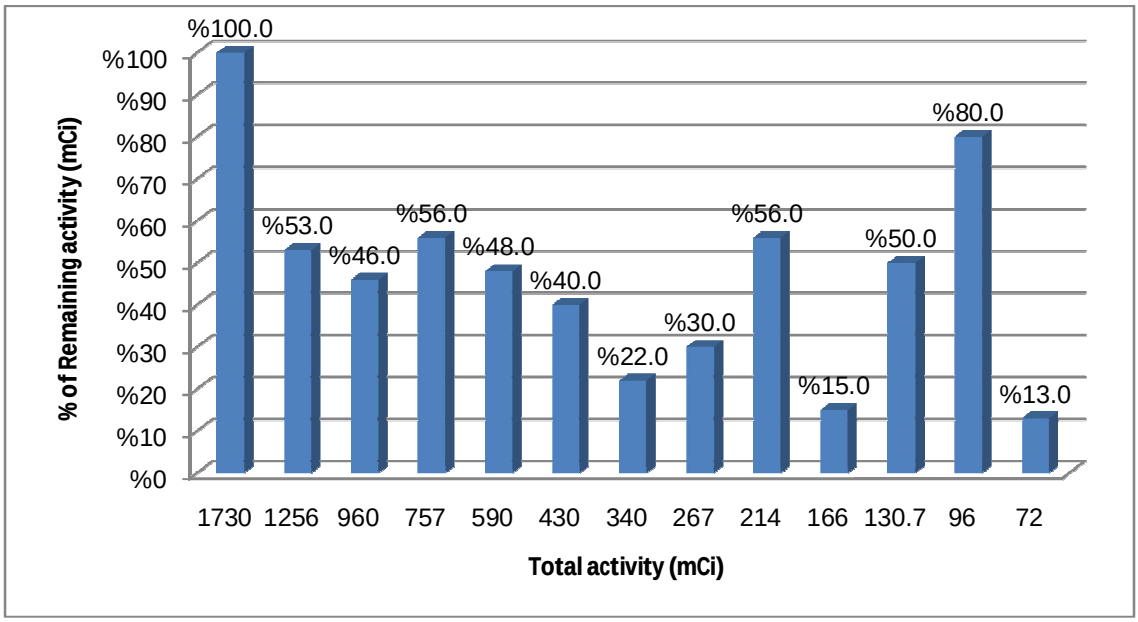


Fig.(4.5) bar graph demonstrating the percentages of remaining activity to the total activity .

Table (4.10) (B) shows the amount and percentage of prepared and unprepared activities of the remaining activities (in mCi) after each elution of the generator.

Elution no.	Remaining activity (mCi)	Prepared (mCi)	Unprepared
1	1730	0	1730 (100%)
2	675	355 (53%)	320 (47%)
3	438.7	207 (47%)	231 (53%)
4	424.76	37.16 (8%)	387.6 (92%)
5	285.38	61.68 (22%)	223.7(78 %)
6	172	114 (66%)	58(58 %)
7	73.7	40. (55%)	33.5 (4%)
8	79.9	15. 51 (19%)	64.75 (81 %)
9	12.1	2.2 (18%)	9.9 (82 %)
10	24.5	3.16(13%)	21.34 (87%)
11	66	34 (52%)	32 (48%)
12	7.89	—	7.8(100%)
13	9.48	—	9.48 (100%)
14	No elution made.	—	—
Average		32%	74%

*The entire elution (no. 13) was allocated for thyroid scans, so there was no preparation (i.e. addition) of pharmaceuticals.

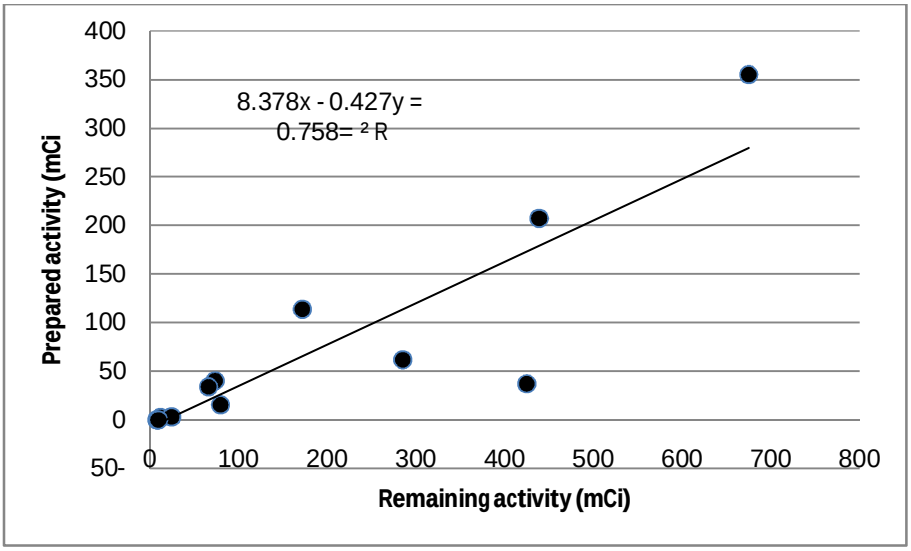


Fig. (4.6) scatter plot diagram demonstrating the prepared activity out of the remaining activity.

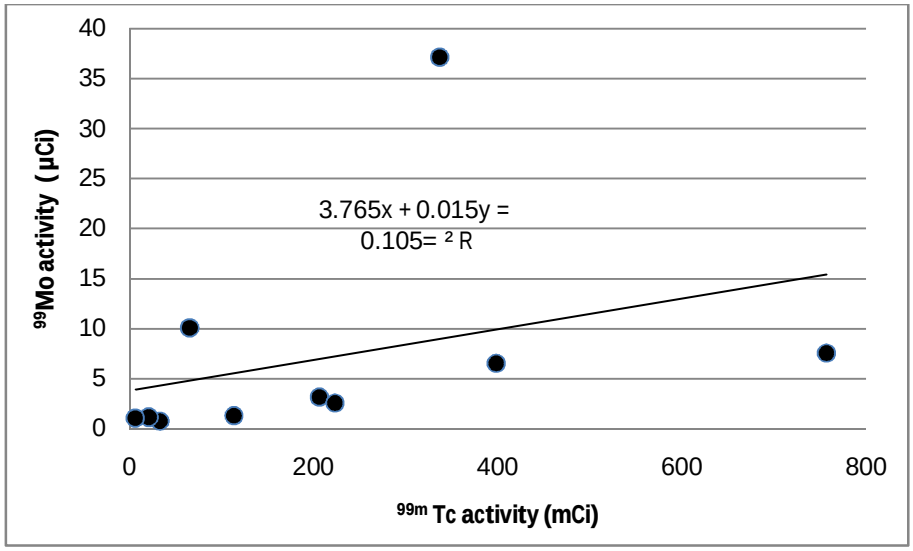
Table (4.11) shows the rabbit test for $Tc^{99m}O_4$ eluate pyrogenicity.

Rabbit	Temperature (C^0)		Total rise (C^0)	normal rise(C^0)
	Before injection	after injection 1h 2h 3h		
A (White)	38.3	38.4 38.6 38.7	0.8	< 0.6
B (black& White)	38.4	38.5 38.6 38.8	0.7	< 0.6
C (black)	39.1	39.2 39.4 39.5	0.8	<0.6
Three rabbits total temperature rise.			2.3	< 1.4
Normal rabbit temperature (38.3^0C – 39.4^0 C). (NCRP publication No 43).				

Table (4.12) shows the ^{99}Mo break through tests in different ^{99m}Tc eluates.

No	^{99m}Tcactivity (mCi)	^{99}Moactivity (µCi)	^{99}Mo/^{99m}Tc	Remarks
1	206.7	3.14	0.015	At elution time
2	337.3	37.16	0.110	At injection time
3	398.6	6.51	0.16	At injection time
4	757.0	7.50	0.009	At elution time
5	33.5	0.71	0.021	At elution time
6	21.36	1.10	0.051	At elution time
7	65.76	10.06	0.152	At injection time
8	6.68	1.01	0.151	At injection time
9	223.7	2.50	0.011	At elution time
10	114.00	1.25	0.010	At elution time

Accepted ratio is ≤ 0.15 at injection time and ≤ 0.038 at elution time. (Sam, 2008, Gobal B. Saha, 2001).



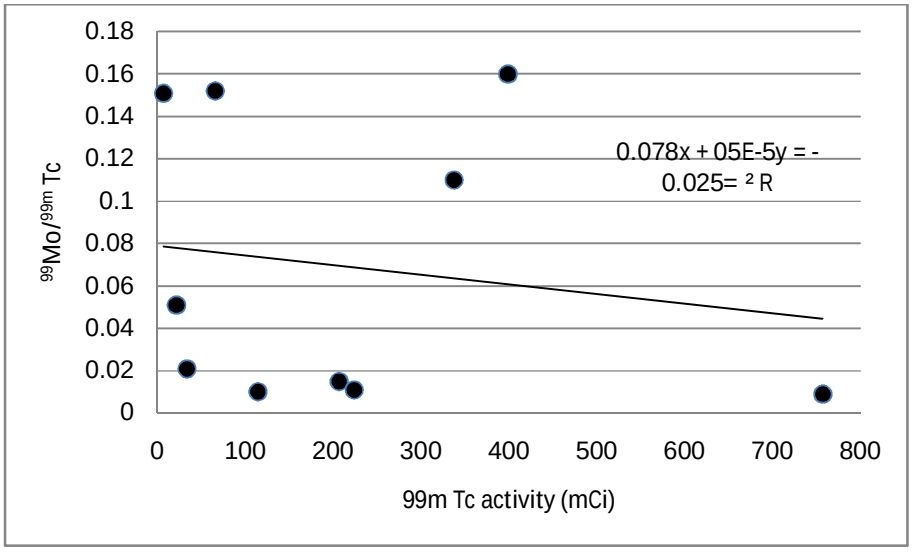


Fig. (4.7) scatter plot diagram showing the Mo breakthrough test.

Table (4.13) measurement of radiation exposure rates in different areas in the N.M department of RICK.

Area	Exp. rate (µSv/h)	Background rate (µSv/h)	Remarks
Hot lab (1) : Inside shield :	21	2.1	2nd elution day
Outside shield:	14	2.15	
Hot lab (2) : Inside shield :	7	7	
Outside shield :	3	2.1	last elution day
Injection room :	19	2	bone dose
	3.5	1.1	thyroid dose
Imaging room :	15	1.7	bone patient
	4.4	1.1	thyroid patient
Patient waiting Area(injected):	30	2.58	(bone +thyroid) patients
Patient toilet :	28	2.1	bone patients
Reception :	3.6	2.4	near waiting area
Staff offices & Rest room :			no radiation detected

Acceptable AAEED = 50 mSv per year.

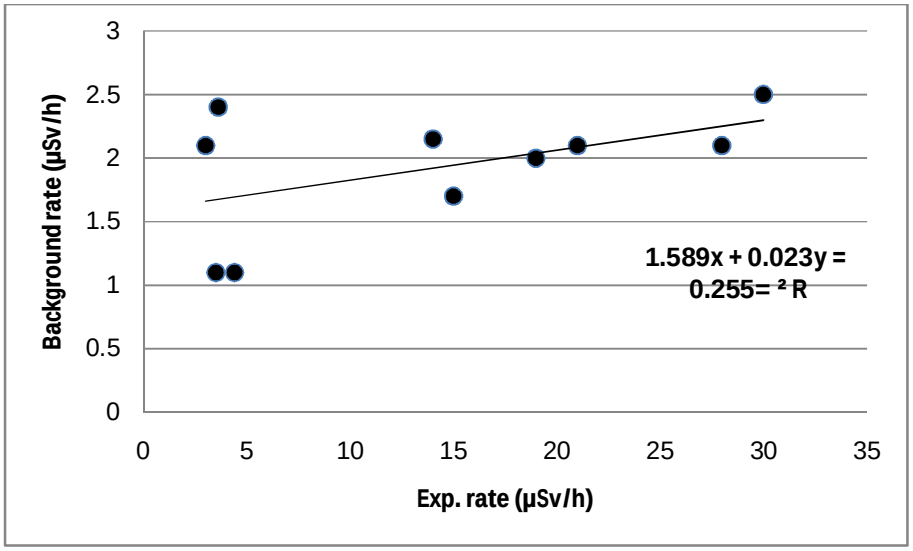


Fig. (4.8) scatter plot diagram showing the exposure rates and background radiation rates at different RICK areas.

Chapter Five

Discussion, Conclusion and Recommendations

5.1 Discussion

5.1.1 Discussion of results obtained through observation:

Table 4.1.1 (A) shows the application of radiation safety rules during the elution and handling of the radiopharmaceuticals. The table contains 17 rules; all of them are significant from the radiation protection standpoint. Nevertheless, the results showed that 8 (47%) of the total stated rules were applied. That means 9 (53%) of the stated rules were either partially applied (3) or not applied at all (6). Partially applied means that the rule was only applied by some staff members, inadequately applied or applied only at some times. The unapplied rules (35.3%) included important ones like wearing personal radiation monitors, changing of gloves, labeling of vials containing radio-activities and covering work surfaces with absorbent materials .This behavior conflicts the global radiation protection rules, which state that the disposable gloves and lab coats ,for instance , must be worn by any one handling isotopes(IAEA 2000& NCRP1975).In RICK, handling of radioactive materials was usually carried out with hands , which exposes the technologist to high radiation doses .Again , this behavior conflicts the protection rule which states that (handling radioactive materials in quantities greater than one mCi should be carried out using remote handling equipment (NCRP1975). The absence of remote handling tools (i.e. tongs) may be the sole reason for this behavior. Rules (1) and (3) were partially applied because the majority of staff members did not wear face masks, and some of them did not wear lab coats although these materials are readily available. This result agrees with the result obtained by Thuwayba et al (2014). Rule (11) was partially applied because the radioactive doses of all listed patients were prepared at one time and put together in one container .The dose decay for the patients who were scheduled to be injected later, was not considered by some staff members. This result is congruent with Adlan result about the same issue (Adlan 2006).Rules (8), (9) and (16) were not applied owing to the absence of facilities .The goal of changing the gloves at regular times(rule 5) , is to minimize spread of contamination (NCRP1975). This rule, however, was not applied.

Table 4.1.2 (A) shows the application of radiation safety rules during the preparation of radiopharmaceuticals. Here, all the stated rules (9 rules) were not fully applied; three of them (33.3%) were partially applied and 6(66.7%) were not applied. Rule (1) was partially applied because the lab coats were not worn by all the staff

members or worn only for short time. Rule (6) was partially applied because the radioactive solution might be put in a shielded container without labeling the vial or the container .Sometimes, a radioactive solution might be inside a syringe or a vial without a lead shield .The use of forceps or tongs for lifting radioactive materials (rule 9) increases the distance between the radiation source and the user (Ibtisam M.A, 2005). Nonetheless, this rule was not applied due to the absence of these tools.

As for table 4.1.3(A), which shows the application of safety procedures before and during the injection of radioactive doses to the patients, only 8 (34.8%) out of the total stated rules (23) were applied, 10 (43.5%) were not applied and 5(21.7%) were partially applied, including rules (1) and (2) which were applied by a few members of the staff.

Rule (6), was partially applied because monitoring of the hands and gloves was not made after each procedure. Rule (8) was partially applied because the syringe needles were not disposed of in a separate container, as the whole syringes, with the needles attached, were disposed of in one container, a matter which conflicts the rules of radioactive waste disposal. Rule (14) was partially applied because all the doses were withdrawn in syringes and the syringes were all put together in one shielded container, and they were not measured again before injection.

In spite of the availability of the required materials , and in spite of its importance in preventing contamination from unsealed radiation sources (Paul E. Christian et al ,2004 & NCRP1975), rule (5)was not applied .This may be attributed to the staff members reliance that there is usually little or no likelihood of contamination occurrence in their department . Rule (18) was not applied, which may be ascribed to the non-availability of the nuclear medicine physician at all times, or just because the staff members became accustomed that the injections were the responsibility of a nurse. Rule (23) was not applied because the radioactive swabs, after injection, were held by the patients to the waiting area or might be disposed of in any area in the department. This result agrees with Adlan result about the same issue (Adlan 2006). Rule (20) was fortunately fully applied , otherwise breast feeding women, if not instructed, might feed their children a radioactive milk, because 10% of secreted pertechnetate is secreted in lactating women milk (N.M Khalid ,2011).

Tables 4.1.4 (A) and (B) show the application of radiation safety rules during patients imaging.

During the patient imaging the technologist will be close to the patient, and studies have shown that the most significant source of whole body exposure is the radiation

emanating from the patient during the imaging procedure (Paul E. Christian et al 2004). According to the aforementioned tables , 2 rules (33.3%) were applied , 3 (50%) were not applied and 1 rule (16.7%) was partially applied .Rule 5 was fortunately fully applied by all the personnel ,as the time , distance and shielding constitute the cardinal principles of radiation protection , and this agrees with Thuwayba study (Thuwayba et al 2014). In general, it is observed by the researcher that the cause for not applying most of the rules was not the lack of facilities, but may be just the customs or laziness.

Table 4.5 shows the application steps of daily operation which should be followed at the nuclear pharmacy. Four steps (57.1%) out of seven were applied, and three steps (42.8%) were not applied which included very important procedures, namely: Q.C tests check of radiation detectors operation condition and infectious waste disposal. Q.C tests are important to protect the patient and to ensure that the radiopharmaceutical localizes in the internal area. A quality control program has, therefore, been stressed by professional groups, governmental agencies and hospital accreditation groups (US. Nuclear Regulatory Commission 1991).

As for the radiation detectors, failure to check them may give wrong doses of radiation and, hence, wrong studies and unnecessary radiation doses to the patients. Infectious waste disposal was not applied because all types of wastes were put together and there was no sequestration of infectious materials from other waste materials.

5.1.2 Discussion of results obtained through tests.

The first elution of the ^{99}Mo-^{99m}Tc generator yields the maximum radioactivity, and hence the maximum radiation dose to the operator, as compared to subsequent elutions.

Table 4.2.1 showed that the hands, which are usually be inside the block shield during the elution and preparation processes, received the highest radiation dose than any other organs (86µSv/h) , as compared to the maximum permissible dose to the hands which is 750mSv/ yr.(NCRP Publication no. 43 , National Council of Radiation Protection report no.93 , USA , 1987) . The eyes and whole body received 4µSv/h and 52 µSv/h respectively, as compared to the maximum permissible dose of 50 mSv/yr for each of the eyes and whole body. This is because both of them were protected by the lead blocks during the elution. These doses were received in µSv/h, whereas the maximum permissible doses were given in mSv/ yr. It is very difficult, therefore, to calculate the doses received by these organs in mSv/yr for comparison

purposes (this result agrees with the results reached by Thuwayba et al 2014, and Maiada 2016). This is because the real working time per hour, per day or per year is not stable, and the same work may be done by different persons throughout the year, in addition to absence from work for any reason. However, an annual dose could be estimated if it is assumed that the elution preparer stays one hour every day for the elution and preparation of the radiopharmaceuticals, and he/ she works five days per week, four weeks per month and ten months per year (exclusion of annual leave), then the annual dose to the hands could be estimated by multiplying the working days per year by the dose received by the hands per hour (1.e per day) as follows:

1 x 5 x 4 x 12 x 86 µSv = 20640 µSv = 20. 640mSv/yr.

This is a very low dose in comparison to the maximum permissible dose to the hands which is 750mSv/ yr. Likewise; the same dose estimation could be applied to the eyes and whole body as follows:

The eyes: 1x5x4x12x4 = 960 µSv= 0.96mSv.

The whole body : 1x5x4x12x52 =12480 µSv= 12.480 mSv/yr.

It is obvious that both the assumed doses were very low as compared to the maximum permissible dose to the eyes and whole body which is 50 mSv/yr for each (Paul E. Christian et al 2004) .The dose estimation made by the researcher was based on daily observation of the staff work in RICK.

It is worth mentioning that the above mentioned doses were particular to the persons who worked in the radiopharmaceuticals elution and preparation, and the elution was sometimes performed by a technologist in the absence or delay of the radio pharmacist, whose work time was estimated as only one hour. For example, the technologist who prepares the radiopharmaceuticals, injects the patients and performs imaging will receive much higher dose than the estimated dose.

Table 4.2.2 shows the radiation dose received by some organs and the whole body during injection of radiopharmaceuticals of bone and thyroid imaging. These organs were selected because the bone dose is the highest dose given in the department (20mCi) and the thyroid is the lowest (5mCi). All other doses lie in between .According to the table, the hands received 68µSv/ h during the bone injection, and 9.5 µSv/ h during thyroid injection .The eyes received 47µSv/ h from bone injection, and 3.5µSv/ h from thyroid injection, and the whole body received 44 µSv/ h from bone dose and 4.2 µSv/ h from thyroid dose. These doses were received from shielded syringes; otherwise they could have been higher. The results obtained here

were almost congruent to the results of Thuwayba et al results (2014). Again it is difficult to compute the maximum permissible dose received by the injector per year, as this depends on the number of patients, amount of doses, working hours…etc. However, according to the above mentioned calculation, it is not likely that the annual dose received by the injector exceeds the annual maximum permissible dose. The low doses received may be attributed to the application of the radiation safety rule which states that high labeling efficiency, short synthesis time and high safety standards are excellent characteristics for routine production on site, where radiopharmaceuticals are administered (IlseZolle, 2007). The actual received dose could have easily been known if the personal radiation monitors were available and worn by the staff members.

Table 4.2.3 shows the results of wipe test to check the presence of radiation contamination on the generator package surface. Two generators were included in the tests .Each test was done using absorbent paper in swabbing an area of 300cm^2 (30cmx10cm) on the package surface, according to the test rule (Gobal B. Saha, 1998). The test swab was then counted using a NaI (Tl) scintillation counter which gave the results shown in the table i.e. 939 dpm /300 cm^2 and 1100 dpm /300cm_2 for the first and second generators respectively. Both results were far less than the maximum allowable rate which is 6600 dpm /300 cm^2 (Goal B. Saha, 1998), which means that the package surfaces of the two generators were free of significant contamination, and could be handled safely.

Table 4.2.4 shows the tests of external radiation exposure from a new generator package. Two generators were used in the tests. The purpose behind the test was to find out whether or not the external radiation emanating from a new generator lied within the permissible limit .Both generators were tested within 3 hours after their arrival to the department, as recommended (Gobal B. Saha, 1998). GM survey meter was used for the exposure measurement, at the package surface and at one meter distance (Gobal B. Saha, 1998).The results of both generators were far less than the maximum permissible value as shown in the table, which means the generators were safe to be dealt with from the external radiation exposure aspects.

Table 4.2.5 (A) shows the remaining (unused) activities after generator elution.

From the protective and economical viewpoints, all the eluted activity should be utilized in radionuclide studies. During the first elutions the activity obtained was usually larger than the number of listed patients in the department and the working gamma cameras. In the nuclear medicine department of RICK, the first elution of any generator is usually sacrificed because, according to the elution performers, it

contains impermissible amount of Mo^{99}breakthrough (impurity). This claim, however, was refuted by the Mo^{99}breakthrough test done by the researcher (see table 4.2.8), there was no justification to abandon these large amounts of activities. As shown on table 4.2.5 (A), an activity of 3999.41mCi remained unused. This activity was capable of doing approximately 800 thyroid scans.

Table 4.2.5 (B) showed the percentage of the prepared activities (in which the chemical agents were added), and the unprepared activities. The percentages of prepared activities ranged between 13% and 66% (elutions numbers 10 and 6 respectively). Here there were two types of loss (loss of eluate and of chemical agents).The percentage of unprepared remaining activities ranged between 4 %(elution number7) and100 % (elutions numbers 1, 12, 13). Elution (1) eluate is usually not used as mentioned above; in elutions 12 and 13 the total eluates were allocated for thyroid scans (i.e. no pharmaceutical had to be added). After elution number 14, the activities were usually very low, and no elutions were performed, so they were not included in the study .The study showed that the average percentages of the prepared and unprepared remaining activities were 32 % and 74% respectively.

Table 4.2.6 shows the rabbit test for Tc^{99m}eluate pyrogenicity.

Pyrogenicity testing is very important, as the presence of pyrogens within the radiopharmaceutical can produce symptoms like fever, chills, leucopenia, joints pain, flushing, sweating and headache within a period of 0.5-2hours after injection. The test should be performed even if the solution is a sterile from bacterial, fungal and yeast growth. This is because pyrogenicity may arise from certain metabolic bi-products of these microorganisms (Sam 2008). A recent pyrogens testing has been adopted, namely LAL testing, as it is more sensitive in detection limit. However, LAL testing is not to replace the rabbit test, but to be used when the rabbit test cannot be easily applied, because LAL test can only detect the presence of endotoxins. Pyrogens may be present which are not of endotoxins origin, and these would only be detected by the rabbit test (P.F.Sharp 1989). Seibert, F.B and co-workers (1923) indicated that rabbit and humans are equally responsive to pyrogens.

The rabbit test was conducted at RICK, in which three mature rabbits were injected with Tc^{99m} – MDP. A dose of o.5 mCi was injected into the ear vein of each rabbit, and the rectal temperature of each rabbit was taken before injection and at 1, 2, 3 hours post injection, using a digital thermometer, and the data were recorded (Sam 2008). The rabbits were selected in three different colors and classified as A, B, and C to avoid any confusion. The test showed temperature elevation in the three rabbits

after 1, 2,3 hours post injection . The total temperature rise in A,B, and C rabbits was 0.8 , 0.7 and 0.8 C^0 respectively , which were higher than the normal range (< 0.6 C^0) The total temperature rise of the three rabbits together was 2.3 C^0 (the acceptable rise is < 1.4C^0). It is more likely that the injected solution contained pyrogens. Absence of aseptic technique in RICK, including the use of laminar airflow enclosures (Paul E, Christian 2004), and the absence of adequate cleaning in the hot lab may be the causes of the presence of these pyrogens. The hot lab was not adequately clean, with some cracks in the ceramic and dust and dirt on the floor. Cleaning was not performed every day. This fact was reached also by Adlan (2007). To prove this, the researcher put an ink smear on the floor of the hot lab. The smear remained unchanged in color or density for more than a month. Cleaning is very important in this field of work, as pyrogens cannot be removed from the radiopharmaceuticals preparations by any sterilization method, and control of pyrogens must be achieved by using only pyrogens-free reagents and maintaining very high pharmaceutical standards throughout the preparation .The preparation must be carried out in the appropriate clean or aseptic facilities (Roy P. parker et al, 1984).

Table4.2.7shows the physical appearance tests of different Tc^{99m} radiopharmaceuticals.

The purpose of the tests was to observe any change in color or presence of particles in the Tc^{99m} O_4solutions. Any change of such nature may reflect changes in the radiopharmaceuticals that would alter its biologic behavior (Gobal p. Saha 2010).

In this test, 7 samples of different volumes from different generators eluates were taken and thoroughly viewed. All samples showed no particles and no change in color.

Table 4.2.8 shows Mo^{99}breakthrough in different Tc^{99m}eluates.

This test is mandatory for any generator eluate (Sam 2008). The presence of Mo^{99}in the eluate gives unnecessary radiation dose to the patient, as Mo^{99} has an energy of 740 -780 Kev (Gopal P. Saha 2001).

Ten samples of different elutions and activities (ranged between 6.46 and 757 mCi) were used in this test, including samples from the first elution. A 4-mm thick lead canister was used to contain the sample vials for activity measurement, using a dose calibrator. According to the world standard level, the Mo^{99} activity in the eluate must not exceed 0.15 µCi Mo^{99} / mCi Tc^{99m}at administration time, and 0.038 at elution time (Sam 2008). The results showed that the Mo^{99} levels in all the measured

amounts were within the accepted values both at elution time and at injection time .The levels in samples number 3 and 6, however, were higher than the accepted levels, which may be due to geometric or other factors .These results proved that there was no justification to leave the activity of the first elution unused (see tables 4.2.5 A and B).

Table 4.2.9 shows the radiation exposure rates in different areas in the nuclear medicine department in RICK.

These measurements were very important from the radiation protection aspects. According to the international rules in this field, areas like the hot lab for dispensing and storage of radiopharmaceuticals should classified as (controlled area), where patients and members of the public are not allowed to access. The dose in the controlled area should not exceed 10µSv /h (IAEA rules). Areas like offices , tea rooms and wards which are accessible to the public ,other patients and staff who are not radiation workers ,should be classified as (uncontrolled areas) , where the dose rate should not exceed 0.5 µSv /h (IAEA rules).

The table showed that the dose rates, during the second elution, were 21 µSv /h inside the lead shield and 14 µSv /h outside the lead shield in the hot lab .Both rates were higher than the permissible rates mentioned above. During the last elution of the generator, where the activity is usually very low, the dose rates in the hot lab were 7 µSv /h and 3 µSv /h inside and outside the shield respectively .Both rates were within the permissible ranges. That means the elution maker should take care during the generator elution and preparation of the radiopharmaceuticals, particularly during the first elutions. This could simply be made, among other procedures, through the application of cardinal principles of radiation protection , i.e. the time ,by spending as short time as possible , the distance , by keeping oneself as far as possible from the radiation source and using the shields whenever possible.

In the injection room, the injector received 19 µSv /h from a bone dose (20 mCi), which was higher than the permissible limit, and 3.5 µSv /h from a thyroid dose (5 mCi), which was within the accepted level. The bones and thyroid doses were selected because they constitute the higher and lower doses respectively. Other organs doses lie in between. Again, the cardinal principles of radiation protection should be fully applied during injection .The injector, usually a nurse, should be informed of this.

Regarding the imaging room, previous studies have shown that the most significant source of whole body exposure is the radiation emanating from the patient during the imaging procedure (Paul E. Christian et al and NCRP report, USA, 1987).This

was proved by this study, which showed that the technologist received 15µSv /h and 4.4 µSv /h from bone patient and thyroid patient respectively .These rates were less than the doses received during the elution and injection of radiopharmaceuticals. This result seemed contradictory to the above mentioned results of previous studies. However, in the imaging room of RICK, the technologist usually stays longer time in positioning and imaging of all patients without shielding, which increases the total dose by the end of the day (see fig. 5.1) below:

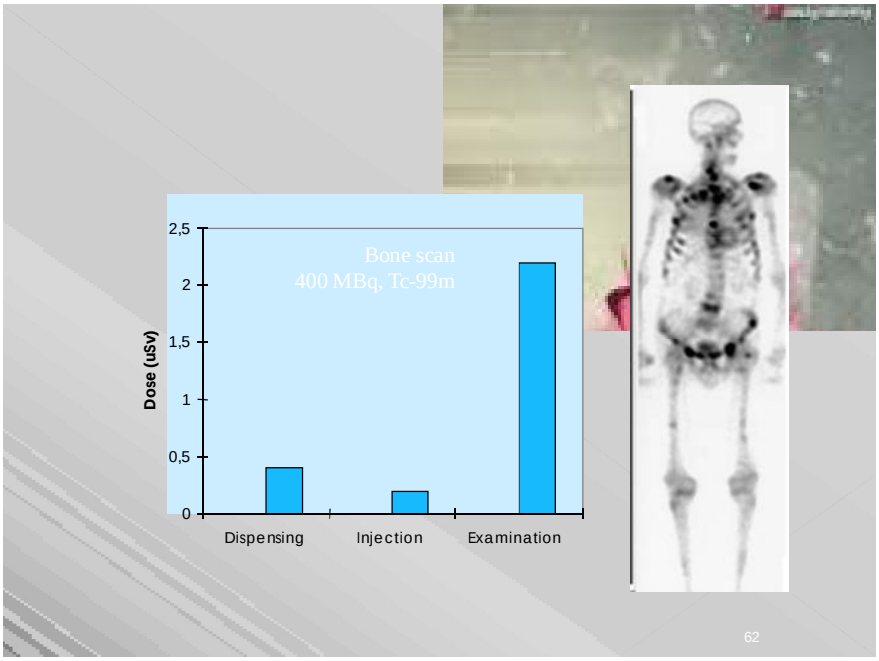


Fig. (5.1) shows the dose received during bone imaging (long column) as compared with the dose received during dispensing and injection of the radiopharmaceutical.

In the waiting area of injected patients (a small room), the dose rate was measured at the center of the room in which 8 patients for bone and thyroid patients were sitting together. The dose rate (30 µSv /h) was the highest rate in the department. The patients received radiation from each other ,and the co-patients or even any other persons who enter that room could receive radiation ,as there was no control on that room .The patients toilet ,as well, recorded a high dose rate(28 µSv/h).This was because there was one toilet for patients and all the patients went there . Other people could receive radiation if they use that toilet, as it was uncontrolled.

As for the reception, the registered dose rate was 3.6 µSv /h, which was higher than the maximum permissible rate in uncontrolled areas (0.5 µSv /h). This may be related to the location of the reception near the injected patients waiting area. The staff offices and rest rooms recorded no radiation exposure rates.

5.2 Conclusion

This study was conducted at RICK, during the period from 2010 to 2016, with the main objective of evaluating the practical procedures performed with respect to dealing with Tc^{99m} radiopharmaceuticals .The evaluation focused mainly on quality control aspects, most of which, if not all, were evaluated for the first time, to the best of the researcher's knowledge . Part of the study results was obtained via observation, including the application of radiation safety rules by the staff members during the generator elution, preparation and handling of radiopharmaceuticals, the dose injection and during the patient imaging.

The observation results showed that some of the rules were applied, some were partially applied and others were not applied at all. The other part of the results was obtained through experiments and tests, and included the measurement of radiation doses received by the hands, eyes and whole body of the operator during the elution of the generator, the injection of the radiopharmaceuticals to the patients and during patients imaging .Wipe tests for radiation contamination on the generator package surface and external radiation exposure from generator package were also conducted. The results of all these tests were compared to the international permissible dose limits .The remaining activities (prepared and unprepared) of some elutions were measured showing that (57%) of the activities were not used at all and discarded as wastes. Rabbits test for the eluate pyrogenicity was performed using three mature rabbits. The rabbits temperatures were measured before and after the injection of small doses of radiopharmaceuticals .The test showed increased rabbits temperature after the injection, which denoted the presence of eluate pyrogens. Eluates physical appearance tests were done and showed no change of eluate color, no turbidity and no presence of visual particles. Tests of Mo^{99}breakthrough for different eluate samples were performed and showed that the presence of Mo^{99} within the eluates did not exceed the permissible levels. Moreover, the radiation exposure rates of all the radiation areas in the nuclear medicine were measured and compared to the internationally acceptable levels. More than fourteen aspects of ^{99m}Tc-radiopharmaceuticals quality control procedures were addressed by this study via observation and experimental tests.

The study included some recommendations which may assist in the establishment of a new policy of dealing with the Tc^{99m} radiopharmaceuticals in the nuclear medicine department of RICK, with respect to the application of radiation protection rules, quality control procedures and other applications which may assist in the improvement of the practice and the outcomes thereof.

5.3 Recommendations

All nuclear medicine staff members are recommended to wear lab coats , disposable gloves and face masks during elution and dispensing of radiopharmaceuticals .

All radiopharmaceuticals containers should be put in shields or shielded areas and clearly labeled.

Personal radiation monitors should be procured to the nuclear medicine department and worn by all workers.

Work surfaces in the hot lab should be covered with absorbent materials to absorb any possible radioactive spillage contamination.

The cardinal principles of radiation protection (i.e. time, distance and shielding) should always be applied in radiation areas.

Aseptic conditions and good cleaning should always be maintained in the hot lab and other areas of the department.

Quality control procedures should be implemented at daily, weekly, monthly and annually basis and all the quality control facilities should be provided at the department.

Radiation areas used by the injected patients, like the waiting area, toilets...etc should be clearly identified by warning signs and notes, and should be accessible only by patients if possible.

The remaining radio- activities after any generator elution should be utilized either by increasing the number of studies, the number of gamma cameras or by selling or donating them to other nuclear medicine centers which may benefit from them .

A generator of lower capacity (less than 10 GBq) should be imported for the time being, until the inoperative gamma cameras are maintained or new cameras are procured. This may also solve the problem of remaining activities.

Additional and modern gamma cameras should be purchased to keep pace with the new nuclear medicine studies and to benefit from the remaining radiation activities and the abundant number of the staff members in the department.

Implementation of the above mentioned recommendations is highly recommended.

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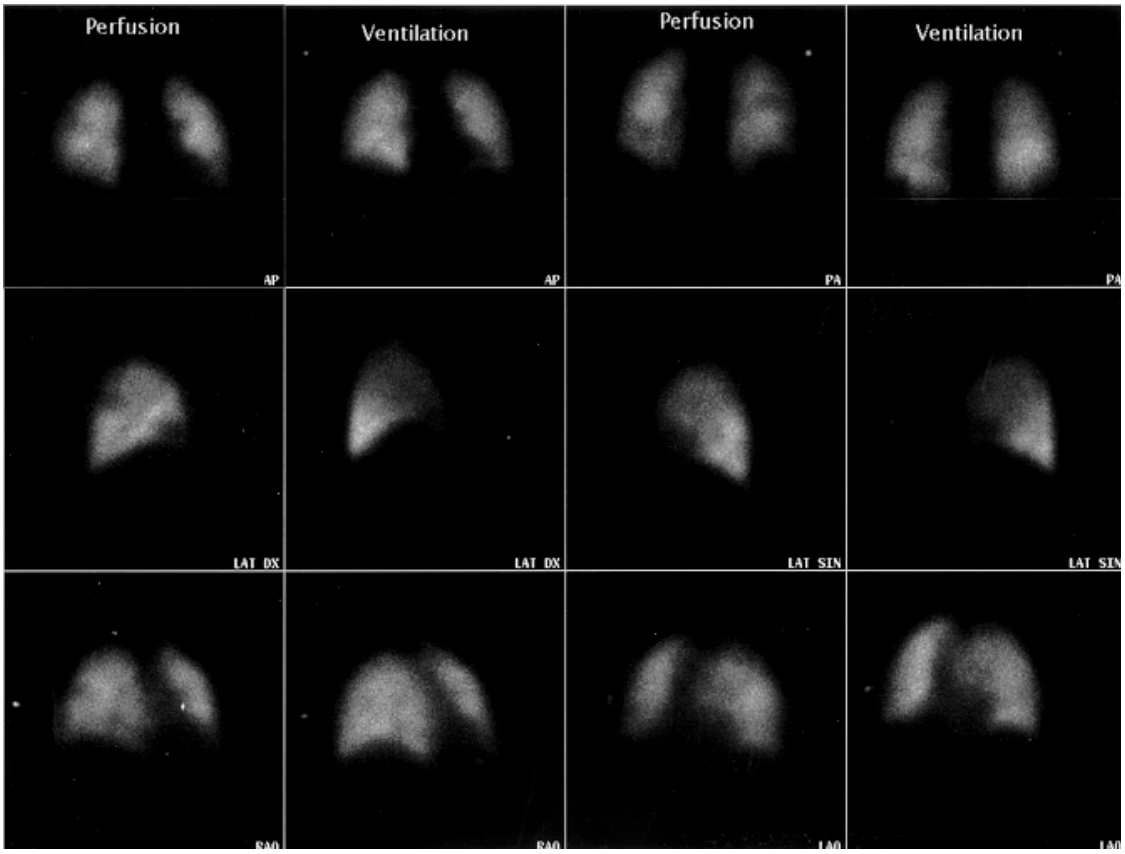
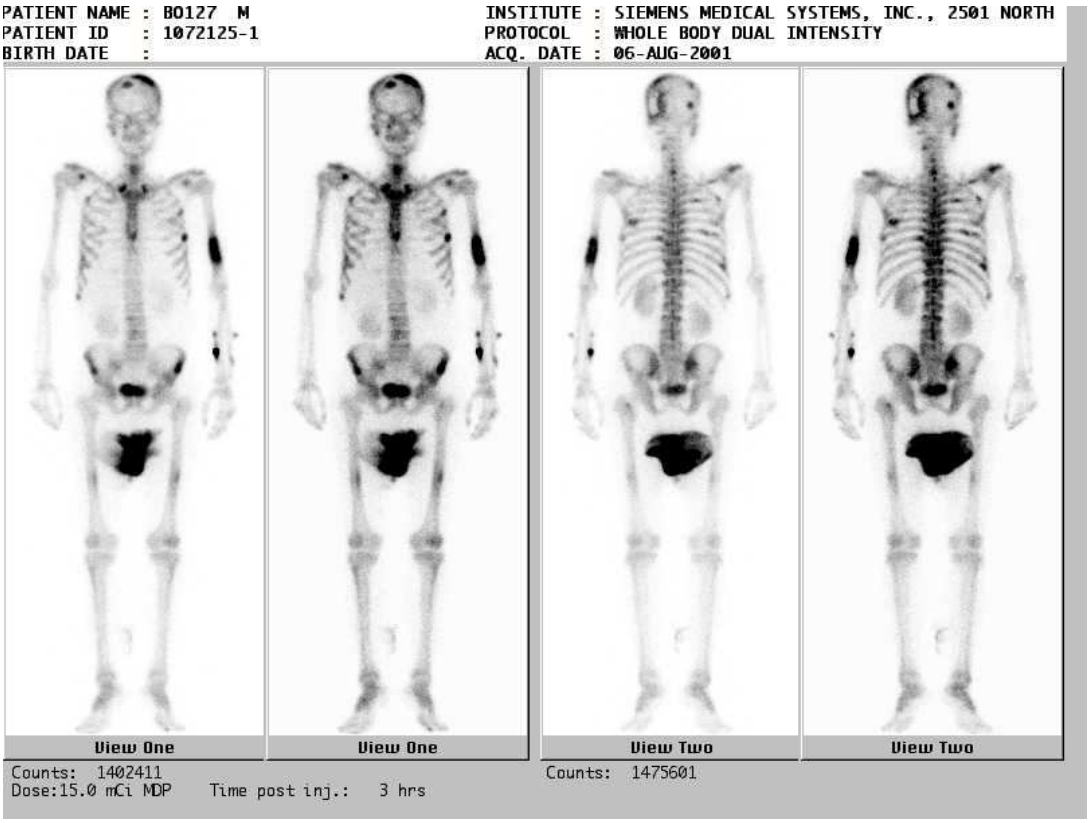
Appendices

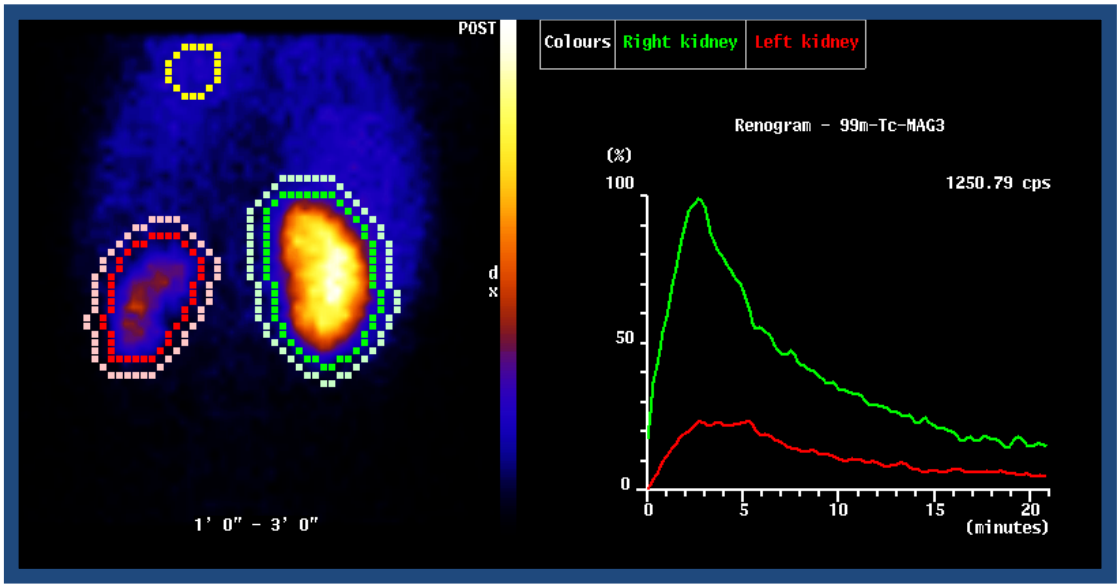
Appendix (1) A radionuclide dose calibrator (Biodex model, Atomlab 5oo)



Appedix (2) : A radionuclide image of the lungs.



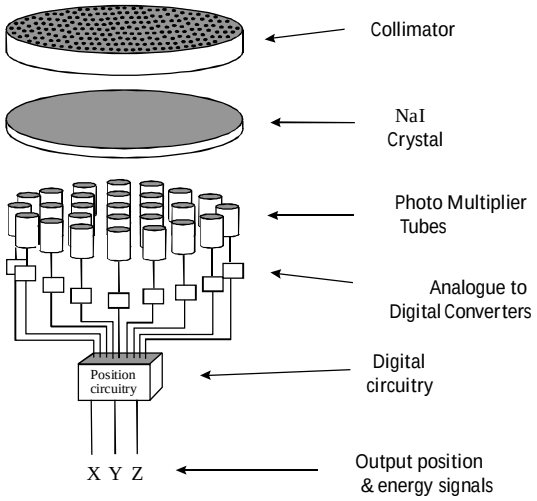
Appendix (3) Radionuclide bone scan showing multipple bone metastases.



Appendix (4) A radionuclide image of dynamic renal scan .

Appendix (5) Radiation survey meter.

The Gamma Camera



Appendix (6) Components of gamma camera

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Appendix (7) Two different types of gamma cameras.