



e-ISSN: 2319-8753 | p-ISSN: 2347-6710

# IJRSET

International Journal of Innovative Research in  
**SCIENCE | ENGINEERING | TECHNOLOGY**



# INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH

IN SCIENCE | ENGINEERING | TECHNOLOGY

Volume 12, Issue 5, May 2023

**ISSN** INTERNATIONAL  
STANDARD  
SERIAL  
NUMBER  
INDIA

Impact Factor: 8.423

# Quality Surveillance & Comparative Analysis of Different Quality Parameters of $^{99}\text{Mo}$ – $^{99\text{m}}\text{Tc}$ Radioisotope Generators in Type Iii Radioisotope Laboratory

Tushar Gupta <sup>1\*</sup>, Arpita Datta <sup>2</sup>, Alpana Goel <sup>3</sup>

U.G. Student, Amity Institute of Nuclear Science and Technology, Amity University Uttar Pradesh, Sector-125 Noida Campus, India<sup>1\*</sup>

Assistant Professor, Amity Institute of Nuclear Science and Technology, Amity University Uttar Pradesh, Sector-125 Noida Campus, India<sup>2</sup>

Head of Department, Amity Institute of Nuclear Science and Technology, Amity University Uttar Pradesh, Sector-125 Noida Campus, India<sup>3</sup>

**ABSTRACT:** The use of radiopharmaceuticals in diagnostics and therapeutics procedures has increased significantly over the years, and the safety and efficacy of these radiopharmaceuticals depend largely on the quality of the radioisotope generators used in their production. Quality surveillance and comparative analysis of different  $^{99}\text{Mo}$ – $^{99\text{m}}\text{Tc}$  radioisotope generators are critical in ensuring the reliability of these radiopharmaceuticals. In the present work, several quality parameters are monitored and analysed, which includes radionuclidic purity, chemical purity, radiochemical purity, pH, and microbial contamination.

Radionuclidic purity refers to the ratio of the desired radioactivity to the total radioactivity of the source. Unwanted activity may be present due to radioactive impurities in the  $^{99}\text{Mo}$ – $^{99\text{m}}\text{Tc}$  generator eluate such as  $^{131}\text{I}$ ,  $^{89}\text{Sr}$  and  $^{103}\text{Ru}$ . While chemical purity refers to the level of non-radioactive impurities present in the generator system such as alumina ( $\text{Al}_2\text{O}_3$ ). Radiochemical purity, on the other hand, refers to the percentage of  $^{99\text{m}}\text{Tc}$  present in the labelled radiopharmaceutical compared to other chemical species in the formulation. The pH refers to the acidity or basicity of the generator eluate. Microbial contamination refers to the harmful bacteria and fungi that might enter with the radiopharmaceutical. Comparative analysis of different generators is essential in selecting the most suitable generator for a specific application. The result of the analysis can be used to compare the quality parameters of different generators and we can select the best quality generator production route, so to achieve the desired quality standards.

**KEYWORDS:** Quality Control, Radioisotope Generator, Sodium Pertechnetate, Radiopharmaceuticals, and Nuclear Medicine

## I. INTRODUCTION

$^{99\text{m}}\text{Tc}$  is the most used radioactive isotope in medical imaging procedures, particularly in nuclear medicine.  $^{99\text{m}}\text{Tc}$  is used in approximately 30 million procedures per year, accounting for 80% of all nuclear medicine diagnostic procedures worldwide [1]. Several reasons for extensively using  $^{99\text{m}}\text{Tc}$  are: It has a half-life of only 6 hours, which means that it decays relatively quick and does not remain in the body for long periods of time. This reduces the risk of radiation exposure to the patient. Technetium can be easily combined with various compounds, allowing it to be used in a wide range of diagnostic tests and imaging procedures. The gamma energy of 142.68 keV is within the optimal energy range for gamma cameras. This energy level is high enough to penetrate through the body and reach the detectors, but not so high that it creates excessive scatter or attenuation in the body. In addition, the energy level is optimal for efficient detection and imaging. This allows for high-quality images to be produced during medical scans. At low energies, the photoelectric effect is the dominant interaction for gamma rays from the metastable state of Technetium. This is because the photoelectric effect is more likely to occur when the gamma-ray energy is similar to the binding energy of the electrons in the material. This can result in gamma-ray being absorbed completely by the

material, which can produce high-quality images in medical imaging. The demand for  $^{99m}\text{Tc}$  can vary depending on several factors, including the number of patients undergoing scans, the availability of  $^{99m}\text{Tc}$  production facilities, and the efficiency of the production process.  $^{99m}\text{Tc}$  is used for diagnostics purposes in nuclear medicine and is attached to a ligand (drug) that directs it to a specific target.

The supply of this radiopharmaceutical must follow strict codes and conducts as we are dealing with the health of the public. The radiological, chemical, and biological regulations must be followed, or the company can face serious legal threats. For this work a radioisotope generator same as Figure: 1 generator system is used to produce the final product (i.e., Sodium Pertechnetate) and several quality control tests are performed on the eluate. Radioisotope generator has proper lead shielding for the safety of the personnel using it, sterile needles & filters for the prevention of any microbial contamination, and the appropriate amount of alumina powder in the glass column to get maximum generator efficiency. The final elution prepared must match up with the standards of the European Pharmacopoeia 7.0 (01/2008:0124) and that is done by performing several physico-chemical and biological tests.

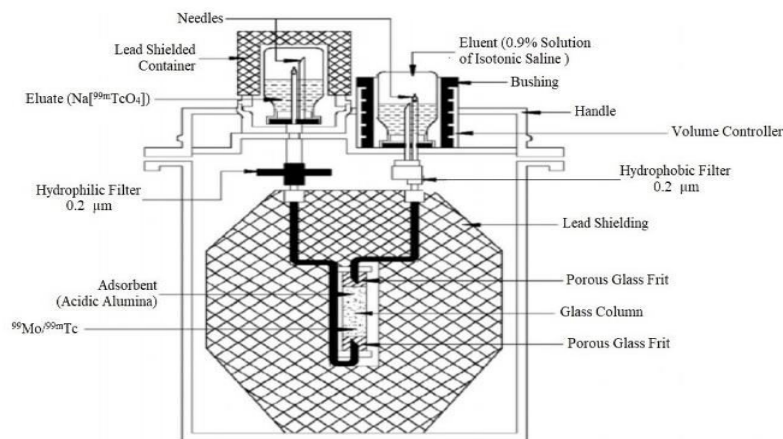


Figure 1: Schematic of a  $^{99}\text{Mo}$ - $^{99m}\text{Tc}$  Dry-Type Generator System [2].

#### • Nuclear Property of $^{99}\text{Mo}$ and $^{99m}\text{Tc}$

$^{99}\text{Mo}$  is a radioactive isotope of molybdenum with a half-life of approximately 66 hours. It decays by beta emission, emitting a high-energy electron with a maximum energy of about 0.78 MeV ( $^{99}\text{Mo} \rightarrow ^{99m}\text{Tc} + \beta^{-1} + \nu_e$ ).  $^{99}\text{Mo}$  has a natural abundance of around 0.24% in natural molybdenum and is typically obtained through 6.1% fission yield in a nuclear reactor, neutron activation of  $^{98}\text{Mo}$  or by particle accelerators using other reactions. It is the parent isotope of  $^{99m}\text{Tc}$ , therefore it is very important to nuclear medicine community, because of its 10 times longer half-life than its decay product [3], transient equilibrium is achieved between parent/daughter. This specific property is very important for the radioisotope generator, as it can be transported to distant facilities. Technetium is the element with the lowest atomic number which has no stable isotope (i.e., every form is radioactive). Technetium is mostly produced synthetically, and some minute amounts found on earth.  $^{99m}\text{Tc}$  is a meta-stable state of  $^{99}\text{Tc}$  isotope with a half-life of 6 hours and gamma-ray energy of 142.68 keV ( $^{99m}\text{Tc} \rightarrow ^{99}\text{Tc} + \gamma$ ). Because it is not found naturally, there are no natural sources of technetium-99m, and it must be produced artificially in a cyclotron or nuclear reactor. This also means that there is no natural way to dispose of technetium-99m, and it must be carefully managed to avoid environmental contamination.

#### • Different $^{99}\text{Mo}$ Production Routes

The near to ideal properties of  $^{99m}\text{Tc}$ -labelled radiopharmaceuticals for diagnostic purposes have heightened the demand for  $^{99}\text{Mo}$ . Only two ways to produce  $^{99}\text{Mo}$  are reactor and accelerators based, and by these two pathways has led us to various diverse methods of production of  $^{99}\text{Mo}$ . Reactor based path depends on the fission of uranium method, where  $^{235}\text{U}$  in Low or High Enriched state undergoes fission to produce a good yield of  $^{99}\text{Mo}$ , and the second method is by the activation of  $^{98}\text{Mo}$  (natural or enriched targets) by neutrons that under goes  $^{98}\text{Mo}(n,\gamma)^{99}\text{Mo}$  reaction. Reactor based pathways are well-established technologies, from production to chemical reprocessing till licensing and approval. Fission based method covers  $\geq 95\%$  of the global demand [4]. The advances in particle accelerators have provided various methods to produce  $^{99}\text{Mo}$ , either by photonuclear reaction or spallation neutron-induced reactions. The advantage in using accelerator-based route is, less radioactive waste generation as compared to fission-based method,



where producing 1Ci of  $^{99}\text{Mo}$  gives approximately 50Ci of waste [5, 6]. But there are several other drawbacks of using accelerator to produce  $^{99}\text{Mo}$ , such as low specific activity, low beam current, lower cross-section, and difficult isotopic separation. Still work is continued for various accelerator-based methods for making it a reliable source of  $^{99}\text{Mo}$ . Most recent development in past few years has led to direct production of  $^{99\text{m}}\text{Tc}$  by the following reactions  $^{100}\text{Mo}(d,3n)^{99\text{m}}\text{Tc}$ ,  $^{98}\text{Mo}(p,\gamma)^{99\text{m}}\text{Tc}$ ,  $^{97}\text{Mo}(d,\gamma)^{99\text{m}}\text{Tc}$ , and  $^{96}\text{Mo}(\alpha,p)^{99\text{m}}\text{Tc}$  in cyclotron [7].

• **Novel Technetium Chemistry**

Technetium is a chemical element, occupying 43rd position in the periodic table. It belongs to the period 5 and VIIB group of transient metals (d-block elements). E.C. of technetium is  $[\text{Kr}] 4d^5 5s^2$ , this provides several options to form complexes with large number of different ligands. Its oxidation state can change from +1 to +7, the O.S. is the main parameter determining the chemical nature of the complexes, most stable is +7 [8]. Pertechnetate ( $N = 4$ , O.S. = +7, and  $Z = -1$ ) is a very stable technetium form in aqueous media. The sodium can weakly bound with pertechnetate to form  $\text{Na}^+(\text{TcO}_4)^-$ , Sodium pertechnetate is highly soluble in water and aqueous solutions [9,10]. This property makes it easy to prepare and administer compounds for medical imaging studies. It is usually prepared as a sterile, pyrogen-free solution that can be injected into a patient’s bloodstream. Sodium pertechnetate can react with a variety of other compounds, including metals and organic compounds. For example, it can react with reducing agents such as stannous ions to form technetium-99m labelled radiopharmaceuticals. It can also react with metal chelating agents to form stable complexes that can be used in medical imaging. Some examples of novel technetium chemistry include new ligands, new labelling methods, new imaging agents, and new therapeutic agents etc. Relatively little is known about the chemistry of technetium compared to other elements. This has led to efforts to develop new technetium compounds and reactions that can improve the effectiveness and safety of radiopharmaceuticals.

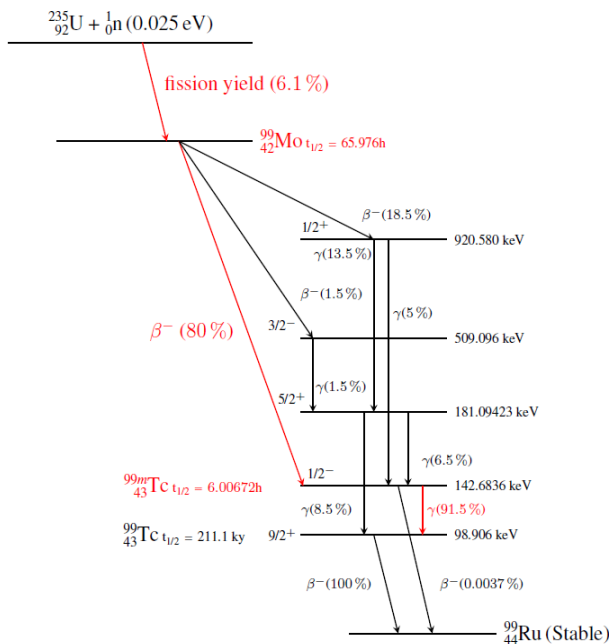


Figure 2: Induced fission of  $^{235}\text{U}$  to ultimately produce  $^{99\text{m}}\text{Tc}$ .

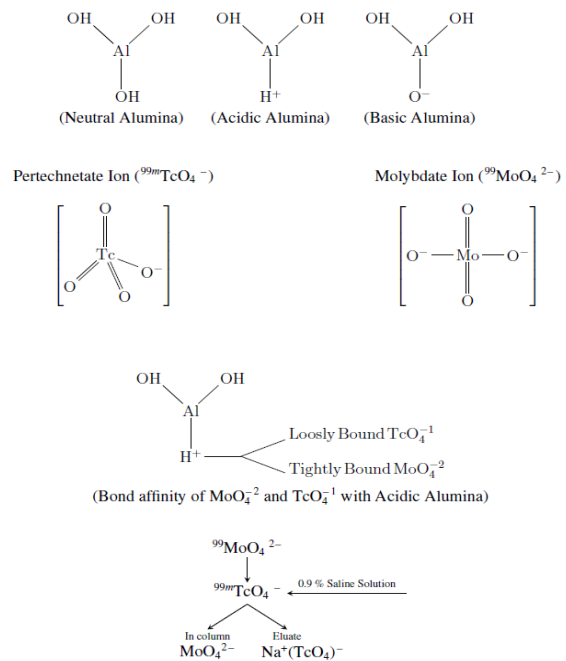


Figure 3: Chemical reactions structure of  $(\text{MoO}_4)^{2-}$  with acidic alumina surface. Washing the column with saline solution will Sodium Pertechnetate in the eluate

The chemical behaviour of sodium pertechnetate is highly dependent on pH [11]. At low pH (less than 3), it exists as the pertechnetic acid ( $\text{HTcO}_4$ ) molecule. At neutral to basic pH (greater than 7), it exists as the sodium pertechnetate ( $\text{NaTcO}_4$ ) ion. The different species that form at various pH levels can affect the stability and reactivity of sodium pertechnetate in different applications. As shown in Figure: 3, molybdenum decays to technetium and pertechnetate is formed. The bond affinity of pertechnetate is greater with sodium (passed during elution in form of saline solution) than acidic alumina, that makes it suitable for elution of  $^{99\text{m}}\text{Tc}$  in form of sodium pertechnetate in a robust and efficient manner. Further,  $\text{Na}^+(\text{TcO}_4)^-$  can be easily used in cold kit for labelling with receptor ligands, metabolic agents, peptides, proteins, antibodies, and antibiotics.

## II. RELATED WORK

### ❖ RADIOISOTOPE HANDLING FACILITY

Three types of laboratories are defined under AERB guidelines based on the inventory and radiotoxicity of the radionuclides handled. The design and safety features would therefore increase with the increasing numbers [12]. SDS life sciences produce radiopharmaceuticals on a commercial level for medical and research purposes, based on these categorizations it is considered a Type III radioisotope lab. Relative radiotoxicity per unit activity of the radionuclide are classified into four groups; Group 1 (Very High Hazards):  $^{210}\text{Pb}$ ,  $^{210}\text{Po}$ ,  $^{226}\text{Ra}$ ,  $^{90}\text{Sr}$ , Group 2 (High Hazard):  $^{125}\text{I}$ ,  $^{133}\text{I}$ ,  $^{99}\text{Mo}$ ,  $^{32}\text{P}$ ,  $^{212}\text{Pb}$ ,  $^{192}\text{Ir}$ ,  $^{60}\text{Co}$ ,  $^{89}\text{Sr}$ ,  $^{90}\text{Y}$ , Group 3 (Moderate Hazard):  $^{82}\text{Br}$ ,  $^{14}\text{C}$ ,  $^{51}\text{Cr}$ ,  $^{18}\text{F}$ ,  $^3\text{H}$ ,  $^{24}\text{Na}$ ,  $^{32}\text{S}$ ,  $^{153}\text{Sm}$ ,  $^{103}\text{Pd}$ ,  $^{99\text{m}}\text{Tc}$ , and Group 4 (Low Hazard):  $^{99}\text{Tc}$ , nat. U, nat. Th.

A Type III laboratory for radioisotope handling is a facility designed for handling and working with moderate ( $^{99\text{m}}\text{Tc}$ ) to high-risk ( $^{99}\text{Mo}$ ) radioactive materials. The radioactive materials are usually contained in unsealed containment or small quantities of radioactive liquids. Type III laboratories follow stringent criteria that are used for research and medical purposes and require strict safety protocols. The facility is designed for the safe handling, storage, and disposal of radioactive materials that have relatively low levels of hazard such as  $^{99}\text{Tc}$ . These laboratories for radioisotope handling are a highly specialized facility that require strict safety protocols to ensure the protection of laboratory staff and the environment when working with moderate to high-risk radioactive materials.

#### • Planning Quality Assurance and Quality Control Activities

Planning Quality Assurance (QA) and Quality Control (QC) activities to produce  $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$  is critical to ensure that the radiopharmaceutical product is safe, effective, and meets regulatory requirements [13]. Here are some recommended activities for QA and QC:

- Establish a quality management system: A quality management system (QMS) is a set of policies, procedures, and processes that ensure consistent quality throughout the production process. The QMS should include procedures for documentation, change control, deviations, and CAPA (Corrective and Preventive Actions).
- Develop and validate analytical methods: Analytical methods are used to test the quality and purity of the radiopharmaceutical product. These methods should be validated to ensure their accuracy, precision, specificity, and sensitivity.
- Perform in-process control: In-process control (IPC) is a set of checks performed during the production process to ensure that the product is on track to meet specifications. IPC includes monitoring critical process parameters (CPPs) and verifying that the product is within specifications at each stage of production.
- Perform release testing: Release testing is performed on the final product to ensure that it meets specifications and is safe for use. Release testing includes tests for radiochemical purity, radionuclidic purity, sterility, endotoxin, and pyrogenicity.

Table 1: Endotoxin Limits for various types of products.

Product	EU for a 70 kg adult	Maximum Injected Volume (ml)	Endotoxin Limit (EU/mL)
Parenteral Drugs	350	V	350/V
Intrathecal Drugs	14	V	14/V
Radiotracers	175	7(adult)	25
Large Volume Parenterals (e.g. Saline )	#	10 per kg	0.5
Water for Injections	#	#	0.25

# Not Defined

- Perform supplier qualification: Suppliers of raw materials, components, and services should be qualified to ensure that they meet the required standards for quality and safety. By implementing these QA and QC activities, the production of  $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$  can be controlled and monitored throughout the production process, ensuring that the final product is safe and effective for use in nuclear medicine.
- Monitor environmental conditions: Environmental monitoring is performed to ensure that the production environment is controlled and that there are no sources of contamination. Environmental monitoring includes air sampling, surface sampling, and water testing.



Table 2: Recommended Limits for Microbial Contamination.

Class	Settle Plates CFU/4hours (90mm)	Contact Plates CFU/Plate (55mm)
A	< 1	< 1
B	5	5
C	50	25
D	100	50

CFU: Colony Forming Units

- Perform equipment qualification and maintenance: Equipment used in the production process should be qualified to ensure that it is suitable for its intended use. Equipment should be maintained and calibrated regularly to ensure that it operates correctly and consistently.
- Perform personnel training: Personnel involved in the production process should be trained in the procedures and processes required to produce the radiopharmaceutical product. Training should include GMP (Good Manufacturing Practice) requirements, safety procedures, and the importance of quality.

By implementing these QA and QC activities, the production of <sup>99</sup>Mo/<sup>99m</sup>Tc can be controlled and monitored throughout the production process, ensuring that the final product is safe and effective for use in nuclear medicine.

• **Good Manufacturing Practices (GMP)**

Good Manufacturing Practices are a set of guidelines that ensure that pharmaceutical products are consistently produced and controlled according to quality standards [14]. The manufacture of generator systems is considered to be conventional pharmaceutical production that is needed to fulfill all requirements of the pharmaceutical industry. In consequence, manufacturers of generator systems have to be licensed for compliance with GMP by the regulatory authorities. Being a Type III facility, all activities are under the stringent control of the quality management system, which is interlinked with the concept of QA, GMP, and QC. As a part of GMP, which applies to the pharmaceutical that is safe for the patient’s use, ISO standards on the other hand regulate the activities of a manufacturer. Having ISO certification for the facility is as important as setting it up within the legal framework.

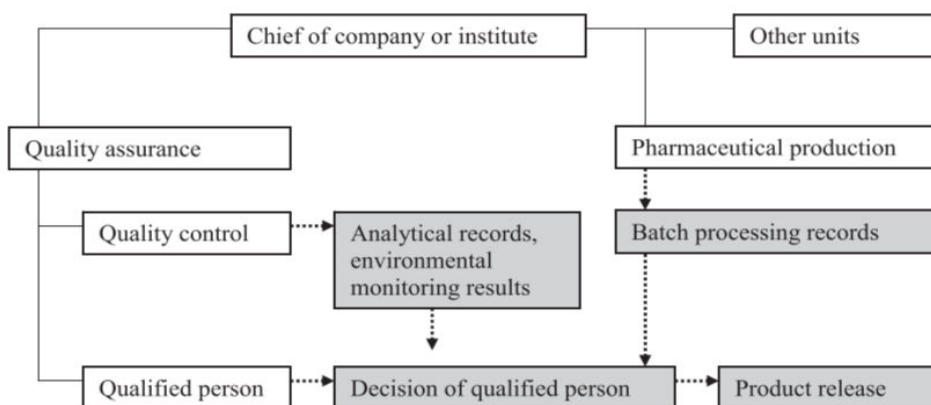


Figure 4: Schematics representation of GMP origination and function.

• **Quality Control (QC)**

QC team works independently of the production and deals with all sampling, testing, and monitoring of the starting & packaging materials and finished product. The QC is carried out according to the given Standard Operating Procedures (SOPs). Each method is validated, re-evaluated, and the instruments are regularly calibrated. Two types of testing are done for ensuring the quality of product (i.e., Sodium Pertechnetate eluate obtained in the evacuated/vacuum vial): physico-chemical and biological tests. Physico-chemical tests are the type of analytical test that examines the physical and chemical properties of a substance. This type of tests is used to determine the characteristics of a substance, such as its composition, structure, and behaviour under different conditions. Whereas biological tests can include methods such as microbiological assays. These tests are used to evaluate the biological activity, potency, purity, and safety of a product, and to identify any potential risks or adverse effects. All the results are then compared against established

specifications to determine whether the product meets the required standards. The data of tests are registered in records which include all the originality required to testify the statement. Based on the data, the QC team will provide an analytical certificate for each production batch; the certificates are clear and brief summaries of the analytical records indicating both the acceptance limits (according to standard documents) and values truly obtained [15]. The QC tests for three different generator systems are documented in the upcoming sections of the project.

### III. METHODOLOGY

#### ❖ QUALITY CONTROL TESTS OF THE SODIUM PERTECHNETATE

(<sup>99m</sup>Tc) Injection (Fission) as per European Pharmacopoeia 7.0 (01/2008:0124) for pharmaceutical parameters and radioactive parameters.

First let's discuss some of the physico-chemical Tests performed on the product:

##### ▪ Appearance Test

The appearance test for Sodium Pertechnetate solution is usually done by visual inspection, behind an L-bench and under proper lighting. Typically, visually inspect is looking for any signs of discoloration, precipitation, or cloudiness. The final elution is collected in a 15ml vacuum vial, desired inspection result should be a clear colourless solution. The appearance test for elution fractions is an important step in ensuring the quality and purity of the eluted substance.

##### ▪ Radionuclide Identification

Identification of the radionuclide is a necessary step and is done by Well-Type NaI (Tl) scintillation detector. A sample that is send for identification is prepared by taking the eluate, putting it on a tissue paper, placed inside a labelled test tube (as shown is Figure: 5). The desired value of gamma-ray energies should be  $140\text{keV} \pm 10\%$  from the sample.

##### ▪ Radiochemical Purity

Radiochemical purity (RCP) is a measure of the degree to which a radiopharmaceutical preparation contains the desired radionuclide in the intended chemical form, and the absence of other chemical forms or contaminants. Procedure to perform the thin layer chromatography (TLC) and calculation of RCP value are: Spot a small amount of the radiopharmaceutical preparation onto a silica gel strip backed with aluminium material, then develop the strip using a suitable solvent system of acetone that separates the desired radiopharmaceutical compound from other chemical forms or contaminants. Capillary action is responsible for carrying the sample mixture along the stationary phase and separating the components of the mixture based on their interaction with the stationary phase. Now measure the distance travelled by the desired radiopharmaceutical compound (Dc) and the distance travelled by all the radioactivity on the plate (Dt). Calculate the retention factor using the following formula: Distance Travelled by the Compound / Distance Travelled by the Solvent Front. This formula is commonly used in TLC and other chromatography techniques to calculate the retention factor of a compound.

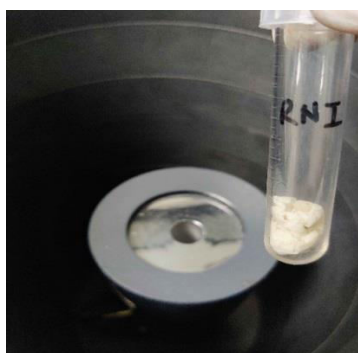


Figure 5: Sample is placed a well type of NaI (Tl) scintillation detector for identification of

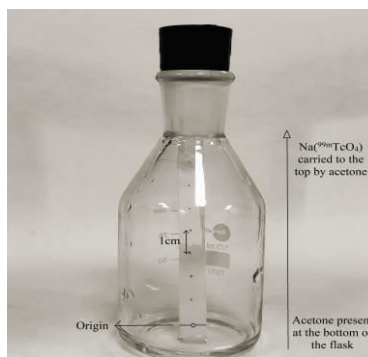


Figure 6: Aluminium-backed material coated with a stationary phase (i.e., silica gel), that is placed inside the solvent system of acetone.

Acetone is visible rising the silica gel strip, whereas our eluate (Sodium Perchnetate) is not. To counter this problem, we use a Well-Type NaI (TI) scintillation detector that was previously used in RNI test. The precalibrated detector take the counts from two different strips and identify whether appropriate amount of eluate has reached on the top strip. The distance travelled by the compound is measured from the origin (where the sample was spotted) to the centre of the spot, and the distance travelled by the solvent front is measured from the origin to point where the solvent front has reached. According to guidelines the Rf value has to be in the range of 0.6-1, therefore we can perform the TLC test by just letting the solvent (acetone) reach at the top and then placing the cut on strip where Rf value will always be greater than 0.6, performing in this manner would help us to effectively save time and reduce the radiation dose to the person performing this test. Rf value is a characteristic property of a compound under specific chromatography conditions, whereas the RCP value reflects the purity of the radiopharmaceutical preparation in terms of the intended chemical form of the radionuclide. While the Rf value is used for qualitative analysis and compound identification, the RCP value is used for quantitative analysis and assessing the quality of radiopharmaceutical preparations, it should be  $\geq 95\%$ .

#### ▪ pH Test

A pH test is a measurement of the acidity or alkalinity of a substance. The pH scale ranges from 0 to 14, with 7 being considered neutral. A pH value below 7 indicates acidity, while a pH value above 7 indicates alkalinity. pH tests can be performed using pH test strips, pH meters, or other specialized equipment. We use Merck indicator paper strip to measure the pH of the Sodium Perchnetate solution. According to the guidelines the pH of the elution should match the pH of the blood, which is between 4 to 8.



Figure 7: pH indicator paper strip.

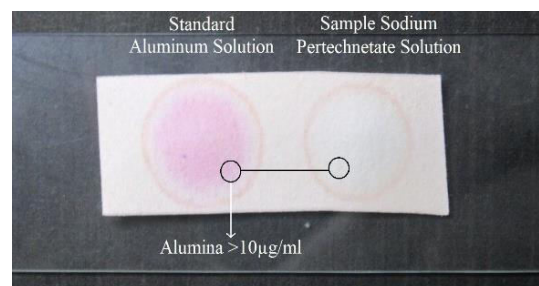


Figure 8: Alumina indicator paper strip.

#### ▪ Aluminium Breakthrough Test

We are using alumina powder in our generator column, therefore, to check for the proper functioning of the generator system we find whether there is any leak of alumina in sample. The final elution sample should comply with the guidelines with respect to limits of alumina. Figure: 8 shows the alumina breakthrough test done using colorimetric spot test, where the indicator paper is first exposed to the standard alumina solution (10µg/ml) and then to our sample.

#### ▪ Molybdenum Breakthrough Test

Molybdenum breakthrough is an important concern when using  $^{99}\text{Mo}$  generators, as it can lead to patient exposure to unwanted radiation. Molybdenum breakthrough occurs when  $^{99}\text{Mo}$  contaminates the  $^{99\text{m}}\text{Tc}$  product. The presence of  $^{99}\text{Mo}$  is referred to as radionuclide impurity and is unacceptable clinically, according to the guidelines the value of Molybdenum should not exceed 10µg/ml. There are two methods to identify molybdenum breakthrough, first is radiometric assay and molybdate ion spot test. Molybdate ion spot test is done by using the solution of 10% KCNS and 10%  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ , volume taken is 100µl of each that is placed inside a test tube and then mixed with first standard solution of Mo ( $\text{MoO}_3$ ) 100µl and then 100µl from sample of final eluate. Preparation methods of all the solutions we need for Molybdate Ion Spot test are: Standard Mo ( $\text{MoO}_3$ ) [10µg/ml]: 1.4gm of  $\text{MoO}_3$  + 2.6ml of 4N NaOH giving  $\text{Na}_2\text{MoO}_4$ , volume is then adjusted to 100ml to get the right concentration of 10µg/ml. 10% KCNS Solution: 1gm of KCNS (solid form) dissolved in 10ml of water for injection. 10% of  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  Solution: 1gm of  $\text{SnCl}_2$  dissolved in 0.3ml of conc. HCl, volume made up to 10ml by adding 9.7ml of water for injection. Standard is prepared such that it would contain near to 10µg/ml molybdenum and will result in colour change of the solution. If no colour change is observed in the sample, it has passed the breakthrough test as shown in the Figure: 9.



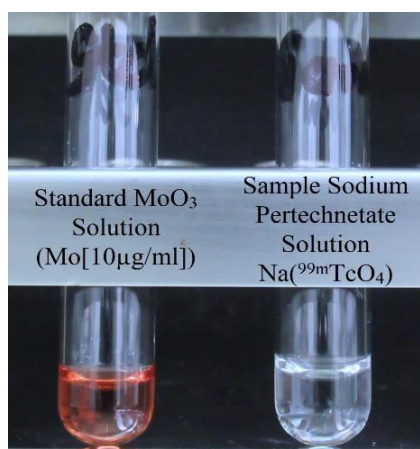


Figure 9: Positive for red and Negative for colourless.



Figure 10: Negative Product Control and Positive Control for different samples. Micro tubes are kept in a dry heat bath maintained at 37 degrees ± 1 degrees for incubation for 1 hour, to obtain results.

**These are Biological Tests performed on the Product:**

▪ **Bacterial Endotoxin Test**

Bacterial endotoxins are toxic substances that are found on the outer membranes of gram-negative bacteria. They are composed of a lipid component (lipid A) and a polysaccharide component (O-antigen) and are released upon the death or lysis of the bacteria. Endotoxins are potent stimulators of the immune system and can cause a range of pathological effects, including fever, inflammation, septic shock, and even death in some cases. They are also responsible for the symptoms of certain infectious diseases caused by gram-negative bacteria, such as meningitis and pneumonia. The detection and removal of endotoxins are critical in the manufacture of sterile pharmaceuticals, medical devices, and other biotechnology products. The Limulus amoebocyte lysate (LAL) assay, derived from horseshoe crab blood, is widely used for the detection of endotoxins in these products [16]. The Limulus amoebocyte lysate (LAL) test is a widely used test for the detection of endotoxins in pharmaceuticals, medical devices, and other biotechnology products. Since the blood of horseshoe crabs contains a clotting factor that reacts specifically with bacterial endotoxins.

The test is performed first getting LAL reagent, that is the blood of horseshoe crabs is collected in a sterile manner and the cells are separated from the plasma. The plasma, which contains the clotting factor, is then processed into a LAL reagent [17]. Preparation of sample must be tested is prepared by diluting it with endotoxin free water or buffer.

Table 3: Test sample preparation as per the Maximum Valid Dilution (MVD)

Sample	EL* [EU/ml]	MVD = EL/λ	In Sample Tube	
			Sample Vol.	Water BET
Na <sup>99m</sup> TcO <sub>4</sub>	25	200	50 µl	10 ml
Isotonic Saline Vials	0.5	4	0.5 ml	2 ml
Vacuum Vials	0.5	4	0.5 ml	2 ml

Add a measured amount of LAL reagent to the sample. Incubation is done by mixing the sample and the LAL reagent is incubated for a specific time and at a specific temperature to allow the clotting factor to react with any endotoxins that may be present in the sample. Finally, after the incubation period, the mixture is observed for the formation of a gel or clot, which indicates the presence of endotoxins in the sample. The strength of the clot is proportional to the amount of endotoxin present in the sample. The LAL test is a sensitive and specific method for the detection of endotoxins and is preferred over traditional methods such as the rabbit pyrogen test. It is also a rapid test that can provide results within a few hours [18].

▪ **Sterility Testing**

Sterility testing using two media involves inoculating a sample of the product onto the culture medium and incubating it under appropriate conditions for a specified period of time. The culture is then observed for the presence of microbial growth, which would indicate a lack of sterility in the product [19]. Fluid Thioglycollate (FTG) media is a type of culture medium used to cultivate and detect the presence of both aerobic and anaerobic microorganisms in various

samples, such as clinical specimens, pharmaceutical products, and food samples. It is a complex medium containing enzymatically hydrolysed casein, yeast extract, dextrose, and sodium thioglycollate, among other components. Soybean Casein Digest (SCD) media is a type of culture medium used to cultivate a wide variety of microorganisms. It contains a mixture of enzymatically hydrolysed soybean and casein proteins, as well as various other nutrients that support the growth of bacteria, yeast, and fungi. By using both FTG and SCD media for sterility testing, laboratories can increase the likelihood of detecting any microorganisms that may be present in different samples (Figure: 11). This can help to ensure the safety and quality of a wide range of products, from saline solution and radiopharmaceuticals to equipment and environmental samples.



(a) Sterile Fluid Thioglycollate (FTG) media for aerobe and anaerobes



(b) Soybean Digest (SCD) media for moulds and yeast

Figure 11: Sterility Tests are carried out on Sample 1 (S<sub>1</sub>) Evacuated Vials (EV), Isotonic Saline Solution (IS), and Sodium Pertechnetate Solution (Tc-G). The Control Kit (CK) on the left is the standard solution for checking colour differences.

▪ **Environmental Monitoring**

Environmental monitoring is the process of regularly testing and evaluating the cleanliness of the manufacturing or laboratory environment to ensure that it is free from microbial contamination. Soybean Casein Digest Agar plates is a common medium used for environmental monitoring because it is able to support the growth of a wide range of microorganisms and can be easily prepared in various forms, such as petri dishes, swabs, or dipslides.



Figure 12: Colonies formation observed on environmental monitoring plates.

We use petri dishes commonly known as contact plates and settle plates are both types of Soybean Casein Digest Agar plates that are typically used in environmental monitoring to assess the level of microbial contamination in the air and on surfaces. Contact plates (55mm dia.) are agar plates that are pressed onto a surface for a specified period of time, typically a few seconds to a minute, to collect any microorganisms present. The plate is then removed and incubated to allow any microorganisms present to grow. The number of colonies that grow on the plate can be counted to determine the level of contamination on the surface. Settle plates (90mm dia.), on the other hand, are agar plates that are left open in the environment to collect airborne microorganisms that settle onto the plate over a specified period of time (i.e., 2 to 4 hr). The plate is then incubated to allow any microorganisms present to grow. The number of colonies that grow on

the plate can be counted to determine the level of contamination in the air. For a given class the number of colonies should be less than the prescribed number (Table: 2). The incubation temperature needed for contact and settle plates depends on the type of microorganisms that are being targeted for detection. Generally, the temperature used for incubation is between 20°C to 37°C. The soybean casein digest agar supplements various nutrients to promote the growth of specific microorganisms (Figure: 12). Contact plates and settle plates are both useful tools in environmental monitoring and can be used to assess the level of contamination on surfaces and in the air, helping to maintain the quality and safety of products in laboratories and manufacturing facilities [20].

IV. EXPERIMENTAL RESULTS

❖ PHYSICO-CHEMICAL TESTS RESULTS

▪ Physical Appearance Test Results for Sodium Pertechnetate Eluate

The acceptable criteria is clear colourless solution for Sodium Pertechnetate elution. The observation denoted in Table 4 shows that all generator system’s elution has passed the test.

Table 4: Appearance Test Results

Generator Number	Appearance Test Observation	Result
1	Clear Colorless Solution	Pass
2	Clear Colorless Solution	Pass
3	Clear Colorless Solution	Pass

▪ Radionuclidic Identification Test Results for Sodium Pertechnetate Eluate

To be sure of which radioisotope is dealt with its identification is must. We use NaI (Tl) well-type scintillation counter for identification. Figure from 13 to 15 are the spectrum of eluate 1 sample. The acceptable criteria of the gamma-ray energy is 140keV±10%. The detector is calibrated before taking the spectrum and channel number 76 corresponds to energy of 140keV. All the three generators, eluate sample’s spectrum confirm the presence of <sup>99m</sup>Tc.

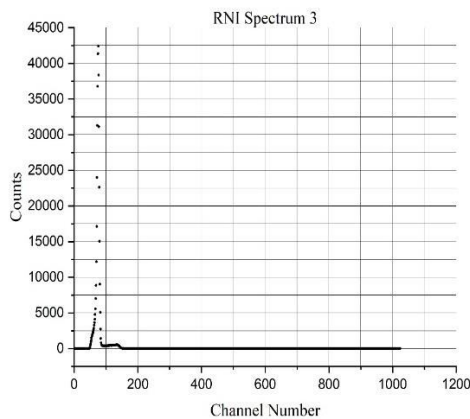


Figure 13: Radionuclidic Identification Result 1

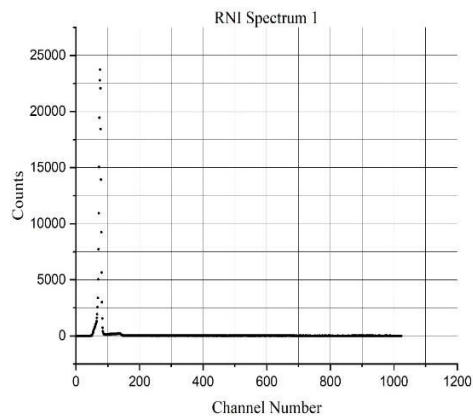


Figure 14: Radionuclidic Identification Result 2

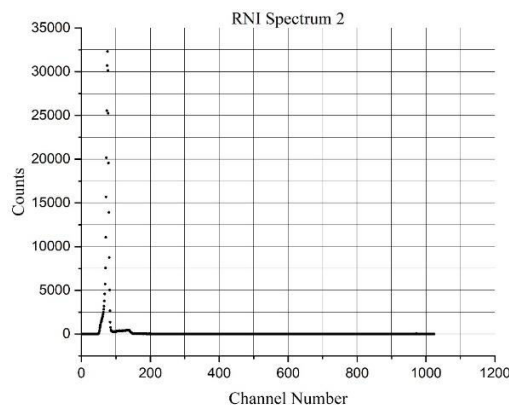


Figure 15: Radionuclidic Identification Result 3

▪ **Radiochemical Purity Test Result for Sodium Pertechnetate Eluate**

Thin layer chromatography is performed for checking whether the intended radiopharmaceutical is in the desired chemical form. Also, for checking qualitatively and quantitatively the presence of impurities in the eluate. As <sup>99m</sup>Tc is begin produced from fission-based process, there is always a possibility for presence of fission-based impurities. Limitation of for different types of impurities is according to European pharmacopeia. Qualitative analysis is done by determining the Rf value, acceptable criteria for the value is between 0.6 - 1. Quantitative analysis is done by the RCP value and its acceptable criteria is ≥ 95%. Three eluate sample of different generator systems are used for plotting the spectrum, shown from Figure 16 to Figure 27, it takes 30 minutes to complete the test. Each eluate will generate four spectrums respectively, labelled as RCP Spectrum 1, 2, and 3, each having two strips i.e., A and B, further each strip is cut in two equal portion I (bottom part) and II (top part). The Rf values are calculated using Formula mentioned in RCP section and Formula to calculate the RCP value is: (Activity of desired compound / Total activity of the sample) X 100%.

Table 5: Rf and RCP result for three different eluate samples

Eluate Sample	Distance traveled by the solvent in cm (Acetone)	Distance traveled by the compound in cm	Counts			
			Strip A (I)	Strip B (I)	Strip A (II)	Strip B (II)
1	8	> 4	91	80	21469	22443
Result	Rf value = 0.6-1		Avg. RCP value = 99.6%			
2	8	> 4	68	70	24825	24160
Result	Rf value = 0.6-1		Avg. RCP value = 99.7%			
3	8	> 4	49	59	21130	20806
Result	Rf value = 0.6-1		Avg. RCP value = 99.7%			



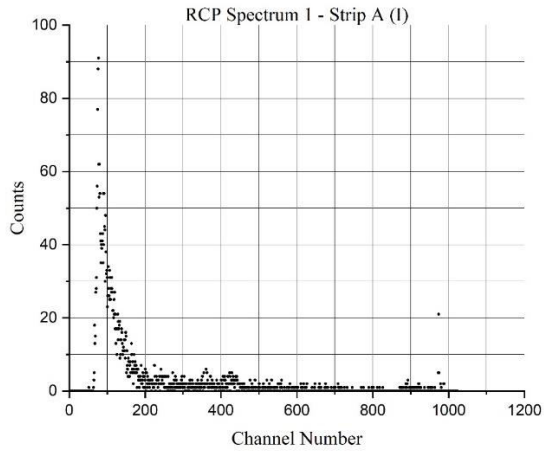


Figure 16: Radiochemical Purity Test Result 1 for Strip 1 Bottom Segment

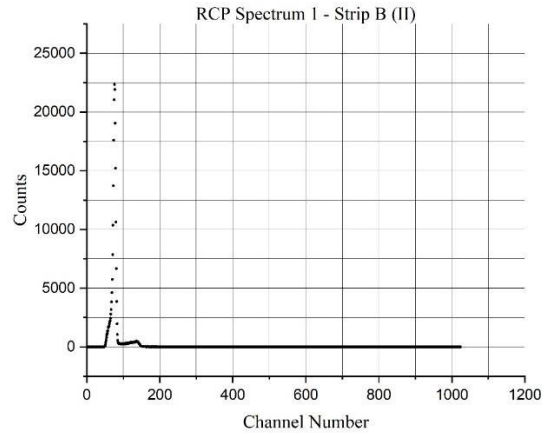


Figure 19: Radiochemical Purity Test Result 1 for Strip 2 Top Segment

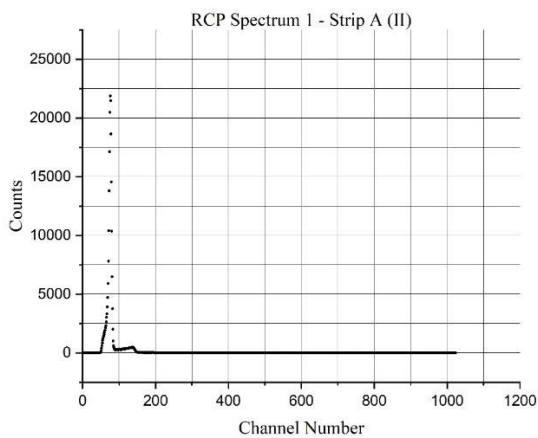


Figure 17: Radiochemical Purity Test Result 1 for Strip 1 Top Segment

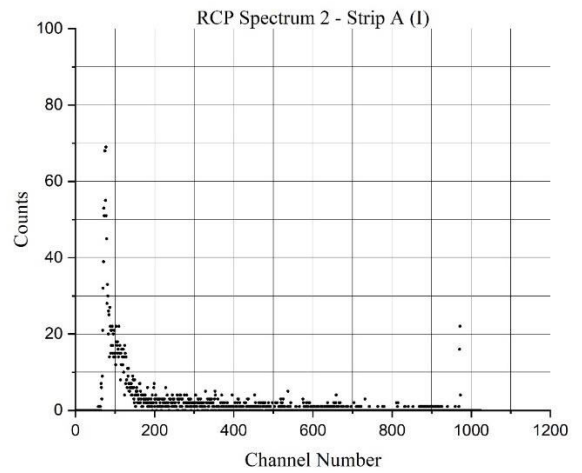


Figure 20: Radiochemical Purity Test Result 2 for Strip 1 Bottom Segment

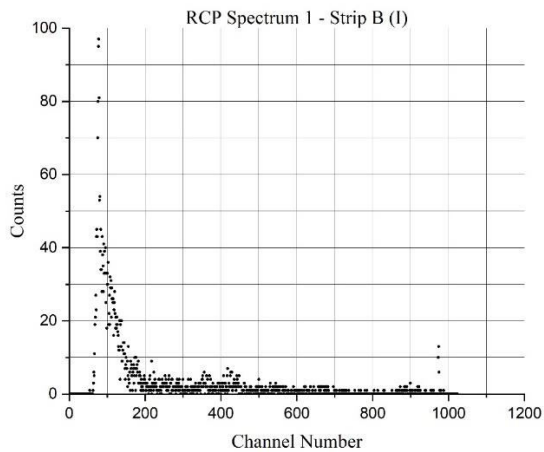


Figure 18: Radiochemical Purity Test Result 1 for Strip 2 Bottom Segment

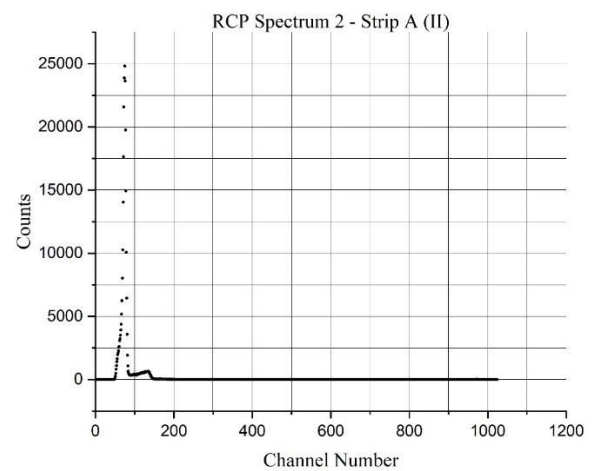




Figure 21: Radiochemical Purity Test Result 2 for Strip 1 Top Segment

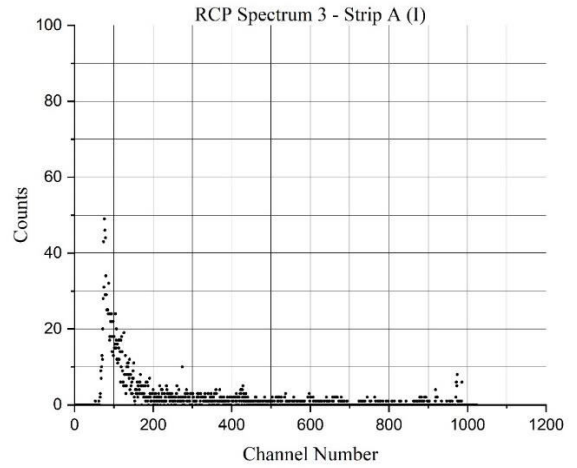
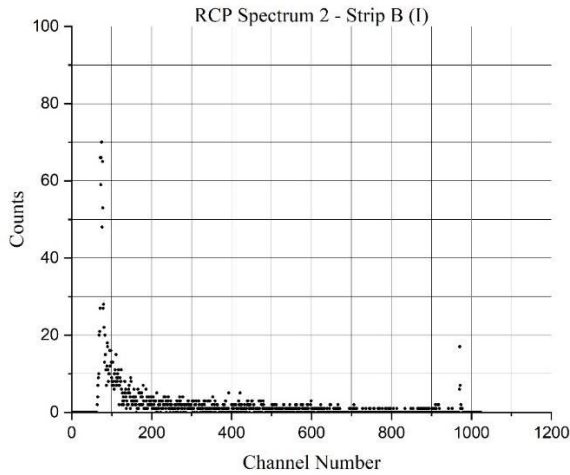


Figure 24: Radiochemical Purity Test Result 3 for Strip 1 Bottom Segment

Figure 22: Radiochemical Purity Test Result 2 for Strip 2 Bottom Segment

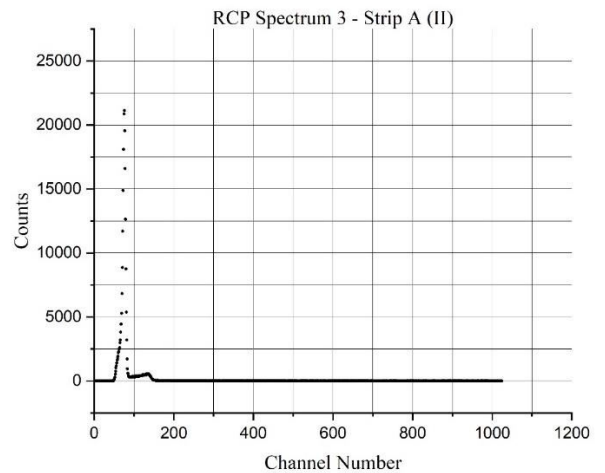
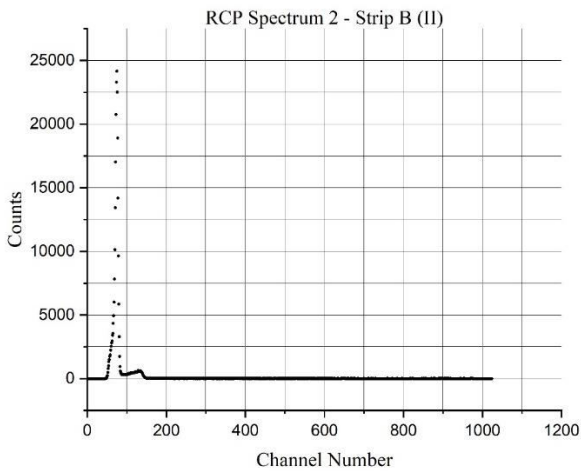


Figure 25: Radiochemical Purity Test Result 3 for Strip 1 Top Segment

Figure 23: Radiochemical Purity Test Result 2 for Strip 2 Top Segment

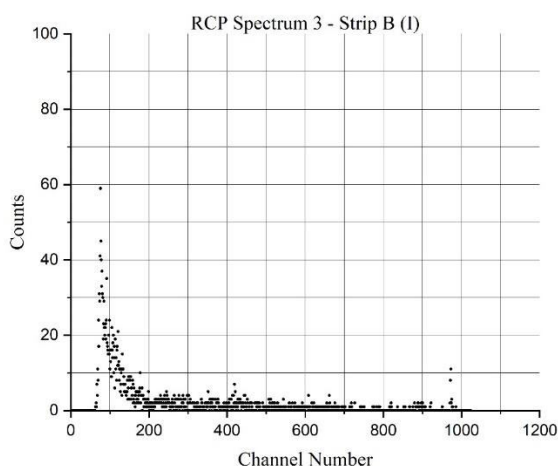


Figure 26: Radiochemical Purity Test Result 3 for Strip 2 Bottom Segment

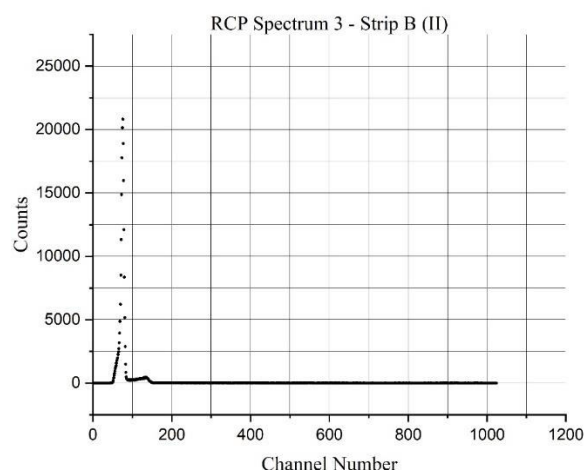


Figure 27: Radiochemical Purity Test Result 3 for Strip 2 Top Segment

▪ **pH Test Results for Sodium Pertechnetate Eluate**

The ideal pH of a radiopharmaceutical should be equal to the pH of blood. According to the guidelines acceptability criteria is between 4-8. Table: 6 show the pH value observed for the three different generator system’s elution that is under acceptability criteria.

Table 6: pH Test Results

Generator Number	Indicator Paper Observation	Test Result
1	5-6 pH	Pass
2	5-6 pH	Pass
3	5-6 pH	Pass

▪ **Alumina Breakthrough Test Results for Sodium Pertechnetate Eluate**

The eluate spot must be less intense than the standard solution (i.e., having pink colour) which ensures us about the eluate containing alumina less than 10µ g/ml. Table: 7 is the observation of test performed on the Sodium Pertechnetate elution for three different generator system and as value matches the acceptability criteria, the test is reported as passed.

Table 7: Alumina Breakthrough Test result for Sodium Pertechnetate eluate

Generator Number	Indicator Paper Observation	Test Result
1	less intense	< 10µg/ml
2	less intense	< 10µg/ml
3	less intense	< 10µg/ml

▪ **Molybdate Ion Spot Test Results for Sodium Pertechnetate Eluate**

The eluate spot must be less intense than the standard solution (i.e., having red colour) which ensure that the eluate contains not more than 10µg/ml of molybdate concentration for passing the acceptability criteria. Table 8 shows the observation for three different generator system’s elution. As no colour is observed for <sup>99m</sup>Tc sample, the test is declared as a success.

Table 8: Colorimetric analysis of Molybdate Ion Spot Test Results

Reagent	MoO <sub>3</sub>	<sup>99m</sup> Tc Sample
MoO <sub>3</sub>	100 $\mu$ l	#
Na( <sup>99m</sup> TcO <sub>4</sub> )	#	100 $\mu$ l
10% SnCl <sub>2</sub>	100 $\mu$ l	100 $\mu$ l
10% KCNS	100 $\mu$ l	100 $\mu$ l
Result 1	Red color solution	Colorless solution
Result 2	Red color solution	Colorless solution
Result 3	Red color solution	Colorless solution

# Not Defined

## ❖ BIOLOGICAL TEST RESULTS

## ▪ Bacterial Endotoxin Test Results for Generator Parts

BET test is usually done by preparing a positive and a negative of the sample, for checking whether the procedure is being followed with caution. The positive will always show the gel clot formation, as we are intentionally adding bacteria just too compared it with the negative. Density of gel clot will be directly proportional amount of bacterial contamination. The acceptability criteria are given by Table 1 and Table 9 shows the BET test performed of different generator system samples to check for contamination.

Table 9: Bacterial Endotoxin Test Results

Sr. No.	Tube	Water BET ( $\mu$ l)	Sample at MVD( $\mu$ l)	CSE 4 $\lambda$ ( $\mu$ l)	Ly sate ( $\mu$ l)	Reaction (Solid Clot)	Result 1	Result 2	Result 3
1	Negative Water Control - C	100			100	NO	Cleared	Cleared	Cleared
2	Positive Water Control - C (0.25EU/ml)	50		50	100	YES			
3	Negative Water Control - B	100			100	NO	Cleared	Cleared	Cleared
4	Positive Water Control (PWC) - B (0.5EU/ml)	50		50	100	YES			
5	Negative Product Control Isotonic Saline Vials	50	50		100	NO	Cleared	Cleared	Cleared
6	Positive Product Control Isotonic Saline Vials (0.5EU/ml)		50	50	100	YES			
7	Negative Product Control Vacuum Vials	50	50		100	NO	Cleared	Cleared	Cleared
8	Positive Product Control Vacuum Vials (0.5EU/ml)		50	50	100	YES			
9	Negative Product Control Sodium Pertechnetate	50	50		100	NO	Cleared	Cleared	Cleared
10	Positive Product Control Sodium Pertechnetate (25EU/ml)		50	50	100	YES			

## ▪ Sterility Test Results for Generator Parts

After 14 days of incubation, if any ambiguity or colour difference is observed of the FTG and SCD media solution with respect to control solution, it will give the indication non-sterile conditions of the generator system samples. Table: 10





show the different sample types and the observations for FTG and SCD media solution, sterility is reported if there is no ambiguity or cloudiness is observed.

Table 10: Sterility Test Results for different samples of generator system

Sample Type	FTG Media Obs.	SCD Media Obs.
Saline	Sterile	Sterile
Vacuum Vial	Sterile	Sterile
Eluate	Sterile	Sterile

▪ **Environment Monitoring Test Results for Different Laboratories**

Table: 2 provides the limitation of contamination in four different Class (i.e., A, B, C, D) room. Our facility has labelled the rooms of different class that are listed: • Room R (O): Security Room/ Entry; Room R1: RSO Room - GM counter, Anemometer, Tele detector, Radiation and Dose survey meter (digital and analogue) , EC-Emergency Cupboard. • R (1C): Change Room - SCS-Shoe Covers Storage, SB-Shoe Barriers, S-Sink, HFCM-Hand Foot and Cloth Monitor; R(1S): Shower Room - Water shower (hot & cold); Room R(1W): Waste Storage Room - FWD and NFWD: Flammable Waste Drums and Non-Flammable Waste Drums (CLASS D). • Room R (2) and R (2A) (Class D): QC/Bio Lab-LFWS (Laminar Flow Workstation) Counting Equipment-Gamma scintillation Counter, (MCA), Work Benches- (for material/equipment storage), LFWS - Laminar Flow Workstation, SSF - Shielded Storage Facility (QC), FH - Fume Hood; F-Fridge, Analytical balance and dispensers, Area Monitor. • Room R (3) (Class D): Preparation Room - LFWS - Laminar Flow Workstation, S - Sink, Conveyers sys: WB - Work Bench (for material / equipment storage) Drying chamber, Steam sterilizer, vacuum unit for vials, Movable trolley with spheres, Double door material locks (R (2) and R (9)); R (4) (CLASS C) :Main Processing Room - Hot cell-1, ALMO- Area monitors for Hot cells, WB- Work bench (for material / equipment storage); Jack hoist table, Double door material lock; Room: R (5) & R (6) Personal Locks . • Room R (7) (CLASS C): Assembling Room - Hot cell-2, Conveyer sys; Work benches, 2 Preparation glove boxes with Laminar Flow Workstation and 2 Glove boxes with LFWS for final assembling. • Room R (8) (CLASS D): Packing Room - Packing Material Storage, DMS- Dose Monitoring Station, PWD - Packing Work Bench, WB- Work bench (for material / equipment storage). • Room R9 (CLASS D): Dispatch Room - WB-Work Bench (for material / equipment storage), LSSF - Lead Shielded Storage Facility. Figure: 28 depicts the CFU on the y-axis and room number on the x-axis such that they are labelled in the given order; R1c=1, R2(a)=2, R2=3, R3=4, R4=5, R7=6, R8=7, and R9=8. All laminar air flow will have (CLASS A) expect laminar air flow of hot cells, which is having the (CLASS B). Figure: 29 depicts the CFU on the y-axis and room number of the laminar flow workbench on the x-axis such that they are labelled in the given order; R2a=1, R3(i)=2, R3(ii)=3, R7(i)=4, and R7(ii)=5.

Environmental monitoring has to be done for laboratories and laminar flow workstation where the generators and its components are prepared. Figure 28 to 29 are representing the colonies forming units observed after 48 hours of incubation time for five different settle and contact plates firstly for the laboratories and then for the laminar flow workbench present at different laboratories. The test was conducted on different days for laboratories and laminar flow workbench. As we did not observe any CFU more than the recommended numbers (given in Table: 2), we have declared our environment is fit for work according to the acceptable criteria.

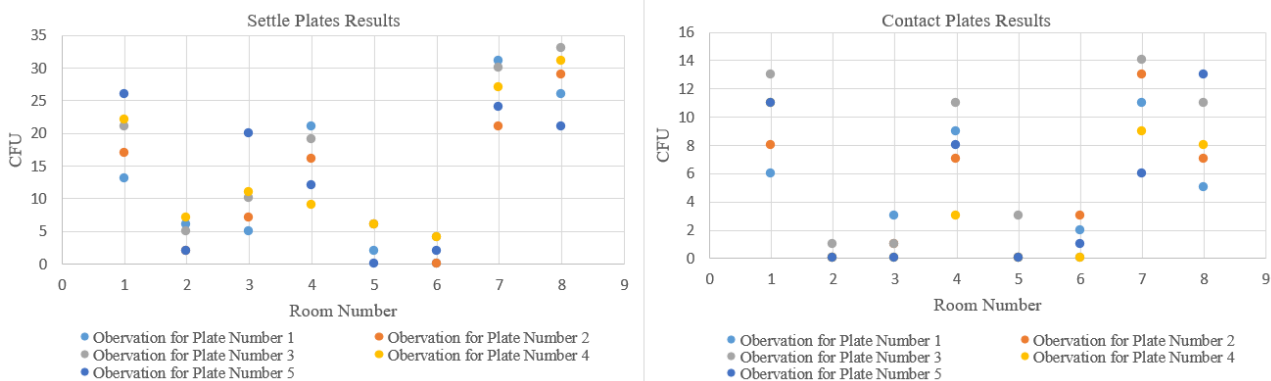


Figure 28: CFU observed for different laboratories of the facility.

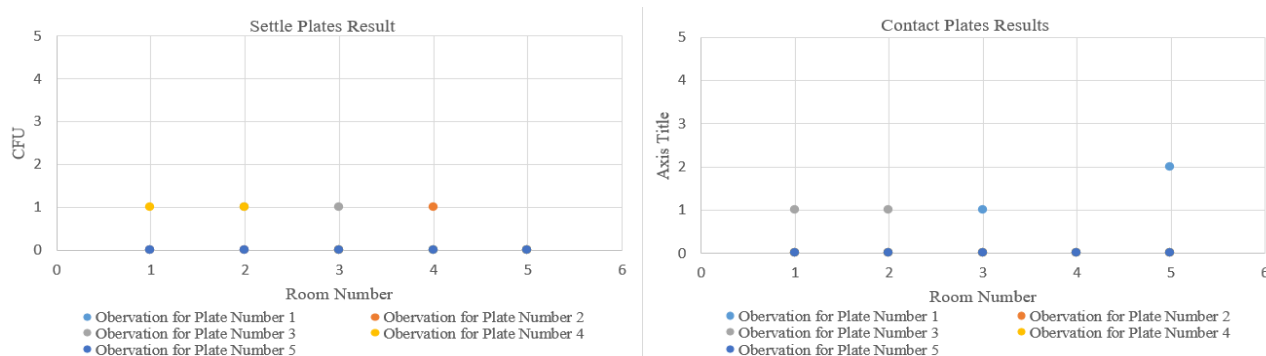


Figure 29: CFU observed for different Laminar Flow Workbench in Labs.

## V. CONCLUSION

$^{99m}\text{Tc}$  being widely used for diagnostics purposes worldwide, the question mark on dealing with both, radioactive and pharmaceutical product, already has a big impact for its potential future use. No compromises in terms of quality have to be in the manufacturer's mind for both, the legal stands and healthcare of general public's perspective. Quality surveillance and comparative analysis of different quality parameters of  $^{99}\text{Mo}$ - $^{99m}\text{Tc}$  radioisotope generators is the essential element for monitoring and evaluating of key quality parameters set by well-defined standards. This paper highlights the importance of quality surveillance in ensuring the safety and effectiveness of radioisotope generators for medical imaging procedures. The results of this study can be used to guide the selection and optimization of generators in nuclear medicine laboratories to ensure consistent and high-quality performance. Further research is needed to investigate the long-term stability and performance of these generators.

## REFERENCES

- [1] J Haroon and E Nichita. "PHWR Reactivity Device Incremental Macroscopic Cross Sections and Reactivities for a Molybdenum-Producing Bundle and a Standard Bundle". In: Nuclear Technology 208.2 (2022), pp. 246–267.
- [2] Shameem Hasan and Mark A Prelas. "Molybdenum-99 production pathways and the sorbents for 99Mo/99mTc generator systems using (n,  $\gamma$ ) 99Mo: a review". In: SN Applied Sciences 2.11 (2020), p. 1782.
- [3] Simon R Cherry et al. Physics in nuclear medicine. Vol. 3. Soc Nuclear Med, 2003.
- [4] Mohamed F Nawar and Andreas T'urler. "New strategies for a sustainable 99mTc supply to meet increasing medical demands: Promising solutions for current problems". In: Frontiers in Chemistry 10 (2022).
- [5] RE Boyd. "Technetium generators: status and prospects". In: Radiochimica Acta 41.2-3 (1987), pp. 59–64.
- [6] R Higgy and DM Fischer. "Safeguards Environmental Sampling Methods to detect 99Mo Production Signatures".
- [7] Manuel C Lagunas-Solar et al. "Cyclotron production of NCA 99mTc and 99Mo. An alternative non-reactor supply source of instant 99mTc and 99Mo  $\rightarrow$  99mTc generators". In: International journal of radiation applications and instrumentation. Part A. Applied radiation and isotopes 42.7 (1991), pp. 643–657.
- [8] Husted, R. "Technetium". Periodic Table of the Elements. <https://www.scientificlib.com/en/Chemistry/Elements/Technetium.html>. 2003.
- [9] K. Schwochau. Technetium: Chemistry and Radiopharmaceutical Applications. Weinheim, Germany: Wiley-VCH. ISBN 978-3-527-29496-1., 2000.
- [10] Philip W Moore. "Technetium-99 in generator systems". In: Journal of nuclear medicine 25.4(1984), pp. 499–502.
- [11] Frederic Poineau et al. "Speciation of heptavalent technetium in sulfuric acid: structural and spectroscopic studies". In: Dalton Transactions 39.37 (2010), pp. 8616–8619.
- [12] Energy Regulatory Board Atomic. Radioisotope handling facilities. Tech. rep. Atomic Energy Regulatory Board, 2015.
- [13] EA Nuclear Medicine et al. "Guidelines on Current Good Radiopharmacy Practice (cGRP) in the Preparation of Radiopharmaceuticals". In: Vienna. EANM (2007).
- [14] World Health Organization. Quality assurance of pharmaceuticals: a compendium of guidelines and related materials. Good manufacturing practices and inspection. Vol. 2. World Health Organization, 2007.
- [15] "TECHNETIUM-99m RADIOPHARMACEUTICALS: MANUFACTURE OF KITS". In: IAEA Series No. 466 (2008).
- [16] Kevin L Williams. Endotoxins: pyrogens, LAL testing and depyrogenation. CRC Press, 2007.



- [17] Charles River. "LAL Test Reagents". [https://www.criver.com/products\\_services/qc-microbial-solutions/endotoxin-testing/lal-reagents-accessories](https://www.criver.com/products_services/qc-microbial-solutions/endotoxin-testing/lal-reagents-accessories).
- [18] Sadaaki Iwanaga. "Biochemical principle of Limulus test for detecting bacterial endotoxins". In: Proceedings of the Japan Academy, Series B 83.4 (2007), pp. 110–119.
- [19] Archana Mukherjee et al. "Studies on batch formulation of a freeze dried kit for the preparation of 99mTc-HYNIC-TATE for imaging neuroendocrine tumors". In: Applied Radiation and Isotopes 145 (2019), pp. 180–186.
- [20] Mahboob Nemati et al. "An overview on novel microbial determination methods in pharmaceutical and food quality control". In: Advanced Pharmaceutical Bulletin 6.3 (2016), p. 301.



**INNO SPACE**  
SJIF Scientific Journal Impact Factor  
**Impact Factor: 8.423**



**ISSN** INTERNATIONAL  
STANDARD  
SERIAL  
NUMBER  
INDIA



# INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH

IN SCIENCE | ENGINEERING | TECHNOLOGY

9940 572 462 6381 907 438 [ijirset@gmail.com](mailto:ijirset@gmail.com)



[www.ijirset.com](http://www.ijirset.com)

Scan to save the contact details