Assessment Of Microbiological Sterility In Radioimmunoassay Laboratory Using SCDM And FTM Materials

Yousif Mohamed Y. Abdallah, Mohamed E.M. Gar-elnebi, Waleed A. Almahi, Nahid M. Matar, Nagi I. Ali

Abstract: This was an experimental study deal with assessment of radiobiological sterility in Radioimmunoassay (RIA) lab. The importance of this study is to highlight the importance of the quality assurance program in nuclear medicine department.. For RIA, the laboratory was tested sterility and apyrogenicity testing determination. The sample of culture media –SCDM and FTM Anaerobic bacteria validation of sterility test with known strains of bacteria, virus and fungi. The SCDM and FTM were put for 24 hours and after that analyzed in microbiological laboratory. The results of sample were 1 colonel yeast cell, 11 colonels of gram negative Bacilli and 3 colonels of Staphococcus detected.

Index Terms: Nuclear medicine, Radioimmunoassay Laboratory, sterility.

1 INTRODUCTION

ONE important aspect of any QA programme is continuous quality improvement. This implies a commitment by the staff to continuously strive to improve the use of unsealed sources in diagnosis and therapy based on new information learned from the QA programme and new techniques developed by the nuclear medicine community at large. Feedback from operating experience and lessons learned from accidents or averted accidents can help to identify potential problems and correct deficiencies, and therefore their systematic use as part of the continuous quality improvement process is to be encouraged**.** The maintenance of management documents and records is an important part of the QA programme, and the management system's documentation needs to be communicated to, understood by, available to and implemented by the appropriate personnel.

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The organization must establish and maintain procedures to control all documents that form part of its management system. This includes those generated internally and those from external sources, such as regulations, standards, other normative documents, and test and/or calibration methods, as well as drawings, software, specifications, instructions and manuals. Ideally, the person responsible for the overall operation of the QA programme, the quality manager (QM), will identify and provide to the QAC a list of tasks related to QA that need written procedures. The QAC will then establish the person(s) responsible for drafting and signing each procedure and for teaching the procedure to the users, where appropriate. The QAC and the QM will maintain a file with copies of all procedures. All changes are to be reviewed and approved by the group that performed the original review, unless other personnel are specifically designated. The designated personnel must have access to pertinent background information upon which to base their review and approval [1-5]. In nuclear medicine when a laboratory subcontracts work, whether for unforeseen reasons (e.g. workload, need for further expertise or temporary incapacity) or on a continuing basis (e.g. through permanent subcontracting, agency or franchising arrangements), competent subcontractors must be selected. A competent subcontractor is one that, for example, complies with the principles included in this report or a similar accepted standard, as well as with the regulatory requirements of the country. The laboratory needs to advise the client of the subcontractor arrangement in writing and, where appropriate, gain the approval of the client, preferably in writing [6-7]. The laboratory is responsible to the client for the subcontractor's work, except in the case where the client or a regulatory authority specifies which subcontractor is to be used. It is advisable for the laboratory to maintain a register of all subcontractors that it uses for tests and/or calibrations and a record of compliance with the principles included here for the work in question. It is highly desirable for the laboratory to have a policy and procedure(s) for the selection and purchase of the services and supplies it uses that affect the quality of the test(s) and/or calibration. Procedures need to be in place for the purchase, receipt (particularly with regard to safety inspection) and storage of radionuclides and consumable materials relevant for the tests and calibrations. It is important to note that some consumable supplies are critical to the accuracy of the measurements of radioactivity[8]. For

example, the geometry, chemical composition and dimensions (especially wall thickness) of the container (vial, syringe, etc.) may have a significant effect on the radioactivity measurement. It is important, therefore, to completely specify such equipment and verify that it meets the requirements upon delivery. For suppliers of radiopharmaceuticals, it is important that the end user be notified of any changes in the container, such as a change in the type of vial in which the drug is delivered. Such a change may affect calibrations derived from previous shipments of the drug in other types of container. The laboratory must ensure that the purchased supplies and radionuclides are not used until they have been inspected or otherwise verified as complying with the standard specifications or requirements defined in methods for the tests and/or calibrations concerned. It is important that records of actions taken to check compliance be maintained [9]. QC measures are necessary to ensure that a product complies with all the requirements and specifications laid out for it. The QC unit should have well documented procedures for QC, which is to be undertaken for each starting material used for production as well as for finished products. It is suggested that the manufacturers refer to national pharmacopoeias, the USP, the EP or any other international pharmacopoeia when designing appropriate QC specifications and methods. All starting materials, including active pharmaceutical ingredients (active substances), excipients and primary packaging materials used for kit production; need to be approved before use [10]. Generally, the starting materials such as buffer salts and reducing agents are used in many types of kit and are to be analyzed when a new bottle is opened. The specifications for such substances are described in various pharmacopoeias. However, it should be borne in mind that 99mTc radiopharmaceuticals are a special class of products in which 'no carrier added' grade 99mTc is used to form a complex with ligands, most often in the presence of a reducing agent such as Sn+2 salts. The presence of even small quantities of competing metal ions or oxidants could cause problems in the formation of the desired radiopharmaceutical. Thus it is difficult to provide complete specifications for all the starting materials with respect to the components that should not be present. Often, the use of high quality materials from reputed manufacturers is adequate to ensure good quality products. QA for the material that forms the radiopharmaceutical (along with the ligand and other materials, which are pretested) is advisable. A QC certificate from the manufacturer should be procured. Although the compliance certificate from the manufacturer may appear to be adequate, compliance with the rules laid out by the local regulatory authorities is desirable. Throughout the world, the laws governing the manufacture and sale of medical and pharmaceutical products are modified from time to time, becoming progressively more stringent and specific. In most countries, when a new product is manufactured for use in humans, all the starting materials are to be tested for their quality. This can be done by having the starting materials analyzed at an approved laboratory; alternatively, the QC analysis can be done in the manufacturer's own laboratory. The quality of all the materials should comply with the specifications in the pharmacopoeias or recommended by the regulatory body of the country. The vials and rubber closures should be purchased from approved manufacturers, and a certificate of quality compliance should be obtained and archived [11-14] Tests for sterility and the absence of pyrogens are used to ensure the microbiological safety of a product. However, since 99mTc radiopharmaceuticals are constituted using the reagents provided in the kit, any breach of sterility or apyrogenicity can in turn be due to the presence of microbial organisms in one or more of the reagents. Hence, in the case of kits, each reagent needs to be certified for microbiological safety. The microbiological safety of the product is established by conducting sterility tests and tests for bacterial endotoxins. These tests are conducted on each component of the kit when a fresh batch is made. Sterility testing Conventional tests for sterility is well established.[15] The tests can be carried out by membrane filtration of the product or by direct inoculation of the culture medium with the product to be examined. Direct inoculation involves aseptic transfer of the contents of the vial into two kinds of growth medium soybean casein digest medium (SCDM) and Fluid Thioglycollate Medium (FTM) to determine if the product is free of viable bacterial and fungal contamination. These broths are incubated for 14 d, as required by the USP (or for the period required by the regulatory requirements of the country), and inspected for evidence of bacterial and fungal growth. Briefly, the contents of the vial to be tested are reconstituted with tested sterile solvent (water or saline, as necessary), and an aliquot (typically 100 µL) is taken with a syringe and inoculated into the media, taking care to use sterile glassware and carrying out the work in a clean work area, such as a laminar flow bench/hood. The agar plates and the tubes are covered, placed in an incubator at 37ºC and monitored daily for any growth for 14 d. The absence of growth indicates adequate sterility of the product. The membrane filtration technique is recommended for filterable aqueous preparation, as is the case with kits. Conventionally, the absence of pyrogens was tested by injecting an aliquot of the preparation into rabbits and watching for any increase in their body temperature. If gram-negative bacteria are present in a product, they are destroyed during sterilization, and the endotoxins are released from their cell walls [16]. A bacterial endotoxin test (BET) using Limulus Amebocyte Lysate (LAL) is now used to determine the presence of endotoxins much faster and with far greater sensitivity than the rabbit based pyrogen test. Briefly, the BET is based on the principle that the bacterial endotoxins react with LAL and form a gel-like precipitate. The pyrogenicity is expressed in BET units; the limits for BET are well established for various products and depend on the volumes generally injected into patients. For example, vehicles such as saline and water for injection have a very low limit of 0.25 BET units, whereas a finished radiopharmaceutical units. This test needs to be carried out in a clean environment in a laminar flow hood. Appropriate amounts of the product to be tested (depending on the limits set) are allowed to react with the LAL reagents, along with positive and negative controls, and are monitored for the formation of gel at the end of the incubation period (typically 30 min). [17-18].

2 MATERIALS AND METHODS

2.1 Instrumentations:

- The available 99Mo-99mTc generator in the department is of dry type, with maximum activity of 7.5 GBq (203 mCi), the 99mTc eluate has to be checked for an eventual breakthrough of ^{99}Mo.
- Digitizer Scanner ability and the facility of printing and computerized reporting system were used.

- Personal Computer, with Intel Pentium IV at 1.25 MHz, 2 GB RAM, 320 GB HDD , 32 x CD-DVD Drive , OS MS – Windows 9x and Printer
- Gamma Counter
- Quality Control tools growth medium soybean casein digest medium (SCDM) and Fluid Thioglycollate Medium (FTM).

For nuclear medicine, each generator was be tested for sterility and apyrogenicity testing. Calculations and analytical methods used to test the acceptable level of the tests were determined.

2.2 Sterility Tests for Radiopharmaceuticals Lab

- Media for aerobic and anaerobic microorganisms
- Fluid thioglycolate (30 – 35°C for 7-14 days)
- Sterility Tests for Radiopharmaceuticals
- Medias for fungus and mold
- Soybean casein (25°C for 7-14 days)

Sterility screening for sterility after final labeling Researchers put culture media –SCDM and FTM Anaerobic bacteria validation of sterility test with known strains of bacteria, virus and fungi.

Steps:
Culture media –SCDM and FTM put in specific areas inside the hot lab (Figure 1)

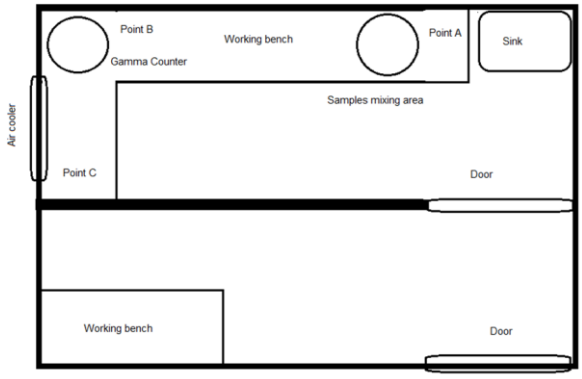

Fig. 1. RIA Lab screening areas

3. THE RESULTS

This was an experimental study deal with assessment of radiobiological sterility in Radioimmunoassay (RIA) lab. The importance of this study is to highlight the importance of the quality assurance program in nuclear medicine department.. For RIA, the laboratory was tested sterility and apyrogenicity testing determination. The sample of culture media –SCDM and FTM Anaerobic bacteria validation of sterility test with known strains of bacteria, virus and fungi. The SCDM and FTM were put for 24 hours and after that analyzed in microbiological laboratory. The results of sample were as shown in table 1 & table 2. And figure 1.

TABLE 1.
NO. OF MICRO-ORGANISM MEASURED IN INSIDE RIA

After 2 hrs.		After 4 hrs.		After 24 hrs.	
SCDM	FTM	SCDM	FTM	SCDM	FTM
0	0	0	0	1	1
0	0	0	0	8	10
0	0	0	0	6	10
0	0	0	0	4	14
0	0	0	0	12	10

TABLE 2.
TYPES OF MICRO-ORGANISM MEASURED IN INSIDE RIA

Microorganism Type	FTM	SCDM
Yeast Cell	1	1
Bacilli (Gram –ve)	11	11
Staphococcus	3	3

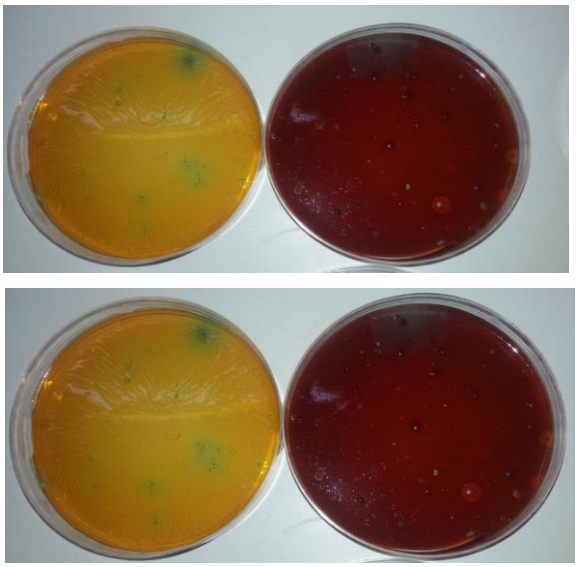

***Fig.2** show microorganism detected in SCDM and FTM culture media*

4. CONCLUSION

The sterity testing should normally be done prior to administration of the end product to patients. This test requires 14 days, so release of the product is allowed due to short half-life of technetium-99. Since this is the case, the user should emphasize good aseptic technique during the preparation and administration of such agents. This was an experimental study deal with assessment of radiobiological sterility in Radioimmunoassay (RIA) lab. The importance of this study is to highlight the importance of the quality assurance program in nuclear medicine department.. For RIA, the laboratory was tested sterility and apyrogenicity testing determination. The sample of culture media –SCDM and FTM Anaerobic bacteria validation of sterility test with known strains of bacteria, virus and fungi. The SCDM and FTM were put for 24 hours and after that analyzed in microbiological laboratory. The results of sample were as shown in table 1 & table2 and figure 1.

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