Sterility Testing and Contamination Analysis of the ^{99m}Tc Eluates from RIPDTEC Mo-99/Tc-99m Chromatographic Column Generator

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ABSTRACT

Metastable Technetium-99 is a widely used radioisotope in nuclear medicine that is administered to patients intravenously for imaging, functional studies of cells, and diagnosis of tumor cells of different body organs. Tc-99m is instantly available from the Mo-99/Tc-99m generator, accounts for more than 80% of all nuclear medicine procedures used for imaging, and is in growing demand worldwide every year. Hence, bulk manufacturing of this generator is carried out under strict aseptic conditions to ensure the sterility of Tc-99m. However, at the production stages of Tc-99m, there is still the possibility of getting contaminated with microbes and their endotoxin, which has a pyrogenic effect. The objective of this study is to assess and analyze the microbial and endotoxin contamination of the 3634 Tc-99m eluate samples collected from RIPDTEC Mo-99/Tc-99m chromatographic column generators. In the case of sterility testing, only 2 samples out of 3634 (0.05% of the total samples) exhibited bacterial growth after 14-day incubation in the media used to detect aerobic bacteria. In contrast, no fungal and anaerobic bacterial growth was observed during the 14-day incubation. In this study, 9 samples out of 3634 (0.25% of the total samples) showed gel clot formation in Tachypleus Amebocytes Lysate (TAL) kits used to detect endotoxin, which indicated the presence of endotoxin in those samples, and the amount was found 0.25-0.50 EU/ml. However, abnormal temperature rise was not observed in the case of any mice injected with eluate samples.

Keywords: Radioisotope, Contamination, Sterility, Bacterial Endotoxin

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INTRODUCTION

Technetium-99m is a metastable nuclear isomer of Technetium-99, symbolized as ^{99m}Tc, a widely used radioisotope for the diagnosis of diseased organs. In nuclear medicine, this isotope is used as a radioactive tracer for approximately 85% of total imaging and functional studies of the brain, myocardium, thyroid, lungs, liver, gallbladder, kidneys, skeleton, blood, and tumors (1–4).

Microbial contamination of pharmaceutical products may occur due to inappropriate handling, unhygienic measures, and a lack of other good manufacturing practices (5). Microbiologically contaminated pharmaceuticals may lead to clinical vulnerability and may cause severe infections in immunocompromised persons (6, 7). On the other hand, endotoxin produced by generally Gram-negative bacteria is a pyrogen and acts as a fever-inducing agent. It is the most common cause of toxic reactions, often resulting from the contamination of pharmaceutical products (8). The use of such contaminated pharmaceuticals, which are the result of poor-quality control and inappropriate handling, is hazardous as drug-borne infections may occur to consumers (9, 10).

In 1943, the U.S. Food & Drug Administration (FDA), the National Institutes of Health (NIH), and several pharmaceutical companies carried out a collaborative study, and they were the first to suggest the inclusion of the rabbit pyrogen test (RPT) in the United States Pharmacopoeia (USP) (11, 12). The current USP monograph, based on the evaluation of the rectal temperature of rabbits before and after intravenous injection of a test solution into the ear, is not substantially different from the original one. The bacterial endotoxin test (BET)—also known as the LAL test—is an alternative in vitro endotoxin assay accepted by the leading regulatory drug agencies (FDA, European Medicines Agency (EMA), Pharmaceuticals and Medical Devices Agency (PMDA),

Figure 1: Collection of eluates from the RIPDTEC Mo-99/Tc-99m chromatographic column generator

Sterility test:

In this study, to test the sterility, three different culture media mentioned in (Table-1) were inoculated with the eluate samples from the RIPDTEC Mo-99/Tc-99m chromatographic column generator (Figure 2). Positive control and negative control were used to validate the test.

Table-1: Media used in this study to test the sterility of samples

Media	Incubation Temperature	Incubation Period	Purpose
Nutrient broth	37°C	14 days	To detect the presence aerobic bacteria in samples
Fluid thioglycolate medium	35°C	14 days	To detect the presence of anaerobic bacteria in samples
Potato dextrose agar	25°C	14 days	To detect the presence of fungus in samples

Figure 2: Inoculation of culture media with eluate samples to detect bacteria and fungus

In-vitro test: Tachypleus Amebocytes Lysate (TAL) test:

Like Limulus Amebocyte Lysate (LAL), Tachypleus Amebocytes Lysate (TAL) has the same principle and procedure. It is used for the qualitative detection of Gram-negative bacterial endotoxins by the gel-clot method. Gram-negative bacterial endotoxin catalyzes the activation of a proenzyme in the Limulus amoebocyte lysate, and the activated enzyme (coagulase) hydrolyses specific bonds within a clotting protein (coagulogen) also present in Limulus amoebocyte lysate and forms a gelatinous clot (19). The gel-clot TAL test is a simple, reproducible test that consists of mixing equal parts of the TAL and test specimen, and the mixture is incubated for 60 minutes at 37°C. The formation of a firm gel clot indicates the presence of endotoxin equal to or more than the TAL reagent's labeled sensitivity. In this study, TAL kits (Endotoxin Assay Kit from Zhanjiang Bokang Marine Biological Co. Ltd., China) were used, which formed the gel clot if the bacterial endotoxin amount in the eluate sample was 0.25 EU/ml or above. Positive control, negative control, and TAL kits, which could detect the endotoxin amount of 0.50 EU/ml or above, were also inoculated with eluate samples to validate the test (Figure 3).

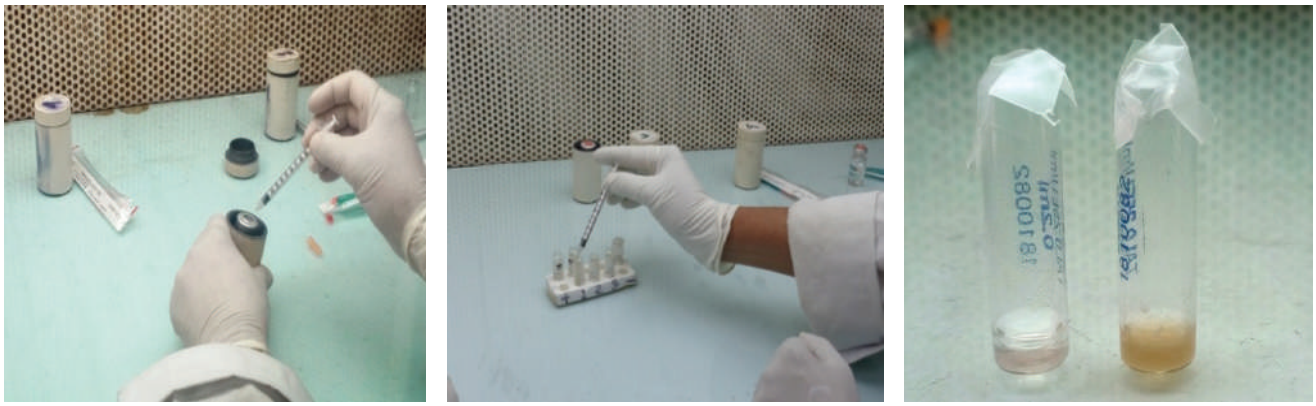

Figure 3: Tachypleus Amebocytes Lysate (TAL) test to detect the endotoxin in samples

In-vivo test: Rabbit pyrogenicity test:

The in-vivo pyrogenicity test is conducted in healthy adult rabbits or mice by measuring the rise in rectal temperature after injecting the eluate samples (13,15). Healthy Albino Swiss mice (each having 25 g weight) used in this study were kept in individual cages at 20°C and had free access to food and water. These mice were also kept fasted for 24 hours before the experiments according to the standard guidelines of US Pharmacopoeia (14). 0.1ml of Tc-99m eluate was injected intravenously into mice through the tail vein, while control mice (injected with saline) were also used and kept in same condition to validate the test. Temperatures were recorded 30 minutes prior to the injection, and post-injection temperatures were also recorded at 30-minute intervals. If the eluate sample is not pyrogenic, the temperature rise in the test mice will not be 0.5°C or above with respect to the temperature of the control mice.

RESULTS

In this study, 3634 eluate samples were analyzed. Only 2 samples, 0.05% of the total samples, showed growth in the media used to detect aerobic bacteria after a 14-day incubation period (Figure 4). (Figure 4), which is 0.05% of the total samples. However, no fungal and anaerobic bacterial growth was observed after the 14-day incubation period (Figure 4).

On the other hand, 9 samples out of 3634 showed gel clot formation in TAL kits, which can detect an endotoxin amount of 0.25 EU/ml or above (Figure 4). However, gel clot formation was not observed in the case of any of these 9 samples when TAL kits were used that could detect the endotoxin amount of 0.50 EU/ml or above. Therefore, results indicated that only 0.25% of the eluate samples were contaminated with endotoxin, and the amount was in the range of 0.25-0.50 EU/ml (Figure 4).

During the in-vivo pyrogenicity test, abnormal temperature rise was not observed in the case of any mice injected with eluate samples (Figure 4).

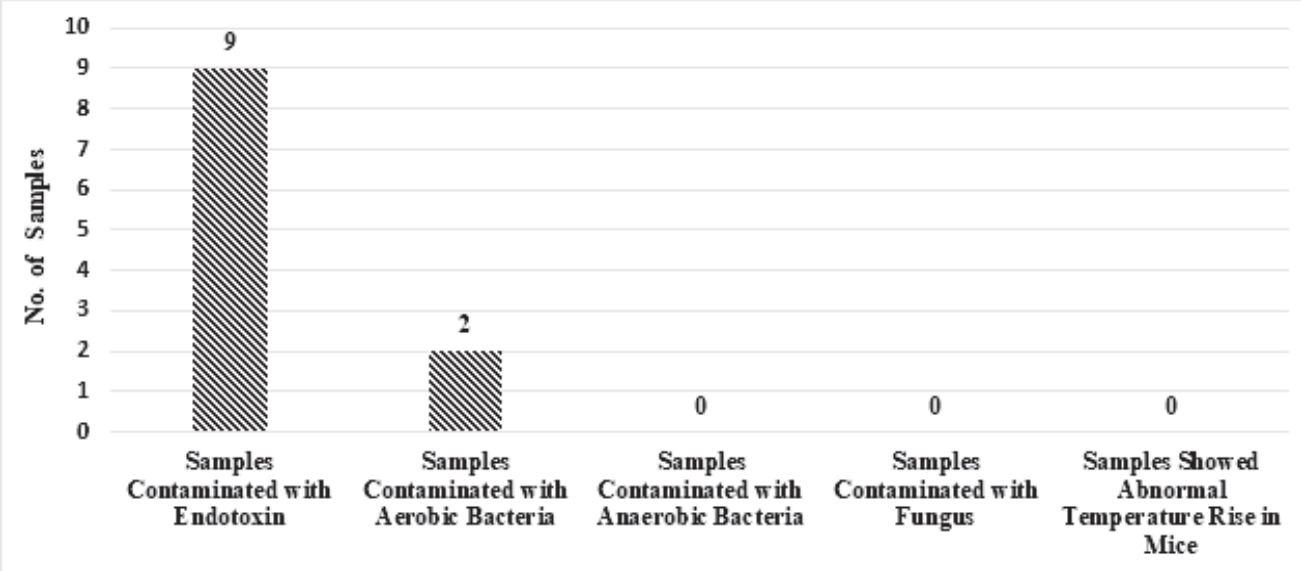

Figure 4: Number of samples out of 3634 showed endotoxin contamination, bacterial contamination, fungal contamination and abnormal temperature rise in mice

DISCUSSION

A total of 3634 Tc-99m generator eluate samples were analyzed in this study. Only 2 samples (0.05% of the total samples) were found contaminated with aerobic bacteria, whereas no fungal and anaerobic bacterial growth was observed after the 14-day incubation period. Therefore, the results of this study suggested that 3632 samples (99.95% of the total samples) were sterile.

The result of the Tachypleus Amebocytes Lysate (TAL) test showed that 9 samples (0.25% of the total samples) were positive for endotoxin contamination, and the amount was found in the range of 0.25-0.50 EU/ml. As the endotoxin amount in those 9 samples was in the range of 0.25-0.50 EU/ml, the amount was even meager and almost at a negligible level. From this study, it was found that 3625 samples (99.75% of the total samples) were free from endotoxin contamination.

Although 9 samples were found positive for endotoxin contamination, only 2 samples were positive for aerobic bacterial contamination, and none of the samples showed any abnormal temperature rise in injected mice. The possible reasons behind this could be that either samples were contaminated with endotoxin of non-viable Gram-negative bacteria or the endotoxin amount, which was in the range of 0.25-0.50 EU/ml, was not good enough to cause the abnormal temperature rise in mice.

Therefore, the results of this study suggested that most of the Tc-99m eluate samples (>99% of the total samples) obtained from the RIPDTEC Mo-99/Tc-99m chromatographic column generator were safe and fulfilled all the necessary safety requirements to be injected intravenously as per (5,14–18).

CONCLUSION

From this study, it can be concluded that the microbial and endotoxin contamination rate of the eluate samples from the RIPDTEC Mo-99/Tc-99m chromatographic column generator was very low, almost at a negligible level, and reflected good manufacturing practices during the production of Tc-99m.

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