STERILITY TESTING OF RADIOPHARMACEUTICALS

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A radiometric method for sterility testing of radiopharmaceuticals has been developed. This technique is rapid and sensitive and may be used for sterility testing of both ^{99m}Tc-labeled compounds and nonradioactive "kit" components.

The sterility testing of radiopharmaceuticals poses unique quality-control problems. First, the total amount of material available for testing is usually limited and may be highly radioactive. Secondly, the shelf life of the material is also limited, often being much shorter than the time required for the official USP procedures. In spite of these major limitations, bacterial contamination of radiopharmaceuticals has not been a significant problem. However, because radiopharmaceuticals are most frequently administered intravenously, testing for sterility is of great importance. Therefore a rapid sterility test utilizing small aliquots of radioactive solution should prove to be useful in the quality-control testing of radiopharmaceuticals and help to assure the continued production and distribution of high-quality products.

A radioactive indicator method for the detection of viable bacteria has been developed (*1,2*). This method is based on the monitoring of $^{14}CO_2$ released from growth media containing ^{14}C-labeled substrates. This method was originally utilized for the detection of bacteria in blood cultures (*3*). The advantages of the method arise from its rapidity and sensitivity. Furthermore, it has been developed as a commercially available, automatic system (*4*) and has been found to detect a very large variety of bacteria (*5*).

The purpose of this paper is to describe the methods for the application of the radiometric to sterility testing of radiopharmaceuticals and to report the results of a comparative study involving 136 sterility tests.

METHODS AND MATERIALS

Each radiopharmaceutical (0.3–1 ml) was inoculated aseptically into an aerobic media and into an anaerobic media. The aerobic media (Johnston vial No. 6A, Johnston Laboratories, Inc., Cockeysville, Md.) contained 30 ml of enriched tryptic soy broth, sodium-polyanethol sulfonate anticoagulant, a magnetic stirring bar, and 1.5 μCi of ^{14}C-substrate. The anaerobic media (Johnston vial No. 74) contained a pre-reduced sulfonate tryptic soy broth with yeast extract, L-cysteine, sodium polyanethol sulfonate, and 1.5 μCi of ^{14}C-substrate. This medium had a redox potential of less than 200 mV.

The inoculated vials were incubated at 35°–36°C. The $^{14}CO_2$ release was assayed periodically utilizing an automatic sampling ionization detector (Bactec, Johnston Laboratories, Inc., Cockeysville, Md.). Aerobic vials were assayed during the day and overnight every 2 hr; then every 24 hr to 5 or 7 days. To assay the vials, the atmospheres were removed and replaced with 10% CO_2 and 90% air. This flushing gas is used for the detection of aerobic and microaerophilic organisms. Anaerobic vials were assayed every 24 hr for 5–7 days. To assay these vials, the atmosphere was removed and replaced with a mixture of 85% nitrogen, 10% CO_2, 5% H.

The first 39 samples of the radiopharmaceuticals were tested in parallel using the radiometric method and the standard USP procedures (*6*). After the evidence from testing a great variety of other samples in addition to the radiopharmaceuticals indicated that the nonsterile cultures both released more than

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5 μCi of $^{14}CO_4$ within 24 hr and eventually became turbid so that bacterial growth was visually evident within 10–12 hr after the inoculation, the USP test was omitted. Subsequent samples were tested by radiometric method and by visual inspection for turbidity.

The automatic sampling device and ionization detector (Bactec) had a linear output scaled from 0–1,000 and was calibrated so that each scale division corresponded to 0.25 ηCi of $^{14}CO_2$. If a sample generated a reading corresponding to 5 ηCi of $^{14}CO_2$ or higher, it was called positive and was inoculated onto a chocolate agar plate (Cat. No. 21267, BioQuest, Cockeysville, Md.) for verification of growth. The agar plate was incubated aerobically or anaerobically according to the source of the suspected positive sample.

Gamma-emitting radiopharmaceuticals that gave higher reading on the instrument and failed to indicate bacterial growth on retesting with the chocolate agar plate method or to show turbidity, suggested that gamma-ray contribution from the sample to background may have been the source of the high $^{14}CO_2$ assay values. To verify this, a simulated study in which a 500-μCi pertechnetate solution was inoculated with Staphylococcus epidermis and $^{14}CO_4$ assay as a function of time was compared with a sterile pertechnetate solution and a sterile preparation of ^{99m}Tc-labeled red blood cells (^{99m}Tc-RBC).

To test further the use of this method for the detection of bacterial growth due to a variety of organisms, a series of 500–600 μCi samples of sodium pertechnetate solutions were inoculated with microorganisms of different growth characteristics. The $^{14}CO_4$ was assayed at 2, 6, and 24 hr and compared with other readings obtained from a sterile pertechnetate control.

RESULTS

The results of the 136 radiopharmaceuticals tested are tabulated in Table 1. No false-negative results were observed. A 4.4% false-positive result rate was observed, however. This was due either to an elevated gamma-ray background, or to a gaseous transfer of a gamma-emitting radionuclide from the vial to the counting chamber. This latter explanation did not apply to ^{99m}Tc-labeled radiopharmaceuticals. It was subsequently observed that the radioactivity of sterile short-lived gamma emitters decreased with time due to radioactive decay whereas that of nonsterile preparations increased with time. This is illustrated by the time–activities curves shown in Fig. 1. Therefore the false-positive rate can be reduced by making multiple determinations to ascertain whether the measurement is increasing or decreasing with time.

TABLE 1. CORRELATION OF THE $^{14}CO_4$ METHOD FOR THE DETECTION OF BACTERIAL CONTAMINATION OF RADIOPHARMACEUTICALS WITH NONRADIOMETRIC METHODS

Result	No. of tests	
Double positive	Aerobic	Anaerobic
$^{14}CO_2$* other +	1	0
$^{14}CO_2$ +, other —	6	6
$^{14}CO_2$ —, other —	129	130
Totals	136	136

* Other methods were USP, chocolate agar plate, turbidity, or a combination of these methods.

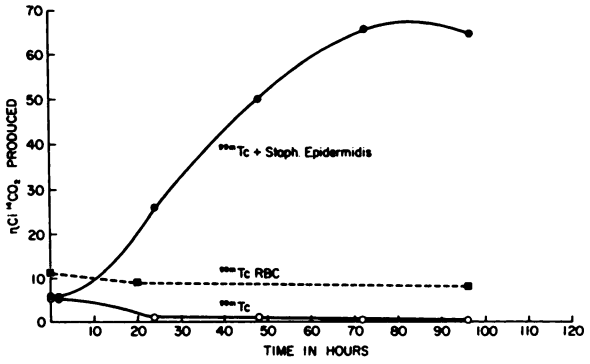

FIG. 1. Time–activity curve for three ^{99m}Tc radiopharmaceuticals. Initial readings are high due to gamma-ray background contribution to $^{14}CO_2$ determinations. When radiopharmaceutical is not sterile, radioactivity reading increases with time. When radiopharmaceutical is sterile, radioactivity reading decreases with time due to radioactive decay of ^{99m}Tc. This decrease results from decrease in gamma-ray background.

The results of a final series of tests are reported in Table 2. Again, it is observed that the change of radioactivity with time is useful in differentiating sterile from nonsterile preparations. Variations in the early readings is an artifact of the high gamma-ray background which depends to a great degree on the geometric arrangement of samples with respect to the detector. This variable can be controlled by assaying only one sample at a time while the other gamma-emitting samples are placed in a shielded container or removed from the room.

Of the micro-organisms tested so far, the anaerobe propionibacterium acnes was the most difficult to detect in the presence of ^{99m}Tc. At 24 hr the radioactivity assay was still decreasing with time although it was significantly higher than the sterile control. Moreover, the growth media had become turbid by 24 hr indicating significant growth.

DISCUSSIONS AND CONCLUSIONS

This method of sterility testing which has been carefully tested using blood cultures has also been found readily applicable to the sterility testing of

TABLE 2. RESULTS OF FINAL TESTS OF RADIOPHARMACEUTICALS INOCULATED INTO AEROBIC AND ANAEROBIC MEDIAS

Organism	Relative radioactivity*			
	0 hr	2 hr	6 hr	24 hr
Aerobes 10^1 organisms				
Control + 530 μCi Tc	13.5	12.3	8.2	3.7
E. coli + 530 μCi Tc	4.7	4.2	4.2	25.0
Pseudomonas + 530 μCi Tc	6.5	6.0	5.0	16.0
S. aureus + 530 μCi Tc	3.5	4.0	3.5	25.0
Anaerobes 10^4–10^5 organisms				
Control + 580 μCi Tc	33.5	27.0	19.5	7.25
Propionibacterium acnes + 580 μCi Tc	27.0	23.3	17.8	15.5
Bacteroides fragilis + 580 μCi Tc	20.5	18.5	14.3	41.3
Clostridium perfringens + 580 μCi Tc	12.0	10.5	41.3	43.8

* Radioactivity is expressed as μCi $^{14}CO_2$ produced. However, part of the radioactivity is due to gamma radiation.

radiopharmaceuticals. Positive results can be obtained for most organisms within 24 hr. In the series of 136 radiopharmaceuticals, no false-negative results were obtained. To eliminate the problem of false-positive results, multiple readings can be used to test for gamma-ray contribution to the $^{14}CO_2$ assay. With ^{99m}Tc compounds, the gamma-ray background is significantly reduced during 24 hr due to radioactive decay. To detect organisms such as propionibacterium acnes, it was necessary to either check the vial for turbidity at 24 hr or to compare its assay with that of a control sterile sample containing an equivalent amount of ^{99m}Tc radioactivity.

This method has not been extensively tested with radioiodinated compounds. It may be expected that the more volatilizable radioiodine will result in a higher percentage of false-negative results. Also, the problem of contamination of the gas chamber with a gamma-emitting radionuclide is a potential disadvantage which has not been encountered with ^{99m}Tc compounds.

Because the ionization chamber is not heavily shielded, the use of this method with high levels of gamma activity (i.e., greater than 500 μCi) poses a significant problem due to the gamma-ray background. This method proves most useful when decayed samples are tested or when nonradioactive components of labeling kits are to be tested.

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