Template Provided By:

Cancer Imaging Program

National Cancer Institute

Standard Operating Procedure

GEN-Q117A

Alternate Procedure for the Sterility Test

1. **Purpose:** To describe the quality control procedure for sterility testing to assure the safety and purity of the final product solution.

2. **Scope:** The sterility procedure will be performed at the sites manufacturing radiopharmaceuticals. The results from this quality control procedure are not available prior to release of the final product batch for human administration. However, if the sterility results are determined to be positive, action will be taken within 24-48 hours as outlined in this procedure.

3. **Responsibilities:**
   - **3.1.** Sites: To follow this procedure as written and to ensure that all results, control information, and raw data are documented in dedicated Quality Control Laboratory Notebooks and recorded on the appropriate testing forms.
   - **3.2.** Each Site is also responsible for providing the following:
     - 3.2.1. A validated standard operating procedure for using the site-specific autoclaves to sterilize media for use in this assay if commercial media are not used.
     - 3.2.2. A standard operating procedure for cleaning and maintaining the laminar flow hoods for aseptic technique.

4. **References**

   USP 28, ‹71›, Sterility Tests
   USP 28 ‹821› Radioactivity
   USP 28 ‹823› Radiopharmaceuticals For Positron Emission Tomography—Compounding

SOP**: GEN-Q117A** Date:_____

Printed Name	Signature	Date
Author:		
Regulatory Approval:		

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USP 28 ‹1116› Microbial Evaluation of Clean Rooms and Other Controlled Environments.
GEN-Q015, "Investigating Out of Specification QC Test Results"
FDA Draft Guidance PET Drug Products—Current Good Manufacturing Practice (CGMP)—September 2005 (CDER)

5. Forms

Form FQC-###, Corresponding "Final Product QC Testing" Form
Form IOS-001, "Investigating Out of Specification (OOS) QC Test Results Form"
Form EQ-001, "General Equipment History Log"

6. Policies

READY-TO-USE MEDIA—Commercially prepared media (hydrated, or dehydrated formulations that can be reconstituted as directed by the manufacturer or distributor) may be used in place of freshly prepared media provided that the requirements of the *Sterility of Media* and *Growth Promotion Test* are met.
Seed lot culture maintenance techniques are to be used so that viable microorganisms are not more than five passages removed from the ATCC cultures.

Sterility Testing Facilities

Clean Room: A facility used for conducting sterility tests. A clean room facility is maintained under microbiological control criteria appropriate for the critical zones in an aseptic processing facility.
Isolators: A type of facility used for conducting sterility tests. Isolators are free-standing environments that allow aseptic manipulations to be made from outside the controlled environment. Isolator systems protect the test article and sterility test supplies from contamination during aseptic handling. Transfer ports from dedicated autoclave or decontamination ports are used. The interior of the isolator must also meet the same microbiological control criteria.
Laminar Flow Hoods: (with horizontal air-flow) A type of facility used for conducting sterility tests. Laminar flow hoods are free-standing environments that allow aseptic manipulations to be made from outside the controlled environment. Laminar Flow Hood systems protect the test article and sterility test supplies from contamination during aseptic handling.
An Approved Clinical Laboratory: This test may be run by a licensed clinical laboratory if the results indicate that the sterility tests are valid, i.e., that the requirements of section 8.5, below are met.

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Handling and Opening Articles/Samples, Reagents, & Supplies

Handle articles/samples carefully to avoid contamination of the test aliquot by microorganisms that may be present on the exterior of the container.

The exterior surfaces of ampules, vials, and bottle closures must be cleansed with a suitable decontaminating agent, and the containers transferred into an environment that prevents recontamination of the exterior surfaces.

Decontaminate outer packaging of purified or sterile supplies (such as syringes, needles, gloves, etc.), and open aseptically.

7. Materials and Equipment

7.1. Thioglycollate Medium

7.2. Alternative Thioglycollate Medium

7.3. Soybean-Casein Digest Medium

7.4. **Test Microorganisms** (Available from the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209)

7.4.1. *Staphylococcus aureus*, ATCC 6538 (Alternative: *Bacillus subtilis*, ATCC 6633)

7.4.2. *Pseudomonas aeruginosa*, ATCC 9027 (Alternative: *Micrococcus luteus* ATCC 9341)

7.4.3. *Clostridium sporogenes*, ATCC 11437 (Alternative: *Bacteroides vulgatus* ATCC 8482, when a nonspore-forming microorganism is desired.)

7.4.4. *Bacillus subtilis*, ATCC 6633

7.4.5. *Candida albicans*, ATCC 10231

7.4.6. *Aspergillus niger*, ATCC 16404

7.5. pH meter

7.6. Preparative Mobile Phase used for the radiopharmaceutical being tested

7.7. Sterile Filters

7.8. Isotonic Saline for Injection, USP

7.9. Incubator, aerobic conditions, 32.5 ± 2.5°C, (with temperature measured at least daily and documentation of the temperature in the equipment history log, **Form EQ-001**, that the temperature was read at least once daily during incubation).

7.10. Anaerobic Incubator, 22.5 ± 2.5°C, (with temperature measured at least daily and documentation of the temperature in the equipment history log, **Form EQ-001**, that the temperature was read at least once daily during incubation).

7.11. Autoclave (with validated site-specific sterilizing procedures)

8. PROCEDURES

Suitability Tests

Growth Promotion Test: Each lot of medium must be tested for its growth-promoting qualities.

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1. Inoculate, in duplicate, respective containers of medium with less than 100 viable microorganisms of each strain listed in Table 1 below.
2. The test medium is satisfactory if visual evidence of growth appears in all inoculated media within 3 days of incubation for bacteria and 5 days of incubation for fungi.

The Growth Promotion Test can be conducted simultaneously with the use of the medium during a sterility test. However, the sterility test is considered invalid if the sterility of the media or growth promotion test fails.

TABLE 1: Test Organisms Suitable for Growth Promotion Test & Validation Tests for Bacteriostasis and Fungistasis

				Incubation (7 Days)	
Tube	**Medium**	**Microorganism**	**Strain**	**Temperature**	**Conditions**
1	**Fluid Thioglycollate**	*Staphylococcus aureus*	**ATCC 6538**	**32.5 ± 2.5°C**	**Aerobic**
2		*Pseudomonas aeruginosa*	**ATCC 9027**		
3		*Clostridium sporogenes*	**ATCC 11437**		
4	**Alternative Thioglycollate**				**Anaerobic**
5	**Soybean Casein Digest**	*Bacillus subtilis*	**ATCC 6633**	**22.5 ± 2.5°C**	**Aerobic**
6		*Candida albicans*	**ATCC 10231**		
7		*Aspergillus niger*	**ATCC 16404**		

Media Storage

Freshly Prepared Media

1. If the freshly prepared media are not used within 2 days, store them at a temperature between 2° and 25°C.
2. If prepared media are stored in unsealed containers, they can be used for one month, provided that they are tested for growth promotion within two weeks of the time of use and that the color indicator requirements are met.
3. If stored in tight containers, the media can be used for one year, provided that they are tested for growth promotion within 3 months of the time of use and that the color indicator requirements are met.

READY-TO-USE MEDIA

1. Commercially prepared media stored in tight containers may be used provided that the requirements of the *Sterility of Media* and the *Growth Promotion Test* are met.

Validation Tests for Bacteriostasis and Fungistasis

Before conducting a sterility test procedure, the product sample must be tested to ensure that the sample is devoid of inherent bacteriostatic and fungistatic activity that would adversely affect the reliability of the test

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and/or the test procedure. This testing is typically required only once for a given sample type, provided that no additional changes to the product, formulation, or manufacturing process occur.

Preparation of Microorganisms

1. Prepare dilute cultures of bacteria and fungi from the strains of microorganisms listed in Table 1, to obtain a final concentration in the product of less than 1000 colony forming units (cfu) per mL. Dilute the cultures using isotonic saline for injection, USP.
2. Repeat step 1, i.e. prepare a dilute culture, for each microorganism used.

Validation Procedure by Immersion (Direct Inoculation) Method

1. Prepare test samples of the preparative mobile phase used with the radiopharmaceutical being tested. Filter the preparative mobile phase prior to dilution using the same type of membrane filter that will be used to sterilize the final product.
2. Prepare the filtered test sample using the same dilution factor and type of containers as will be used in the sterility test. Use isotonic saline for injection, USP for all dilutions.
3. Using a volume of less than 0.2 mL, inoculate the test sample with less than 100 cfu of the microorganism to be tested.
4. Inoculate the test medium with the test samples.
5. Repeat the procedure for each appropriate microorganism and medium specified (refer to Table 1). Do each microorganism test in triplicate.
6. Incubate the containers at the appropriate conditions for up to 5 days for both bacteria and fungi.

Interpretation of Results

1. If the growth of each test organism in the test containers is visually comparable to growth in the positive control, use the same conditions, i.e. type of membrane filter and dilution ratio, and medium when conducting the sterility test.
2. If the growth of the test organisms in the test containers is not visually comparable to that in the positive control, the concentration of test sample used is bacteriostatic or fungistatic. Repeat the validation test, using a more dilute test sample.

   NOTE: A change in the type of membrane filter used for sterilization of the final product and use of neutralizing agents, if available, may reduce the antimicrobial effect of the test sample.

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3. If growth of a test organism in test containers is visually comparable to that in the positive control, proceed with the sterility test.

Sterility Testing by Immersion (Direct Inoculation) Method

Obtain a sample of the final product.

Use aseptic technique to dilute the final product sample to the validated concentration using isotonic saline for injection, USP.

Remove an aliquot of the diluted final product and store at 2-8°C in the event of a failed sterility test result necessitating a repeat test or further investigation.

Inoculate the test media with the test samples. Due to the radiation exposure to personnel, this test must be initiated within 24-48 hours of sterile filtration of the final product but not when radiation levels are high.

Incubate the test samples for not less than 14 days at 32.5 ± 2.5°C for the Fluid Thioglycollate Medium or at 22.5 ± 2.5°C for the Soybean-Casein Digest Medium. Observe the media on a periodic basis over the 14 days of incubation.

Visually inspect the media for growth periodically during the 14-day period.

Continue incubation of the containers for a total of not less than 14 days from the time of inoculation.

If the test specimen is positive before 14 days of incubation, further incubation is not necessary.

Interpretation of Test Results

The final product solution meets the requirements of the sterility test when no microbial growth is observed.

If microbial growth is observed and confirmed microscopically, the final product does not meet the requirements for sterility. The physician of record must be notified within 24 hours and an investigation initiated to determine the microorganisms involved and the cause of the positive sterility result as per **GEN-Q015**, "Investigating Out of Specification QC Test Results."

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