SOP for Inoculating ATS with Bacteria

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1.0 Purpose:

1.1 To provide detailed instructions on how to inoculate **A**rtificial **T**est **S**oil (ATS) with bacteria for use in the Evaluation of Cleaning, Disinfection and/or Sterilization of both Narrow Lumens and Surfaces.

2.0 Scope:

2.1 This document includes the materials and steps necessary to Inoculate Artificial Test Soil with bacteria.

3.0 Materials:

3.1 Sterile snap cap tubes (eppendorf)
3.2 Sterile inoculation loops
3.3 Racks to hold tubes
3.4 Cuvettes appropriate for the Spectrophotometric Method
3.5 0.5 McFarland Barium Sulfate standard for the Turbidimetric Method
3.6 Viable bacterial culture (24-48 hr old cultures on appropriate media)
3.7 Sterile tubes appropriate for centrifugation
3.8 Sterile pipettes (of appropriate volume)

4.0 Equipment:

4.1 Spectrophotometer with 530 nm filter (for Spectrophotometric Method)
4.2 Vortex (variable speed preferred)
4.3 Biological safety cabinet
4.4 Bunsen burner or Bacti-Cinerator for flaming loops
4.5 Refrigerated centrifuge capable of achieving 3500 RPM
4.6 Pipette Aid

5.0 Reagents:

5.1 Sterile water or Phosphate Buffered Saline (PBS, 0.01M, pH 7.5)

6.0 Responsibility:

6.1 It is the responsibility of the supervising personnel to ensure that the laboratory personnel performing these tasks are trained appropriately and that proper procedures are followed.
6.2 It is the responsibility of laboratory personnel to accurately record all required data and results.
6.3 It is the responsibility of laboratory personnel to document any deviation from these procedures, and consult supervising personnel on the matter.

7.0 Definitions:

7.1 ATS: Artificial Test Soil
7.2 McF: McFarland
7.3 BSC: Biological Safety Cabinet
7.4 PBS: Phosphate Buffered Saline

8.0 References:

8.1 Refer to appropriate SOPs for Spectrophotometer Operation and handling of Biohazardous Materials.

9.0 Safety Considerations:

9.1 Consider all bacteria to be a potential biohazard. Any handling of bacterial suspensions should be handled under the appropriate BSC to protect the worker from aerosols.

9.2 Refer to appropriate MSDS sheets for handling and safety considerations when using ATS.

10.0 Procedure:

10.1 *Preparation of a 10^8 cfu/mL bacterial suspension using the Spectrophotometric Method*

10.1.1 Warm up spectrophotometer 30 minutes (at 530 nm).

10.1.2 Using an appropriate bacterial culture (grown on correct media at correct temperature), make a suspension of the bacteria into an aliquot of sterile water or PBS in a sterile snap cap tube. Make the suspension in a total volume greater than what will be required for soiling of lumens or surfaces.

10.1.3 Transfer appropriate volume of bacterial suspension into a cuvette and read the Absorbance at 530 nm on the spectrophotometer. Different bacteria will give a 10^8 cfu/ml at different absorbancies (e.g. *Enterococcus faecalis* at an absorbance reading of 0.370 should produce a viable count of ~1 x 10^8 cfu/mL).

10.2 *Preparation of a 10^8 cfu/mL Bacterial Suspension Using the Turbidometric Method*

10.2.1 Using an appropriate bacterial culture (grown on correct media at correct temperature), make a suspension of the bacteria into an aliquot of sterile water or PBS in a sterile snap cap tube. Make the suspension in a total volume greater than what will be required for soiling of lumens or surfaces.

10.2.2 Compare the turbidity visually to a 0.5 mcF Barium Sulfate Standard. Make adjustments to achieve equal turbidity to the 0.5 mcF tube. If the turbidity of the suspension is greater than the standard, add more bacteria or add more diluent if the turbidity of the bacterial suspension is more than the standard.

10.3 *Inoculation of Artificial Test Soil with 10^8 cfu/mL of bacteria*

10.3.1 Once a 10^8 cfu/mL suspension of bacteria has been achieved using either the spectrophotometric or turbidometric method, transfer the appropriate volume required for soiling of device to a sterile centrifugation tube.

10.3.2 Spin the entire volume at 3500 rpm at 4 degrees Celsius for 15 minutes to produce a bacterial pellet.

10.3.3 Remove the supernatant and discard (in appropriate Biohazard-waste disposal container).

10.3.4 Resuspend the bacterial pellet in an equal volume of ATS. Mix well.

10.3.5 Soil lumen or surface by applying bacterial ATS suspension.

NOTE:

- *For narrow lumens*:

Feed the soil into the device and let dry 1-2 hours. Drain out the excess ATS-bacteria fluid. Harvesting this lumen after drying (should produce a recoverable bioburden of ~10^6 – 10^7 cfu/mL).

- *For surfaces:*

Inoculate the surface with 10uL of the 1 x 10^8 cfu/ml bacterial ATS suspension for a final inoculation of 10^6 total cfu of bacteria per surface area inoculated.

- The soiled device can now be used to proceed with the specific test process (e.g. device cleaning).

10.4 *Inoculation of Artificial Test Soil with >1 Type of Bacteria*

10.4.1 If mixing 2 types of bacteria together, make the required volume of each type of bacteria separately at 1 x 10^8 cfu/mL concentration in sterile water or PBS. (See sections 10.1 & 10.2).

10.4.2 Spin the same full required volume of each to produce a pellet.

10.4.3 Discard supernatant, then resuspend the 2 pellets with ATS so that the total volume of the 2 suspensions combined equals the required volume for soiling (e.g. resuspend each pellet in ½ of required volume, so that when pooled, the combined volume will be the required volume for soiling)

11.0 Records:

11.1 Appropriate records should be retained (e.g. lab book) per archiving procedures for your lab.

12.0 Attachments:

12.1 N/A