STANDARD OPERATING PROCEDURE

Title: Bioburden Testing of Raw Materials, Subassemblies and Final Assemblies

Effective Date: _____

Approvals (Signature and Date):

_____	_____	_____
Responsible Department Head	Technical Authority	QA/QC

1. PURPOSE

1.1 To define a general procedure to determine the bioburden of raw materials, subassemblies and final assemblies.

2. SCOPE

2.1 This procedure is to be used for determination of bioburden levels as well as guidelines for organism characterization in raw materials, subassemblies and final assemblies.

3. RESPONSIBILITY

3.1 It is the responsibility of the QC department to perform bioburden testing. It is the responsibility of department supervisor to ensure that all persons performing bioburden testing have been properly trained in aseptic technique, the use of the Milliflex 100 system, the use of gas flame and agar plating techniques.

4. REFERENCES AND APPLICABLE DOCUMENTS

4.1 USP 23 <71> Sterility Test, Membrane Filtration

4.2 *Standard Methods for the Examination of Water and Wastewater*-American Public Health Association, 18th Edition, Part 9000

4.3 09-0089-SOP-1.0, *Operation and Maintenance of the Leica Quebec Darkfield Colony Counter*

5. MATERIALS AND EQUIPMENT

5.1 Class 100 horizontal laminar flow hood with vacuum and gas flame

5.2 35° ± 2° C Incubator

5.3 44° - 46°C Circulating water bath

5.4 Millipore three place vacuum manifold

5.5 Millipore Milliflex 100 mL funnels (Cat. # MXHA-WG1-24 or MXHA-BG1-24)

5.6 Prefilled Tryptic Soy Agar cassettes (Millipore Cat.# MXSM-CTS-40)

5.7 15-20 mL Tryptic Soy Agar (TSA) Pour Tubes

5.8 15-20 mL TSA with Tween 80 Pour Tubes

5.9 100 x 15 mm Sterile Plastic Pour Plates (Petri Dishes 57 cm^2)

5.10 Forceps in tube of 70% isopropyl alcohol (IPA)

5.11 Glass spreading rod in beaker of 70 % IPA

5.12 Sterile PBS.

5.13 70% IPA swabs

5.14 1000 µL Pipettor

5.15 Pipet tips

5.16 70% IPA in spray bottle

5.17 Quebec Darkfield Colony Counter

5.18 Sterile USP Fluid D (neutralizing rinse fluid-Peptone Water 0.1% with Polysorbate 80)

6. HEALTH AND SAFETY CONSIDERATIONS

6.1 Use Safety glasses when working with 70% IPA.

6.2 Use caution when working with gas flame. Do not spray 70% IPA near flame.

6.3 Dispose of all objects that have been in contact with viable organisms into biosafety bag.

7. DOCUMENTATION REQUIREMENTS

7.1 Bioburden Test Report, Attachment A.

8. GENERAL PROCEDURE

8.1 The maintenance of sterility and proper aseptic technique is essential in this test. This test is to be performed in a class 100 biological safety cabinet. The hood blower and UV lamps must be turned on for a minimum of 30 minutes prior to use. The UV lamps should be turned off immediately before use. Any items that are introduced into the hood should be sprayed with 70% IPA.

8.2 There are 2 methods used to determine bioburden levels: membrane filtration and pour plate. The method of choice is determined by the sample size received from production: membrane filtration is used for aqueous solutions or extraction fluid amounts over 2 mL, the pour plate method is used for aqueous solutions amounts between 0.1 and 2 mL.

8.3 Test samples that contain a preservative such as Sodium Benzoate must be tested with a neutralizing agent such as lecithin and polysorbate 80. Membrane filtration samples should be filtered, then the filter must be rinsed with 200 mL of USP fluid D. Pour plate samples should be poured with TSA that contains polysorbate 80.

8.4 After determining test method, enter all appropriate information on Bioburden Data Sheet.

9. MEMBRANE FILTRATION PROCEDURE

9.1 Remove the correct number of Tryptic Soy Agar cassettes from the refrigerator and bring them to ambient temperature prior to initiating test.

9.2 Don hair cover, particle mask, latex gloves and disposable sleeves. Spray sleeves and hands thoroughly with 70% IPA before entering hood.

9.3 Spray hood with 70% IPA and wipe down with lint-free cloth. Check to ensure that all hoses are attached from manifold to waste flask and from waste flask to vacuum nozzle inside hood.

9.4 Spray media cassettes with 70% IPA and bring into hood.

9.5 Remove a package of funnels and label a funnel lid with test sample information for each sample to be tested. Spray the package of funnels with 70% IPA and bring into hood.

9.6 Swab each of the three vacuum ports with 70 % IPA.

9.7 Gently lift a funnel off of the package, being careful not to dislodge its cover, and, using sterile forceps, pick up the filter screen from the package and place onto vacuum port.

9.8 Place the funnel firmly on top of the filter. Use care to line up properly and avoid touching any other surface. Repeat for other vacuum ports as necessary.

9.9 Remove the cover of the funnel and gently pour in the sample. An innoculum of 10-100 CFUs of one of the following ATCC organisms as a positive control: Pseudomonas aeruginosa 9027, Bacillus subtilis 6633 or Staph. aureus 6538 as a positive control, and sterile water or PBS is used as a negative control.

9.10 Replace the funnel cover slightly askew to allow air to pass through. If the cover is on tightly it will cause a build up of vacuum pressure, and the filter will rupture.

9.11 Turn on the vacuum until the sample has passed through the filter completely, then turn vacuum off. If test sample contains a preservative, it must be rinsed with 200 mL of USP Fluid D.

9.12 Remove funnel from the manifold, being careful not to remove the filter screen with it. The filter screen must not be attached to the filter.

9.13 Remove the protective backing from a filled media cassette, and attach the cassette to the funnel. Push firmly until the two click together forming a tight seal.

9.14 Break off the funnel cup using a steady, rocking motion, then place the funnel cover onto the cassette. Repeat for other samples on manifold.

9.15 Swab each vacuum port on manifold with 70% IPA before repeating procedure for remaining samples.

9.16 When all samples have been run, place cassettes in incubator at 35° ± 2° C for 7 days.

9.17 Empty and rinse waste flask, then clean hood and all work surfaces thoroughly with 70% IPA.

9.18 Read cassettes after 48-72 hours and at 7 days and record results on data sheet. Results are expressed as colony forming units (CFU) per mL for aqueous solutions and as CFU per assembly for items that have undergone an extraction.

9.19 Plate Counting Procedure (Millipore Milliflex-100 System):

9.19.1 Read cassettes promptly after incubation. The Quebec Colony Counter may be used for illumination and counting if necessary.

9.19.2 Count the total number of distinct colonies. Count impinging colonies that differ in appearance, such as morphology or color, as individual colonies. If spreading colonies are encountered, count each of the following types as one: spreaders that form a film; a chain of colonies caused by the disintegration of a bacterial clump; and spreaders that grow along the edge of the filter.

9.19.3 Count all colonies on the membrane when there are 1 to 2 or fewer colonies per square. For 3 or greater colonies per square, count 10 representative squares. Multiply the total 10 square count by 21 to obtain counts/plate. Greater than 3 counts per square only apply when the colonies are distinguishable and there is no crowding.

9.19.4 Divide the final count/plate by the dilution value if used. If average counts are used, report results as estimated colony-forming units. Make estimated counts only when there are discrete, separated colonies without spreaders.

9.19.5 If colonies are crowding and not distinguishable, report results as > 630 divided by the dilution value if used. Convert results to colony-forming units per mL or unit.

9.20 Discard used cassettes into biohazard waste bag.