STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sterility Testing By Membrane Filtration Method	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 PURPOSE:

To lay down procedure for sterility testing of samples by membrane filtration method.

2.0 SCOPE:

Applicable to sterility testing of samples purporting to be sterile at Quality Control Microbiology Laboratory.

3.0 RESPONSIBILITY

Trained Quality Control Microbiology Officer is responsible for performing the sterility test and Microbiology Laboratory In Charge is responsible to ensure that the SOP is followed properly.

4.0 PROCEDURE

4.1 General Instructions and Precautions

4.1.1 Follow SOP, for entry exit to Microbiological testing and Sterility Testing areas.

4.1.2 Ensure that all the sterile or sterilized items are within their expiry/use before date and the packets/wrapping are integral. If any packs or wrapping are found damaged or integrity is compromised, then do not use such materials.

4.1.3 Ensure that the all the media and diluents to be used are pre-incubated. Check the media for contamination or physical damages. In case of Fluid Thioglycollate Medium, if the more than upper one-third of the medium has acquired pink colour (Resazurin ring), then do not use such media containers.

4.1.4 Check the physical parameters (Temperature, Humidity and Differential Pressure) of the area and ensure they are within limits before starting the activities.

4.1.5 During each sterility test session, perform environmental monitoring as per SOP.

4.2 Transfer of Materials to Sterility Testing Area and cRABS

4.2.1 Materials such as media, rinsing fluid or diluents, test accessories viz., forceps, scissors, equinox drain tray with tubing, drain collection container, etc required for sterility testing shall be sterilized in autoclave, unloaded and stored in sterile side (buffer room).

4.2.2 All other materials shall be transferred as follows:

4.2.2.1 Keep all the required materials related to sterility testing such as samples, canisters and other sterile accessories in pass box in Incubator room opening to LAL test room.

4.2.2.2 From the LAL room unload the materials and keep in LAF.

4.2.2.3 Remove the flip off seals of the product vials and disinfect the outer surface of vials using filtered disinfectant solution.

4.2.2.4 Disinfect the outer surface of the canister packs and other sterile accessories packs using filtered disinfectant solution.

4.2.2.5 Transfer all the materials into dynamic pass box opening to buffer room. Ensure the dynamic pass box is in ON condition.

4.2.2.6 From the buffer room, unload the materials and transfer to sterility testing room.

4.2.3 Operate cRABS as per SOP.

4.2.4 Take all the required materials like canisters, media containers, sterilized accessories, filtered disinfectant solution & 70% IPA and product samples to sterility testing room.

4.2.5 Keep all materials except for product samples in cRABS pass box.

4.2.6 Close the cRABS pass box and disinfect the outer surface of material with filtered 70% IPA by wiping. Turn on the UV light and expose the materials for not less than 15 minutes.

4.2.7 After minimum 15 minutes, transfer the materials from the cRABS pass box to cRABS through the channel provided.

4.2.8 In case of samples, keep them in cRABS pass box, close the pass box and disinfectant the outer surface of the samples with filtered 70% IPA by wiping. Do not turn ON the UV light. Allow the disinfectant to dry.

4.2.9 Transfer the samples from the cRABS pass box to cRABS through the channel provided.

4.2.10 Before test, ensure that the disinfectant on each article is dried properly and the packs are integral.

4.2.11 Connect the drain collection container to cRABS drain port securely.

4.3 Sample Requirements

4.3.1 Number of Containers

- Batch of Not More Than 100 Containers – 10% or 4 Containers, whichever is greater
- Batch of More than 100 but not more than 500 containers – 10 Containers
- Batch of More than 500 containers – 2% or 20 containers, whichever is lesser

4.3.2 Volume of Sample to be tested

Content of Container	Minimum Quantity to be tested for each medium
Less than 1 ml	Total Content
1 to 40 ml	Half the contents but not less than 1 ml
More than 40 ml but less than 100 ml	20 ml
100 ml or more	10% of contents, but not less than 20 ml

Note: In case fill volume greater than 10 ml then consider the leftover sample from 3 vials for bacterial endotoxin test (To be pooled before test)

4.4 Sterility Testing by Steritest Equinox System

4.4.1 Operate the Steritest Equinox system as per SOP. Ensure all the connections are made as per requirements.

4.4.2 Install the sterilized drain tray with tubing attached on the right side of the Steritest Equinox Pump. Engage it sideways onto its support until it clicks securely into place. Place the tubing to pipe connected to drain collection container.

4.4.3 Press the on/off button to switch on the pump. The startup screen appears and the pump head opens.

4.4.4 After initialisation select the Manual-testing mode by turning the knob to highlight the mode and press the knob to confirm the selection.

4.4.5 Remove the Steritest canisters from its packaging and place the two canisters upright on the drain tray.

4.4.6 Install the tubing in the pump head by holding the tubing by both hands and gently pulling downward in the slot. Check that the two tubing hoses are positioned on either side of the bossed on the right and left sides of the cover

4.4.7 Ensure that the tubing is correctly positioned by sliding from left to right and right to left. If the tubing does not slide freely, repeat the operation.

4.4.8 Adjust the tubing by sliding such that the tubing is not excessively bent on the canister side and sufficient length is available for free operation on the other side.

4.4.9 Close the pump head by pressing the **open/close** button.

4.4.10 Turn the knob to change the pump rotation speed. Turn the knob clockwise to increase the speed and anticlockwise to reduce the speed. Set the pump at 55 or lower for rinsing, product filtration and addition of SCDM and less than 45 for addition of FTM.

4.4.11 Press the knob or use footswitch for ON/OFF of the pump during testing.

4.4.12 Open the pouch containing caps, plugs and tubing clamps and place them inside the empty canister plastic tray.

4.4.13 If the product to be tested is lyophilized product, then remove the protective cap from the LVP-type vent needle of diluting tubing. Aseptically insert the LVP type needle into the bottle containing sterile diluting fluid. Close the roller clamp of the diluting tube if open. Keep the diluting fluid bottle in bottle holder in inverted position.

4.4.14 Pre wetting of Membrane

4.4.14.1 Remove the protective cap from the product-testing needle and insert the needle into the bottle containing 100 ml of rinsing or diluting fluid.

4.4.14.2 Turn pump on with foot switch and invert the bottle, transfer the entire contents of the bottle to both canisters

4.4.14.3 Aseptically place a red cap onto each canister’s air vent to allow the rinsing fluid to start filtering through the membranes.

4.4.15 Product Testing – Liquid in Vials

4.4.15.1 After pre wetting of the membrane, remove the needle from the rinsing fluid bottle and insert into first product vial.

4.4.15.2 Invert the vial and turn pump on with the footswitch and transfer the contents to the canisters.

4.4.15.3 Remove the needle from the first vial, insert into second vial, invert and turn pump on with the footswitch and transfer the entire contents to the canisters.

4.4.15.4 Repeat the above steps until the last sample vial of the batch is filtered.

Note: If required insert air vent needles to the product vial to allow transfer of air and avoid creation of vacuum.

4.4.16 Product Testing – soluble powder in vials (Lyophilized Product)

4.4.16.1 After pre wetting of the membrane, remove the needle from the rinsing fluid bottle and insert into first product vial.

4.4.16.2 Keep/Hold the vial upright and open the roller clamp of the diluting fluid tubing and allow the required volume of fluid to reconstitute the product and close the clamp.

4.4.16.3 Shake the vial and completely reconstitute the product.

4.4.16.4 Invert the vial and turn pump on with the footswitch and transfer the entire contents to the canisters.

4.4.16.5 Remove the needle from the first vial and insert into second product vial. Repeat the above reconstitution and filtration steps.

4.4.16.6 Repeat the above steps until the last sample vial of the batch is filtered.

4.4.17 Rinsing of Tubing, Canisters and Membranes

4.4.17.1 Insert the needle in to first bottle of rinsing fluid (0.1% Peptone Water) and place the bottle upright. If required insert air vent needles to the product vial to allow transfer of air and avoid creation of vacuum.

4.4.17.2 Remove the red caps from the top air vents of the canisters and place in canister pouches or on work bench.

4.4.17.3 Turn on the pump and hold the bottle inverted or place inverted in bottle holder.

4.4.17.4 Pump 100 ml of rinsing fluid to each canister and then turn off the pump. Place the rinsing fluid upright on the workbench.

4.4.17.5 Aseptically replace the red caps onto the top air vents of the canisters. Keep the needles in the bottle.

4.4.17.6 If appropriate, take out the canisters from the drain tray and gently swirl to remove any adhering product residues from the internal walls of the canister.

4.4.17.7 Turn on the pump and allow the 100 ml of rinse fluid to filter slowly through the canisters are empty.

4.4.17.8 Repeat the above steps for total 3 x 100 of rinsing fluid filters through the tubing, canisters and membranes.

Note: The rinsing fluid and volume of rinsing fluid shall be changed as per validation if different from above.

4.4.18 Addition of Media to Canisters

4.4.18.1 Remove red caps from the air vents of the canisters.

4.4.18.2 Lift one of the canisters from the drain tray, aseptically place a yellow plug into bottom outlet port of the canister. Secure the yellow plug firmly by twisting a half turn while pushing it. Return the canister to the drain tray and repeat the procedure for the other canister.

4.4.18.3 Clamp off one tubing line with the clamps attached to the tubing. Place this clamp as close as possible to Y-connector of the needle spiking device.

4.4.18.4 Remove the needle from the rinsing fluid bottle and insert into first media bottle. If required insert air vent needle into the media bottle to allow transfer of air.

4.4.18.5 Turn on the pump, hold the bottle inverted or place inverted in bottle holder. (Keep the pump speed to lower than 45 for Fluid Thioglycollate Medium to reduce aeration).

4.4.18.6 When 100 ml media is transferred to the canister, keep the bottle upright on the work surface and turn off the pump when the tubing is clear of media

4.4.18.7 Unclamp the clamped tubing and clamp the other tubing line (where the media just transferred through) as before.

4.4.18.8 Remove the needle first media bottle and insert into second media bottle. Transfer the media to the second canister, and then turn off the pump when tubing is clear of media as before.

4.4.18.9 Clamp the both tubing lines approximately 6 cm from the canister inlets.

4.4.18.10 Open the pump by pressing open/close button and remove the tubing from the pump head.

4.4.18.11 With sterile scissors, cut the tubing approximately 2 cm above the clamp.

4.4.18.12 Fold over and insert the tubing into the top air vents of the canisters. Remove the canisters from the drain tray and set them aside.

4.4.18.13 Remove the needles from bottles and put the tubing into the discard bin.

4.4.18.14 Label the canisters with Product name, Batch No/A.R No, Media Lot No, Date and Analyst initials. Record the details in annexure I and II.

4.5 Negative Product Control Test

4.5.1 Perform one negative control test during each sterility test session as control for media and diluents sterility and conditions during test session.

4.5.2 Perform the testing by considering 100 ml of 0.1% sterile peptone water as product and performing maximum manipulations performed during the session as follows:

4.5.2.1 If the maximum manipulations performed during the test session are for lyophilized product, then all steps involved in lyophilized product testing shall be performed for negative product control.

4.5.2.2 If the maximum manipulations performed during the test session are for liquid product then all steps involved in liquid product shall be performed for negative product control.

4.6 Transfer and Disposal of Material

4.6.1 After completion of test, transfer all accessories, spent sample containers, empty media diluents containers to autoclave area through pass box from Buffer Room to Autoclave Room.

4.6.2 Transfer the canisters for incubation through dynamic pass box from buffer room to LAL Test Room and then through pass box from LAL Test Room to Incubator Room.

4.6.3 Clean the cRABS with filtered disinfectant agent. Disinfect the port and tubing for drain collection by transferring sufficient volume of disinfectant solution through the port and tubing such that all the surface comes in contact with the disinfectant solution.

4.6.4 Close the valve if the drain collection tubing, disconnect the SS container by opening the TC clamp. Transfer the container to autoclave through pass box from buffer room.

4.6.5 Deactivate the solution in the container by addition of appropriate deactivating agent and dispose as per.

4.7 Incubation and Observation

4.7.1 Incubate Soybean Casein Digest Medium (SCM) sterility test canisters at 22.5 ± 2.5℃ and Fluid Thioglycollate Medium (FTG) sterility test canisters at 32.5 ± 2.5℃ for 14 days.

4.7.2 Examine on each working day for the evidence of microbial growth throughout the 14 days incubation period and record the results. If the 14^{th} day is weekly off or holiday then observe on next working day.

4.7.3 During observation, any evidence of clear growth in terms of turbidity, cloudiness, precipitation, cotton ball type growth, clear medium with twister raising from the bottom when swirled compared to negative control, then remove such canisters and proceed for identification/investigation as per SOP on the day of such observation.

4.7.4 After completion of incubation, dispose the media as per SOP.

4.8 Acceptance Criteria

4.8.1 If there is no evidence of microbial growth in any of the sterility test media at the conclusion of the incubation period, the sample / product under test complies with the requirements for sterility.

4.8.2 If evidence of microbial growth is found in either of the media, the sample / product being tested does not comply with the test for sterility, unless it can be clearly demonstrated that the test was invalid for causes unrelated to the sample / product being tested. The test may be considered invalid only if one or more of the following conditions apply:

4.8.2.1 The microbiological monitoring of the sterility testing area shows a fault.

4.8.2.2 A review of the testing procedure used during the test in question reveals a fault.

4.8.2.3 Microbial growth is found in the negative controls.

4.8.2.4 After determination of the identity of the microorganisms isolated from the test, the growth of this species (or these species) may be ascribed unequivocally to faults with respect to the material and or the technique used in conducting the sterility test procedure.

4.8.3 If the test is declared invalid, the test must be repeated with the same number of units as in the original test.

4.8.4 If no evidence of microbial growth is found in the repeat test, the product examined complies with the test for sterility.

4.8.5 If microbial growth is found in the repeat test, the product examined does not comply with the test for sterility.

5.0 ABBRIVATIONS AND DEFINITIONS

SOP	Standard Operating Procedure
QCM	Quality Control Microbiology
QAD	Quality Assurance Department
Rev.	Revision
No.	Number
cRABs	Closed Restricted Access Barrier System
%	Percent
UV	Ultra violet
°C	Degree centigrade
mL	Milli litre

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SCA	Soyabean Casein Digest Agar
SCM	Soyabean Casein Digest Medium

6.0 REFERENCE DOCUMENTS

SOP	Entry And Exit Procedure For Routine Microbiology Testing Area Sterility Area
SOP	Monitoring Of Microbiology Laboratory
SOP	Disposal Of Contaminated Material
SOP	Handling Of Out Of Specification Results In Microbiological Testing
SOP	Handling Of Cytotoxic Drugs And Related Wastes.
USP <71>	Sterility Tests
IP 2.2.11	Sterility
Phar. Eur 2.6.1	Sterility
JP 4.06	Sterility Test

7.0 ANNEXURE / ATTACHMENTS

Appendix I: Flow chart of sterility
Annexure I: Form 1 - Sterility sample entry record
Annexure II: Form 2 - Sterility test data sheet

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

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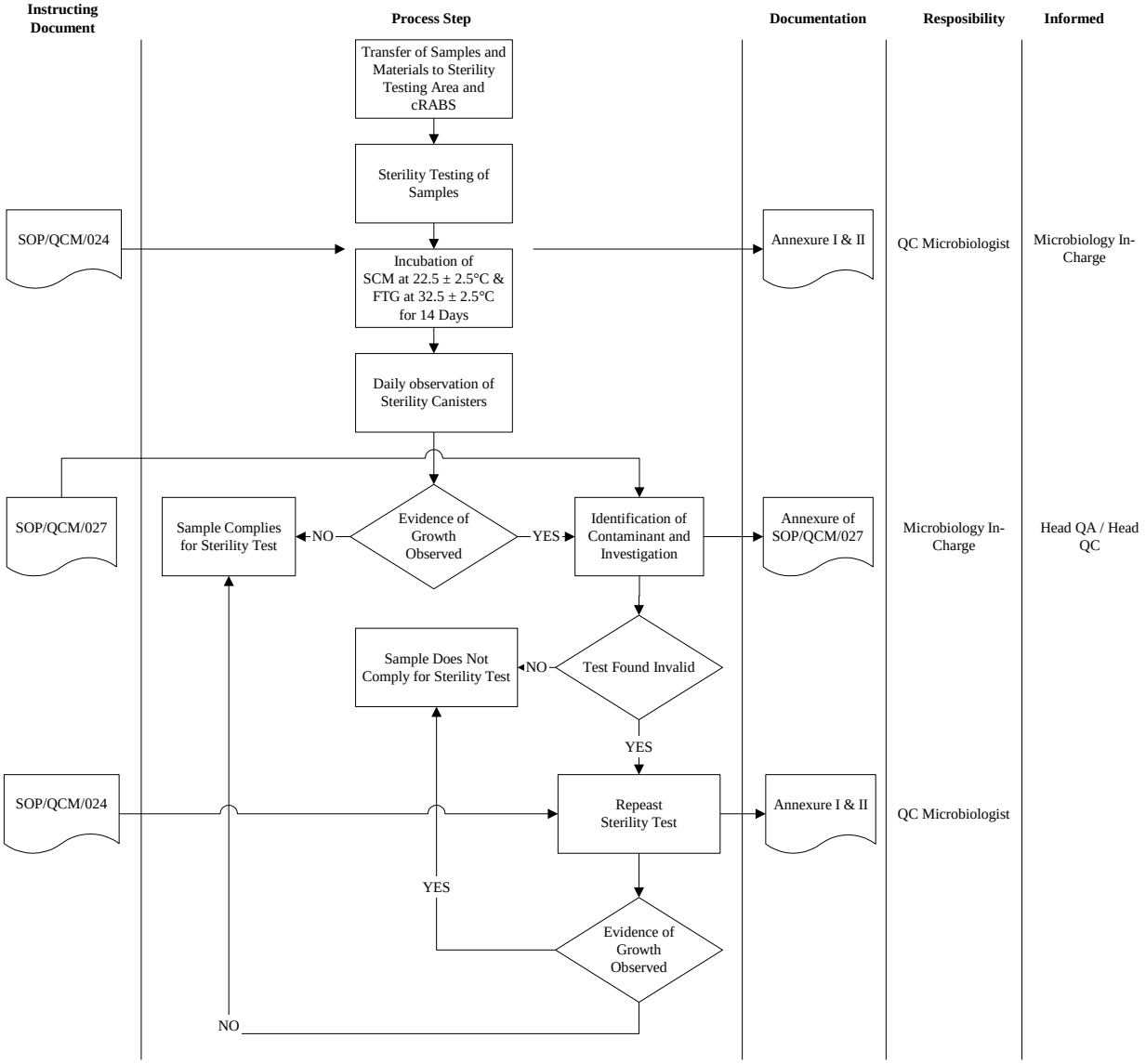
APPENDIX - I
FLOW CHART OF STERILITY TEST



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8.0 REVISION LOG

Revision Number	Effective Date	Reason for Revision