PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sterility test using manifold for dilution study and Stability	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

TABLE OF CONTENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sterility test using manifold for dilution study and Stability	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 PURPOSE:

To lay down the procedure to perform sterility test by membrane filtration using filtration manifold to confirm the absence of microbial contamination in the product.

2.0 SCOPE:

It is applicable to the microbiology lab for sterility testing samples of dilution study and stability samples.

3.0 RESPONSIBILITY:

Microbiology personnel

4.0 PROCEDURE:

4.1 Precaution taken during sterility

4.1.1 Check the temperature, humidity and differential pressure. They shall be with in the limit.

4.1.2 Check the cleaning status of the area and sterility testing cRABS.

4.1.3 Check the fluid thioglycolate medium if more than the upper one-third of the medium has acquired a pink colour the media tube shall not be used for the test.

4.1.4 Check the pre-incubated sterility media for any contamination visually.

4.1.5 Check all the accessories are autoclaved properly.

4.1.6 Each prepared lot of the Soyabean casein digest medium and fluid thioglycolate medium shall be tested for growth promotion test.

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sterility test using manifold for dilution study and Stability	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

4.2 Man movement

4.2.1 For preparation of sterility enter according to SOP.

4.2.2 For performing sterility test enter according to SOP.

4.3 Sterility test Preparation

4.3.1 Keep all the material required for sterility test such as product vial/bag, forceps, membrane, sporicidal agent in the pass box (LAL test room to Incubator).

4.3.2 Enter the room according to SOP.

4.3.3 Take all the material into the LAF and disinfect the outer side of the vials/bags by spraying the sporicidal agent.

Note: Prepare the disinfectant according to SOP.
If wrapping of sterilized item is found torned/compromised, do not use the same. Item need to be re-sterilized.

4.3.4 Transfer all the material into pass box (Buffer room to LAL test room).

4.4 Sterility testing procedure

4.4.1 Enter the 'sterility test area' as per SOP.

4.4.2 Enter into the buffer area and collect all the material from the pass box (Buffer room to LAL test room) and transfer it into cRABS pass box.

4.4.3 Unload the required accessories for sterility from autoclave unloading area.

4.4.4 Following material should be transferred in cRABS pass box

- Sterility samples, Forceps and scissors.
- Sporocidal agent and sterile lint free mop.
- Rack containing tubes of 100 ml of Fluid Thioglycollate Medium and 100 ml of Soyabean Casein Digest Medium tubes.
- Containers of 100 ml sterile 0.1% peptone water.
- Membrane filtration units, containing 0.45 µ porosity, 47 mm diameter, or presterilised filters can be used.

- Filtration manifold.

4.4.5 Transfer the containers of sample to be tested into the cRABS. Transfer the contents of containers to funnel of membrane filtration aseptically and filter according to the following procedure.

4.4.6 Open the vacuum valve & pre wet the membrane of the funnel with peptone water after that filter the contents of each container through membrane filter by partially removing the funnel lid of membrane filtration unit and pour the contents into the funnel. Repeat the procedure with the remaining containers of the lot tested. Filter the contents by applying vacuum.

4.4.7 Rinse membrane filter with three 100 ml portions of sterile 0.1% peptone water, by adding 0.1% peptone water to funnel of membrane filtration unit and filter it out.

4.4.8 Aseptically cut the membrane into two equal half and transfer one half into FTM tube and other half into SCM tube

4.4.9 Label the tubes with the details Media lot number, Name of the product, A.R number, sign / date.

4.4.10 Perform negative control test for a set of sterility test as a control of rinsing fluid sterility.

4.4.11 Pour 100 ml portions of sterile 0.1% peptone water into one membrane filtration unit. Filter fluid through the membrane filter by applying vacuum.

4.4.12 After filtering the contents of container rinse the membrane filter. Close the vacuum valve. Open the membrane filtration unit.

4.4.13 Cut the membrane filter into two half with sterilised scissor.

4.4.14 Transfer one half the membrane filter to tube containing 100 ml Fluid Thioglycollate Medium and the other half to tube containing 100 ml Soyabean Casein Digest Medium, using sterile forceps.

4.4.15 Transfer all equipments; media tubes, and tested containers of sample from work chamber of cRABS to the pass box of the cRABS and from pass box of the cRABS to pass box (Autoclave room to Buffer room). Clean the cRABS with sporicidal agent.

4.4.16 Incubate the tube of fluid thioglycollate medium at 30-35°C and tube of soyabean casein digest medium at 20° to 25°C for 14 days. Observe the tube on all working days up to 14 days if holiday is there observe the tube on next working day.

4.5 During routine visual observation of the sterility tube (Both FTG and SCM) any turbidity/cloudiness, Cotton ball type of growth, medium with precipitate, clear medium but when swirled gently a twister raising from the bottom as compared against negative control (clear) by swirling gently, identify the canister and record the day of observation

4.6 Incubate the same tube further on the same temperature condition for completion of the incubation period.

4.7 After completion of incubation period swirl the tube thoroughly and subculture it on Soyabean casein digest agar plate and incubate for 5 days at 30-35°C

4.8 Observe the plate for growth, If no evidence of growth is found, the product meets the requirement of test for sterility.

4.9 If growth is observed follow SOP for handling out of specification results in microbiological testing and identify the contaminant using biomeriux as per SOP.

4.10 If evidence of microbial growth is found, the product to be examined does not comply with the test for sterility, unless it can be clearly demonstrated that the test was invalid for causes unrelated to the product to be examined.

4.11 The test may be considered invalid only when one or more of the following conditions are fulfilled.

- The data of the microbial monitoring of the sterility facility shows a fault.

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sterility test using manifold for dilution study and Stability	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

- A review of the testing procedure used during the test in question reveals a fault.
- Microbial growth is observed in negative controls.
- After determination of the identity of the microorganisms isolated from the test, the growth of these species or these species may be ascribed unequivocally to faults with respect to material and/or the technique used in conducting the sterility test procedure.

4.12 If the test is declared to be invalid, repeat the sterility test using same number of representative samples as the original sterility test & employ the same method of testing. Incubate tubes for period of 14 days.

4.13 If no evidence of growth is found, the product meets the requirement of test for sterility. Record the observation in Annexure - I

4.14 If evidence of growth is found in the retest the sterile product fails to meet sterility test requirements and shall be rejected.

4.15 The liquid product waste shall be deactivated according to SOP.

4.16 The media tube after incubation shall be discarded according to SOP.

4.17 Enter the sample details in Annexure-I of SOP.

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Title: Sterility test using manifold for dilution study and Stability	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

5.0 ABBREVIATIONS AND DEFINITIONS

SOP	Standard Operating Procedure
QCM	Quality Control Microbiology
QAD	Quality Assurance Department
Rev.	Revision
No.	Number
QADF	Quality Assurance Department First Floor
LAF	Laminar Air Flow
ML	Milli litre
µ	Micron
FTM	Fluid Thioglycollate Medium
SCM	Soyabean Casein Digest Medium
°C	Degree Centigrade
%	Percentage
cRABS	Closed restricted access barrier system

Sterile: Free from viable microorganism

6.0 REFERENCE DOCUMENTS

SOP Disposal of contaminated material
SOP Handling of cyto toxic drugs and related wastes
SOP Identification of microbial cultures using biomeriux identification system.
SOP Handling of out of specification results in microbiological testing
SOP Entry and Exit procedure for Routine microbiology testing area, Sterility area
SOP Cleaning and sanitization of microbiological area

7.0 ANNEXURE / ATTACHMENTS

Appendix I: Flow chart of sterility
Annexure I: Form 1 - Sterility test data sheet (Manifold method)

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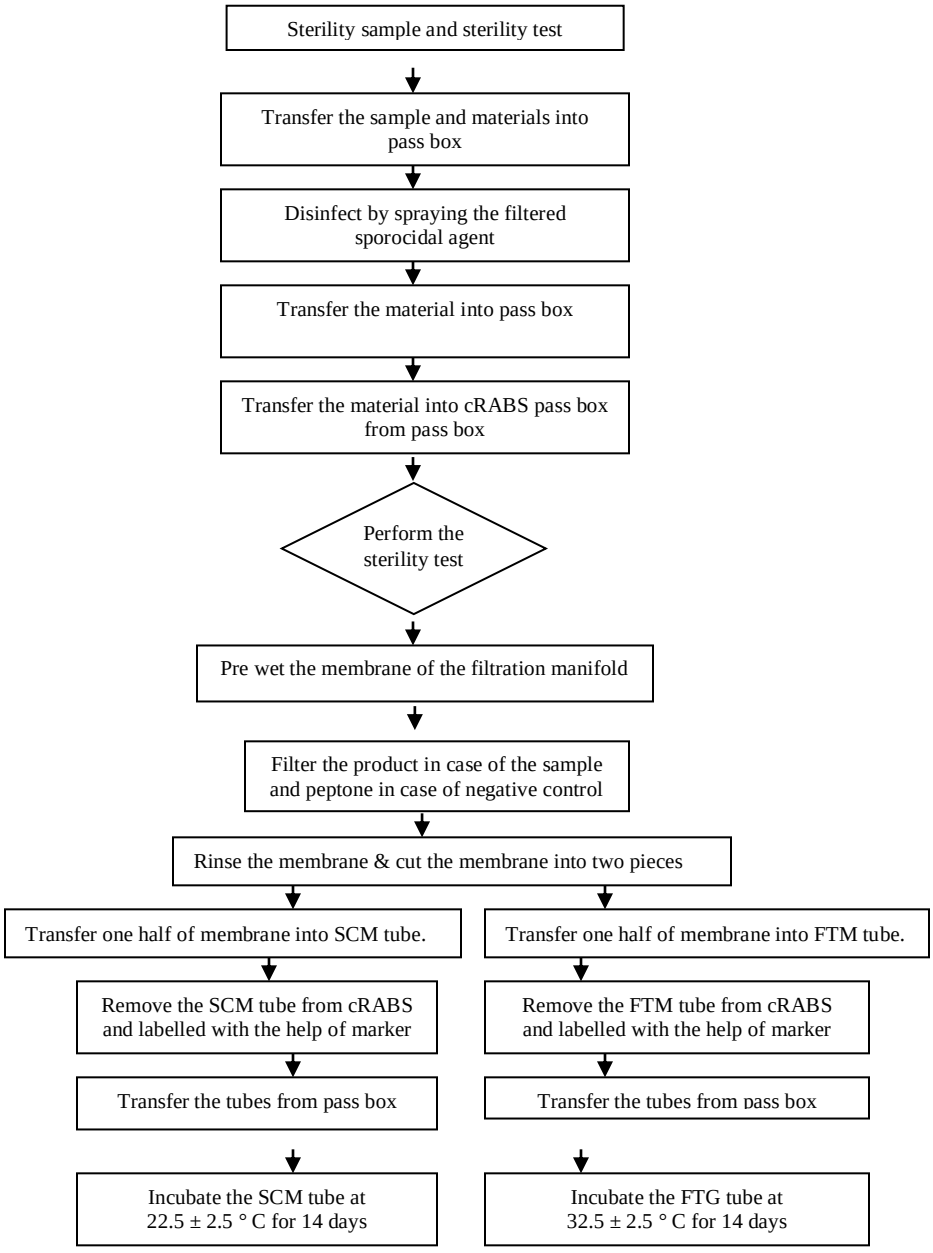
Title: Sterility test using manifold for dilution study and Stability	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

Appendix-I - Flow chart of Sterility



STANDARD OPERATING PROCEDURE	
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Title: Sterility test using manifold for dilution study and Stability	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

8.0 REVISION LOG

Revision Number	Effective Date	Reason for Revision