STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sterility Test by Closed System	**Effective Date:**
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1.0 OBJECTIVE:

To lay down a procedure for Sterility Test by Closed System.

2.0 SCOPE:

This SOP is applicable for Sterility Test by Closed System. Make: MDI, in Microbiology lap of Quality Control Department at……..

3.0 RESPONSIBILITY:

Officer/Executive – Microbiology.

4.0 ACCOUNTIBILITY:

Head – QC

5.0 ABBREVIATIONS:

IPA	Isopropyl Alcohol
Ltd.	Limited
No.	Number
QC	Quality Control
QA	Quality Assurance
SOP	Standard Operating Procedure

6.0 PROCEDURE:

6.1 REQUREMENTS:

6.1.1 Sterilized Fluid Thioglycollate Medium in 100 ml bottle.

6.1.2 Sterilized Soybean Casein Digest Medium in 100 ml bottle.

6.1.3 Sterilized Rinsing Fluid (0.1% Peptone).

6.1.4 Compact Test Instrument with Canisters and accessories.

6.1.5 Sterile wipes.

6.1.6 Laminar Air Flow.

6.1.7 Scissor, forceps.

6.1.8 After receiving the sample for sterility test, record the details of sample as per sop number ….. in sample receiving register e.g. Date of receipt, Product Name, Batch No., in Annexure – 1 of SOP number …….. Titled "**Sample Receipt and Analysis for Sterility Test**".

6.1.9 Collect the samples to be tested for sterility test: sanitize the external surface of all samples subjected for sterility test by using sporocidal disinfectant available in SS sample tray and mark the SS sample tray with details of sample e.g. product name, batch number, and lot number and Transfer desired SS sample tray through dynamic pass box (…….) in sterility room.

6.1.10 Transfer, pre incubated SCM & FTM bottle, SCA plates, DNA plates, rinsing fluid 0.1 % peptone water bottle, Air sampler (if required) and other required material to dynamic pass box (……) of buffer zone.

6.1.11 Before enter in the sterility area ensure pressure differential should be within limit.

6.1.12 Enter in sterility area as per SOP No. ……., Title Entry, and Exit and Gowning Procedure for Sterility Area.

6.1.13 Check the temperature & relative humidity of Sterility Area as per SOP No. ……..and status of cleaning and fogging of sterility area before start the sterility test. If cleaning and fogging has been done and temperature and relative humidity within limit, perform the Sterility test, if not clean and fog the area.

6.1.14 Mop the LAF platform with 0.22 µ filtered 70 % IPA & start the LAF as per SOP No. …….. for the operation of LAF. Ensure LAF should be "switched ON" before 30 minutes prior the test if it is in switched OFF condition.

6.1.15 After completion of sterilization cycle, unload the sterilized articles from autoclave to mobile LAF and transfer sterilized articles contained in mobile LAF from cooling zone to LAF of sterility Room.

6.1.16 Frequency sanitize the hand and LAF working station with 0.22 µ filtered 70 % IPA during sterility test.

6.1.17 Before starting sterility test expose the SCA plates at specified location throughout the testing as per SOP No. …… and Personnel monitoring shall be carried out after the completion of sterility test by contact

plate method as per SOP No. ……. After personnel monitoring. Sanitize the hand gloves and contact site boiler suit with 0.22 µ filtered 70 % IPA by removing it in Air lock – V.

6.2 PRE-STERILIZED CANISTERS & DILUTORS:

6.2.1 Use the specific canister of MDI or Sartorius or Millipore with respect to the catalogue numbers as per sample requirement.

6.2.2 Review the COA received from the manufacturer for all details with respect to filter, sterilization and expiry. Maintain the COA for every lot.

6.3 TEST:

6.3.1 Enter in sterility area as per SOP No. …….. , title Entry, Exit and Gowning Procedure for Sterility Area.

6.3.2 Sterilize the exterior parts of the Steritest Compact Instrument, LAF bench with 0.22 µ filtered v/v 70 % IPA.

6.3.3 Transfer the sealed canister packs, rinsing fluid, containers, reconstitution bottles and other related test aids to the sterility testing LAF.

6.3.4 Remove the canister/diluter from the sealed pack.

6.3.5 Place the canister over the drain tray. Open the clamp completely and introduce canisters tubing from left to right and closed the clamp.

6.3.6 Insert the needle of transfer tube into bottle containing sterilized fluid solution.

6.3.7 Transfer 100 ml of sterilized fluid to both the canister (50 ml each) by using peristaltic Steripump for pre-wetting purpose. Closed the canisters vent by using the cap. Filter 100 ml of sterilized fluid with the help of steripump as a pre wetting step for membrane.

6.4 RAW MATERIAL (DRY POWDER):

6.4.1 Dissolve the contents of the sterile product (6 grams) by transferring 100 ml sterile fluid – A/ sterile diluents solution into the sample vial.

6.4.2 Reconstitute the product sample for complete dissolution of the product.

6.4.3 Insert sample tube needle into bottle containing reconstituted product solution.

6.4.4 Transfer the entire contents and simultaneously filter the sample with the help of the steripump immediately

without any hold up of the product in the canisters.

6.4.5 Remove the vent and insert sample tube needle to bottle containing sterilized fluid – A.

6.4.6 Transfer the sterilized fluid –A to the canisters of 100 ml to each canisters with the help of steripump. This volume is for rinsing the membrane for neutralization.

6.4.7 Recap the vent and filter it with the help of steripump.

6.4.8 Repeat the step 5.4.6 & 5.4.7 for 2 times to have a rinse of 2 × 100 ml for each canister or as per requirements.

6.4.9 After rinsing of membrane, closed the lower aperture or outlet port of the canister with the help of rubber closure provide in the kit.

6.4.10 Closed the second arm of sample tube by clamping it with clamp provided.

6.4.11 Insert sample tube needle to bottle containing sterilized 100 ml fluid Thioglycollate Medium.

6.4.12 Transfer the sterilized fluid Thioglycollate Medium to one canister with the help of steripump at lower steripump speed.

6.4.13 Open the clamp of the second arm and fix it on the first arm of the sample tube.

6.4.14 Insert sample tube needle to bottle containing sterilized 100 ml Soybean Casein Digest Medium.

6.4.15 Transfer the sterilized Soybean Casein Digest Medium to the second canister with the help of stripump.

6.4.16 Clamp both the arms of the sample tube with clamps provided in kit.

6.4.17 Cut the tubing with scissor and connect the respective tube to their vent filters to separate and integrate the canisters.

6.4.18 Label the SCDM/FTM canister as per Annexure II.

6.4.19. Incubate fluid Thioglycollate Medium canister at 32.5 + 2.5 ˚C and Soybean Casein Digest Medium canister at 22.5 + 2.5 ˚C for NLT 14 days.

6.4.20 Record the observation of sterility test in Annexure – I, Titled "**Sterility Test by Closed Method**".

6.5 DRY POWDER FINISH PRODUCT:

6.5.1 Reconstitute the product sample for the complete dissolution of the product.

6.5.2 Insert sample tube needle into the bottle containing Reconstituted product solution.

6.5.3 Transfer the entire contents and simultaneously filter sample with the help of the steripump immediately

without any hold up of the product in the canisters.

6.5.4 Remove the vent and insert sample tube needle to bottle containing sterilized fluid – A.

6.5.5 Transfer the sterilized fluid –A to the canisters of 100 ml to each canisters with the help of steripump. This volume is for rinsing the membrane for neutralization.

6.5.6 Recap the vent and filter it with the help of steripump.

6.5.7 Repeat the step 5.5.4 & 5.5.5 for two times to have a rinse of 2 × 100 ml for each canister.

6.5.8 After rinsing of the membrane, closed the lower aperture or outlet port of the canister with the help of rubber closure provide in the kit.

6.5.9 Closed the second arm of sample tube by clamping it with clamp provided.

6.5.10 Insert sample tube needle to bottle containing sterilized 100 ml fluid Thioglycollate Medium.

6.5.11 Transfer the sterilized fluid Thioglycollate Medium to one canister with the help of steripump at lower steripump speed.

6.5.12 Open the clamp of the second arm and fix it on the first arm of the sample tube.

6.5.13 Insert sample tube needle to bottle containing sterilized 100 ml Soybean Casein Digest Medium.

6.5.14 Transfer the sterilized Soybean Casein Digest Medium to the second canister with the help of stripump.

6.5.15 Clamp both the arms of the sample tube with clamps provided in kit.

6.5.16 Cut the tubing with scissor and connect the respective tube to their vent filters to separate and integrate the canisters.

6.5.17 Label the SCDM / FTM canister as per Annexure II.

6.5.18 Incubate fluid Thioglycollate Medium canister at 32.5 ±2.5 ˚C and Soybean Casein Digest Medium canister at 22.5± 2.5˚C for NLT 14 days.

6.5.19 Record the observation of sterility test in Annexure – I, Titled "**Sterility test by Closed Method**".

6.6 FINISH PRODUCT VIAL, LDPE BOTTLE AND EYE/EAR DROPS:

6.6.1 Insert sample tube needle into the Vial, LDPE bottle and Eye/Ear Drops bottle containing product solution.

6.6.2 Transfer the entire contents and simultaneously filter sample with the help of the steripump immediately without any hold up of the product in the canisters.

6.6.3 Remove the vent and insert sample tube needle to bottle containing sterilized fluid – A.

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6.6.4 Transfer the sterilized fluid –A to the canisters of 100 ml to each canisters with the help of steripump. This volume is for rinsing the membrane for neutralization.

6.6.5 Recap the vent and filter it with the help of steripump.

6.6.6 Repeat the step 5.6.3 & 5.6.4 for two times to have a rinse of 2 × 100 ml for each canister.

6.6.7 After rinsing of the membrane, closed the lower aperture or outlet port of the canister with the help of rubber closure provide in the kit.

6.6.8 Closed the second arm of sample tube by clamping it with clamp provided.

6.6.9 Insert sample tube needle to bottle containing sterilized 100 ml fluid Thioglycollate Medium.

6.5.10 Transfer the sterilized fluid Thioglycollate Medium to one canister with the help of steripump at lower steripump speed.

6.5.11 Open the clamp of the second arm and fix it on the first arm of the sample tube.

6.5.12 Insert sample tube needle to bottle containing sterilized 100 ml Soybean Casein Digest Medium.

6.5.13 Transfer the sterilized Soybean Casein Digest Medium to the second canister with the help of stripump.

6.5.14 Clamp both the arms of the sample tube with clamps provided in kit.

6.6.15 Cut the tubing with scissor and connect the respective tube to their vent filters to separate and integrate the canisters.

6.6.16 Label the SCDM /FTM canister as per Annexure II.

6.6.17 Incubate fluid Thioglycollate Medium canister at 32.5 ± 2.5 ˚C and Soybean Casein Digest Medium canister at 22.5±2.5 ˚C for NLT 14 days.

6.6 18 Record the observation of sterility test in Annexure – I, Titled "**Sterility test by Closed Method**".

6.7 NEGATIVE CONTROL OF CANISTER AND ACCESSORIES:

6.7.1 Perform negative control of canister supplied by the manufacturer with both the medium by passing 100 ml of Soybean Casein Digest Medium and Fluid Thioglycollate Medium.

6.7.2 Remove the canister/diluter from the sealed pack.

6.7.3 Place the canister over the drain tray. Open the clamp completely and introduce canisters tubing from left to right and closed the clamp.

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6.7.4 Insert the needle into bottle containing sterilized fluid A- solution.

6.7.5 Transfer 100 ml of sterilized fluid to both the canister (50 ml each) by using peristaltic steripump.

6.7.6 After rinsing of the membrane, closed the lower aperture or outlet port of the canister with the help of rubber closure provide in the kit.

6.7.7 Closed the second arm of sample tube by clamping it with clamp provided.

6.7.8 Insert sample tube needle to bottle containing sterilized 100 ml fluid Thioglycollate Medium.

6.7.9 Transfer the sterilized fluid Thioglycollate Medium to one canister with the help of steripump at lower steripump speed.

6.7.10 Open the clamp of the second arm and fix it on the first arm of the sample tube.

6.7.11 Insert sample tube needle to bottle containing sterilized 100 ml Soybean Casein Digest Medium.

6.7.12 Transfer the sterilized Soybean Casein Digest Medium to the second canister with the help of stripump.

6.7.13 Clamp both the arms of the sample tube with clamps provided in kit.

6.7.14 Cut the tubing with scissor and connect the respective tube to their vent filters to separate and integrate the canisters.

6.7.15 Incubate fluid Thioglycollate Medium canister at 32.5±2.5 ˚C and Soybean Casein Digest Medium canister at 22.5±2.5 ˚C for NLT 14 days.

6.8 TEST PRECAUTIONS:

6.8.1 Time of contact between the antibiotic and the membrane should be less (recommended stripump speed 60 – 80).

6.8.2 Rinsing of the membrane to be done at lower speed (recommended strip ump speed 30 – 40).

6.8.3 While rinsing, open the vent and transfer about 100 ml of rinsing fluid, after filling close the vent and start the stripump. This is to facilitate the efficient and total inner surface area rinsing of the filter.

6.8.4 Pumping of fluid Thioglycollate Medium to be done at lower speed (35 -40) to reduce aeration.

6.8.5 SS drain plate of compact instrument to be sterilized prior to test and shall be intermittently sanitized during testing of samples.

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6.9 OBSERVATION AND INTERPRETATION OF RESULTS:

6.9.1 Observe all the canisters and controls on daily basis for evidence or absence of microbial growth.

6.9.2 If no evidence of microbial growth is found, the product sample being examined passes the tests for sterility.

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

6.9.4 The test may be considered invalid only if one of the following conditions are fulfilled.

6.9.4.1 The data of microbiological monitoring of the sterility testing facility show a fault.

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

6.9.4.3 Microbial growth is found in the negative controls.

6.9.4.4 After identification of the microorganisms isolated from the test, the growth of the this species may be ascribed unequivocally to faults with respect to the material and or the technique used in conducting the sterility test.

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

6.10 EQUAL SPLITTING TEST:

6.10.1 Set the steripump and connect it to power supply.

6.10.2 Remove the canister from the sealed pack.

6.10.3 Place the canister over the drain tray. Open the clamp completely and introduce canisters tubing from left to right and closed the clamp.

6.10.4 Insert the needle of transfer tube into bottle containing 100 ml pre sterilized sterilize purified water.

6.10.5 Remove the rubber plug from canister vent.

6.10.6 Switch on the steripump.

6.10.7 Invert the 100 ml glass bottle and place properly on the bar.

6.10.8 Allow the fluid to flow through the tubing to canisters.

6.10.9 Switch off the pump when entire water sample has transferred into the canisters.

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6.10.10 Remove the needle from the bottle and [lace it safely.

6.10.11 Remove the outlet plugs and each canister on measuring cylinder.

6.10.12 Apply rubber plugs on the vents of the canisters.

6.10.13 Start the pump so that water in canisters start moving into the measuring cylinders, and all water is driven out.

6.10.14 Note the volume of filtered water in each measuring cylinder.

6.10.15 Perform the equal splitting test at three points at 40, 60 and 80 speed of steripump.

6.10.16 Repeat the exercise three times at every set point of speed and compare the volumes.

6.10.17 Record the observations of Equal splitting test in Annexure III, Titled "Equal Splitting Test Report".

6.10.18 **Acceptance criteria:** If the volume of sterile purified water between two canisters remains within ± 10 % test is passed.

6.10.19 Frequency of Equal Splitting test: Once in a year ± one month.

7.0 Annexures:

Annexures No.	Title of Annexure	Format No.
Annexure - I	Sterility test report closed method	
Annexure - II	Label for SCM/FTM canister for sterility observation	
Annexure - III	Equal Splitting Test Report	

ENCLOSURES: SOP Training Record.

8.0 DISTRIBUTION:

- Controlled Copy No. 01 Quality Assurance
- Controlled Copy No. 01 Microbiology Laboratory
- Master Copy Quality Assurance

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9.0 REFERNCES:

Not Applicable

10.0 REVISION HISTORY:

Revision No.	Change control No.	Details of Change	Reason for Change	Effective Date	Updated By

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

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ANNEXURE – 1

STERILITY TEST REPORT BY CLOSED METHOD

Product Name		**A.R. No.**	
Batch No.		**Mfg. Date**	
Date of Sampling		**Expiry Date:**	
Date of Receiving		**Sampling Quantity**	
Date of Testing		**Tested By**	
Method Used	**Closed Method**	**Date of Release**	
Canister Details	**Lot No.**		
	Expiry Date:		

MEDIA CONTROL

Media Used	**Fluid Thioglycollate Medium(FTM)**	**Soybean Casein Digest Medium(SCM)**
Autoclave Media Ref. No.		
Incubating Temperature		
Incubator ID No.		

OBSERVATIONS

Date	**Fluid Thioglycollate Medium**		**Soybean Casein Digest Medium**		**Observed By**	**Checked By**
	Test Sample	**Negative Control**	**Test Sample**	**Negative Control**		

+ ve = Growth observed - ve = No Growth observed

Remarks: Rosazuring ring in FTM verified/ not verified and sterility test complies/does not comply as per specification.

Released By: Reviewed By:

Date: Date:

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Annexure – II

LABEL FOR SCM/FTM CANISTER FOR STERILITY OBSERVATION	
Product Name	
Batch No.	
Media Reference No.	
Date of Testing	
Date of Release	
Tested By	

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ANNEXURE – III

EQUAL SPLITTING TEST REPORT

Date of testing		**Tested By**	
Canister Detail	**Lot No.**		
	Expiry Date		

OBSERVIATIONS

Data at 40 speed unit of Steripump

Run No.	Volume in Canister 1 X	Volume in Canister 1 Y	Difference z=(X-Y)	% Difference (Z/X)*100	Pass/Fail
1st					
2nd					
3rd					

Data at 60 speed unit of Steripump

Run No.	Volume in Canister 1 X	Volume in Canister 1 Y	Difference z=(X-Y)	% Difference (Z/X)*100	Pass/Fail
1st					
2nd					
3rd					

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Data at 80 speed unit of Steripump

Run No.	Volume in Canister 1 X	Volume in Canister 1 Y	Difference z=(X-Y)	% Difference (Z/X)*100	Pass/Fail
1st					
2nd					
3rd					

Acceptance Criteria: If the volume of sterile purified water between two canisters remains within ± 10 % test is passed.

Remarks: Equal splitting test complies/does not comply as per acceptance criteria.

Tested By: **Checked By:** **Approved By:**

Sign & Date **Sign & Date** **Sign & Date**