STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sterility Test by Closed System	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

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 Lab of Quality Control Department.

3.0 RESPONSIBILITY:

Officer / Executive - Microbiology

4.0 ACCOUNTABILITY:

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 REQUIREMENTS:

6.1.1 Sterilized Fluid Thioglycollate Medium in 100 ml bottle.

6.1.2 Sterilized Soyabean 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 samples for sterility test, record the details of sample as per sop in sample receiving register e.g. Date of receipt, Product Name, Batch No., in **Annexure-I of SOP**, Titled **"Sample Receipt and Analysis Record for Sterility Test"**.

6.1.9 Collect all samples to be tested for the sterility test; sanitize the external surface of all samples subjected for sterility test by using Sporicidal 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**,** title Entry, Exit and Gowning Procedure for Sterility Area.

6.1.13 Check the temperature & Relative Humidity of Sterility Area as per SOP 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 0.22µ filtered70% IPA & Start the LAF as per SOP 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 Frequently 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 and Personnel monitoring shall be carried out after the completion of sterility test by contact plate method as per SOP. After personnel monitoring, sanitize the hand gloves and contact site of boiler suit with 0.22µ filtered70% 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**,** title Entry, Exit and Gowning Procedure for Sterility Area.

6.3.2 Sanitize the exterior parts of the Steritest Compact Instrument, LAF bench with 0.22µ 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 /dilutor from the sealed pack.

6.3.5 Place the canisters 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 canisters [50 ml each] by using peristaltic Steripump for pre-wetting purpose. Closed the canister vent by using the cap. Filter 100 ml of sterilized fluid with the help of Steripump as a pre-wetting step for the 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 the complete dissolution of the product.

6.4.3 Insert sample tube needle into the 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 canister 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 the Steripump.

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

6.4.9 After rinsing of the membrane, closed the lower aperture or outlet port of the canisters with the help of rubber closure provided 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 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 Steripump.

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 observations of sterility test in **Annexure-I**, Titled "**Sterility Test Report 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 the 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 canister 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 the Steripump.

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

6.5.8 After rinsing of the membrane, closed the lower aperture of the canisters with the help of rubber closure provided 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 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 Steripump.

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 observations of sterility test in **Annexure-I**, Titled **"Sterility Test Report 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 the sample with the help of the Steripump immediately without any hold up of the product in the canisters.

6.6.3 Remove the vent, insert sample tube needle to bottle containing sterilized fluid -A.

6.6.4 Transfer the sterilized fluid-A to the canisters of 100 ml to each canister 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 the Steripump.

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

6.6.7 After rinsing of the membrane, closed the lower aperture of the canisters with the help of rubber closure provided 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.

6.6.10 Transfer the sterilized Fluid Thioglycollate Medium to one canister with the help of Steripump at lower Steripump speed.

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

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

6.6.13 Transfer the sterilized Soybean Casein Digest Medium to the second canister with the help of Steripump.

6.6.14 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 observations of sterility test in **Annexure-I**, Titled **"Sterility Test Report 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 Soya bean Casein Digest Medium and Fluid Thioglycollate Medium.

6.7.2 Remove the canister /dilutor from the sealed pack.

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

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

6.7.5 Transfer 100 ml sterilized fluid-A to both the canisters [50 ml each] by using peristaltic Steripump.

6.7.6 After rinsing of the membrane, closed the lower aperture of the canisters with the help of rubber closure provided 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 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 Steripump.

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 antibiotics and the membrane should be less [Recommended Steripump speed: 60-80].

6.8.2 Rinsing of the membrane to be done at lower speed (Recommended Steripump 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 Steripump. 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.

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 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 examined.

6.9.4 The test may be considered invalid only if one or more 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 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 canisters over the drain tray. Open the clamp completely and introduce canisters tubing from left to right and close the clamp.

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

6.10.5 Remove the rubber plug from canister vent.

6.10.6 Switch on the steripump.

6.10.7 Invert the 100ml 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.

6.10.10 Remove the needle from the bottle and place it safely.

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

6.10.12 Apply rubber plugs on the vents of the canisters.

6.10.13 Start the pump so that water in the 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:

ANNEXURE No.	TITLE OF ANNEXURE	FORMAT No.
Annexure-I	Sterility test report by 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. 02 — Microbiology Laboratory
- Master Copy — Quality Assurance

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9.0 REFERENCES:

Not Applicable

10.0 REVISION HISTORY:

CHANGE HISTORY LOG

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

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

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ANNEXURE – I
STERILITY TEST REPORT BY CLOSED METHOD

Product Name		A. R. No.	
Batch No.		Mfg. Date	
Date of Sampling		Expiry Date	
Date of receiving		Sampled Quantity	
Date of Testing		Tested By	
Method Used	Closed method	Date of Release	
Canister Detail	Lot No.		
	Expiry Date		

MEDIA CONTROL

Medium used	Fluid Thioglycollate Medium (FTM)	Soyabean Casein Digest Medium(SCM)
Autoclave Media Ref. No.	FTM/	SCM/
Incubation Temperature	32.5 ± 2.5°C	22.5 ± 2.5°C
Incubator ID No.		

OBSERVATIONS

Date	Fluid Thioglycollate Medium		Soyabean Casein Digest Medium		Observed by	Checked by
	Test Sample	Negative Control	Test Sample	Negative Control		

+ve = Growth observed –ve = No Growth observed

Remarks: Rosazurine 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		

OBSERVATIONS

Data at 40 speed unit of Steripump

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

Data at 60 speed unit of Steripump

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

Data at 80 speed unit of Steripump

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

sop

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

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