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

1.0 OBJECTIVE:
To lay down the procedure for Sterility Test.

2.0 SCOPE:
This SOP is applicable for Sterility Test in Microbiology Laboratory of Quality Control Area.

3.0 RESPONSIBILITY:
Officer / Executive Microbiologist– QC

4.0 ACCOUNTABILITY:
Head – QC

5.0 PROCEDURE:

5.1 STERILITY TEST BY DIRECT INOCULATION METHOD:

5.1.1 After receiving the samples for sterility test, record the details of sample in sample receiving register e.g. Product Name, Batch No., GRN No., Batch Size, Mfg. date, Exp. Date etc. in **Annexure-I**, titled **"Sample Receiving Record for Sterility Test"**.

5.1.2 Collect all samples to be tested for the sterility test; wipe the surface of individual sample container by spraying 70 % filtered (0.2 µ) IPA. Collect all the samples in previously sanitized sample container and affix the label of details of sample e.g. product name, batch no. and lot no.

5.1.3 Transfer the desired samples container, pre incubated SCM & FTM Tubes, SCA Plates, contact plates, Air sampler and other required material to dynamic pass box of cooling zone.

5.1.4 Check the pressure differential of sterility room.

5.1.5 Enter in Sterility Room as per SOP title Entry, Exit and Gowning Procedure for Sterility Area.

5.1.6 Check the temperature & Relative Humidity of Sterility Room as per SOP and status of cleaning and fumigation of sterility room before start the sterility test. If cleaning and fogging has been done and temperature and humidity within limit perform the sterility test, if not, clean and fog the area. After fogging hold the area for at least 12 hours before performing the sterility test.

5.1.7 Mop the LAF platform with 70% filtered IPA & Start LAF as per SOP for the operation of LAF before 30 minutes prior the test.

5.1.8 After completion of sterilization cycle unload the sterilized articles from autoclave to cooling zone on SS trolley/SS Container and transfer from cooling zone to LAF of Sterility Room.

5.1.9 Before starting sterility test expose the SCA plates and Surface monitoring, Air sampling as per **SOP** at specified locations throughout the testing. Personnel monitoring must be carried out after

the completion of sterility test by finger dab and contact plates method as per **SOP**. After personnel monitoring sanitize hand gloves and contact site of boiler suit with 70% filtered (0.2 µ) IPA by removing it in Air lock –V.

5.1.10 Transfer the prescribed quantity of sample to be examined prescribed in Table 1 & Table 2 directly into the culture medium so that the volume of the product should not be more than 10% of the volume of the medium.

5.1.11 In case of oils preparation and oily solutions use media with 1% w/v of polysorbate 80 or other suitable emulsifying agent, in an appropriate concentration, shown not to have any antimicrobial properties under the conditions of test.

5.1.12 If product is solid transfer the quantity of a dry solid or prepare a suspension of the product by adding sterile diluent to the immediate container, corresponding to not less than the quantity indicated in Table 1 & Table 2. Transfer the material so obtained to 100 ml of FTM and mix. Similarly transfer the same quantity to 100 ml of SCM and mix.

5.1.13 In case of sterile devices, articles can be immersed intact or disassembled. To ensure that device pathways are also in contact with media, immerse the appropriate number of units per medium in a volume of medium sufficient to immerse the device completely.

5.1.14 Label the SCM / FTM tubes / container with following information:

Product Name _____
Batch No. _____
Media Reference No. _____
Date of Testing _____
Date of Release _____
Tested By _____

5.1.15 After completion of work transfer all inoculated media and other materials to dynamic pass box of incubation room and exit from sterility room as per SOP for Entry/Exit and Gowning Procedure for Sterility Room.

5.1.16 Collect the FTM, SCM tubes from dynamic pass box of incubation room and incubate FTM tubes at 32.5°C ± 2.5°C and SCM tubes at 22.5°C ± 2.5°C for 14 days. Incubate SCA and contact plates for environmental and personal monitoring at 22.5°C ± 2.5°C for 72 hrs and further 32.5°C ± 2.5°C for 48 hrs.

5.1.17 If material being examined renders the medium turbid so that the presence or absence of microbial growth can not be determined by visual examination, transfer aseptically 1ml portion of the medium to the another container / tube of 100 ml FTM, SCM medium after 14th day of incubation.

5.1.18 Continue incubation of transfer tubes for NLT 4 additional days after transfer and for a total of NLT 18 days.

5.1.19 Carry out the positive control by using any one suitable microorganism given in SOP for Growth Promotion Test. Positive control shall be perform in Assay Room.

5.1.20 Record the observations of sterility test in **Annexure-II**, titled **"Sterility Test Report by Direct Inoculation Method"**.

5.1.21 Results /Acceptance Criteria.

5.1.21.1 During the incubation period examine the medium for macroscopic evidence of microbial growth. If no evidence of growth is found, sample being examined complies with the test for sterility.

5.1.21.2 If the evidence of growth is found, sample being 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. The test may be considered invalid under the following conditions:

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

5.1.21.4 Review the testing procedure of sterility.

5.1.21.5 Microbial growth is found in the negative controls.

5.1.21.6 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

5.1.21.7 If the test is declared to be invalid, it shall be repeated with the same number of units as in the original test. If no evidence of microbial growth is found in the repeat test, the product complies with sterility test.

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

5.2 STERILITY TEST BY MEMBRANE FILTRATION METHOD:

5.2.1 After receiving the samples for sterility test, record the details of samples in sample receiving register e. g. Product Name, Batch No., GRN No., Batch Size, Mfg. date, Exp. Date etc. in **Annexure-I**, Titled **"Sample Receiving Record for Sterility Test"**.

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

5.2.2 The entire sample to be tested for the sterility test shall be collected and the outer surface of individual sample container will be wiped by spraying 70 % (0.2 µ) filtered IPA. All the samples shall be collected in the previously sanitized sample container and affix the label having details of sample e.g. Product name, batch no. and lot no.

5.2.3 Transfer the desired samples container, pre incubated SCM & FTM Tubes, SCA Plates, contact plates, Air sampler and other required material to dynamic pass box of cooling zone.

5.2.4 Check the pressure differential of sterility room.

5.2.5 Enter in Sterility Room as per SOP for Entry/ Exit and gowning procedure for sterility room.

5.2.6 Check the temperature & Relative Humidity of Sterility Room as per SOP and status of cleaning and fumigation of sterility room before start the sterility test. If cleaning and fogging has been done and temperature and humidity within limit perform the sterility test, if not, clean and fog the area. After fogging hold the area for at least 12 hours before performing the sterility test.

5.2.7 Mop the LAF platform with 70% filtered (0.2µ) IPA & Start LAF as per SOP for the operation of LAF before 30 minutes prior the test.

5.2.8 Load the autoclave with items (Filtration Manifolds, filtration holders, forceps, scissors, hand gloves, sterility garments) and run the cycle of autoclave as per current SOP. After completion of sterilization cycle unload the sterilized articles from autoclave to cooling zone on SS trolley/SS Container and transfer from cooling zone to LAF of Sterility Room

5.2.9 Also transfer the pre incubated SCA plates & contact plates, pre incubated FTM & SCM tubes, air sampler, rinsing fluids and other accessories and sample from cooling zone to LAF of Sterility Room

5.2.10 Before starting sterility test expose the SCA plates and Surface monitoring, Air sampling as per SOP at specified locations throughout the testing. Personnel monitoring must be carried out after the completion of sterility test by finger dab and contact plates method as per SOP. After personnel monitoring sanitize hand gloves and contact site of boiler suit with 70% filtered (0.2 µ) IPA by removing it in Air lock –V.

5.2.11 Assemble the filtration unit and connect the manifold assembly with reservoir properly with silicon rubber tubing and reservoir to vacuum pump with silicon rubber tubing. Filtration flask can also be use. Start the flame and aseptically put the membrane filter (pour size not more than 0.45 µm and diameter 47 mm) between the filtration cup and receptacle.

5.2.12 Select the membrane filter based on the characteristics of the product if the product having antimicrobial activity use cellulose nitrate with hydrophobic edge membrane, for aqueous, oil and weakly alcohol use cellulose nitrate membrane and for strong alcoholic solutions use cellulose acetate membrane.

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

5.2.13 Prior the test wet the membrane filter by adding 15-20 ml of fluid A (0.1 % peptone water) and filters the fluid by employing vacuum. Take the quantity of the container as per Table no. 1. Aseptically cut the tip of bottle/vial and ampoule with sterile SS cutter/scissor in front of gas burner and immediately transfer the required quantity of the sample (as per Table no. 2) to filtration flask, immediately filter the solution with the help of vacuum and wash the membrane filter with 3 X 100 (Number of washing and fluid as per validation study) ml fluid A or fluid D.

5.2.14 In case of dry powder injection, dissolved the prescribed quantity according to the table 1 & 2 of sample into the sterile 100 ml of fluid A or fluid D or 0.9% normal saline as per sample. Wet the membrane filter with approx. 15-20 ml of fluid A and filter the solution with the help of vacuum and wash the membrane filter with 3 X 100 ml (Number of washing and fluid as per validation study) fluid A or fluid D.

5.2.15 For raw material prepared the pooled sample from the total number of container and performed the sterility test, dissolved the 6 gm of sample into the sterile 100 ml of fluid A or fluid D or 0.9% normal saline as per sample. Wet the membrane filter with approx. 15-20 ml of fluid A and filter the solution with the help of vacuum and wash the membrane filter with 3 X 100 ml (Number of washing and fluid as per validation study) fluid A or fluid D.

5.2.16 For rubber bung/flip off seal/glass vials/primary packing materials of three pieces /ffs take 20 nos. and transfer aseptically in to the conical flask containing 100 ml of fluid A or 0.9% normal saline solution, rinse the sample after rinsing aseptically filter the sample with sterile filtration assembly with the help of vacuum and wash the membrane filter with 100ml fluid A or fluid D.

5.2.17 After completion of filtration, switch OFF the vacuum and lift the SS cup carefully. Aseptically cut the membrane filter into two halves with sterile SS forceps and scissors and transfer one half to SCM tube and other one half to FTM tube with Sterile Forceps. Label the SCM / FTM tubes / container with following information:

Product Name _____
Batch No. _____
Media Reference No. _____
Date of Testing _____
Date of Release _____
Tested By _____

5.2.18 Frequently sanitize the hand and LAF working station with 70% (0.2 µ) filtered IPA during sterility test.

5.2.19 Similarly prepare negative control by filtering 100 ml of sterile fluid A by using another filtration assembly. Aseptically cut the membrane filter into two halves with sterile SS forceps and scissor and transfer one half to SCM tube and other one half to FTM tube with sterile forceps. These

tubes will serve as negative control so that label the tubes as "negative control" along with date of test and date of release.

5.2.20 Carry out the positive control by inoculating 100 ml of (fluid A or D) with not more than 100 cfu of any one suitable microorganism given in SOP for Growth Promotion Test and wash the membrane filter and after completion of washing switch OFF the vacuum pump and lift the membrane filter with sterilized forceps and aseptically transfer into FTM tube. Repeat the same procedure to SCM tube, label these tube as "positive control". Positive control shall be perform in Microbial Assay Room.

5.2.21 Label the SCM / FTM tubes / container with following information:

Product Name	_____
Batch No.	_____
Media Reference No.	_____
Date of Testing	_____
Date of Release	_____
Tested By	_____

5.2.22 After completion of work transfer all inoculated media and other materials to dynamic pass box of incubation room and exit from sterility room as per SOP for Entry/ exit and Gowning Procedure for Sterility Room.

5.2.23 Collect the FTM, SCM tubes from dynamic pass box of incubation Room and incubate FTM tubes at 32.5°C ± 2.5°C and SCM tubes at 22.5°C ± 2.5°C for 14 days. Incubate SCA and contact plates for environmental and personal monitoring at 22.5°C ± 2.5°C for 72 hrs and further 32.5°C ± 2.5°C for 48 hrs.

5.2.24 Daily visual observation of all tested sample tubes including both negative control of FTM, SCM for Positive control incubate the FTM Tube for 3 days & SCM Tube for 5 days.

5.2.25 Record the observations of sterility test in **Annexure-III**, Titled **"Sterility Test Report by Membrane Filtration Method"**.

5.2.26 Results /Acceptance Criteria

5.2.26.1 During the incubation period examine the medium for macroscopic evidence of microbial growth. If no evidence of growth is found, sample being examined complies with the test for sterility.

5.2.26.2 If the evidence of growth is found, sample being 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. The test may be considered invalid under the following conditions:

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

5.2.26.2.2 Review the testing procedure of sterility.

5.2.26.2.3 Microbial growth is found in the negative controls.

5.2.26.3 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

5.2.26.4 If the test is declared to be invalid, it shall be repeated with the same number of units as in the original test. If no evidence of microbial growth is found in the repeat test, the product complies with sterility test.

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

Table-1

Minimum Quantity to Be Used for Each Culture Medium

Quantity in each container of Injectables preparation	Minimum quantity to be used for each culture medium
For Liquids	
Less than 1 ml	Total contents of a container
1 ml to 40 ml	Half the contents of a container but not less than 1 ml
40 ml to 100 ml	20 ml
Greater than 100 ml	10% of the contents of a container but not less than 20 ml
Antibiotic liquids	1 ml
Insoluble preparations, creams and ointments to be suspended or emulsified	Use the content of each container to provide not less than 200 mg
For Solids	
Less than 50 mg	Total contents of a container
50 mg or more but less than 300 mg	Half the contents of a container but not less than 50 mg
300 mg to 5 g	150 mg
Greater than 5 g	500 mg
Other medical devices	The whole device, cut in to pieces or disassembled

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

Table-2
Minimum Number of Articles to Be Used for Each Culture Medium

Number of item in the Batch	Minimum number of articles to be used for each culture medium
Parenteral preparation	
Not more than 100 containers	10 % or 4 containers, whichever is the greater
More than 100 but not more than 500 containers	10 containers
More than 500 containers	2 % or 20 containers, whichever is less
For large volume Parenteral	2 % or 10 containers, whichever is less
Antibiotic solids	
Pharmacy bulk packages(<5g)	20 containers
Pharmacy bulk packages(≥5g)	6 containers
Bulks and Blends	6 g
Ophthalmic and other non Injectables preparation	
Not more than 200 containers	5 % or 2 containers, whichever is the greater
More than 200 containers	10 containers
Devices	
Catgut and other surgical sutures for veterinary use	2 % or 5packages, whichever is the greater, up to a maximum total of 20 packages
Not more than 100 articles	10 % or 4 articles, whichever is the greater
More than 100 but not more than 500 articles	10 articles
Bulk solid products	
Up to 4 containers	Each container
More than 4 but not more than 50 containers	20 % or 4 containers, whichever is the greater
More than 50 containers	2 % or 10 containers, whichever is the greater

6.0 REFERENCES:

- United State Pharmacopoeia-38 <71> Sterility Test
- Indian Pharmacopoeia 2014
- British Pharmacopoeia 2015
- PIC/S

7.0 ANNEXURES:

ANNEXURE No.	TITLE OF ANNEXURE	FORMAT No.
Annexure – I	Sample Receiving Record For Sterility Test	
Annexure – II	Sterility Test Report by Direct Inoculation Method	
Annexure - III	Sterility Test Report by Membrane Filtration Method	

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

ENCLOSURES: SOP Training Record

8.0 DISTRIBUTION:

- Controlled Copy No. 01 — Quality Assurance Department
- Controlled Copy No. 02 — Quality Control Department
- Master Copy — Quality Assurance Department

9.0 ABBREVIATIONS:

CQA	Corporate Quality Assurance
CFU	Colony Forming Unit
Exp.	Expiry
FTM	Fluid Thioglycollate Medium
Ltd.	Limited
LAF	Laminar Air Flow
Mfg.	Manufacturing
No.	Number
QA	Quality Assurance
QC	Quality Control
SOP	Standard Operating Procedure
SCM	Soyabean Casein Digest Medium

10.0 REVISION HISTORY:

CHANGE HISTORY LOG

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

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

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

ANNEXURE – I
SAMPLE RECEIPT AND ANALYSIS RECORD FOR STERILITY TEST

Product/ Material Name	Batch No.	Lot No.	GRN No.	Batch Size	Mfg. Date	Exp. Date	Sample Quantity	Analytical Report No	Sampling Date	Analysis Date & Sign	Release Date & sign	Checked By (Sign & Date)	Remarks

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

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

ANNEXURE – II

STERILITY TEST REPORT BY DIRECT INOCULATION METHOD

Product Name		A. R. No.	
Batch No.		Mfg. Date	
Lot No.		Expiry Date	
Batch Size		Sampled Quantity	
Date of Sampling		Tested By	
Date of Testing			
Method Used	Direct Inoculation	Date of Release	

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.	PC/INS/S-39/	PC/INS/S-39/
GPT Report No.	GPT/FTM/	GPT/SCM/

OBSERVATIONS:

Date	Fluid Thioglycollate Medium			Soyabean Casein Digest Medium			Observed by	Reviewed by	Remarks
	Test Sample	Negative Control	Positive Control	Test Sample	Negative Control	Positive Control			

+ve = Growth observed –ve = No Growth observed

Remarks: The Sterility test complies / does not comply as per specification.

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

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

Product Name		A. R. No.	
Medium used	Fluid Thioglycollate Medium (FTM)	Soyabean Casein Digest medium (SCM)	
Autoclaved Media Ref. No.	FTM/	SCM/	
Incubation Temperature	32.5 ± 2.5°C	22.5 ± 2.5°C	
Incubator ID No.			
GPT Report No.	GPT/FTM/	GPT/SCM/	
Autoclaved Media Ref. No. after 14th day incubation	FTM/	SCM/	

OBSERVATIONS AFTER 14TH DAY INCUBATION

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

+ve = Growth observed –ve = No Growth observed

Remarks: The Sterility test complies / does not comply as per specification.

Microbiologist: **Checked By:** **Approved By:**

Date: **Date:** **Date:**

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

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

ANNEXURE – III

STERILITY TEST REPORT BY MEMBRANE FILTRATION METHOD

Product Name		A. R. No.	
Batch No.		Mfg. Date	
Lot No.		Expiry Date	
Batch Size		Sampled Quantity	
Date of Sampling		Tested By	
Date of Testing			
Method Used	Membrane Filtration	Date of Release	
Membrane Filter Details	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.		
GPT Report No.	GPT/FTM/	GPT/SCM/

OBSERVATIONS

Date	Fluid Thioglycollate Medium			Soyabean Casein Digest Medium			Observed by	Reviewed by	Remarks
	Test Sample	Negative Control	Positive Control	Test Sample	Negative Control	Positive Control			

+ve = Growth observed –ve = No Growth observed

Remarks: The Sterility test complies / does not comply as per specification

Microbiologist: **Checked By:** **Approved By:**

Date: **Date:** **Date:**