PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Sterility Testing	**Effective Date:**
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1.0 OBJECTIVE

1.1 To lay down the Procedure for sterility testing.

2.0 SCOPE

2.1 This procedure is applicable for sterility test of finished product, raw material, in-process products and sterilized primary packaging material in Microbiology Laboratory.

3.0 RESPONSIBILITY

3.1 Microbiologist is responsible for performing the sterility test, daily observation and final releasing of results.

4.0 ACCOUNTABILITY

4.1 Head Microbiology

5.0 EHS CONSIDERATIONS

5.1 Handle the microbial cultures very carefully with gloved hands and nose mask as infection may occur.

5.2 Do not disturb the laminarity of LAF while performing the operation.

5.3 Do not inhale the culture.

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6.0 PROCEDURE

6.1 Media Preparation:

6.1.1 Culture media and other fluids should be prepared as per SOP of culture media preparation SOP.

6.2 Number of articles to be tested:

6.2.1 Sterile bulk API: Minimum 6 gram of composite sample of the batch.

6.2.2 Dry powder and Lyophilized vials: Minimum 20 vials per batch.

6.2.3 Sterilized rubber stopper: Minimum 10 numbers per batch.

6.2.4 Sterilized empty vials: Minimum 10 vials per batch.

6.2.5 Sterilized rubber gloves: 01 pair from one lot.

6.2.6 In-process samples: as per sampling plan.

6.3 Method to be used: Membrane filtration by **o**pen method of sterility testing and direct inoculation method.

6.4 Procedure for Sterile bulk API:

6.4.1 Arrange the sample vial containing approximately 6 gm of sample to be tested for sterility test.

6.4.2 Arrange the media tubes and mark with the name of sample, date of testing and date of release with marker pen.

6.4.3 Sanitize the surface of the sample vial using 0.2µm filtered 70% IPA and keep it in the pass-box in MLT room to autoclave unloading area of sterility area.

6.4.4 Enter the sterility testing area as per SOP of Entry and exit of sterility area as per SOP.

6.4.5 Switch on the LAF at least 20 minutes before performing the test.

6.4.6 Expose the culture media plates in the respective locations and perform the active air sampling after sterility testing (if sterility testing performed).

6.4.7 Wipe the sterility room LAF platform with mop soaked in 0.2µm filtered 70% IPA solution.

6.4.8 Clean the exterior of sample vial with 0.2µm filtered 70% IPA solution and transfer to LAF platform.

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6.4.9 Arrange the material on the LAF platform and ignite the flame.

6.4.10 Now take the sample vial (containing 6 gm of the pooled sample) to be tested, open with vial opener near the flame and transfer the sample into conical flask containing 200 ml sterilized peptone water aseptically.

6.4.11 Dissolve the whole content in peptone water till a clear solution will be obtained.

6.4.12 Now assemble the filtration cup on to the sterilized manifold aseptically.

6.4.13 Connect the manifold to oil free vacuum pump using a sterilized silicon tube and switch on the vacuum pump.

6.4.14 Take an individually packed pre-sterilized 0.45 µm pore size and 47 mm diameter filter membrane. Open and place on the sieve in the filtration cup aseptically.

6.4.15 Now aseptically transfer 100 ml peptone water to the filtration cup of assembled filtration unit for wetting the filtration membrane.

6.4.16 Turn on the valve present in the manifold to start the filtration.

6.4.17 After completion of pre-wetting of membrane filter the sample solution by transferring the sample solution to filtration cup.

6.4.18 After completion of sample filtration post wash the membrane with 3 x 100 ml sterile Fluid A.

6.4.19 Turn off the valve present in the manifold and remove the cup.

6.4.20 Now take sterilized scissors and forceps, heat in flame, cool and cut the membrane in two equal halves aseptically.

6.4.21 Now aseptically transfer one half to SCDM media tube and another half to FTM media tube near the flame.

6.4.22 Incubate one un-inoculated tube of each media along with the test samples as another negative control.

6.4.23 Performed personnel monitoring, after completion of work in sterility testing room.

6.4.24 Switch off the flame and vacuum pump.

6.4.25 Transfer the sterility culture media tubes and petri-plates through the pass box from autoclave unloading room to MLT room.

6.4.26 Transfer the used glassware and other un-cleaned material to pass box in sterile corridor for further transfer to cleaning room.

6.4.27 Wipe the LAF platform with 0.2µm filtered 70% IPA and come out from the sterility area.

6.4.28 Perform positive control by filtering 1 ml of aliquot from dilution giving count of 10-100 cfu/ml of recommended organism (Refer Table 01) and rinsing with 100 ml sterile peptone water in LAF in MLT room. Perform one organism for one media at least.

6.4.29 Incubate SCDM media tube containing membrane at 22.5±2.5°C for 14 days. Incubate FTM media tube containing membrane at 32.5±2.5°C for 14 days.

6.4.30 Observe the tubes for turbidity / growth after every 24 hours.

6.5 Procedure for Dry powder and Lyophilized vials:

6.5.1 Arrange the sample vials containing to be tested for sterility test.

6.5.2 Arrange the media tubes and mark with the name of sample, date of testing and date of release with marker pen.

6.5.3 Sanitize the surface of the sample vials using 0.2µm filtered 70% IPA and keep it in the pass-box in MLT room to autoclave unloading area of sterility area.

6.5.4 Enter the sterility testing area as per SOP of Entry and exit of sterility area as per SOP.

6.5.5 Switch on the LAF at least 20 minutes before performing the test.

6.5.6 Expose the culture media plates in the respective locations and perform the active air sampling after sterility testing (if sterility testing performed).

6.5.7 Wipe the sterility room LAF platform with 0.2µm filtered 70% IPA.

6.5.8 Clean the exterior of sample vials with 0.2µm filtered 70% IPA solution and transfer to LAF platform.

6.5.9 Arrange the material on the LAF platform and ignite the flame.

6.5.10 Reconstitute 20 vials with sterile 0.1% peptone with the help of a sterile disposable syringe by adding 10 ml sterile 0.1% peptone in each vial. Shake and mix well.

6.5.11 Open vials using a de-crimper and collect the reconstituted content in a sterile conical flask.

6.5.12 Now assemble the filtration cup on to the sterilized manifold aseptically.

6.5.13 Connect the manifold to oil free vacuum pump using a sterilized silicon tube and switch on the vacuum pump.

6.5.14 Take an individually packed pre-sterilized 0.45 µm pore size and 47 mm diameter filter membrane. Open and place on the sieve in the filtration cup aseptically.

6.5.15 Now aseptically transfer 100 ml peptone water to the filtration cup of assembled filtration unit for wetting the filtration membrane.

6.5.16 Turn on the valve present in the manifold to start the filtration.

6.5.17 After completion of pre-wetting of membrane filter the sample solution by transferring the sample solution to filtration cup.

6.5.18 After completion of sample filtration post wash the membrane with 3 X 100 ml sterile Fluid A.

6.5.19 Turn off the valve present in the manifold and remove the cup.

6.5.20 Now take sterilized scissors and forceps, heat in flame, cool and cut the membrane in two equal halves aseptically.

6.5.21 Now aseptically transfer one half to SCDM media tube and another half to FTM media tube near the flame.

6.5.22 Incubate one un-inoculated tube of each media along with the test samples as another negative control.

6.5.23 Performed personnel monitoring, after completion of work in sterility testing room.

6.5.24 Switch off the flame and vacuum pump.

6.5.25 Transfer the sterility culture media tubes and petriplates through the pass box from autoclave unloading room to MLT room.

6.5.26 Transfer the used glassware and other un-cleaned material to pass box in sterile corridor for further transfer to cleaning room.

6.5.27 Wipe the LAF platform with 0.2µm filtered 70% IPA and come out from the sterility area.

6.5.28 Perform positive control by filtering 1 ml of aliquot from dilution giving count of 10-100 cfu/ml of recommended organism (Refer Table 01) and rinsing with 100 ml sterile peptone water in LAF in MLT room. Perform one organism for one media at least.

6.5.29 Incubate SCDM media tube containing membrane at 22.5±2.5°C for 14 days. Incubate FTM media tube containing membrane at 32.5±2.5°C for 14 days.

6.5.30 Observe the tubes for turbidity / growth after every 24 hours.

6.6 Procedure for sterilized empty vials:

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6.6.1 Arrange the sterilized bottle containing sterilized sampled empty glass vials to be tested for sterility test.

6.6.2 Arrange the media tubes and mark with the name of sample, date of testing and date of release with marker pen.

6.6.3 Sanitize the surface of the sterilized bottle using 0.2µm filtered 70% IPA and keep it in the pass-box in MLT room to autoclave unloading area of sterility area.

6.6.4 Enter the sterility testing area as per SOP of Entry and exit of sterility area as per SOP.

6.6.5 Switch on the LAF at least 20 minutes before performing the test.

6.6.6 Expose the culture media plates in the respective locations and perform the active air sampling after sterility testing (if sterility testing performed).

6.6.7 Wipe the sterility room LAF platform with 0.2µm filtered 70% IPA.

6.6.8 Arrange the material on the LAF platform and ignite the flame.

6.6.9 Open the bottle containing the empty sterilized vials and with the help of a sterilized forceps, transfer 5 number samples to SCDM media tube and another 5 number samples to FTM media tube near the flame.

6.6.10 Incubate one un-inoculated tube of each media along with the test samples as another negative control.

6.6.11 Incubate one un-inoculated tube of each media along with the test samples as another negative control.

6.6.12 Performed personnel monitoring, after completion of work in the sterility room.

6.6.13 Switch off the flame.

6.6.14 Transfer the sterility culture media tubes and petriplates through the pass box from autoclave unloading room to MLT room.

6.6.15 Transfer the used glassware and other un-cleaned material to pass box in sterile corridor for further transfer to washing room.

6.6.16 Wipe the LAF platform with 0.2µm filtered 70% IPA and come out from the sterility area.

6.6.17 Perform positive control by filtering 1 ml of aliquot from dilution giving count of 10-100 cfu/ml of recommended organism (Refer Table 01) and rinsing with 100 ml sterile peptone water in LAF in MLT room. Perform one organism for one media at least.

6.6.18 Incubate SCDM media tube containing membrane at 22.5±2.5°C for 14 days. Incubate FTM media tube containing membrane at 32.5±2.5°C for 14 days.

6.6.19 Observe the tubes for turbidity / growth after every 24 hours.

6.7 Procedure for sterilized rubber bungs:

6.7.1 Arrange the sterilized bottle containing sterilized sampled sterilized rubber bungs to be tested for sterility test.

6.7.2 Arrange the media tubes and mark with the name of sample, date of testing and date of release with marker pen.

6.7.3 Sanitize the surface of the sterilized bottle using 0.2µm filtered 70% IPA and keep it in the pass-box in MLT room to autoclave unloading area of sterility area.

6.7.4 Enter the sterility testing area as per SOP of Entry and exit of sterility area as per SOP.

6.7.5 Switch on the LAF at least 20 minutes before performing the test.

6.7.6 Expose the culture media plates in the respective locations and perform the active air sampling after sterility testing (if sterility testing performed).

6.7.7 Wipe the sterility room LAF platform with 0.2µm filtered 70% IPA.

6.7.8 Arrange the material on the LAF platform and ignite the flame.

6.7.9 Open the bottle containing sterilized rubber bungs and with the help of a sterilized forceps, transfer 5 number samples to SCDM media tube and another 5 number samples to FTM media tube near the flame.

6.7.10 Incubate one un-inoculated tube of each media along with the test samples as another negative control.

6.7.11 Incubate one un-inoculated tube of each media along with the test samples as another negative control.

6.7.12 Performed personnel monitoring, after completion of work in the sterility room.

6.7.13 Switch off the flame.

6.7.14 Transfer the sterility culture media tubes and petriplates through the pass box from autoclave unloading room to MLT room.

6.7.15 Transfer the used glassware and other un-cleaned material to pass box in sterile corridor for further transfer to washing room.

6.7.16 Wipe the LAF platform with 0.2µm filtered 70% IPA and come out from the sterility area.

6.7.17 Perform positive control by filtering 1 ml of aliquot from dilution giving count of 10-100 cfu/ml of recommended organism (Refer Table 01) and rinsing with 100 ml sterile peptone water in LAF in MLT room. Perform one organism for one media at least.

6.7.18 Incubate SCDM media tube containing membrane at 22.5±2.5°C for 14 days. Incubate FTM media tube containing membrane at 32.5±2.5°C for 14 days.

6.7.19 Observe the tubes for turbidity / growth after every 24 hours.

6.8 Procedure for sterilized rubber gloves:

6.8.1 Arrange the sterilized rubber gloves packet to be tested for sterility test.

6.8.2 Arrange the media tubes and mark with the name of sample, date of testing and date of release with marker pen.

6.8.3 Sanitize the surface of the packet using 0.2µm filtered 70% IPA and keep it in the pass-box in MLT room to autoclave unloading area of sterility area.

6.8.4 Enter the sterility testing area as per SOP of Entry and exit of sterility area as per SOP.

6.8.5 Switch on the LAF at least 20 minutes before performing the test.

6.8.6 Expose the culture media plates in the respective locations and perform the active air sampling after sterility testing (if sterility testing performed).

6.8.7 Wipe the sterility room LAF platform with 0.2µm filtered 70% IPA.

6.8.8 Arrange the material on the LAF platform and ignite the flame.

6.8.9 Open the packet containing sterilized rubber gloves.

6.8.10 With the help of a sterilized forceps, transfer one piece (left hand fully) of rubber glove to SCDM media tube and another piece (right hand fully) to FTM media tube near the flame.

6.8.11 Incubate one un-inoculated tube of each media along with the test samples as another negative control.

6.8.12 Incubate one un-inoculated tube of each media along with the test samples as another negative control.

6.8.13 Performed personnel monitoring after completion of work in the sterility room.

6.8.14 Switch off the flame.

6.8.15 Transfer the sterility culture media tubes and petriplates through the pass box from autoclave unloading room to MLT room.

6.8.16 Transfer the used glassware and other un-cleaned material to pass box in sterile corridor for further transfer to washing room.

6.8.17 Wipe the LAF platform with 0.2µm filtered 70% IPA and come out from the sterility area.

6.8.18 Perform positive control by filtering 1 ml of aliquot from dilution giving count of 10-100 cfu/ml of recommended organism (Refer Table 01) and rinsing with 100 ml sterile peptone water in LAF in MLT room. Perform one organism for one media at least.

6.8.19 Incubate SCDM media tube containing membrane at 22.5±2.5°C for 14 days. Incubate FTM media tube containing membrane at 32.5±2.5°C for 14 days.

6.8.20 Observe the tubes for turbidity / growth after every 24 hours.

6.9 Interpretation:

6.9.1 If no growth is observed in sample and negative control tubes within 14 days of incubation samples passes the test for sterility.

6.9.2 Positive control tubes of bacterial culture should exhibit growth within 3 days and that of yeast and mould within 5 days of incubation.

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

Table 01

Organism used for Positive Control Test			
S.No.	**Test Organism**	**Suitable Medium**	**Incubation Temperature**
1.	*Pseudomonas aeruginosa*	FTM	32.5 ±2.5°C
2.	*Staphylococcus aureus*	FTM	32.5 ±2.5°C
3.	*Clostridium sporogenes*	FTM	32.5 ±2.5°C
4.	*Candida albicans*	SCDM	22.5 ±2.5°C
5.	*Aspergillus niger*	SCDM	22.5 ±2.5°C
6.	*Bacillus subtilis*	SCDM	22.5 ±2.5°C

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7.0 DEFINITIONS AND ABBREVIATIONS

7.1 SCDM - Soybean Casein Digest Medium

7.2 FTM - Fluid Thioglycollate Medium

7.3 ml - Milliliter

7.4 gm - Gram

7.5 MLT - Microbial Limit Test

7.6 LAF - Laminar Air Flow

7.7 IPA - Iso Propyl Alcohol

8.0 REFERENCE

8.1 USP monograph <71> Sterility Testing

9.0 ANNEXURES

9.1 Annexure I : Sterility testing log book

9.2 Annexure II : Sterility test report

10.0 DISTRIBUTION DETAILS

10.1 Controlled copy of this SOP shall be distributed to Quality Assurance and Microbiology.

11.0 REVISION HISTORY

Supersedes SOP No.	Change Control No.	Reason for revision

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Annexure I

Sterility Testing Log Book

S.No.	Date of Sterility Test	Sample Analyzed	Batch No.	Date of Release	Done By	Reviewed By	Remarks

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Annexure II
Sterility Test Report

Date:		Amount of Sample taken:	
Name of Product:		Batch No. :	
Filter lot no:		LAF ID:	
Method: Membrane Filtration			
Incubation started on:		Incubation completed on :	
Media used	Fluid Thioglycollate Medium	Soyabean Casein Digest Medium	Peptone Water
Media Number	FTM/	SCDM/	PW/
Incubator ID			
Incubation Temperature	30 to 35^{0}C	20 to 25^{0}C	NA
Incubation Period	14 Days	14 Days	NA

S. No.	Sample name	Media	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
		FTM														
		SCDM														
		FTM														
		SCDM														
		FTM														
		SCDM														
		FTM														
		SCDM														
		FTM														
		SCDM														
		FTM														
		SCDM														
		FTM														
		SCDM														
Blank		FTM														
		SCDM														
Positive control		FTM														
		SCDM														
Observed by																
Checked by																

NG=No Growth, G=Growth

Remarks: The sample complies / does not comply with the test for sterility as per specifications.

Analyzed By: Reviewed By:

Date: Date: