VALIDATION PROTOCOL

FOR

STERILITY METHOD VALIDATION

Sterility Method Validation

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PREPARED BY:

NAME	DESIGNATION	SIGNATURE	DATE

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1.0 PROTOCOL APPROVAL

This document is prepared by the Microbiology Department for Sterility Method Validation. Hence this document before being effective shall be approved by the QC Head & QA Head. If any modification shall be desired it design as addendum and approved before execution.

PREPARED BY:

NAME	DESIGNATION	SIGNATURE	DATE

REVIEWED BY:

NAME	DESIGNATION	SIGNATURE	DATE

APPROVED BY:

NAME	DESIGNATION	SIGNATURE	DATE

Sterility Method Validation

2.0 OBJECTIVE

This protocol is designed to validate that any Bacteriostasis and Fungistasis activity inherent in the products; to be tested does not adversely affect the reliability of the test and the test procedure to be followed is suitable for testing the products as per the pharmacopoeial methods.

3.0 SCOPE

The scope of this protocol shall be limited to validate sterility method validation for all finished products and Sterile powder for injection.

4.0 RESPONSIBILITY

Responsibility of different department/personnel involved in different activities related to the valuation study.

Functions	Responsibility
Microbiology	Preparation of protocol & report
Microbiology	Review of the protocol & Execution
Quality assurance	Review & Approval of protocol & report
Quality assurance	Approval of the executed protocol and report

5.0 REFERENCE DOCUMENT

Following documents are referred during preparation of the protocol:

Document Name	Document Number
USP monograph Sterility Testing	Chapter No. 71
General Test Procedure (Sterility testing)	…….

6.0 METHODOLOGY

6.1 Pre-Requisites

6.1.1 Culture Organisms to be used -

a) *Staphylococcus aureus* ATCC 6538
b) Pseudomonas aeruginosa ATCC 9027
c) Clostridium sporogenes ATCC 11437
d) Bacillus spizizenii ATCC 6633
e) Candida albicans ATCC 10231
f) Aspergillus brasiliensis ATCC 16404
g) Environmental isolate - I
h) Environmental isolate - II

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6.1.2 **Preparation of 10 - 100 CFU / 0.1 ml -** Prepare the 10 - 100 CFU / 0.1ml suspension of culture organisms as per the SOP (Working culture dilution preparation).

6.1.3 **Media and Diluting/Rinsing fluid to be used -**
Fluid Thioglycollate Medium.
Soyabean Casein Digest Medium.
0.1% Peptone water / Water for injection.

6.1.4 **Preparation of Media and Diluting/Rinsing fluid -** Prepare and sterilize the media and Diluting/Rinsing fluid as per the SOP (Culture Media Preparation and Sterilization).

6.1.5 In-Case of antibiotic product we have to use β-lactamase enzyme in SCDM and FTM medium.

6.1.6 **Accessories, Material & Equipments to be used -**
Sterilized forceps, scissors, filtration funnel, conical flask, silicon tube, mopper & manifold and BOD incubator, LAF.

6.1.7 **Sample**
Perform the Sterility method validation for three batches of the each product.

6.2 **System/ Process description:**
The validation will be performed under the following subheadings:

- Bacteriostasis and Fungistasis Test (PPC)
- Sterility testing of product (NPC)
- Negative Control Test (NC)
- Positive Control Test (PC)
- Negative Control of Media

6.3 **Bacteriostasis and Fungistasis Test (Positive Product Control):**

6.3.1 Take required number of product containers for testing as per respective GTP.

6.3.2 Perform the sterility test of the product as per respective GTP (Current version of the GTP to be used).

6.3.3 Pre-wet the membrane With 100 ml 0.1 % peptone water to facilitate the filtration.

6.3.4 Take the sample container (in case of sterile bulk API containing 6 gm of the pooled sample) to be tested, open with container opener and transfer the sample into conical flask containing 100 ml sterilized 0.1 % peptone water or suitable diluents aseptically.

6.3.5 In-case of dry powder and lyophilized vials; reconstitute 20 vials with sterile 0.1% peptone; shake and mix well.

6.3.6 Dissolve the whole content in peptone water or suitable diluents till a clear solution will be obtained.

6.3.7 Then Filter the product.

6.3.8 Wash the membrane with 2 x 100 ml of 0.1% Peptone water.

6.3.9 Rinse the 3rd 100 ml through membrane filter with 0.1% peptone water which is previously inoculated with 10 - 100 CFU / 0.1ml of the culture organisms listed below in table - I.

6.3.10 Inoculate the membrane into FTM or SCDM tube.

6.3.11 Repeat the above exercise with all other culture organisms and incubate the tube at specified temperature and time as shown in table - I.

6.4 **Sterility testing of product (Negative Product Control):**

Take required number of product containers for testing.

Perform the sterility test of the product as per respective GTP (Current version of the GTP to be used) and incubate the tubes at specified temperature and time as shown in table - I.

6.5 **Negative Control Test:**

Filter 3 x 100 ml portions of 0.1% peptone water through membrane filter and transfer it to Fluid Thioglycollate Medium and Soyabean Casein Digest Medium respectively.

Incubate the tubes at specified temperature and time, which act as a negative control as shown in table - I.

6.6 **Positive Control Test:**

Filter 2 x 100 ml portions of 0.1% peptone water through membrane filter and finally filter with 100 ml of 0.1% peptone water which is previously inoculated with 10-100 CFU / 0.1ml of the culture organisms listed below in table - I and transfer in Fluid Thioglycollate Medium and Soyabean Casein Digest Medium.

Repeat the above exercise with all other culture organisms and incubate the tubes at specified temperature and time as shown in table - I.

6.7 **Negative Control of Media:**

After sterilization incubate one Fluid Thioglycollate Medium and one Soyabean Casein Digest Medium as a negative control at specified temperature and time as shown in table - I.

6.8 **Post GPT:** After completion of sterility testing of product (Negative product control); inoculate (10-100 cfu) of organism in 100 ml of Fluid thioglycollate Medium with *Staphylococcus aureus* ATCC 6538 and incubate at 30-35°C for not more than 3 days. Inoculate (10-100 cfu) of organism in 100 ml of Soybean Casein Digest Medium with *Candida albicans* ATCC 10231and incubate at 20-25°C for not more than 3 days.

Table - I

Incubation Temperature and Time with respect to Media and Culture Organisms

S.No.	Medium	Culture Organisms	Incubation Temperature	Incubation Time
1.	**Bacteriostasis and Fungistasis Test (PPC) and Positive Control Test(PC)**			
a.	Fluid Thioglycollate	*Staphylococcus aureus* ATCC 6538	30°C - 35°C	48-72 Hrs.
		Pseudomonas aeruginosa ATCC 9027		48-72 Hrs.

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S.No.	Medium	Culture Organisms	Incubation Temperature	Incubation Time
	Medium (FTM)	*Clostridium sporogenes* ATCC 11437		48-72 Hrs.
		Environmental isolate - I		48-72 Hrs.
		Environmental isolate - II		48-72 Hrs.
b.	Soyabean Casein Digest Medium (SCDM)	*Bacillus subtilis* ATCC 6633	20°C - 25°C	48-72 Hrs.
		Candida albicans ATCC 10231		48-72 Hrs.
		Aspergillus niger ATCC 16404		5 Days
		Environmental isolate - I		48-72 Hrs.
		Environmental isolate - II		48-72 Hrs.
2.	**Negative Product Control(NPC)**			
a.	Fluid Thioglycollate Medium (FTM)		30°C - 35°C	14 days
b.	Soyabean Casein Digest Medium (SCDM)		20°C - 25°C	14 days
3.	**Negative Control Test (NC)**			
a.	Fluid Thioglycollate Medium (FTM)		30°C - 35°C	14 days
b.	Soyabean Casein Digest Medium (SCDM)		20°C - 25°C	14 days
4.	**Media Negative Control**			
a.	Fluid Thioglycollate Medium (FTM)		30°C - 35°C	14 days
b.	Soyabean Casein Digest Medium (SCDM)		20°C - 25°C	14 days

6.9 Record the test results of product as per respective data sheet.

7.0 VALIDATION PARAMETERS AND ACCEPTANCE CRITERIA:

S.No.	Test	objective	Acceptance criteria
1.	PPC (Positive Product Control)	To check any Inhibition properties of product	Should show growth within 3 days for bacteria and 5 days for fungi.
2.	NPC (Negative Product Control)	Sterility of Product	Should show no growth till 14 days
3.	PC(Positive Control)	Suitability of media	Should show growth within 3 days for bacteria and 5 days for fungi
4.	NC (Negative Control)	Process Negative control	Should show no growth till 14 days
5.	Media Negative Control	Sterility of media	Should show no growth till 14 days

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7.1 If clearly visible growth of microorganisms is obtained in the test sample (with product) after the incubation, visually comparable to that in the control sample (without product), either the product possesses no antimicrobial activity under the conditions of the test or such activity has been satisfactorily eliminated. The test for sterility may then be carried out without further modification.

7.2 If clearly visible growth is not obtained in the presence of the product to be tested, visually comparable to that in the control sample without product, the product possesses antimicrobial activity that has not been satisfactorily eliminated under the conditions of the test. Modify the conditions such as increase in rinsing but not more than 5x100 ml per filter in order to eliminate the antimicrobial activity, and repeat the validation test.

7.3 **Revalidation**

Revalidation shall be carried out in case of:

7.3.1 Whenever there is a change in the experimental conditions of the test.

7.3.2 When the test for sterility has to be carried out on a new product validation to be carried out on three consecutive batches.

7.3.3 Whenever there is change in manufacturing process.

8.0 SUMMARY OF VALIDATION REPORT:

The validation report shall consist of a summary document, in the narrative form, which briefly describes the work as well as conditions regarding acceptability. This validation report shall also include the raw data, which shall be completed at the time of validation activity as per annexure.

9.0 APPROVAL OF VALIDATION REPORT:

All validation parameters should comply with acceptance criteria as per protocol, reviewed and signed by Quality assurance.

10.0 ANNEXURE:

Following documents (Annexure) are enclosed as a part of protocol and shall be pre-approved as a part of main protocol.

S.No.	Document	Title	Data sheet Number
1.	Data Sheet -1	Observation Sheet for Sterility Method Validation	

11.0 ABBREVIATIONS:

Abbreviation	Terms
SCDM	Soyabean Casein Digest Medium
FTM	Fluid Thioglycollate Medium
ATCC	American type of culture collection
CFU	Colony Forming Unit

12.0 CHANGE HISTORY DETAILS:

Version no.	Reason for revision	CRF no.	Effective date
00	First Issue	Not Applicable	