Pharma GUIDE Hub.com	**Sterility Test Validation Protocol**	**Protocol No.**	
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AREA: Q.C - MICROBIOLOGY			

DEPARTMENT	**QC -MICROBIOLOGY**
PRODUCT NAME	
EFFECTIVE DATE	

CHANGE HISTORY				
Date	**Supersede**	**CCR No.**	**Changes Made**	**Revision No.**
	Nil	NA	Original Issue	00

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1.0 PROTOCOL APPROVAL

Signing of this approval page of protocol indicates agreement with the method validation approach described in this document. If any modification in the method validation approach becomes necessary, a revision through change control shall be prepared, checked and approved. This protocol cannot be executed until approved by following personnel.

Department	Name	Designation	Signature/Date
Prepared by			
Quality Control Microbiology			
Reviewed by			
Quality Control Microbiology			
Approved by			
Quality Assurance			
Authorised by			
HOD - QA			

2.0 OBJECTIVE:

The protocol is designed so as to validate that any Bacteriostatic and Fungistatic 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 pharmacoepial methods.

3.0 SCOPE

This protocol is applicable to sterile raw material and finished products, which are tested for sterility.

4.0 VALIDATION DESCRIPTION

The purpose of this protocol is to ensure that any Bacteriostatic and fungistatic activity inherent in the Product does not adversely affects the reliability of the test procedure.

5.0 RESPONSIBILITIES AND IDENTIFICATION OF EXECUTION TEAM

5.1 Responsibilities: The group comprising of representatives from each of the following departments and they shall be responsible for the overall compliance with this protocol.

Department	Responsibility
Quality Control Microbiology	Execute, Participate and provide necessary support for the validation activity. Preparation and review of the validation Report of the documents and its compliance to meet the **acceptance criteria** of the protocol.
Quality Assurance	Monitoring the validation activities, compilation, review and authorization of the method validation report and its compliance to meet the **acceptance criteria** of the protocol.

5.2 Identification of Executors: All the identified executors involved with this Protocol are to Record Name, Designation, Signature and Date.

6.0 TEST PROCEDURES

Before proceeding for validations following materials are required.

- Sterile product sample.
- Sterile distilled water.
- Sterile Molten Soyabean Casein Digest Agar.
- Poured SCDA plates.
- Sterile forceps.
- Sterile membrane filtration units.
- Sterile membranes.
- Vortex Mixer.
- 0.1 % peptone water.
- Sterile Fluid Thioglycollate Medium.
- Sterile Soyabean Casein Digest Medium.

- Cultures required suspensions of ***Staphylococcus aureus, Pseudomonas aeruginosa, Clostridium sporogenes, Bacillus subtilis, Candida albicans, Aspergillus Niger***.

The validation will be performed under the following subheadings.

6.1 Preparation of 10 –100 cells / ml: Prepare the 10 – 100 cells / ml solution as per the SOP XXX.

6.2 To Determine Sterility of the Product.

6.2.1.1 After sampling, the sterile product sample will bring to microbiology lab.

6.2.1.2 Microbiologist will place the sterile product sample within the pass box after decontaminating the outer surface of the pouch with disinfectant solution.

6.2.1.3 Enter the sterility testing room as per the SOP for sterile area entry and exit procedure for sterility testing lab.

6.2.1.4 After entering in the sterility testing room, clean the LAF surface with 70 % filtered IPA

6.2.1.5 Place the Sterile product Samples within the LAF.

6.2.1.6 Perform the sterility test of the sample by following procedure described in XXX.

6.2.1.7 Operate the sterility test apparatus Equinox as per XXXXX.

6.2.1.8 After performance Incubate Soyabean Casein Digest Medium at 20 – 25°C for 14 days and Fluid Thioglycollate Medium at 30 – 35°C for 14 days.

6.2.1.9 Observe the medium tubes/canisters daily for any growth.

6.2.1.10 Record the observations daily in the format given as annexure-1.

6.3 To Determine the Bacteriostasis and Fungistasis nature of the Product.

6.3.1.1 Perform the whole exercise under the LAF in Sterility testing area.

6.3.1.2 Take the sterile sample as per the XXXXX and dissolve the sample in 200 ml of 0.1 % peptone water.

6.3.1.3 Perform the sterility test for the above sample by following procedure described in SLT112.

6.3.1.4 Rinse the membrane with 2 X 100 ml portions of the sterile 0.1 % peptone water.

6.3.1.5 Inoculate the final rinse with 10 - 100 CFU/ mL of the test microorganisms.

6.3.1.6 After filtration aseptically transfer the membrane on to the SCDM / FTGM depending on the organism being tested.

6.3.1.7 Perform the whole exercise with all the organisms listed below.

6.3.1.8 Note down the results in Annexure-1.

6.4 Positive Control:

6.4.1.1 Filter 2 X 100 ml portions of 0.1 % peptone water through 0.45µ filter membrane.

6.4.1.2 Finally rinse the membrane with the third 100 ml 0.1 % sterile peptone water inoculated with 10 – 100 cells / ml of any culture listed above.

6.4.1.3 Repeat the above exercise with all the other organisms.

6.4.1.4 Inoculate the membrane into medium.

S.No.	Medium	Organisms	Strain Number
01	Fluid Thioglycollate	*Staphylococcus aureus*	
		Pseudomonas aeruginosa	
		Clostridium sporogenes	
02	Soyabean Casein Digest	*Bacillus subtilis*	
		Candida albicans	
		Aspergillus niger	

6.5 Negative Control:

6.5.1.1 Filter 3 X 100 ml portions of 0.1 % peptone water through 0.45µ filter membrane.

6.5.1.2 Cut the membranes into two halves and inoculate each half separately into specified medium.

6.6 Incubation of Tubes:

6.6.1.1 Incubate test for sterility sample tubes of Fluid Thioglycollate Medium at 32.5 ± 2.5°C and for Soyabean Caesin digest Medium incubate at 22.5 ± 2.5°C for 14 days.

6.6.1.2 The negative control should be incubated for 14 days, observed daily and recorded.

6.6.1.3 Incubate the Positive control tubes for 3 days in case of bacteria and 5 days incase of fungi.

6.7 Acceptance Criteria:

6.7.1.1 The growth of each test organisms in the test sample (with the product) is visually comparable to the growth of the positive control.

6.7.1.2 The negative control should not show any growth during the 14 days of incubation.

7.0 RECORDING OF OBSERVATIONS

Record the observations after the execution of each test procedures, in the annexure–1.

8.0 DISCREPANCY AND CORRECTIVE ACTION REPORT

Document any discrepancies observed during the validation in annexure -1. Include the corrective actions of the same. When all the discrepancies are satisfactorily resolved or an approved action plan is developed which ensures that the discrepancy will be resolved.

9.0 COMPILATION, REVIEW AND SUMMARY REPORT

Compile and review that all test procedures have been completed, reconciled and attached to this protocol. Verify that the approvals for deviations have been taken and are resolved appropriately to the satisfaction.

Testing method validation shall be considered acceptable when all the conditions specified in the test procedures have been met.

Prepare the summary report in the annexure –2 and submit this for review, approval and authorization to Validation Core Team.

10.0 APPENDIX

10.1 Abbreviations and definitions

Abbreviation	Definitions

10.2 References.

10.2.2 Preparation Of Culture Suspensions.

10.2.3 Sterility Testing.

10.2.4 Growth Promotion test.

10.2.5 Sterility Testing Area, Entry And Exit Procedure SOP.

10.2.6 Operation Of Equinox; Make: Millipore. SOP.

10.3 Annexure

10.3.1.1 Annexure – 1 (Recording Of Observations For Validation).

10.3.1.2 Annexure – 2 (Sterility Test Validation Report).

11.0 REVALIDATION CRITERIA:

Revalidation shall be carried out in case of

11.1 If the manufacturing process is changed.

11.2 If there is any change in testing procedure which is being used for sterility testing.