



PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Disinfectant Efficacy Testing Procedure	Effective Date:
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1.0 Objective

To lay down a procedure for evaluate efficacy of sanitizing and disinfectant solutions used for cleaning, sanitization and disinfection of clean area and equipment in manufacturing and Microbiology laboratory.

2.0 Scope

This Standard Operating Procedure is applicable to Microbiology Section of Quality Control Department.

3.0 Responsibility

Executive/ Officer-Microbiology : Shall be responsible for following the Disinfectant Efficacy Testing procedure.

Head-QC/Designee : Shall be responsible for the compliance of this SOP.

4.0 Abbreviations and Definitions

SOP : Standard Operating Procedure

QC : Quality Control

SCDA : Soyabean Casein Digest Agar

SS : Stain less steal

CFU : Colony forming unit

5.0 Procedure

5.1 Direct Contact Method

5.1.1 Prepare the culture suspension from the original culture as per the SOP for "Preparation of Standardized Cell Suspension".

5.1.2 Record the observations for inoculum preparation in Annexure-1.

5.1.3 Select the dilution which yields 10^5 to 10^6 cells per mL



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- 5.1.4 Prepare 10 mL of test dilution to be tested with sterile water.
- 5.1.5 Vortex the tube for 1 minute.
- 5.1.6 Add 0.1 mL of any one culture containing 10^5 to 10^6 cells per mL into the decided concentration of disinfectant.
- 5.1.7 The final concentration shall be 10^4 to 10^5 cells per tube. Consider the preparation for 0 min.
- 5.1.8 Prepare the same above for other 3 different sets for 3 different time intervals.
- 5.1.9 Give a contact time of 0 min, 5 min, 10 min and 15 min.
- 5.1.10 After the specified contact time, filter the samples through a 0.45μ membrane filter.
- 5.1.11 Give three washings of 100 mL each with sterile water.
- 5.1.12 After filtration place the membrane filter on a pre incubated plate of Soyabean casein digest agar with the help of a sterile forcep.
- 5.1.13 Incubate the bacterial culture at 30-35 °C for 24 to 48 hours and fungal cultures at 20-25 °C for 72 to 120 hours.
- 5.1.14 After incubation count the number of colonies present on the membrane filter.
- 5.1.15 Note down the number of colonies as observed counts after the exposure time in Annexure-2.
- 5.1.16 Select the plates, which have least to Nil counts.
- 5.1.17 Proceed in the same manner taking all the cultures to be tested.
- 5.1.18 Contact time for the usage of the disinfectant shall be set on the basis of the results, which have least counts.
- 5.1.19 Record all the observation for Direct Contact Method in Annexure -2.



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5.2 Surface Spray /Wipe Method

- 5.2.1 Prepare the culture suspension from the original culture as per the SOP for "Preparation of Standardized Cell Suspension".
- 5.2.2 Record the observations for inoculum preparation in Annexure-1.
- 5.2.3 Select the dilution which yields 10^5 to 10^6 cells per ml
- 5.2.4 Take plate of different materials such as SS, Epoxy, and glass having a surface area of 25 cm^2 .
- 5.2.5 Place 0.1 mL of the previously determined suspension having 10^5 to 10^6 cells per mL on the plates of different surfaces mentioned above.
- 5.2.6 Spread the culture on the surface with the help of a sterile spreader.
- 5.2.7 Allow it to stand on the LAF bench for drying.
- 5.2.8 After drying spray the disinfectant or disinfect the surface by wipe method.
- 5.2.9 Give a contact time of 0 min, 5 min, 10 min and 15 min.
- 5.2.10 The exact procedure for sanitization followed in the clean room shall be followed.
- 5.2.11 With the help of a sterile moistened swab, swab the surface gently covering all the area of the surface.
- 5.2.12 Place the swab sticks in a test tube having sterile saline solution.
- 5.2.13 Vortex the test tube gently for 5minutes.
- 5.2.14 Aseptically filter the samples through a 0.45μ membrane filter.
- 5.2.15 Give three washings of 100 mL each with sterile water.
- 5.2.16 After filtration place the membrane filter on a pre-incubated plate of Soyabean casein digest agar with the help of a sterile.



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5.2.17 Incubate the bacterial culture at 30-35 °C for 24 to 48 hours and fungal cultures at 20-25 °C for 72 to 120 hours.

5.2.18 After incubation count the number of colonies present on the membrane.

5.2.19 Record all the observations for Surface Spray/Wipe method in Annexure-3.

5.2.20 Proceed in the same manner taking all the cultures to be tested with the all mentioned disinfectants.

5.3 Acceptance Criteria:

5.3.1 The decrease in the microbial load to the exposed disinfectant shall be minimum of 4-log reduction for non-spore forming microorganisms and Yeast, minimum of 3-log reduction shall achieve for Spore forming organisms and molds with the decided concentration.

5.3.2 Determine the contact period where the above said population log reduction of microorganisms achieved.

6.0 Forms and Records

6.1 Inoculum Preparation Record : Annexure-1

6.2 Growth Observation Record for Direct Contact Method : Annexure-2

6.3 Growth Observation Record for Spray Wipe Method : Annexure-3

7.0 Reference

7.1 SOP: "Preparation of Standardized Cell Suspension".

8.0 Distribution

8.1 Master Copy : Documentation Cell (Quality Assurance)

8.2 Controlled Copy : Quality Control, Quality Assurance,
Administration and Housekeeping



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9.0 History

Date	Revision Number	Reason for Revision