| STANDARD OPERATING PROCEDURE | |
|---|---|
| **Department:** Quality Assurance | **SOP No.:** |
| **Title:** Microbial Swab Recovery Validation | **Effective Date:** |
| **Supersedes:** Nil | **Review Date:** |
| **Issue Date:** | **Page No.:** |

## 1.0 PURPOSE:

The purpose of this SOP is to describe the procedure for microbial swab recovery validation.

## 2.0 SCOPE:

This Standard Operating Procedure is applicable at Microbiology Department.

## 3.0 REFERENCES:

3.1 In – house

## 4.0 RESPONSIBILITY:

4.1 Officer or Executive of Microbiology department shall be responsible for preparation of new or revision of existing SOPs.

4.2 Head of the department / designee of respective areas & QA shall be responsible for reviewing the SOPs.

4.3 Plant Head and Head-Quality shall be responsible for approval of SOP.

4.4 QA shall be responsible for distribution and control of SOP's to various departments.

## 5.0 ABBREVIATIONS:

5.1 ATCC : American type culture collection
5.2 CC : Change Control
5.3 cm : Centimeter
5.4 CFU : Colony Forming Unit
5.5 °C : Degree Celsius
5.6 LAF : Laminar Air Flow
5.7 ml : Millilitre
5.8 mm : Milimeter
5.9 NA : Not Applicable
5.10 No. : Number
5.11 NCTC : National Collection of Type Cultures
5.12 QA : Quality Assurance
5.13 QC : Quality Control
5.14 SOP : Standard Operating Procedure
5.15 SCDA : Soyabean Casein Digest Agar
5.16 % : Percentage
5.17 µm : Micrometer

## 6.0 DEFINITION:

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6.1 **Standard Operating Procedure (SOP):** A written authorized procedure, which gives instructions for performing operations

## 7.0 PROCEDURE:

### 7.1 Pre-requisites:

7.1.1 Sterilized media
7.1.2 Micropipette
7.1.3 Sterile swab
7.1.4 Cell Suspension
7.1.5 Stainless steel surface (5x5 cm or 10x10 cm)
7.1.6 Sterile Petriplate

### 7.2 Sampling:

7.2.1 Take the sterilized normal 0.9% saline water or buffered peptone water and sterilized swab to the LAF area. Aseptically fill 3-5 ml sterilized normal 0.9% saline water or buffered peptone water in the test-tube and place one swab stick in each test-tube.

7.2.2 Take the Three areas of 10x10cm or 5 x 5 cm square on S.S. template surface.

7.2.3 Add 0.1ml of the cell suspension containing approximately $10^2$ cfu/ml (for Bacteria & Yeast/Mold) of any one selected microorganism on the each (3nos.) template surface area and spread equally with an L-spreader.

7.2.4 Take precaution not to over spill the applied challenge inoculum from the template surface.

7.2.5 Hold the template in Horizontal position for drying.

7.2.6 Recover the challenge inoculum by swab method (Using gentle strokes, rub the swab over the coupon surface horizontally & vertical ten times) on the three surfaces for one challenge organism with individual swab sticks.

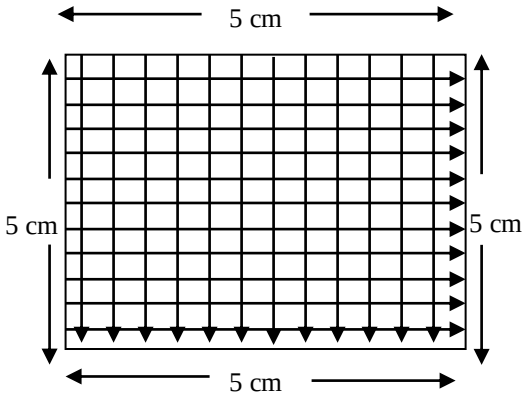


**Fig.-1-** Direction for Swab sampling

7.2.7 Place the swab immediately in to a tube containing 3-5 ml purified water or normal 0.9% saline water and close the tubes.

7.2.8 Repeat the procedure for all specified microorganisms (*Escherichia coli* (ATCC8739), *Staphylococcus aureus*(ATCC6538), *Candida albicans* (ATCC10231), *Aspergillus brasiliensis* (ATCC16404), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella abony* (NCTC 6017) & EM Isolates) on each separate surface coupons respectively.

7.2.9 Apply the same concentration (0.1ml) of inoculum on every plate surface.

**7.3 Testing:**

7.3.1 Vortex the tube containing swab for 20-30 seconds and proceed by filtration method.

7.3.2 Arrange filter assembly, attach the vacuum pump and prewet the 0.45 µ membrane filter which is having 47 mm diameter with 50 ml of sterile Buffer peptone water or normal 0.9% saline water before analysis.

7.3.3 Transfer the vortexed saline on to the membrane filter.

7.3.4 Pour 3-5 ml sterile Buffer peptone water or normal 0.9% saline water in to the test tubes to remove the traces of the remaining of the sample and transfer on the membrane filter.

7.3.5 Rinse the membrane with 2x50 ml of sterile buffer peptone water on to the membrane filter.

7.3.6 After Rinsing remove the membrane filter with the help of sterile forceps and aseptically transfer the membrane filter on pre-incubated SCDA with neutralizer (if required) plate for microbial growth.

7.3.7 Incubate the plates at 22.5ºC ± 2.5°C for 72 hours followed by 32.5°C ± 2.5°C for 48 hours.

7.3.8 After the completion of incubation period take out the plates from the incubator and count the number of colony forming units (cfu).

7.3.9 Record the results as per format given in Annexure-1.

7.3.10 Perform the test of every challenge inoculum on three different S.S. Plate.

**7.4 Interpretation and Results:** Calculate the percentage of microorganism recovery by the following formula:

$$\% \text{ of Microorganism recovery} = \frac{\text{Observed Count (Swab)} \times 100}{\text{Inoculums count}}$$

**7.5** Record the results as per format given in Annexure-1.

**7.6** Perform the analysis within two hours.

**7.7** **Acceptance Criteria:** Swab recovery should be more than 70%.

**7.8** Swab recovery test shall be performed whenever new swab is received / Batch No. or lot No. is changed / Make or Vendor is changed.

## 8.0 DISTRIBUTION:

8.1 Quality Assurance

8.2 Quality Control

## 9.0 ANNEXURE:

9.1 Annexure- 1: Microbial Swab Recovery Validation Record.

## 10.0 REVISION HISTORY:

| Version Number | Revision Details | Effective Date | Ref. Change Control Number |
|---|---|---|---|
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