PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Preparation of Microbial Inoculum	**Effective Date:**
Supersedes: Nil	**Review Date:**
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1.0 Objective

To describe the procedure for preparation of microbial inoculum (10 to 100 CFU/ml).

2.0 Scope

This SOP is applicable to pharmaceutical formulation plant.

3.0 Responsibility

Microbiologist/ QC-Officer : Responsible for sampling of water from different user points.

Head-QC/Designee : Responsible for compliance of the SOP.

4.0 Abbreviations & Definitions

SOP	: Standard Operating Procedure
QC	: Quality Control
QA	: Quality Assurance
SCDA	: Soybean Casein Digest Agar
SDA	: Sabouraud Dextrose Agar
LAF	: Laminar Air Flow

5.0 Procedure

5.1 Prepare the required quantity of Soyabean Casein Digest media (SCDA) and Sabouraud Dextrose Agar (SDA) media in a conical flask.

5.2 Reconstitute the media with the required volume of purified water.

5.3 Boil the media in the water bath to uniformly dissolve the media.

5.4 Dispense 15 ml of the media in a clean 18 mm rimless test tube.

5.5 Plug the tubes with cotton plug and wrap the cotton plug of the tube with crepe paper and label the tubes for type of media, autoclave lot no and date of sterilization.

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5.6 Similarly prepare normal saline solution (0.9% w/v sodium chloride solution) for harvesting of bacterial cultures, and 0.1% w/v peptone solution containing 0.5% Tween 80 solution for harvesting of fungal and yeast cultures.

5.7 Transfer 9 ml of the normal saline solution and 0.1% w/v peptone solution in required number of test tubes and label the tubes for type of solution, autoclave lot no and date of sterilization.

5.8 Plug the test tube with cotton plug and wrap the plug with crepe paper.

5.9 Steam sterilize the media slants, normal saline solution tubes and peptone solution tubes as per the validated autoclave cycle.

5.10 After steam sterilization remove the media slants, normal saline solution tubes and peptone solution tubes from the autoclave.

5.11 Place the media tubes under laminar airflow (LAF) at approximately 30º from the surface.

5.12 Allow the media to solidify.

5.13 After the solidification of the media slants, transfer the SCDA slants, normal saline solution tubes and peptone solution tubes to the incubator for incubation at 30 to 35ºC for 48 hours and SDA slants at 20 to 25ºC for 48 hours for checking of any contamination.

5.14 Streak the surface of the SCDA slant with the bacterial culture and SDA slant with fungal culture.

5.15 Incubate the SCDA slants at 30 to 35ºC for 48 hours and SDA slants at 20 to 25ºC for 48 hours for *Candida albicans* and *Aspergillus niger* culture for 3- 5 days. Incubate the slant of *Clostridium sporegenes* anaerobically at 30 to 35ºC for 48 hours.

5.16 Prepare culture suspension by washing and scraping the surface of the slant by means of sterile inoculating loop in 10 ml of 0.9% saline for Bacteria and fungal and yeast culture with 0.1% w/v peptone solution containing 0.5% Tween 80 solution.

5.17 Transfer the culture suspension in a sterile test tube.

5.18 Collect the suspension in a sterile test tube.

5.19 Vortex the culture suspension to obtain a uniform suspension.

5.20 Carry out serial dilution so as to obtain a culture suspension of 10-100 cfu/ml by following the steps given below.

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5.20.1 Transfer 1 ml of the suspension to 9 ml sterile normal saline solution – 10^1 Dilution.

5.20.2 1 ml of 10^1 Dilution to 9 ml sterile normal saline solution – 10^2 Dilution.

5.20.3 1 ml of 10^2 Dilution to 9 ml sterile normal saline solution – 10^3 Dilution.

5.20.4 1 ml of 10^3 Dilution to 9 ml sterile normal saline solution – 10^4 Dilution.

5.20.5 1 ml of 10^4 Dilution to 9 ml sterile normal saline solution – 10^5 Dilution.

5.20.6 1 ml of 10^5 Dilution to 9 ml sterile normal saline solution – 10^6 Dilution.

5.20.7 1 ml of 10^6 Dilution to 9 ml sterile normal saline solution – 10^7 Dilution.

5.20.8 1 ml of 10^7 Dilution to 9 ml sterile normal saline solution – 10^8 Dilution.

Note: For preparation of *Clostridium sporogenes* microbial dilution add 100 µl of immersion oil on the surface of the normal saline solution.

5.21 Pipette out 1 ml of the each microbial inoculum from last three dilution tubes into sterile petriplate in duplicate.

5.22 Incubate the SCDA plates at 30 to 35ºC for 48 hours for bacterial cultures and SDA plates at 20 to 25ºC for 48 hours for *Candida albicans* and for 5 days for *Aspergillus niger* culture. Incubate the SCDA plates of *Clostridium sporegenes* anaerobically at 30 to 35ºC for 48 hours.

5.23 Till the observation of the microbial counts preserve all the dilution tubes at 2 to 8ºC.

5.24 After incubation count the colonies and note the microbial count in the format attached as Annexure I.

5.25 Note the dilution, which is giving a microbial count in between 10 to 100 CFU/ml.

5.26 Preserve the previous dilution which is giving 10 to 100 CFU/ml. This dilution shall be preserved for microbial inoculum and from this dilution 100µl of the suspension shall be used for testing. Eg., if the 10^7 dilution is giving microbial count in between 10 to 100 CFU/ml the 10^6 dilution tubes shall be preserved and 100µl of the suspension shall be used to give microbial count in between 10 to 100 CFU/ml.

5.27 Label the suspension tubes as per the label given below

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CULTURE SUSPENSION	
SOP No	**:**
Name of Microorganism	**:**
Strain N o	**:**
Counts	**:**
Dilution	**:**
Prepared on ;	**:**
Due On	**:**
Prepared By	**:**

5.28 Preserve the diluted suspension in refrigerator at temperature 2- 8ºC for 15 days.

5.29 The frequency for preparation of the microbial suspension shall be fortnightly.

6.0 Forms and Records

6.1 Preparation of Microbial Inoculum : Annexure-1

7.0 Distribution

7.1 Master Copy : Documentation cell (Quality Assurance)

7.2 Controlled Copies : Quality Control, Quality Assurance

8.0 History

Date	Revision Number	Reason for Revision