



PHARMA DEVILS
MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Procedure for Working Culture Dilution Preparation	Effective Date:
Supersedes: Nil	Review Date:
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1.0 OBJECTIVE

1.1 To lay down the Procedure for working culture dilution preparation.

2.0 SCOPE

2.1 This procedure is applicable for Microbiology Laboratory.

3.0 RESPONSIBILITY

3.1 Microbiologist is responsible working culture dilution preparation.

4.0 ACCOUNATABILITY

4.1 Head Microbiology

5.0 EHS CONSIDERATIONS

5.1 Always wear nose mask, sterile hand gloves during culture handling.



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6.0 PROCEDURE

- 6.1 Operate the LAF as per SOP of Operation of LAF.
- 6.2 Take a loop full of the culture from weekly slant culture and transfer in 50 ml sterile 0.9% NaCl saline solution. Mark this as stock solution & properly vortex.
- 6.3 Take 05 ml from stock solution; add in other tube containing 45 ml sterile 0.9% NaCl saline solution. Mark this as 10^{-1} dilution & properly vortex.
- 6.4 Take 05 ml from above solution of 10^{-1} dilution; add in other tube containing 45 ml of sterile 0.9% NaCl saline solution. Mark this as 10^{-2} & properly vortex.
- 6.5 Similarly make serial dilutions up to 10^{-8} & properly vortex.
- 6.6 Vortex thoroughly every dilution before transferring into next tube.
- 6.7 Pipette out 1.0 ml of each dilution from 10^{-3} to 10^{-8} tubes into sterile petriplates in duplicates.
- 6.8 Add approximately 20-25 ml sterile SCDA (previously cooled to about 45°C) into each plate and mix well. For anaerobic bacteria shall be used RCA and SCA for fungus.
- 6.9 Leave the plates in laminar air flow for solidification.
- 6.10 Incubate bacterial culture plates at $32.5 \pm 2.5^{\circ}\text{C}$, yeast and fungal cultures at $22.5 \pm 2.5^{\circ}\text{C}$ for 2 to 5 days.
- 6.11 After Incubation, count the number of colonies in each dilution plate and take average.
- 6.12 Select the dilution having 10- 100 CFU / ml and use for other microbiological activities.
- 6.13 Proper labeling of selective dilution as per Annexure II.
- 6.14 Store this dilution at $2^{\circ} - 8^{\circ}\text{C}$ and use within 10 days.

7.0 DEFINITIONS AND ABBREVIATIONS

- 7.1 CFU - Colony Forming Unit.
- 7.2 SCDA - Soybean Casein Digest Agar.
- 7.3 ml - Milliliter
- 7.4 $^{\circ}\text{C}$ - Degree Celsius



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8.0 REFERENCE

8.1 NA

9.0 ANNEXURES

9.1 Annexure I : Working Culture Dilution Preparation Record.

9.2 Annexure II : Working Culture Dilution Label

10.0 DISTRIBUTION DETAILS

10.1 Controlled copy of this SOP shall be distributed to Quality Assurance and Microbiology.

11.0 REVISION HISTORY

Supersedes SOP No.	Change Control No.	Reason for revision



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ANNEXURE II Working Culture Dilution Label

	Working Culture Dilution
Culture Name	
Strain No.	
Culture code	
Serial dilution no.	
Date of preparation	
Date of expiry	
Prepared by	