



STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Procedure for preparation of Microbial Suspension	Effective Date:
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1.0 OBJECTIVE:

To lay down a procedure for Preparation of Microbial Suspension.

2.0 SCOPE:

This SOP is applicable for Preparation of Microbial Suspension for Microbiological Analysis in Microbiology Laboratory of Quality Control Area.

3.0 RESPONSIBILITY:

Operating Person – Microbiology

4.0 ACCOUNTABILITY:

Head – QC

5.0 ABBREVIATIONS:

°C	Degree Celsius
CFU	Colony Forming Unit
Ltd.	Limited
ml	Milliliter
No.	Number
QA	Quality Assurance
QC	Quality Control
SOP	Standard Operating Procedure
ATCC	American Type Culture Collection
NCIM	National Collection of Industrial Microorganisms
SCA	Soyabean casein digest agar
SDA	Sabouraud Chloramphenicol Agar
GPT	Growth Promotion Test
CFU	Colony Forming Unit
EM	Environmental Monitoring
NA	Not Applicable

6.0 PROCEDURE:

- 6.1** Transfer weekly working culture from the refrigerator into the microbiological assay room under bio safety cabinet and kept at room temperature.
- 6.2** After attaining at room temperature; take a loop full culture of required microorganisms and inoculate into 10 ml of 0.9% sterile normal saline solution and perform the serial dilution of each tube of microorganism.
- 6.3** Transfer the 1ml inoculums into 9 ml 0.9% of sterile Normal saline solution (Sodium Chloride Solution) to obtain a test dilution of 10^{-1} except *Aspergillus brasiliensis*.



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- 6.4** For *Aspergillus brasiliensis*; transfer the loop full culture in to 10 ml of 0.9% sterile normal saline solution containing 0.05% Polysorbate 80.
- 6.5** Transfer 1.0 ml inoculum into 9.0 ml of 0.9% sterile normal saline solution for preparation of 10^{-1} dilution.
- 6.6** Similarly prepare serial dilution of the culture suspension to obtain dilution of 10^{-2} , 10^{-3} , 10^{-4} and respectively as shown in **Table-I**.
- 6.7** Determine the microbial count (For bacterial culture) in dilution by pour plate method, inoculate 1 ml of culture in two (in duplicate) sterile petri plates and pour 20-25 ml sterilized Soyabean casein digest agar and for *methylobacterium* pour sterilized R₂A media and rotate the plate gently in clockwise and anticlockwise direction for proper mixing of inoculum, Allow to solidify and incubate the soyabean casein digest agar plates at 30-35 °C for 24-48 hours and R₂A plates at 30-35 °C for 48-72 hours. Incubate the media plates of *Clostridia sporogenes* in anaerobic jar.
- 6.8** Determine the microbial count (For fungal culture) in dilution by pour plate method, inoculate 1 ml of culture in two (in duplicate) sterile Petri plates and pour 20-25 ml sterilized Sabouraud Chloramphenicol Agar and rotate the plate gently in clockwise and anticlockwise direction for proper mixing of inoculum, Allow to solidify and incubate at 20-25 °C for 48- 72 hours.
- 6.9** Perform the same activity of all dilution e.g. 10^{-5} , 10^{-6} , 10^{-7} and 10^{-8} for bacteria and 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} and 10^{-8} for yeast and mold. Label the details on tubes with marker e.g. name of inoculum, dilution and prepared date.
- 6.10** Store the dilution at 2-8°C.
- 6.11** After completion of incubation, count the colonies in plates with colony counter and select the dilution with 10-100 CFU/ml.
- 6.12** Select the dilution with containing 10-100 CFU/0.1 ml for routine GPT and its previous dilution for storage and label the stock inoculum tubes as annexure-II “Label for stock inoculum” and store the inoculum tubes at 2-8°C.
- 6.13** If the dilution stock of 10-100 CFU/0.1 ml is exhausted then prepare fresh stock from the previous dilution stored by adding 1 ml in 9 ml of sterile normal saline.
- 6.14** Record the observations in “**Inoculum Preparation Report**” as per **Annexure-I**.

Table-I



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DESCRIPTION OF DILUTIONS

Dilution Number	Sample	Diluents (Normal Saline)	Dilution
A	1.0 ml of stock	9.0 ml	1×10^{-1}
B	1.0 ml of A	9.0 ml	1×10^{-2}
C	1.0 ml of B	9.0 ml	1×10^{-3}
D	1.0 ml of C	9.0 ml	1×10^{-4}
E	1.0 ml of D	9.0 ml	1×10^{-5}
F	1.0 ml of E	9.0 ml	1×10^{-6}
G	1.0 ml of F	9.0 ml	1×10^{-7}
H	1.0 ml of G	9.0 ml	1×10^{-8}

6.15 Storage & Usage:

- 6.15.1** Appropriate dilution of culture suspensions shall be stored in refrigerator at 2-8°C, used up to one week after release.
- 6.15.2** After completion of one week, culture shall be discarded as per procedure for disposal of media.
- 6.15.3** Prepared culture suspension shall be used for growth promotion test, inhibitory test and validation.
- 6.15.4** Prepare the culture suspension of following microorganisms as per mentioned in table-2, if any other microorganism culture suspension required; same shall be prepared as per procedure mentioned above.

Table-2

S.No.	Name of Organism	ATCC No.	NCIM No.
1.	<i>Staphylococcus aureus</i>	6538	5345
2.	<i>Escherichia coli</i>	8739	2065
3.	<i>Pseudomonas aeruginosa</i>	9027	2200
4.	<i>Bacillus subtilis</i>	6633	2063
5.	<i>Clostridia sporogenes</i>	19404	5125
6.	<i>Salmonella spp.</i>	14028/ 6017	2257/5643
7.	<i>Shigella boydii</i>	8700/9207	5709
8.	<i>E. coli mutant</i>	14169/11105	2567/2068
9.	<i>Candida albicans</i>	10231	3471



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10.	<i>Aspergillus brasiliensis</i>	16404	1196
11.	In House Isolate (<i>EM</i>)	NA	NA
12.	In House Isolate (Water)	NA	NA

7.0 ANNEXURES:

ANNEXURE No.	TITLE OF ANNEXURE	FORMAT No.
Annexure – I	Inoculum Preparation Report	
Annexure – II	Label for stock inoculum	

ENCLOSURES: SOP Training Record

8.0 DISTRIBUTION:

- Controlled Copy No. 01 Quality Assurance
- Controlled Copy No. 02 Microbiology Laboratory
- Master Copy Quality Assurance

9.0 REFERENCES:

United State Pharmacopoeia 40
Indian Pharmacopoeia 2018

10.0 REVISION HISTORY:

CHANGE HISTORY LOG

Revision No.	Change Control No.	Details of Changes	Reason for Change	Effective Date	Updated By



PHARMA DEVILS
MICROBIOLOGY DEPARTMENT

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In House Isolate (Water)														
<i>E. coli mutant ATCC 14169</i>														
<i>Candida albicans ATCC10231</i>														
<i>Aspergillus brasiliensis ATCC16404</i>														
Negative control	SCA													
	R ₂ A													
	SDA													

**Checked By
(Sign & Date)**



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ANNEXURE – II

LABEL FOR STOCK INOCULUM	
Name of Inoculum	
Dilution	
Prepared on	
Use From	
Valid up to	
Prepared by	