



PHARMA DEVILS
MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Procurement and Maintenance of Microbiological Cultures	Effective Date:
Supersedes: Nil	Review Date:
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1.0 Objective

To lay down a procedure for Procurement and Maintenance of Microbiological Cultures.

2.0 Scope

This SOP is applicable to Microbiology section of formulation plant.

3.0 Responsibility

Microbiologist : Shall be responsible for raising the purchase requisition for Procurement and Maintaining of Microbiological Cultures.
Head-QC/Designee : Shall be responsible for implementation and compliance of this SOP.

4.0 Abbreviations and Definitions

SOP : Standard Operating Procedure
QC : Quality Control
LAF : Laminar Air Flow
SLC : Seed Lot Culture
WC : Working Culture
MTCC : Microbial Type Culture Collection (Institute of Microbial Technology Sector-39, Chandigarh).
NCIM : National Collection of Industrial Microorganisms, Pune
ATCC : American Type Culture Collection
NCTC : National Collection of Type Cultures
NCYC : National Collection of Yeast Cultures
Authorized Sources : Center which maintains microbial cultures, traceable to National/ International Microbial Reference Culture.



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5.0 Procedure

5.1 Procurement of Cultures

- 5.1.1 Prepare the list of ATCC/ NCTC / NCYC / MTCC / NICM cultures required in Microbiology section as per the details mentioned in Annexure-1.
- 5.1.2 Raise the purchase requisition for the cultures to be procured, required cultures shall be procured once in a year.
- 5.1.3 All cultures shall be procured from the authorized sources with certificate based on permissible sub culturing periods.
- 5.1.4 Ensure that the cultures shall not be more than 2 passages removed from the reference.
- 5.1.5 Upon receipt of the cultures, enter the details in culture inward record as per Annexure-2 and store them in refrigerator between 2° and 8°C.

5.2 Reconstitution of Freeze Dried Cultures:

- 5.2.1 Disinfect the surface of ampoule or vial or slant or loops using 70 % IPA and flame.
- 5.2.2 Transfer the ampoule or vial or slant or loops under LAF and open the culture aseptically.
- 5.2.3 Add 0.5 ml to 1.0 ml of sterile water to the vial / ampoule/ slant to reconstituting the lyophilized/slant cultures or reconstitute the cultures as per the recommendation of the provider.
- 5.2.4 This culture will serve as mother Culture.
- 5.2.5 Record the details in Culture Maintenance Record as per Annexure-5.

5.3 Revival and Maintenance of Cultures

- 5.3.1 Streak the mother culture on agar plates for confirmation of purity and simultaneously inoculate the total content of vial/ ampoule in 100 ml of sterilized Soyabean casein digest medium.



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- 5.3.2 After transfer the mother culture in to the medium, dispose the remaining content and vial as per SOP No. BF-QC-156 “Disposal of Used Microbial Media and Culture” and record the details in Annexure-7.
- 5.3.3 Agar plate shall be used for purity check and the liquid medium shall be used for preparation of Seed lot cultures.
- 5.3.4 Incubate the media containing cultures of bacteria at $32.5 \pm 2.5^\circ\text{C}$ for 24-48 hrs, cultures of molds at $22.5 \pm 2.5^\circ\text{C}$ for 72 hrs and cultures of yeasts at $22.5 \pm 2.5^\circ\text{C}$ for 24- 48 hrs.
- 5.3.5 Media and incubation conditions shall be followed for different cultures as recommended in Annexure-1.
- 5.3.6 After completion of incubation check the purity of culture by colonial characteristics, microscopic examination, staining and biochemical characterization and record the observations in Annexure- 8.
- 5.3.7 Add 10% v/v sterile glycerol in culture suspension in 1:1 ratio, mix well and dispense 2 ml into the sterile cryo vial prepare 14 such vials which serves as Seed lot Culture (SLC).
- 5.3.8 Maintain the cultures as per the Schematic Flow for Sub culturing as per Annexure-4.
- 5.3.9 Mark culture ID number as SLC-1, SLC-2, and SLC-3 and so on and store the cryo vials at not more than -10°C .
- 5.3.10 Label each cryo vial of SLC with the details like name of culture, strain no., passage no., culture ID. date of sub culturing, sub cultured by and use before as per Annexure-3.
- 5.3.11 12 cryo vials of seed lot culture shall be used for sub culturing up to 12 months (yearly) and 2 cryo vials shall be kept as stock which shall be used if any vial get damaged or spillage.



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- 5.3.12 Ensure that the cryo vials shall not be used after one year. Discard remaining two cryo vials after completion of yearly sub culturing and record the details in Annexure-7.
- 5.3.13 For first month subculture five slants of agar medium from the cryo vial of SLC-1 and mark culture ID numbers as SLC-1WC-1, SLC-1WC-2, SLC-1WC-3, SLC-1WC-4, and SLC-1WC-5. Simultaneously streak on the plates of agar medium for purity check.
- 5.3.14 Incubate the slants and plates containing cultures of bacteria at $32.5 \pm 2.5^\circ\text{C}$ for 24-48 hrs, cultures of molds at $22.5 \pm 2.5^\circ\text{C}$ for 72 hrs and cultures of yeasts at $22.5 \pm 2.5^\circ\text{C}$ for 24-48 hrs.
- 5.3.15 When proper growth observed on the transferred slants discard SLC-1 following the SOP titled "Disposal of Used Microbial Media and Culture" and record the details in Annexure-7.
- 5.3.16 Label each slant of working culture with the details like name of culture, strain no., passage no., seed lot culture no., culture ID., date of sub culturing, sub cultured by and use before as per Annexure-6 and store the working cultures at $2 - 8^\circ\text{C}$.
- 5.3.17 Use one working culture for each week for routine lab work up to one month. Discard the working culture at the end of week before using new working culture.
- 5.3.18 Fifth working culture shall also be discarded at the end of the month if it remains unused and details shall be recorded in Annexure-7.
- 5.3.19 Start the same procedure with SLC-2 and so on, well before completing the cycle of previous SLC to get the working cultures ready to use for next month.
- 5.3.20 Check the purity of seed lot culture and working culture at the time of use by colonial characteristics, microscopic examination, staining and biochemical examination and record the observations in Annexure-8.
- 5.3.21 Details of sub culturing shall be recorded in Annexure-5 at every step of sub-culturing.



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5.3.22 Tentative Schedule for Maintenance of Microbial Cultures shall be prepared for sub culturing at the time of first revival of new cultures as per Annexure-9.

5.3.23 Ensure that the inoculums used shall not be more than 5 passage removed from the certified reference cultures.

6.0 Forms and Records

- 6.1 List of Microbial Cultures : Annexure-1
- 6.2 Microbial Culture Inward Record : Annexure-2
- 6.3 Seed Lot Culture Label : Annexure-3
- 6.4 Schematic flow for Sub-Culturing : Annexure-4
- 6.5 Culture Maintenance Record : Annexure-5
- 6.6 Working Culture Label : Annexure-6
- 6.7 Culture Disposal Record : Annexure-7
- 6.8 Purity Check of Microbial Culture : Annexure-8
- 6.9 Tentative Schedule for Maintenance of Microbial Cultures : Annexure-9

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