PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Management of Microbial Cultures	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 PURPOSE:

To lay down the procedure for management of Microbial Cultures.

2.0 SCOPE:

This Standard Operating Procedure is applicable at Microbiology Department.

3.0 REFERENCES:

3.1 CQ guideline: Guideline for the procurement, transfer, preservation & disposal of microbiological cultures.

3.2 SOP: Disposal of used media and cultures.

3.3 SOP: Isolation and identification of microorganisms.

3.4 USP/IP.

4.0 RESPONSIBILITY:

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

4.2 Head of the Department/Designee of respective areas & QA shall be responsible for reviewing the SOP's.

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 SOPs to various departments.

5.0 ABBREVIATIONS:

5.1 ATCC : American Type Culture Collection

5.2 CC : Change Control

5.3 °C : Degree Celsius

5.4 IPA : Isopropyl alcohol

5.5 LAF : Laminar Air Flow

5.6 MTCC : Microbial Type Culture Collection

5.7 NA : Not Applicable

5.8 NA : Not Applicable

5.9 NCTC : National Collection of Type Cultures

5.10 NCYC : National Collection Of Yeast Culture

5.11 No. : Number

5.12 QA : Quality Assurance

5.13 QC : Quality Control

5.14 SCDA : Soyabean Casein Digest Agar

5.15 SDA : Sabouraud Dextrose Agar

5.16 SOP : Standard Operating Procedure

5.17 v/v : Volume by Volume

6.0 DEFINITION:

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

7.0 PROCEDURE:

7.1 Procurement of Culture

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7.1.1 Prepare the list of ATCC/NCTC/NCYC/MTCC cultures required in Microbiology section as per the details mentioned in Annexure-1.

7.1.2 Raise the purchase requisition for the cultures to be procured, required cultures shall be procured once in a year.

7.1.3 All cultures shall be procured from the authorized sources with certificate based on permissible sub culturing periods.

7.1.4 Ensure that the cultures shall not be more than 2 passages removed from the reference.

7.1.5 Upon receipt of the cultures, enter the details along with in house identification no. in culture inward record as per Annexure-2 and store them in refrigerator between 2º-8ºC or as per manufacturer recommendation.

For Example: E. coli received on 10/02/22 then given in house number should be E.coli100222

7.2 Reconstitution of Freeze Dried Cultures:

7.2.1 Sanitized the surface of ampoule or vial or slant or loops using 70 % IPA.

7.2.2 Transfer the ampoule or vial or slant or loops under LAF/Biosafety cabinet and open the culture aseptically.

7.2.3 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.

7.2.4 This culture will serve as mother Culture.

7.2.5 Record the details in Culture Maintenance Record as per Annexure-3.

7.3 Revival and Maintenance of Cultures

7.3.1 Streak the mother culture on agar plates for confirmation of purity as per SOP "Isolation and identification of microorganisms" (Annexure-4) or direct by automated identification system and simultaneously inoculate the total content of vial/ ampoule in 100 ml of sterilized Soyabean casein digest medium.

7.3.2 After transfer the mother culture in to the medium, dispose the remaining content and vial as per current version of SOP "Disposal of used media and cultures." and record the details in Annexure-8.

7.3.3 Agar plate shall be used for purity check and the liquid medium shall be used for preparation of Seed lot cultures.

7.3.4 Incubate the media containing cultures of bacteria at 32.5±2.5ºC for 24-48 hrs, cultures of molds at 22.5± 2.5ºC for 72-120 hrs and cultures of yeasts at 22.5± 2.5ºC for 24- 48 hrs.

7.3.5 Media and incubation conditions shall be followed for different cultures as recommended in Annexure-1.

7.3.6 After completion of incubation check the purity as per SOP "Isolation and identification of microorganisms" of culture by colonial characteristics, microscopic examination, staining and biochemical characterization or through automated identification System (BD Phoenix).

7.3.7 Add 10% v/v sterile glycerol in culture suspension in 1:1 ratio, mix well and dispense 2-3 ml into the sterile cryo vial prepare 14 such vials which serves as Seed lot Culture (SLC).

7.3.8 Mark culture ID number as SLC-1, SLC-2, and SLC-3 and so on and store the cryo vials (Cryoprotective medium) at -30°C or below until use.

7.3.9 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 the Annexure-6.

7.3.10 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.

7.3.11 Ensure that the cryo vials shall not be used after one year. Discard remaining two cryo vials after completion of yearly sub culturing as per current version of SOP "Disposal of used media and cultures".

7.4 Sub culturing

7.4.1 Maintain the cultures as per the Schematic Flow for Sub culturing as per Annexure-5.

7.4.2 For first month subculture take 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-1 WC-3, SLC-1WC-4, and SLC-1WC-5. Simultaneously streak on the plates of agar medium for purity check as per SOP "Isolation and identification of microorganisms" of culture by colonial characteristics, microscopic examination, staining and biochemical characterization or through automated identification System (BD Phoenix).

7.4.3 Incubate the slants and plates containing cultures of bacteria at 32.5±2.5ºC for 24-48 hrs, cultures of molds at 22.5± 2.5ºC for 72-120 hrs. and cultures of yeast at 22.5± 2.5ºC for 24- 48 hrs.

7.4.4 When proper growth observed on the transferred slants discard SLC-1 following the current version of SOP "Disposal of used media and cultures" and record the details in Annexure-8.

7.4.5 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-7 and store the working cultures at 2 - 8 ºC.

7.4.6 Use one working culture for each week for routine lab work up to one month. Discard the working culture at the end of week or before using new working culture.

7.4.7 Fifth working culture shall also be discarded at the end of the month if it remains unused and details shall be recorded in Annexure-8.

7.4.8 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.

7.4.9 Check the purity of seed lot culture and working culture as per SOP "Isolation and identification of microorganisms" at the time of use by colonial characteristics, microscopic examination, staining and biochemical examination and record the observations in Annexure-4 or through automated identification system (BD Phoenix).

7.4.10 Details of sub culturing shall be recorded in Annexure-3 at every step of sub-culturing.

7.4.11 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.

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

8.0 DISTRIBUTION:

8.1 Quality Assurance

8.2 Quality Control

9.0 ANNEXURES:

9.1 Annexure-1 : List of Microbial Cultures

9.2 Annexure-2 : Microbial Culture Inward Record

9.3 Annexure-3 : Culture Maintenance Record

9.4 Annexure-4 : Purity Check of Microbial Culture

9.5 Annexure-5 : Schematic flow for Sub-Culturing

9.6 Annexure-6 : Seed Lot Culture Label

9.7 Annexure-7 : Working Culture Label

9.8 Annexure-8 : Culture Disposal Record

9.9 Annexure-9 : Tentative Schedule for Maintenance of Microbial Cultures

10.0 REVISION HISTORY:

Version Number	Revision Details	Effective Date	Ref. Change Control Number