



STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Maintenance, Handling and Sub-culturing of Microorganisms	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

1.0 OBJECTIVE:

To lay down a procedure for Maintenance, Handling & Subculturing of Microorganisms.

2.0 SCOPE:

This SOP is applicable for Maintenance, Handling & Subculturing of Microorganisms in Microbiology Lab.

3.0 RESPONSIBILITY:

Officer / Executive – Microbiology

4.0 ACCOUNTABILITY:

Head – QC

5.0 ABBREVIATIONS:

°C	:	Degree Celsius
ATCC	:	American Type Culture Collection
Hrs	:	Hours
LAF	:	Laminar Air Flow
NaCl	:	Sodium Chloride
NCIM	:	National Collection of Industrial Microorganisms
No.	:	Number
QA	:	Quality Assurance
QC	:	Quality Control
SOP	:	Standard Operating Procedure

6.0 PROCEDURE:

6.1 Microbial Cultures (Lyophilized or Slant) must be procured from any Approved Government Source or our subsidiary with their Authentic Certificate.

6.2 Store the pure cultures, mother culture, monthly culture & weekly culture at 2-8°C till further use.

6.3 Use Preincubated slants for sub culturing of microorganism with labeled of following details.

Media Ref No.:

Prepared By:

6.4 MAINTENANCE OF LYOPHILIZED CULTURES :

6.4.1 Prepare 0.9% Normal Saline (1 ml for each culture) and sterilize it by autoclaving.



STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Maintenance, Handling and Sub-culturing of Microorganisms	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

6.4.2 Score the middle of the ampoule above the cotton plug with ampoule cutter. Disinfect the ampoule with 70% IPA damped gauze. Wrap sterile gauze around the ampoule and break it carefully using sterile scissors & sterile forceps.

6.4.3 Transfer the whole content of the ampoule in 1 ml 0.9% Normal Saline. Store the culture suspension tubes at 2-8°C till sub culturing carried out.

6.4.4 Transfer the culture suspension tubes and fresh slants to biosafety cabinet of microbial assay room and streak a loopful suspension from tube to a fresh set of three slants and treat as P-1 generation (if received 0 passage) and streak a loopful suspension from tube to SCA media plates for bacterial culture and SDA/SBD plate for fungal culture for purity check. Check the purity at species level. Incubate at specified temperature and period as given in Table-1. Label the slants as per **Annexure-III, Titled “Culture Label”** and mention the Passage No. P1Mc1, P1Mc2 and P1Mc3.

Where,

P1Mc1	=	Master cultures, for first six months
P1Mc2	=	Master cultures, for last six months
P1Mc3	=	Master cultures (kept as an extra set)
P	=	Passage
Mc	=	Master culture

6.4.5 After ensuring the purity of mother culture, create P-2 generation (if received 0 passage). For the preparation of monthly cultures for first six months, transfer a loop full culture from P1Mc1 to a fresh set of six slants and treat as P-2 generation (if received 0 passage). Label the slants as earlier and mention the Passage No. P2M1, P2M2, P2M3, P2M4, P2M5 and P2M6 (signifying Passage 2, Month1 and so on). Similarly prepare for last six months by transferring a loop full culture from P1Mc2 to a fresh set of six slants. Label the slants and mention the Passage No. P2M7, P2M8, P2M9, P2M10, P2M11 and P2M12. Incubate all the slants at specified temperature and period as given in Table-1. If one of the above cultures gets contaminated or other specified region, use P1Mc3 for sub culturing.

Where,

P2M1 to P2M12	=	Monthly cultures, each for one month
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6.4.6 For the preparation of first month working cultures, transfer a loopful culture from P2M1 to fresh five slants and treat as P-3 generation (if received 0 passage). Label the slants as P3M1W1, P3M1W2, P3M1W3, P3M1W4 and P3M1W5 (signifying Passage 3, Month1, Week 1 and so on). For second month transfer a loopful culture from P2M2 to fresh five slants and label P3M2W1, P3M2W2, P3M2W3, P3M2W4 and P3M2W5. Similarly prepare for third month by transferring a loopful culture from P2M3 to fresh five slants and label the slants as P3M3W1, P3M3W2, P3M3W3, P3M3W4 and P3M3W5. Incubate all the slants at specified temperature and period as given in Table-1.

Where,



STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Maintenance, Handling and Sub-culturing of Microorganisms	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

P3M1W1 to P3M1W5	=	Working cultures for first month
P3M2W1 to P3M2W5	=	Working cultures for second month
P3M3W1 to P3M3W5	=	Working cultures for third month
P3M4W1 to P3M4W5	=	Working cultures for fourth month
P3M5W1 to P3M5W5	=	Working cultures for fifth month
P3M6W1 to P3M6W5	=	Working cultures for sixth month
P3M7W1 to P3M7W5	=	Working cultures for seventh month
P3M8W1 to P3M8W5	=	Working cultures for eighth month
P3M9W1 to P3M9W5	=	Working cultures for ninth month
P3M10W1 to P3M10W5	=	Working cultures for tenth month
P3M11W1 to P3M11W5	=	Working cultures for eleventh month
P3M12W1 to P3M12W5	=	Working cultures for twelfth month

6.4.7 For daily microbiological analysis for first month use P3M1W1 for first week, P3M1W2 for second week, P3M1W3 for third week, P3M1W4 for fourth week and P3M1W5 for fifth week of the month.

6.5 MAINTENANCE OF CULTURES IN SLANT / BUTT FORM:

6.5.1 After ensuring the purity of received mother culture, transfer the mother culture and fresh slants to biosafety cabinet of microbial assay room and streak a loop full growth to a fresh set of three slants and treat as P-2 generation (if received 1 passage). Incubate P-2 generation at specified temperature and period as given in Table-1. Label the slants as given below and mention the Passage No. P2Mc1, P2Mc2 and P2Mc3.

Where,

P2Mc1	=	Master cultures, for first six months
P2Mc2	=	Master cultures, for last six months
P2Mc3	=	Master cultures (kept as an extra set)

6.5.2 For the preparation of monthly cultures for first six months, transfer a loopful culture from P2Mc1 to a fresh set of six slants and treat as P-3 generation (if received 1 passage). Label the slants as earlier and mention the Passage No. P3M1, P3M2, P3M3, P3M4, P3M5 and P3M6 (signifying Passage 2, Month1 and so on). Similarly prepare for last six months by transferring a loopful culture from P2Mc2 to a fresh set of six slants. Label the slants and mention the Passage No. P3M7, P3M8, P3M9, P3M10, P3M11 and P3M12. Incubate all the slants at specified temperature and period as given in Table-1. If one of the above cultures gets contaminated or other specified region, use P2Mc3 (kept as an extra set) for sub culturing.

Where,

P3M1 to P3M12	=	Monthly cultures, each for one month
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STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Maintenance, Handling and Sub-culturing of Microorganisms	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

6.5.3 For the preparation of first month working cultures transfer a loopful culture from P3M1 to fresh five slants and treat as P-4 generation(if received 1 passage). Label the slants as P4M1W1, P4M1W2, P4M1W3, P4M1W4 and P4M1W5 (signifying Passage 4, Month1, Week 1 and so on). For second month transfer a loopful culture from P3M2 to fresh five slants and label P4M2W1, P4M2W2, P4M2W3, P4M2W4 and P4M2W5. Similarly prepare for third month by transferring a loopful culture from P2M3 to fresh five slants and label the slants as P4M3W1, P4M3W2, P4M3W3, P4M3W4 and P4M3W5. Incubate all the slants at specified temperature and period as given in Table-1.

Where,

P4M1W1 to P4M1W5	=	Working cultures for first month
P4M2W1 to P4M2W5	=	Working cultures for second month
P4M3W1 to P4M3W5	=	Working cultures for third month
P4M4W1 to P4M4W5	=	Working cultures for fourth month
P4M5W1 to P4M5W5	=	Working cultures for fifth month
P4M6W1 to P4M6W5	=	Working cultures for sixth month
P4M7W1 to P4M7W5	=	Working cultures for seventh month
P4M8W1 to P4M8W5	=	Working cultures for eighth month
P4M9W1 to P4M9W5	=	Working cultures for ninth month
P4M10W1 to P4M10W5	=	Working cultures for tenth month
P4M11W1 to P4M11W5	=	Working cultures for eleventh month
P4M12W1 to P4M12W5	=	Working cultures for twelfth month

6.5.4 For daily microbiological analysis for first month use P4M1W1 for first week, P4M1W2 for second week, P4M1W3 for third week, P4M1W4 for fourth week and P4M1W5 for fifth week respectively.

6.5.5 After completion of work or period of duration, discard the microbial culture slant as per Respective SOP Titled “**Procedure for Discarding of Microbiological Waste**”.

6.5.6 Purity of culture shall be check at species level.

6.5.7 Record the Sub Culturing Details in format “**Microbial Strains Sub Culturing Record**” as shown in **Annexure-I**.

6.5.8 Record the used microbial strains Details in format “**Microbial Strains Used Record**” as shown in **Annexure-II**.

6.5.9 Schematic Flow Diagram Details Maintenance of Lyophilized Cultures in format “**Schematic Flow Diagram for Maintenance of Lyophilized Cultures**” as shown in **Annexure-IV**.

6.5.10 Schematic Flow Diagram Details Maintenance of Cultures in Slant or Butt in format “**Schematic Flow Diagram for Maintenance of Cultures in Slant or Butt**” as shown in **Annexure-V**.

6.5.11 Validity of culture is one year.



STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Maintenance, Handling and Sub-culturing of Microorganisms	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

6.5.12 Mother culture shall not be used for more than five passages.

6.5.13 Affix the culture label on each sub-cultured tube as per **Annexure-III**.

Table –I

S.No.	Culture Name	ATCC / NCIM No.	Maintenance Media	Incubation Conditions		Storage Condition
				Temperature	Period	
1.	<i>Bacillus subtilis</i>	6633/2063	Nutrient Agar	32.5 ± 2.5 ⁰ C	24 – 48 hrs.	2-8 ⁰ C
2.	<i>Staphylococcus aureus</i>	6538/5345	Nutrient Agar	32.5 ± 2.5 ⁰ C	24 – 48 hrs.	2-8 ⁰ C
3.	<i>Salmonella species</i>	6017(NCTC)/ 2257 14028/5643	Nutrient Agar	32.5 ± 2.5 ⁰ C	24 – 48 hrs.	2-8 ⁰ C
4.	<i>Pseudomonas aeruginosa</i>	9027/2200	Nutrient Agar	32.5 ± 2.5 ⁰ C	24 – 48 hrs.	2-8 ⁰ C
5.	<i>Escherichia coli</i>	8739 /2065	Nutrient Agar	32.5 ± 2.5 ⁰ C	24 – 48 hrs.	2-8 ⁰ C
6.	<i>Escherichia coli</i> (Mutant)	11105/2068 14169/2567	B12 Culture Agar	32.5 ± 2.5 ⁰ C	24 – 48 hrs.	2-8 ⁰ C
7.	<i>Staphylococcus epidermidis</i>	12228/2493	Nutrient Agar	32.5 ± 2.5 ⁰ C	24 – 48 hrs.	2-8 ⁰ C
8.	<i>Shigella boydii</i>	9207 8700/5709	Nutrient Agar	32.5 ± 2.5 ⁰ C	24 – 48 hrs.	2-8 ⁰ C
9.	<i>Candida albicans</i>	10231/3471	SDA/SBD	22.5 ± 2.5 ⁰ C	72 – 120 hrs.	2-8 ⁰ C
10.	<i>Aspergillus brasiliensis</i>	16404/1196	SDA/SBD	22.5 ± 2.5 ⁰ C	72 – 120 hrs.	2-8 ⁰ C
11.	<i>Clostridium sporogenes</i>	19404/5125	Columbia Agar	32.5 ± 2.5 ⁰ C	24 – 48 hrs.	2-8 ⁰ C
12.	<i>Brevundimonas diminuta</i>	19146/5216	Nutrient Agar	32.5 ± 2.5 ⁰ C	24 – 48 hrs.	2-8 ⁰ C
13.	<i>Bordetella bronchiseptica</i>	4617/5389	Nutrient Agar	32.5 ± 2.5 ⁰ C	24 – 48 hrs.	2-8 ⁰ C
14.	<i>Burkholderia cepacia</i>	25416	Nutrient Agar	32.5 ± 2.5 ⁰ C	24 – 48 hrs.	2-8 ⁰ C

Note: Passage number shall be assigned on the basis of received passage number of pure culture.

Passage: A passage number is the number of times a cell culture has been subcultured, and knowing the passage number can make or break an experiment.



STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Maintenance, Handling and Sub-culturing of Microorganisms	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

7.0 ANNEXURES:

ANNEXURE No.	TITLE OF ANNEXURE	FORMAT No.
Annexure – I	Microbial Strains Sub Culturing Record	
Annexure - II	Microbial Strains Uses Log	
Annexure - III	Culture Label	
Annexure - IV	Schematic Flow Diagram For Maintenance of Lyophilized Cultures	
Annexure - V	Schematic Flow Diagram For Maintenance of Cultures In Slant or Butt	
Annexure - VI	Microbial Strains Receipt Record	

ENCLOSURES: SOP Training Record.

8.0 DISTRIBUTION:

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

9.0 REFERENCES:

In House, Certificate of Authentication from culture source (from where procured).

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

STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Maintenance, Handling and Sub-culturing of Microorganisms	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

ANNEXURE-III

CULTURE LABEL	
Culture Name	:
ATCC / NCIM No.	:
Passage No.	:
Subculturing Date	:
Use From	:
Use Before	:
Subcultured By	:



STANDARD OPERATING PROCEDURE

Department: Microbiology

SOP No.:

Title: Maintenance, Handling and Sub-culturing of Microorganisms

Effective Date:

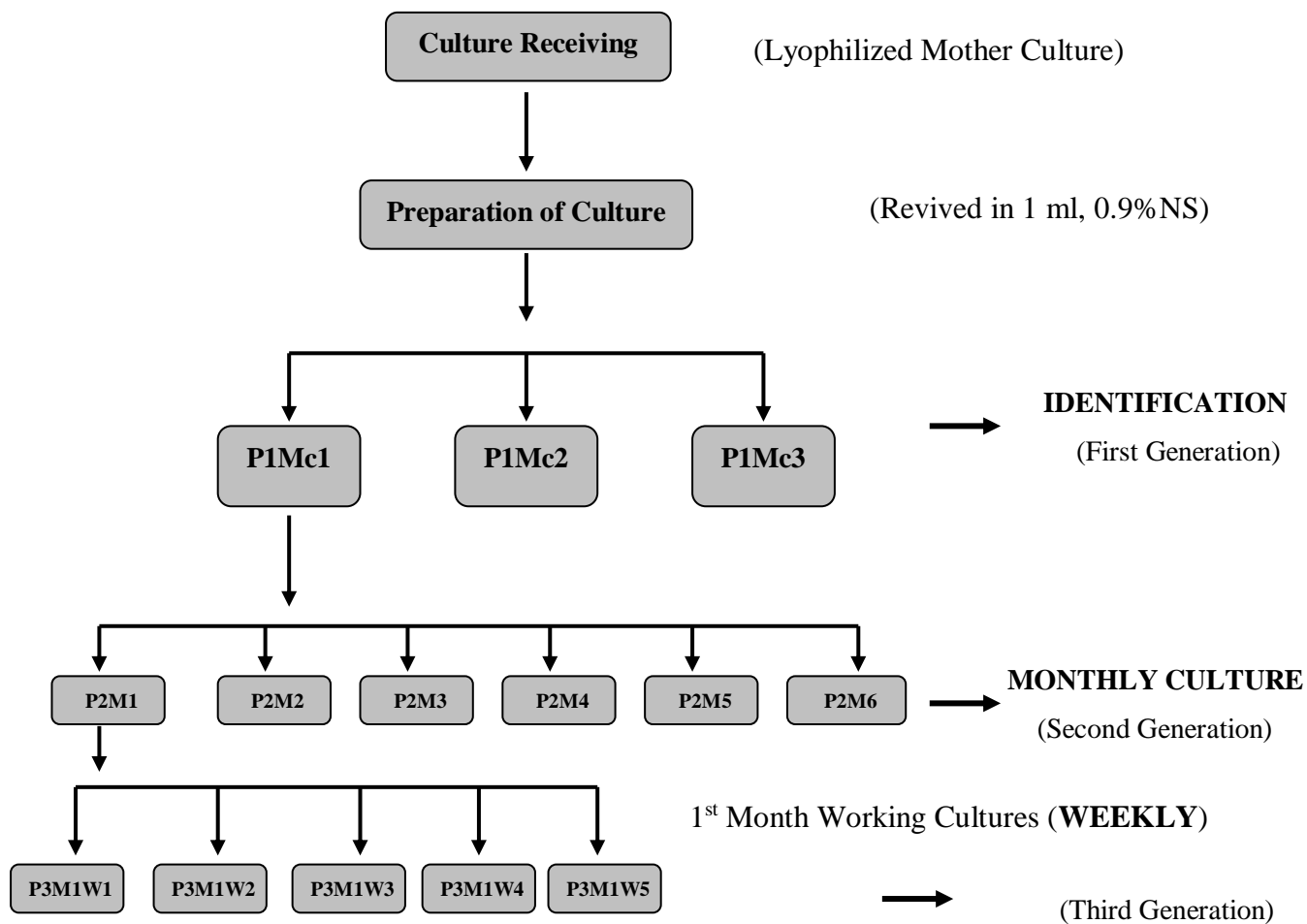
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Review Date:

Issue Date:

Page No.:

ANNEXURE-IV
SCHEMATIC FLOW DIAGRAM FOR MAINTENANCE OF LYOPHILIZED CULTURES





STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Maintenance, Handling and Sub-culturing of Microorganisms	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

ANNEXURE-V
SCHEMATIC FLOW DIAGRAM FOR MAINTENANCE OF CULTURES IN SLANT OR BUTT

