1.0 OBJECTIVE

To lay down the procedure for Receipt, Storage, Reconciliation, Revival, Preparation and Maintenance of Microbial Cultures.

2.0 SCOPE

This SOP is applicable for Receipt, Storage, Reconciliation, Revival, Preparation and Maintenance of Microbial Cultures in Microbiology Laboratory of pharma micro hub Pvt Ltd.

3.0 RESPONSIBILITY

3.1 Microbiologist is responsible for the Receipt, Storage, Reconciliation, Revival, Preparation and Maintenance of Microbial Cultures.

3.2 Head – Microbiology or designee is responsible for the overall compliance of this SOP.

4.0 PROCEDURE

4.1 **Precautions:**

4.1.1 Handle the culture strains in Bio-safety cabinet.

4.1.2 Ensure the personnel handling the culture strains are adequately trained on Operation of bio-safety and aseptic techniques.

4.1.3 In case of any spillage of microbial strains or culture suspension, clean the biosafety cabinet with the sporicidal disinfectant.

4.1.4 Wear nose mask and sterile hand gloves before starting analysis.

4.1.5 Do not use the cultures after the expiry date given by the supplier.

4.1.6 Ensure Biosafety cabinet is cleaned with sporicidal disinfectant before usage.

4.2 **Procurement and Receipt:**

4.2.1 Procure the lyophilized Reference Strains from ATCC (American Type Culture Collection) or its equivalent strains as per the requirement.

4.2.2 Upon receipt, check the correctness of the label information of each of the reference strains. In case of any discrepancy, notify the Head Microbiology/ Designee and the Supplier. Do not use the strain.

4.2.3 Ensure the CoA (Certificate of Analysis) received along with the culture strains. Check the CoA for the details like Name of the Strain, strain Number, passage number, Storage Conditions and Date of Expiration. COA shall be reviewed, signed as a proof of review and filed.

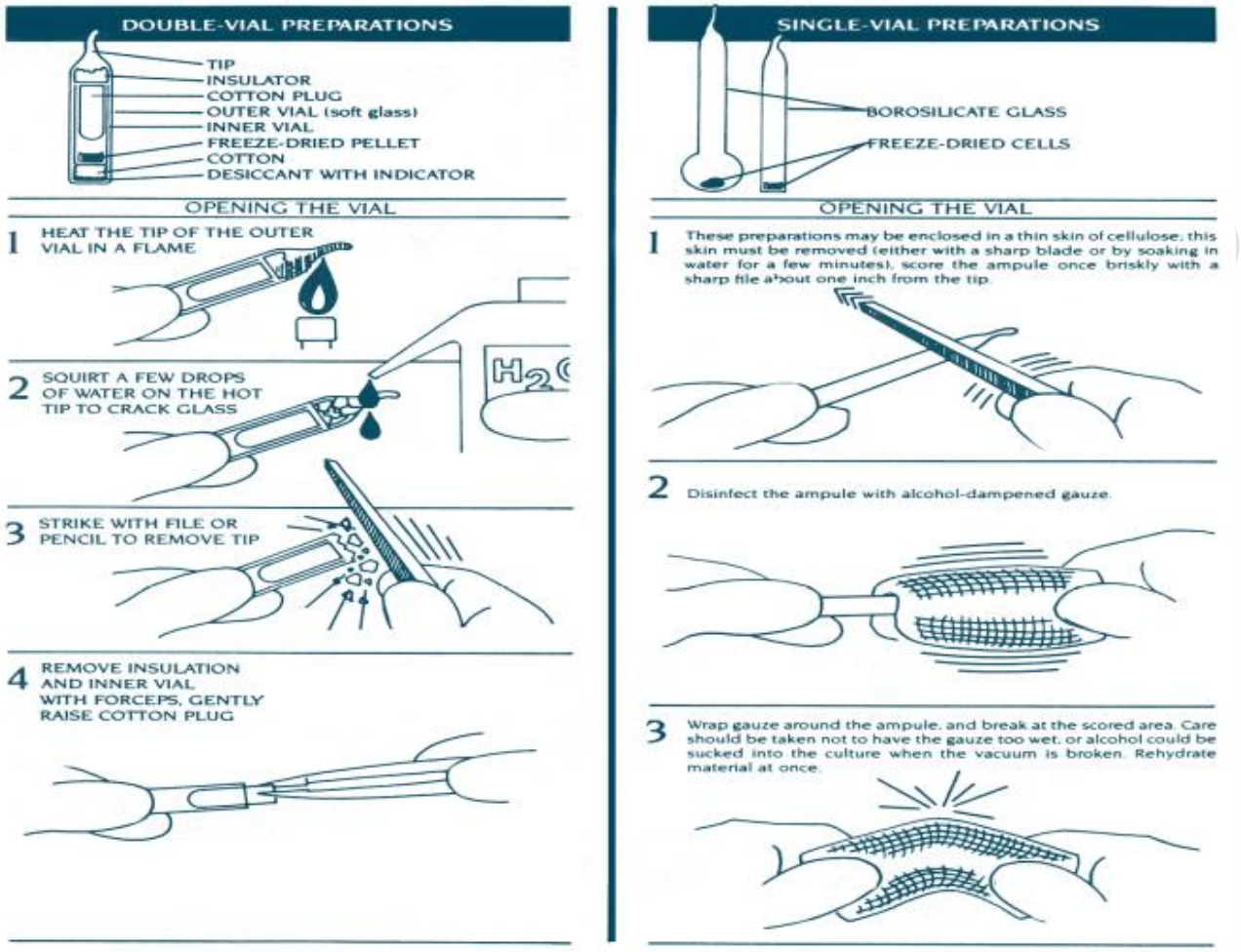
4.2.4 If the physical condition of the box and packing are satisfactory, then only accept the culture and transfer to Microbiology department. If it is not satisfactory send back to the vendor.

4.2.5 Label the received culture as per the Culture Receipt Label.

4.2.6 Enter the receipt details of cultures in the culture stock card.

4.3 **Labelling and Storage:**

4.3.1 Store all the lyophilized cultures in the refrigerator at 2-8°C or as per the Vendor's recommendation.



4.4 **Revival of master culture (Lyophilized form):**

4.4.1 **Revival of culture received in double vial preparation:**

4.4.1.1 Heat the vial outer layer tip in flame.

4.4.1.2 Add a few drops of water on the flame exposed side to crack the glass.

4.4.1.3 Strike the glass with sterile forceps or sterile scissors.

4.4.1.4 Remove the insulation and inner vial, with sterile forceps and gently raise the cotton plug.

4.4.1.5 For further procedure for preparation refer point No.5.5.

4.4.2 **Revival of culture received in single vial preparation:**

4.4.2.1 Open the culture pack and sanitize the ampoule outer surface with wet mop moistened with sterile 70% v/v IPA and break open the ampoule with sterile forceps or sterile scissors.

4.4.2.2 Wrap thick sterile cotton wool (or gauge) around the ampoule and break the ampoule carefully.

4.4.2.3 Care should be taken while opening the ampoule as hasty opening will release fine particles of dried organisms into the air.

4.4.2.4 Take the sterile forceps and aseptically remove the pointed top of the ampoule and cotton plug.

4.4.2.5 Transfer the cotton and broken part of ampoule/Vial in the beaker containing sporicidal disinfectant.

4.4.2.6 For further procedure for preparation refer point No.5.5.

4.5 **Preparation of Cryo vials :**

4.5.1 Allow the freeze-dried strains to attain room temperature before revival.

4.5.2 Disinfect the outer surfaces of the lyophilized culture ampoules/vials using the 70%v/v Iso-propyl alcohol.

4.5.3 In case of vials, open the Aluminum seal first followed by the rubber stopper aseptically.

4.5.4 In case of the ampoules, break open the unit follow the instructions as mentioned in 5.4.

4.5.5 Aseptically add 0.3 mL of sterile Soyabean casein digest broth and mix well.

4.5.6 Take a loop full of culture from above and streak onto agar plate containing soyabean casein digest agar. Transfer the whole mixture to a sterile test tube containing 20 mL of Soyabean casein digest broth. The agar plate shall be used for purity check and the broth is used as a backup in case the streaked plate does not show growth. Incubate both SCDB and SCDA at 30-35°C for 24 – 48 hrs. This is considered as the first transfer (T1).

4.5.7 Check the agar plate for purity by Gram staining as per the SOP. Pick up isolated colony and perform gram staining followed by species level identification. Simultaneously pick isolated colony and inoculate into 100mL of the Soyabean casein digest medium with 10 % glycerol. Incubate the tube at 30-35°C for 24 – 48 hrs. This is the second transfer. (T2). Document the details in Culture Maintenance Record.

4.5.8 In case of anaerobes, incubate the plate in anaerobic jar to maintain anaerobic conditions.

4.5.9 In case of molds, use SDB for enrichment, use SDA for sub culturing further incubate the plates at 20-25°C for 3 to 5 days.

4.5.10 In case of Fungal Streak loop full of culture on to the Agar Slant and further incubate the slants at 20-25°C for 3 to 5 days.

4.5.11 Post completion of incubation period, verify the visual growth and transfer 1 mL of culture into sterile cryovials. Label the vials as per the Cryovial Label and store at -30°C or below.

4.5.12 During the usage of cryovials, purity of the culture to be verified by streaking a loop full of culture from cryovial parallelly on to suitable media.

4.5.13 Verify the purity of the culture by staining and check the plate for the pure culture. If mixed colonies found on the plate, then subject the colonies for species level identification for further confirmation.

4.5.14 If cryovial found contaminated discard the entire lot and prepare freshly.

Note: The frozen cryovials should not be used for more than one year.

4.6 **Cryovial preparation for In-house isolates**:

4.6.1 Take the colony from the master plate and streak on to SCDA/R2A plate (Use R2A for water isolates) (consider this as T1 passage) and subject it for identification.

4.6.2 Post completion of identification, take an isolated colony from T1 plate and streak on to the SCDA/R2A plate (Use R2A for water isolates) and inoculate a loop full of culture in to sterile 100 mL SCDM with 10 % glycerol (consider this as T2 passage). Incubate both tube and plate at 30-35°C for 24 to 48 hrs. Agar plate shall be used for purity check and broth tube shall be further used for cryovial preparation.

4.6.3 In case of molds, use SDB for enrichment, use SDA for sub culturing further incubate the plates at 20-25°C for 3 to 5 days.

4.6.4 In case of Fungal Streak loop full of culture on to the Agar Slant and further incubate the slants at 20-25°C for 3 to 5 days.

4.6.5 Post completion of incubation period, verify the visual growth and transfer 1 mL of culture into sterile cryovials. Label the vials as per the Cryovial Label and store at -30°C or below.

4.6.6 During the usage of cryovials, purity of the culture to be verified by streaking a loop full of culture from cryovial parallelly on to suitable media.

4.6.7 Verify the purity of the culture by staining and check the plate for the pure culture. If mixed colonies found on the plate, then subject the colonies for species level identification for further confirmation.

4.6.8 If cryovial found contaminated discard the entire lot and prepare freshly.

Note: The frozen cryovials should not be used for more than one year.

4.7 **Determination of inoculum concentration**.

4.7.1 Take 0.1 mL or loop full of culture from the cryovials (T2 cultures) and inoculate in to the 10 mL SCDM (for enrichment) and streak a loop full of culture on to Soyabean casein digest agar plate (for purity check, in case of cryovial). Use SDB for yeast and molds.

4.7.2 Document the purity check and gram staining details in culture maintenance record.

4.7.3 Incubate bacterial culture at 30-35℃ for 18 – 24 hrs and yeast & mold cultures at 20-25℃ for 24- 48 hrs.

4.7.4 Determine the inoculum concentration to obtain NMT 100 CFU/0.1 mL by making serial dilutions from the above enrichment tube. Take 1 mL from the enrichment tube and add into 9 mL of sterile saline solution (10^{-1} dilution) likewise do the serial dilution up to 10^{-8}.

Dilution Level	Volume of sterile saline	Volume of culture
10^{-1}	9 mL	1 mL from enrichment tube
10^{-2}	9 mL	1 mL from 10^{-1}
10^{-3}	9 mL	1 mL from 10^{-2}
10^{-4}	9 mL	1 mL from 10^{-3}
10^{-5}	9 mL	1 mL from 10^{-4}
10^{-6}	9 mL	1 mL from 10^{-5}
10^{-7}	9 mL	1 mL from 10^{-6}
10^{-8}	9 mL	1 mL from 10^{-7}

4.7.5 Plate out the dilutions from 10^{-4} to 10^{-7} (or to obtain NMT 100 cfu) in duplicates using Soyabean casein digest agar.

Note: Plating can be done by pour plate method using 1 mL of above suspension or spread plate method by using 0.1 mL of above suspension.

4.7.6 Use R2A media for water isolates and use SDA in case of yeast & molds.

4.7.7 Incubate the plates at 30-35℃ for 18 – 72 hrs for bacteria and at 20-25℃ for 3 to 5 days for yeast & molds. Document the details in Record for Determination of inoculum concentration.

4.7.8 Select a suspension of NMT 100 CFU/0.1 mL for routine laboratory use and label the dilutions as per the culture suspension label.

4.7.9 Assign the reference number as mentioned below to each inoculum determination.

For example: IS/YY/0001

IS-Inoculum Suspension

YY denotes last two digits of current year.

NNNN denotes serial number starting from 0001

4.7.10 Preserve the in-house suspension (NMT 100 CFU / 0.1 mL) of each organism at 2-8℃ for not more than 5 days from the date of standardization.

Note: The frozen cryovials should not be used for more than one year.

5.0 GLOSSARY

Nil

6.0 ABBREVIATIONS

6.1 °C : Degree centigrade

6.2 CFU : Colony Forming Unit

6.3 COA : Certificate of Analysis.

6.4 hrs : Hours.

6.5 MB : Microbiology

6.6 mL : mili litter

6.7 NMT : Not More Than

6.8 NLT : Not Less Than

6.9 QA : Quality Assurance

6.10 SCDA : Soyabean Casein Digest Agar

6.11 SCDB : Soyabean Casein Digest Medium

6.12 SDA : Sabouraud Dextrose Agar

6.13 SDB : Sabouraud Dextrose Broth

6.14 SOP : Standard Operating Procedure

7.0 FORMAT(S)

S.No.	Formats
1	Culture maintenance Record
2	Record of Determination of Inoculum Concentration
3	Cryovial Label
4	Culture Suspension Label
5	Culture Receipt Label
6	Culture Stock card

8.0 REFERENCES

8.1 Handling of Culture and Media Spillage in Microbiology Laboratory

8.2 Procedure for Isolation Identification and Handling of Isolates.

9.0 CHANGE HISTORY

9.1 New SOP

END OF DOCUMENT

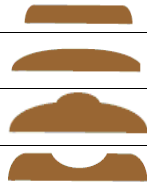
TITLE	CULTURE MAINTENANCE RECORD

Name of the Culture		Date of Receipt/ Identification	
Source		Expiry Date	
ATCC/ Equivalent ATCC No./Isolate No.			
Total Number of Vials received			

FIRST TRANSFER (T1) – REHYDRATION OF LYOPHILISED CULTURE

Media for Rehydration		Lot No./Expiry Date	
Quantity of Media used			
Media for purity check		Lot No./Expiry Date	
Incubation Temperature		Incubator ID	
Incubation start date/time		Done By(Sign/Date)	
Incubation end date/time		Observed By (Sign/Date)	
Observation	Growth/No growth	Gram Stain	
Reviewed By(Sign/Date):			

OBSERVATION OF COLONY CHARACTERISTICS:

Colour :			
S.No	Morphology		Observation
1	Shape		
a.	Circular		
b.	Irregular		
c.	Filamentous		
d.	Rhizoid		
e	Spindle		
2.	Elevation		
a.	Flat		
b.	Raised		
c.	Umbonate		
d.	Crateriform		
e.	Convex		
f.	Pulvinate		
3.	Margin		
a.	Entire		
b.	Undulate		
c.	Lobate		

d.	Curled		
e.	Filiform		
4.	Size		
a.	Punctiform		
b.	Small		
c.	Medium		
d.	Large		
5.	Surface		
a.	Smooth		
b.	Rough		
c.	Wrinkled		
d.	Dry powder		
Done by (Sign/Date)		Reviewed by (Sign/Date)	

PURITY CHECK BY ID SYSTEM

Name of the Organism: _____

Instrument used for Identification/Instrument ID.: _____

Done by:
Sign/date:

Reviewed by:
Sign/date:

SECOND TRANSFER (T2) FOR CRYOVIAL PREPARATION

Name of the Media		Lot No./Expiry Date	
Quantity of Media used for Cryovial Preparation			
Incubation Temperature		Incubator ID	
Incubation start date		Done By (Sign/Date)	
Incubation end date		Observation	Growth/No growth
No. of Cryovials Prepared			
Date Of Preparation		Valid Up To	
Prepared by (Sign/Date)		Reviewed by (Sign/Date)	

TITLE	CULTURE MAINTENANCE RECORD

Cryovial No.	Media Used for purity check	Media Lot No.	Done by	Observation	Gram stain	Observed by	Reviewed by

TITLE	RECORD OF DETERMINATION OF INOCULUM CONCENTRATION

1. Sub culturing Details:

Date of sub culturing		Date of observation	
Name of the organism		Inoculum ID number	
Enrichment media Name		Lot number	
Source		Valid up to	
Incubation start date & time		Incubation end date & time	

Added _____ of culture from cryovial / plate and incubated at _____ temperature and growth observed / not observed.

Done by:
Sign & Date

Observed by:
Sign & Date

2. Culture Suspension Details:

Date of serial dilution		Date of release	
Media used		Lot No & Exp date	
Diluent used		Lot No & valid up to	
Incubation start date & time		Incubation end date & time	
Analyzed by (Sign & Date)			

Dilution level	Plate count		Average	Observed by
	Plate-1	Plate-2		

Selected dilution & Concentration: _____

Remarks:

Reviewed by:
Sign & Date

TITLE	CRYOVIAL LABEL

CRYOVIAL LABEL	
Name of Organism	
Date of Preparation	
Vial Number	
Expiry Date	
Prepared by	

TITLE	CULTURE SUSPENSION LABEL

Culture Suspension	
Name of Organism	
Culture Suspension Ref. No:	
Dilution/Concentration	
Valid Up to	
Storage condition	2-8℃
Prepared By	

TITLE	CULTURE RECEIPT LABEL

CULTURE RECEIPT LABEL	
Name of Organism	
Strain Number	
Transfer /Passage Number	
Number of vials	
Date of Receipt	
Storage Condition	
Received by	

TITLE	CULTURE STOCK CARD

Name of Organism:		Strain No:	
Date of Receipt		Quantity Received	
Storage Temperature		Expiry Date:	
Received By (Sign & Date)			

Date of Issuance	Used Quantity	Used For	Balance Quantity	Used By	Reviewed By	Remarks