STANDARD OPERATING PROCEDURE

Title: Handling, Maintenance and Use of Microbial Culture				
SOP No.:		**Department:**	Microbiology	
		Effective Date:		
Revision No.:	00	**Revision Date:**		
Supersede Revision No.:	Nil	**Page No.:**	1 of 12	

1.0 OBJECTIVE

To lay down procedure for handling, maintenance and use of microbial cultures.

2.0 SCOPE

This SOP is applicable for all the microbial cultures used in microbiology laboratory.

3.0 RESPONSIBILITY

Prepared by - Executive Microbiology

Checked by - Assistant Manager Microbiology / QC

Approved by - Head QA, QC

4.0 PROCEDURE

4.1 Procurement and Maintenance of Lyophilized Cultures

4.1.1 Cultures can be procured from any of the following authorized suppliers -

4.1.1.1 **ATCC:** American Type Culture Collection, 10801, University Blvd, Manassas, VA20110, USA.

4.1.1.2 **NCIM:** National Collection of Industrial Microorganisms, National Chemical Laboratory,

Pune, 411 008, INDIA.

4.1.1.3 **MTCC:** IMTECH Institute of Microbial Technology, Sector 39 - A, Chandigarh, INDIA.

4.1.1.4 **NCTC:** National Collection of Type Cultures, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT, UK.

4.1.2 On receipt of a lyophilized reference culture from an approved vendor, assign a Lot Number and serial number to the culture.

4.1.3 The Lot number / Serial number will be "**A/S-1**" if the culture is received for the first time; "**B/S-1**" for second time; "**C/S-1**" for third time and so on……

4.1.4 After receiving the lyophilized cultures from the supplier and assigning lot number enter the relevant details into the culture information record sheet as per Annexure -I.

41.5 Sterilize cleaned 10 ml stoppered test tubes (double wrapped with aluminum foil) by dry heat sterilizer at validated temperature for validated time.

4.1.6 After sterilization arrange the required items in the laminar airflow unit.

4.1.7 Take out all the ampoules having lyophilized cultures and place it in the laminar airflow unit.

4.1.8 Wipe the outer surface of the ampoule with 70 % filtered IPA taking care not to reach inside the ampoule.

4.1.9 Cut the ampoule at the neck portion of the ampoule.

4.1.10 With the help of a sterile forceps take out the cotton plug from the ampoule.

4.1.11 Transfer the lyophilized culture from the ampoule to the sterilized stoppered test tube carefully and close the stopper of the test tube.

4.1.12 Wrap the test tube with Para film.

4.1.13 Store the transferred lyophilized culture test tubes at - 10^0C - 30^0C in the deep freezer.

4.1.14 Label the lyophilized culture test tubes as per Annexure - II.

4.1.15 Frequency - Whenever new lyophilized culture is received.

4.1.16 Use Before Date: Lyophilized culture can be used for one year from the date of transferring into lyophilized test tube.

4.2 Reviving of Lyophilized Cultures

4.2.1 For reviving of lyophilized cultures prepare the media as per Table - I.

Table -I
Reviving of Lyophilized Culture
Growth Medium, Incubation Temperature and Time

Culture Organisms	MTCC No.	Growth Medium	Incubation temperature/time
Escherichia coli	MTCC 1687	Soyabean Casein Digest Medium (SCDM)	32.5 ± 2.5°C for 48 - 72 hrs
Salmonella choleraesuis	MTCC 3858	Soyabean Casein Digest Medium (SCDM)	32.5 ± 2.5°C for 48 - 72 hrs
Staphylococcus aureus	MTCC 737	Soyabean Casein Digest Medium (SCDM)	32.5 ± 2.5°C for 48 - 72 hrs
Pseudomonas aeruginosa	MTCC 1688	Soyabean Casein Digest Medium (SCDM)	32.5 ± 2.5°C for 48 - 72 hrs
Bacillus subtilis	MTCC 441	Soyabean Casein Digest Medium (SCDM)	32.5 ± 2.5°C for 48 - 72 hrs
Candida albicans	MTCC 227	Soyabean Casein Digest Medium (SCDM)	22.5 ± 2.5°C for 72 - 120 hrs
Aspergillus niger	MTCC 1344	Soyabean Casein Digest Medium (SCDM)	22.5 ± 2.5°C for 72 - 120 hrs
Clostridium sporogenes	MTCC 1349	Fluid Thioglycollate Medium (FTGM)	32.5 ± 2.5°C for 48 - 72 hrs
Staphylococcus epidermidis	MTCC 3615	Soyabean Casein Digest Medium (SCDM)	32.5 ± 2.5°C for 48 - 72 hrs

4.2.2 Prepare and sterilized the media mentioned in Table - I as per SOP.

4.2.2 After sterilization arrange the required items in the laminar airflow unit along with the prepared and sterilized media as per Table - I.

4.2.3 Take out all the test tubes having lyophilized cultures and place it in the laminar airflow unit.

4.2.4 Wipe the outer surface of the test tubes with 70 % filtered IPA taking care not to reach the test tubes.

4.2.5 Red hot the transferring loop and cools it.

4.2.6 Take out a small amount of lyophilized culture from the test tube respectively and inoculate it in soyabean casein digest medium for all the aerobic bacterial, yeast / molds cultures.

4.2.7 In case of anaerobic organisms inoculate the small amount of lyophilized culture in fluid thioglycollate medium.

4.2.8 After transferring from the lyophilized culture from the test tubes again seal the test tube with Para film and assign the serial number.

4.2.9 The Lot number / Serial number will be "A/S-2" if the culture is received for the first time; "B/S-2" for second time; "C/S-2" for third time and so on……

4.2.10 Store the above test tube at - 10^0C to - 30^0C in the deep freezer.

4.2.11 Incubate the bacterial and fungal culture at respective temperature and time as mentioned in Table - I.

4.2.12 After completion of incubation period spread out

4.2.13 After the growth is observed in all the tubes for bacteria and fungi spread out a loop full of suspension from each tube to culture media plates as mentioned in table - II.

4.2.14 Incubate the bacterial and fungal culture at respective temperature and time as mentioned in Table – II

4.2.15 After completion of incubation period check for the purity of the culture by doing simple Gram staining techniques.

4.2.16 After checking the purity store the above inoculated medium as stock solution for further sub culturing on to sterile slants (stock and working culture).

4.2.17 Enter the details of reviving, incubation period, medium used, temperature and gram staining of cultures in Annexure - III.

4.2.18 Frequency - Whenever new lyophilized culture is received and after completion of five passage of culture organisms to get again first passage.

4.3 Sub Culturing

4.3.1 Prepare sufficient number of slants as per Table - II so that each organism will have two slants.

Table -II
Maintenance of Cultures
Growth Medium, Incubation Temperature and Time

Culture Organisms	MTCC No.	Growth Medium	Incubation temperature/time
Escherichia coli	MTCC 1687	Soyabean Casein Digest Agar (SCDA)	32.5 ± 2.5°C for 48 - 72 hrs
Salmonella choleraesuis	MTCC 3858	Soyabean Casein Digest Agar (SCDA)	32.5 ± 2.5°C for 48 - 72 hrs
Staphylococcus aureus	MTCC 737	Soyabean Casein Digest Agar (SCDA)	32.5 ± 2.5°C for 48 - 72 hrs
Pseudomonas aeruginosa	MTCC 1688	Soyabean Casein Digest Agar (SCDA)	32.5 ± 2.5°C for 48 - 72 hrs
Bacillus subtilis	MTCC 441	Soyabean Casein Digest Agar (SCDA)	32.5 ± 2.5°C for 48 - 72 hrs
Candida albicans	MTCC 227	Sabourauds dextrose Agar (SDA)	22.5 ± 2.5°C for 72 - 120 hrs
Aspergillus niger	MTCC 1344	Potato Dextrose Agar (PDA)	22.5 ± 2.5°C for 72 - 120 hrs
Clostridium sporogenes	MTCC 1349	Cooked Meat Medium (CMM)	32.5 ± 2.5°C for 48 - 72 hrs
Staphylococcus epidermides	MTCC 3615	Soyabean Casein Digest Agar (SCDA)	32.5 ± 2.5°C for 48 - 72 hrs

4.3.2 Prepare and sterilize the media mentioned in Table - II as per SOP.

4.3.3 Preincubate the prepared slants at 32.5 ± 2.5°C for 24 to 48 hours.

4.3.4 After the Preincubation, examine the slants and discard the contaminated slants.

4.3.5 Perform the entire activity under laminar airflow unit.

4.3.6 Maintain two tubes (slants) for each organism.

4.3.7 Remove the stock solution from refrigerator and allow it to come to room temperature.

4.3.8 Red-hot the transferring loops and cools it.

4.3.9 Take a loopful of culture from the stock solution and streak on the slanting portion of the new fresh slant.

4.3.10 Repeat the step using another new fresh slant.

4.3.11 Label one slant as 'Stock Culture' and other as 'Working Culture'.

4.3.12 Use the 'Stock Culture' for further sub culturing and 'Working Culture' for routine lab use.

4.3.13 Label the 'Stock Culture' and 'Working Culture' slant as per Annexure - IV.

4.3.15 Incubate the bacterial and fungal culture at respective temperature and time as mentioned in table - II.

4.3.16 After completion of incubation store the stock culture and working culture in refrigerator at 2 to 8°C.

4.3.17 Frequency: Once in 15 days

4.4 Assigning Passage Number to Subcultures

4.4.1 On receipt of a lyophilized reference culture from an Approved vendor, assign a Lot Number to the culture.

4.4.2 The Lot Number will be "A/S-1" if the culture is received for the first time; "B/S-1" for second time; "C/S-1" for third time and so on……..

4.4.3 To the Ist subcultures, starting from the reference cultures, assign the Passage Number as A-S / S-1 /P-1,

Where - A-S - (A- Lot No. of the lyophilized culture and S - Stock culture)

S-1 - Serial number of the lyophilized culture of lot A.

P-1 - Passage number.

4.4.4 This subculture may be further sub-cultured up to 4 times (Total Not more than 5 times starting from Reference Cultures).

4.4.5 The Passage Number for 2nd Subculture will be assigned as A-S /S-1/P-2, and can go upto A-S/S-1/P-5.

4.4.6 After the 5 Passages, again start the subculture from the reference culture and assign the Passage number as A-S/S-2/P-1 and can go up to A-S /S-2/P-5.

4.4.7 In the same way label the working culture as A-W / S-1 /P-1,

Where - A-W - (A- Lot No. of the lyophilized culture and W - Working culture)

S-1 - Serial number of the Lyophilized culture of lot A.

P-1 - Passage number

4.4.8 Record the details in sub culturing record as per Annexure - V.

5.0 SAFETY & PRECAUTIONS

5.1 Handle the culture very care fully and in case of any spillage handle the spillage as per SOP.

5.2 Discard the used or expired microbial culture by validated disposal procedure in autoclave as per SOP.

6.0 REVISION HISTORY

Revision No.	Reason for Revision	Superseded from & date
00	First Issue	--------

7.0 REFERENCES

SOP .

8.0 ABBREVIATIONS

SOP	:	Standard Operating Procedure
No.	:	Number
mL	:	Milliliter
%	:	Percentage
°C	:	Degree Centigrade
IPA	:	Isopropyl Alcohol

9.0 ANNEXURES

Annexure - I : Culture information record sheet

Annexure - II : Lyophilized culture label

Annexure - III : Record sheet for reviving of lyophilized cultures

Annexure - IV : Working / Stock culture label

Annexure - V : Sub culturing record sheet

ANNEXURE - I
CULTURE INFORMATION RECORD SHEET

Name of the organism	Source	MTCC No./ Equivalent ATCC No.	Date of Receipt	Preservation Temperature (°C)	Date of Preservation	Preservation Done By	Lot No. /Sr. No.	Remarks

Checked By:
(Date & Sign)

ANNEXURE - II
LYOPHILIZED CULTURE LABEL

Lyophilized Culture Label

Name of organism: _____

Source: _____

MTCC/ATCC no.: _____

Date of receipt: _____

Date of preservation: _____

Assigned lot no./Sr no.: _____

Use Before date: _____ Sign: _____

ANNEXURE - III
RECORD SHEET FOR REVIVING OF LYOPHYLIZED CULTURES

Name of the Organism	Lot No./ Sr. No.	Media used for reviving	Sterilized Media Lot No.	Inoculation Done on/by (Date & Sign)	Incubation Temperature & time	Observation	Observation Done on/by (Date & Sign)	Gram Staining Observation	Gram Staining Observation Done on/by (Date & Sign)

Checked By:
(Date & Sign)

ANNEXURE - IV

WORKING / STOCK CULTURE LABEL

Working / Stock Culture Label

Name of organism: _____

MTCC/ATCC no.: _____

Date of sub culturing: _____

Result obtain on: _____

Passage no.: _____

Valid up to: _____Use Before date: _____

Due date of sub culturing: _____ Sign: _____

ANNEXURE - V

RECORD SHEET FOR REVIVING OF LYOPHYLIZED CULTURES

Name of the Organism: _____ MTCC/ATCC No: _____

Lot No./Sr. No. Of Lyophilized Culture: _____

Date of Subculturing	Media used for Subculturing	Sterilized Media Lot No.	Incubation Temperature & time	Result Obtain on (Date & Sign)	Sub culturing detail			Passage No.		Checked On/by (Date & Sign)
					Valid up to	Use before date	Due date of sub culturing	Stock Culture	Working culture	