



# PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

## STANDARD OPERATING PROCEDURE

**Title:** Procedure for Procurement, Transfer, Preservation and Disposal of Microbiological Culture

<b>SOP No.:</b>		<b>Department:</b>	Microbiology
		<b>Effective Date:</b>	
<b>Revision No.:</b>	00	<b>Revision Date:</b>	
<b>Supersede Revision No.:</b>	Nil	<b>Page No.:</b>	1 of 11

1. **Purpose:** The purpose of this SOP is to describe procedure for Procurement, Transfer, Preservation & Disposal of microbiological cultures.
2. **Scope:** This procedure is applicable to Bacterial and Fungal cultures used in the microbiology laboratory.
3. **References, Attachments & Annexures:**
  - 3.1 **References:**
    - 3.1.1 USP Chapter <61> (Microbiological examination of non sterile products:Microbial enumeration test)
    - 3.1.2 USP Chapter <51> (Anti Microbial Effectiveness Testing )
    - 3.1.3 SOP “Receipt, Storage, Preparation,Growth Promotion Test,Use And Disposal of Microbiological Media”
  - 3.2 **Attachments:**
    - 3.2.1 Attachment -1: Culture Transfer Record
    - 3.2.2 Attachment -2:Flow chart- culture transfer procedure for lyophilized form culture
    - 3.2.3 Attachment -3: Culture Qualification Record
    - 3.2.4 Attachment-4:Flow chart- culture transfer procedure for unlyophilized (slant/stab) form culture.
  - 3.3 **Annexures:** None
4. **Responsibilities:**
  - 4.1 **Microbiologist:**
    - 4.1.1 To perform the activity as per SOP
    - 4.1.2 To maintain the records as per SOP
  - 4.2 **QC Head or designee:**
    - 4.2.1 To check the SOP
    - 4.2.2 To give the training to all concern persons
  - 4.3 **Quality Assurance:**
    - 4.3.1 To check the SOP



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4.3.2 To ensure proper implementation of SOP

4.4 **Regulatory Affairs, Quality Head , Plant Head:**

4.4.1 To review and approve the SOP.

### 5. Distribution:

5.1 Quality Assurance

5.2 Quality control (Microbiology Section).

### 6. Abbreviations & Definition of Terms:

#### 6.1 Abbreviations:

6.1.1 I.M.T : Institute of Microbial Technology

6.1.2 C.D.L: Central Drugs Laboratory

6.1.3 MTCC : Microbial Type Culture Collection

6.1.4 NTCC : National Type Culture Collection Center.

6.1.5 NCIM : National Collection of Industrial Microorganisms

6.1.6 NCL: National Chemical Laboratory

6.1.7 SDA : Sabdouraud Dextrose Agar

6.1.8 SCDA : Soyabean Casein Digest Agar

6.1.9 ATCC : American type culture collection

6.1.10 NCTC : National type culture collection

#### 6.2 Definition of Terms:

6.2.1 **SOP** : Standard Operating Procedure

6.2.2 **Colony** : A colony is a pile or mass of a sufficiently large number of cells, growing on or in solid medium that they are visible to the naked eye.

6.2.3 **Passage** : One passage is defined as the transfer of organisms from an established culture to fresh medium. Any form of subculturing is considered to be a transfer/passage.



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### 7. Procedure:

#### 7.1 Culture procurement:

- 7.1.1 Procure the microbial culture required for test methods either from .....
- 7.1.2 The culture received may be either in lyophilized or unlyophilized form which shall be termed as mother culture.
- 7.1.3 Each and every culture received should be given in house identification number. For example :E. coli received on 07/09/24 (DD/MM/YY), then given in - house number should be E.coli070924.
- 7.1.4 The viable microorganisms used in the test must not be more than five passages removed from the original culture.
- 7.1.5 Use culture media *as per supplier's recommendation.*

#### 7.2 Culture revival & Purity check (Culture qualification):

- 7.2.1 The culture received are pure culture where all cells in the population are identical in sense that they come from same parent cell.
- 7.2.2 Mother culture shall be revived as per supplier's instruction .
- 7.2.3 Every fourth month new mother cultures of each culture shall be required for lyophilized form culture.
- 7.2.4 Every third month new mother cultures of each culture shall be required for unlyophilized (slat/swab) form culture.
- 7.2.5 As per supplier's instruction, streak drop/s of the suspension onto recommended agar medium in petri plates for checking morphological colony characteristics.
- 7.2.6 Streaking on agar slants shall be done simultaneously to prepare stock cultures.
- 7.2.7 Check the purity of the culture by gram staining, morphological & biochemical tests or using identification.
- 7.2.8 Record the results in culture qualification record *as per Attachment-3*

#### 7.3 Culture transfer & preservation for lyophilized form culture:

- 7.3.1 Frequency: Stock culture: Once in a month.

#### 7.4 Procedure for First Month:

- 7.4.1 Take a loopful of culture from mother culture & streak on two slants of required media as per supplier's recommendation. If supplier's recommendation is not given then follow *as mentioned in below table.*

**Note:** If we are not following supplier's recommendation's then we can follow below table.

- 7.4.2 Label these slants as 1A and 1B. (First passage)
- 7.4.3 Identify 1A as primary working culture and 1B as stock culture.
- 7.4.4 Incubate these slants for time and temperature as mentioned in below table.



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Cultures medium	Organisms	Incubation temperature and time
Tryptone soya Agar slants (SCDA)/ Plate count agar slants / Nutrient Agar	<i>Bacillus subtilis</i> <i>Escherichia coli</i> <i>Salmonella abony</i> <i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i>	30-35 <sup>0</sup> C for 18-24 hrs
Sabourauds Dextrose Agar (SDA)	<i>Candida albicans</i>	20 <sup>0</sup> C - 25 <sup>0</sup> C for 44 -52 hrs
Sabourauds Dextrose Agar (SDA)	<i>Aspergillus niger</i>	20 <sup>0</sup> C - 25 <sup>0</sup> C for 6 to10 days.

- 7.4.5 Check the slants for growth.
- 7.4.6 If growth is confluent then wrap the tube 1B (stock culture) with parafilm and store in refrigerator at 2-8 °C which shall be used in next month.
- 7.4.7 Proceed as mentioned below for preparation of secondary working cultures from 1A.
- 7.4.8 From 1A (primary working culture) subculture in a broth or slant media as and when required.
- 7.4.9 If the culture is required second time in the same month, make fresh transfer from primary working culture. The primary working culture to be used up to one month
- 7.4.10 Identify first sub-transfer as 1A/1 and second sub-transfer as 1A/2 (secondary working cultures)
- 7.4.11 Follow the incubation and preservation procedure as mentioned for stock culture.
- 7.4.12 Dispose the secondary working culture after use.
- 7.4.13 Record the culture transfer and disposal details as per attachment-1.

### 7.5 Procedure for second month:

- 7.5.1 At the end of the first month, remove the 1B (stock culture) from refrigerator and allow it to attain room temperature.
- 7.5.2 Take a loopful of culture from 1B and streak on two slants of required media.
- 7.5.3 Label these slants as 2A and 2B.
- 7.5.4 Identify 2A as primary working culture and 2B as stock culture.
- 7.5.5 Incubate these slants for time and temperature as mentioned in the above table.
- 7.5.6 Check the slants for growth.



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- 7.5.7 If growth is confluent then wrap the tube 2B (stock culture) with parafilm and store in refrigerator at 2-8°C which shall be used in next month.
- 7.5.8 Dispose the primary working culture (1A) and stock culture(1B) at the end of the first month/ start of second month. Record the disposal details in attachment-1.
- 7.5.9 Proceed as mentioned below for preparation of secondary working cultures from 2A.
- 7.5.10 From 2A (primary working culture) subculture in a broth or slant media as and when required.
- 7.5.11 If the culture is required second time in the same month, make fresh transfer from primary working culture. The primary working culture to be used up to one month.
- 7.5.12 Identify first sub-transfer as 2A/1 second sub-transfer as 2A/2 (secondary working cultures)
- 7.5.13 Dispose the secondary working culture after use.

**7.6 Procedure for third and fourth month:**

- 7.6.1 Procedure described for second month shall be followed for remaining months.
- 7.6.2 Numbering system for stock culture, primary culture and secondary culture shall be followed as mentioned in the flow chart (Attachment-2 & 4) for the remaining months
- 7.6.3 Maintain the culture transfer and disposal record for each culture as per attachment-1.
- 7.6.4 Do not make more then four sub-transfers from the mother culture since the viable microorganisms used in the test must not be more than five passages removed from the original ATCC culture.
- 7.6.5 Working and stock culture should be kept separately.

**7.7 Culture transfer & preservation for unlyophilized (slant/stab)form culture:**

- 7.7.1 Frequency: Stock culture: Once in a month.
- 7.7.2 The first passage will be considered at suppliers end , and the subsequent passage will be followed as per the procedure mentioned under point 7.4
- 7.7.3 Numbering system for stock culture, primary culture & secondary culture shall be followed as mentioned in the flow chart (Attachment-2 & 4)

**Note:**In manufacturing locations where cultures are used only for GPT and are not used for any microbiological assay & for sterile product , in that case the mother culture can be used up to 8 months provided it meets the requirement on requalification. Qualify the mother culture initially on receipt & store it at 2°C to 8°C for 4 months.After 4 months requalify it & if it meets the requirement for qualification then use for further transfer.



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7.8 **Disposal of cultures:** As per SOP No. BSQM/006 “Receipt, Storage, Preparation, Growth Promotion Test, Use And Disposal of Microbiological Media”





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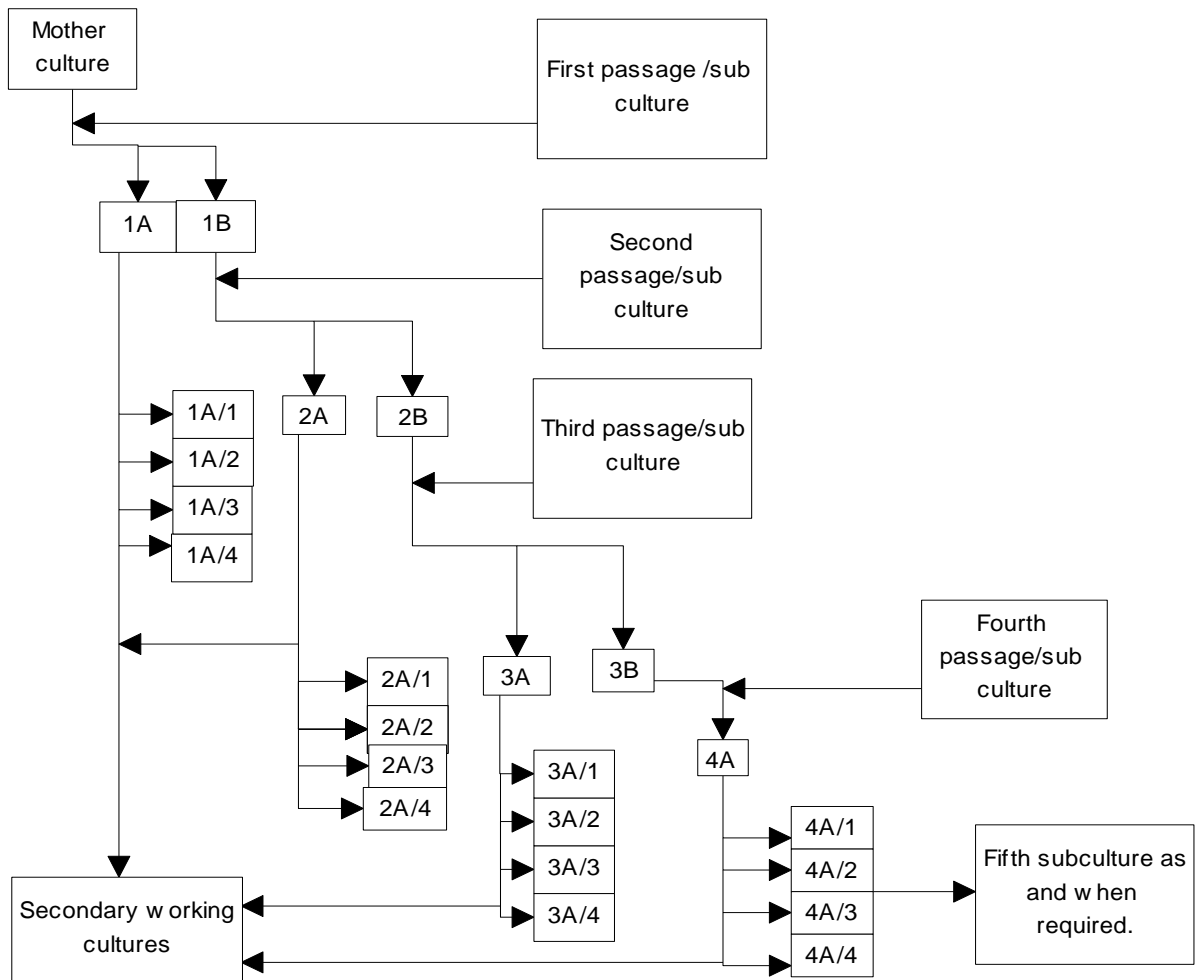
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### Attachment -2 Culture Transfer Record

#### Flow chart- culture transfer procedure for Lyophilized form culture







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### Attachment 3 Culture Qualification Record

**Name of Culture:** \_\_\_\_\_

**ATCC/NCIM/other No.:** \_\_\_\_\_

**1. Morphological details of culture:**

- a) Color:
- b) Shape
- c) Opacity

**2. Purity checking by staining:**

- (a) Gram stains Lot No.:
- (b) Gram's stain detail:
- (c) Lacto phenol cotton blue Lot No.:
- (d) Lacto phenol cotton blue stain detail:

**3. Identification test by inoculation on selective/differential media:**

Name of Media: (1) \_\_\_\_\_ (2) \_\_\_\_\_ (3) \_\_\_\_\_  
Media Lot No. \_\_\_\_\_  
Colony characteristic \_\_\_\_\_

Negative control: \_\_\_\_\_

**4. Biochemical Test:**

- a) Name of biochemical test:
- b) Reagent/Kit used for test:
- c) Reagent/Kit Lot No.:
- d) Result of test:

**Remarks:**

Microbiologist sign /date	Checked By /date	Approved By/date



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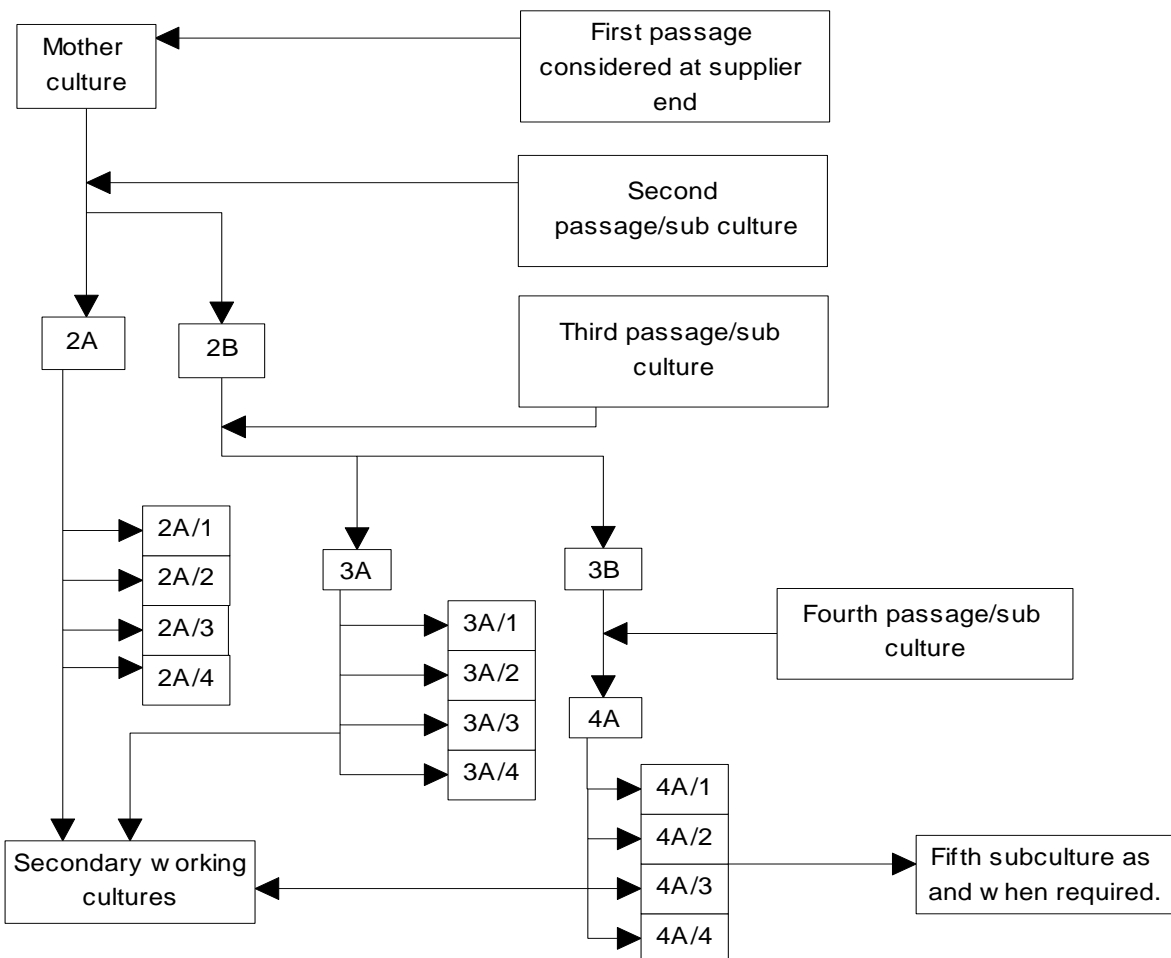
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### Attachment 4

#### Flow chart- culture transfer procedure for unlyophilized (Slant/stab) form





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