PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

Title: Receipt, Storage, Preparation,Growth Promotion Test,Use And Disposal of Microbiological Media

SOP No.:		**Department:**	Microbiology
		Effective Date:	
Revision No.:	00	**Revision Date:**	
Supersede Revision No.:	Nil	**Page No.:**	1 of 14

1. **Purpose:** The purpose of this SOP (Standard Operating Procedure) is to define the Procedure for the receipt, storage, preparation, growth promotion test,use and disposal of microbiological media used during microbiological analysis.

2. **Scope:** This SOP (Standard Operating Procedure) is applicable to microbiological media used by microbiology.

3. **References,Attachments & Annexures:**
   - 3.1 **References:**
     - 3.1.1 USP
     - 3.1.2 SOP : Microbial Culture Suspension Preparation, Cell Enumeration, Use, Storage and Destruction.
   - 3.2 **Attachments:**
     - 3.2.1 Attachment-1:Media consumption record
     - 3.2.2 Attachment-2:Media disposal record
     - 3.2.3 Attachment-3:Media Preparation,Sterilization and GPT Record
     - 3.2.4 Attachment-4: List of media with storage condition
     - 3.2.5 Attachment-5: Growth promotion recovery test of media
     - 3.2.6 Attachment-6: Media stock record
   - 3.3 **Annexures:**
     - 3.3.1 Annexure-1:List of media,organisms and incubation conditions for GPT.

4. **Responsibilities:**
   - 4.1 **Microbiologist:**
     - 4.1.1 To perform the activity as per SOP.
     - 4.1.2 To maintain all the records as per SOP.
   - 4.2 **QC Head or designee:**
     - 4.2.1 To check the SOP.
     - 4.2.2 To give training to all concerned persons before implementation of SOP.
     - 4.2.3 To ensure proper documentation as per SOP.
   - 4.3 **Quality Assurance:**
     - 4.3.1 To check the SOP.

4.3.2 To ensure the implementation of system as per 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 & Definitions of Terms:

6.1 **Abbreviations:**

6.1.1 **GPT :** Growth promotion test.

6.1.2 **CFU :** Colony forming unit.

6.1.3 **SOP :** Standard Operating Procedure

6.1.4 **ATCC :** American type culture collection.

6.1.5 **R2A :** Reasoner's 2A

6.1.6 **NCTC :** National collection type culture.

6.1.7 **USP :** United state pharmacopoeia.

6.1.8 **Lbs :** The traditional symbol stands for *libra*, the Latin word for the unit pound

6.1.9 **ATCC :** American Type Collection Culture

6.2 **Definitions of Terms:**

6.2.1 **Growth promotion test (GPT):** Also referred to as fertility or nutritive properties test, which is performed on the media used during different tests like sterility test, microbial limit test, preservative efficacy test etc to demonstrate that it is capable of supporting the growth of micro-organisms.

6.2.2 **Colony forming unit (CFU):** Visible outcome of growth of micro-organisms arising from a single or multiple cells.

7. Procedure:

7.1 Receipt of media:

7.1.1 On the receipt of the media, check for the expiry date of the media and mention the date of receipt on the label of media container.

7.1.2 Date of opening with sign shall be written while opening the container.

7.1.3 After receipt of the media,make necessary entries in media stock record as per attachment-6 and maintain the record of media consumption as per attachment-1..

7.1.4 After opening the media container, the media shall be used up to shelf life defined on media container.

7.2 Storage of dehydrated media:

7.2.1 The dehydrated media are highly hygroscopic and shall be stored as per the supplier's storage condition mentioned on label.

7.2.2 Temperature monitoring of media storage room shall be done with minimum/maximum digital thermo hygrometer and record shall be maintained.

7.3 Media preparation:

7.3.1 Dissolve the specified amount as mentioned on the label of dehydrated media in required volume of purified water as mentioned on the label of dehydrated media container.

7.3.2 Add 1% w/v of glycerol AR in the agar media before sterilization to avoid drying of media during exposure/ incubation.

7.3.3 Plug the mouth of the conical flask containing dissolved media in case of agar medium with non-absorbent cotton plugs or stericaps and wrap with aluminium foil.

7.3.4 After adding the dehydrated media in the purified water it should be sterilized within 1 hour.

7.3.5 For slant preparation, distribute about 5 - 7 ml of molten media in each tube and plug it.

Note: While distribution of media before sterilization, ensure that agar is molten / dissolved and mixed properly which otherwise shall affect solidification process.

7.3.6 For the liquid media preparation, appropriate volume shall be dispensed either in test tube or in conical flask as required.

7.3.7 Test tube or flask mouth shall be closed using non-absorbent cotton plugs /stericaps or plastic caps/stainless steel caps.

7.4 pH check:

7.4.1 Transfer about 10 to 15 ml of dissolved media in test tube or beaker.

7.4.2 Check pH of the medium before sterilization, if required adjust the pH with the help of 1N or 0.1N Hcl or NaOH solutions.

7.4.3 Record pH check/adjustment details in media preparation record.

7.5 **Media sterilization and pouring:**

7.5.1 Sterilize the media by autoclaving at 121°C, 15 lbs. pressure for 15 minutes. or as per media preparation method described by media manufacturer.

Note : For media, where the instruction is given "DO NOT AUTOCLAVE", keep in free-floating steam or in boiling water bath as mentioned on media container.

7.5.2 After sterilization,check the pH of the medium (in separate test tube).

7.5.3 Make necessary entries in media preparation,sterilization and GPT record as per attachment-3.

7.5.4 For agar plates, mix the sterile medium ensuring absence of bubble formation. When it reaches to about 45-50^{0}C temperature (to avoid the formation water droplets on the lids of petri plates) pour approximately 15-20 ml sterile agar media in sterile petri plates under LAF wearing mask and sanitized hand and allow to solidify.

7.5.5 For slants, keep the tubes in slanting position and allow to solidify.

7.5.6 Follow below procedure for RODAC plate preparation if required.

7.5.7 Wear sterile gloves and nose mask before working under the laminar air flow unit.

7.5.8 Disinfect the external surface of the plastic bag of the presterilized empty sterile RODAC plates in laminar airflow. Allow the bag to air dry.

7.5.9 Open the plastic bag and takeout the empty sterile RODAC plate avoiding contact to inner side of the plates.

7.5.10 Pour the prepared medium gently into each RODAC plate till the formation of raised convex surface. Approx 14-15ml of medium shall be required for RODAC plate having 55 mm diameter.

7.5.11 Keep the lid of the RODAC plate open till the media gets solidified.

7.5.12 After solidification, cover the lid on to the RODAC plate and label.

7.6 **Media lot numbering :**

7.6.1 Allot media lot number using prefix M (stands for media),last two digit of year(13 for 2013),and four digit serial number (0001). For Example:First lot number of the media prepared in year 2023 shall be M/23/0001.

7.7 **Pre-incubation:**

7.7.1 Pre-incubation shall be done to those media which are used in pathogen testing,environmental monitoring, water analysis (by membrane filtration),

cleaning validation.(ie. Wherever the pre-incubated media is required, use only pre-incubated media.)

7.7.2 After the media is solidified/cooled container containing media plates/tubes shall be identified with status label as " Media under incubation".

7.7.3 Preincubation for the media to be used for environmental monitoring shall be at 30-35°C for minimum 24 hrs. incubation time.

7.8 **Storage of prepared media:**

7.8.1 Prepared media shall be stored at room temperature after pre incubation.

7.9 **Growth promotion test (GPT) of media:**

7.9.1 GPT is to be performed whenever a new media container is opened for use.

7.9.2 This test is applicable to the media using the cultures and incubation conditions given in Annnexure-1.

7.9.3 Prepare medium as per manufacturer's instruction and separately inoculate ≤100 viable microorganisms/ml in test containers of the medium under test.

7.9.4 For liquid media inoculate the broth with appropriate amount of culture suspension (culture suspension having ≤100 cfu/ml organisms) of selected dilution of the respective organism .

7.9.5 For of agar media, inoculate the media with appropriate amount of culture suspension (culture suspension shall contains ≤100cfu/ml organisms) of selected dilution of the respective organism by pour plate method.

7.9.6 Incubate the plates or tubes as per Annexure-1.

7.9.7 The growth promotion test of the medium complies if it meets acceptance criteria given in attachment-3.

7.9.8 Record the results in media preparation record.

7.9.9 GPT recovery analyzed for every new lot of media.

7.9.9.1 Prepare culture suspension and select the dilution that shows less than 100 CFUs for test organism as per SOP: Microbial Culture Suspension Preparation, Cell Enumeration, Use, Storage and Destruction.

7.9.9.2 For Solid media, add less than 100 CFUs onto the surface, with respective microorganisms, or use the microorganisms that are required for selective media, and spread using a sterile spreader.

7.9.9.3 Incubate the media as per pharmacoepial or routine testing requirements.

7.9.9.4 Record the result in attachment 5. For the media which used for quantification (like Soyabean casein digest agar and Saboraud Dextrose Agar), the % recovery of microorganisms must not differ

by a factor greater than 2 from the value for a standardized inoculum. , i.e not less than 50 % and not more than 200 %.

7.9.9.5 If growth promotion test is not found to be satisfactory, discard the remaining media and invalid the on going test.

7.10 **Use:**

7.10.1 Before use carefully examine the plates for contamination, uneven filling or bubbles on the surface of agar,color changes, and cracks due to loss of moisture. Discard such defective plates or tubes.

7.10.2 Media prepation should be daily basis.

7.11 **Disposal:**

7.11.1 Prepare 5 % v/v Dettol solution in S.S bucket or.container.

7.11.2 Wear nose mask and hand gloves.

7.11.3 Transfer all the used media into autoclave.

7.11.4 If the media is in test tubes as agar slants then keep it in test tube stand and Keep the test tube stand in autoclave.

7.11.5 Run the cycle at 121°C for 30 minutes as per instrument operating procedure.

7.11.6 After autoclave transfer the content into the S.S. Bucket or container having above sanitization solution and cover it for 1-2 hrs.

7.11.7 Allow the medium to cool and dispose off the medium in effluent treatment plant.

7.11.8 Wash glassware's thoroughly with potable water followed by 2% soap solution and purified water and dry in hot air oven.

7.11.9 Record the details in media disposal record.(Attachment 2)

7.11.10Take out the media for disposal to the washing room.

Attachment – 1
Media Consumption Record

Name of Media:	**Code no :**
Lot no :	**Expiry date :**

Date	Quantity Withdrawn	Balance Quantity	Sign

Attachment-2
Media Disposal Record

Autoclave Code No._____

Date	Sterilization run No.	Material decontaminate	Cycle Start Time	Cycle End Time	Disposed by	Checked by	Autoclave Indicator Used

Attachment-3
Media Preparation, Sterilization and GPT Record

Name Of the Media :
Manufacturer's Lot/Batch No :
Preparation Lot No :
Date of Media Preparation :

Activity	Sign.
Balance Code No._____ Calibration due on:_____ Weighed _____gm of dehydrated media. Suspended in _____ ml of purified water and heated to dissolve (if	
pH meter Code No: _____ Calibration due on pH of the medium before sterilization/before heating : pH of the medium after sterilization/after heating :	
Sterilized by autoclaving at _____°C for ____minute.	
Sterilization Run No: _____ . Autoclave Code No:_____	
Final distribution:_____ plates/flasks	
_____Tubes/Slants.	
_____RODAC plates.	
Growth Promotion Test:Culture used & ID No.: Observation:Growth/No Growth,(plates) Turbidity/No Turbidity(tubes/flask) cfu / ml : _____	
Remarks :	
Checked by : Date	

Attachment- 4

List of Media with Storage condition

Medium	Storage condition	pH Limit

Prepared By: **Date:**	**Checked By:** **Date:**

Attachment- 5

Growth promotion recovery test of media

Name of the media:_____	
Media Lot No :_____	**Media preparation date:**_____
Incubation temperature_____	**Incubator No.**_____

Name of the Microorganism	CFU added	Recovered CFU	% Recovery	Sign/ Date	Checked By

Attachment- 6

Media Stock record

Name of Media:	**Code no:**
Expiry date:	**Manufacturer's name:**

Receiving Date	Received Quantity	Media Lot No.	Date of GPT	Balance quantity	Sign

Annexure -1
List of media, organisms and incubation conditions for GPT

Date	Media	Organism	Incubation condition	Observe for	Analyzed By	Checked By

8. History:

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