



**STANDARD OPERATING PROCEDURE**

<b>Department:</b> Microbiology	<b>SOP No.:</b>
<b>Title:</b> Growth Promotion Test and Inhibition Test of Media	<b>Effective Date:</b>
<b>Supersedes:</b> Nil	<b>Review Date:</b>
<b>Issue Date:</b>	<b>Page No.:</b>

- 1. Purpose:** To lay down the procedure for evaluating the growth promotion and Inhibition property of the sterilized media used for microbiological testing.
- 2. Scope:** This Standard Operating Procedure is applicable at microbiology section of Quality Control department.
- 3. References & Annexures:**
  - 3.1 References:**
    - 3.1.1 CQ guideline: Guideline for receipt, storage, preparation, growth promotion test, use and disposal of microbiological media
    - 3.1.2 USP
  - 3.2 Annexures:**
    - 3.2.1 Annexure- 1: Media Growth Promotion and Inhibition Test for Agar Media.
    - 3.2.2 Annexure- 2: Newly Received Agar Media-Comparative Test Report.
    - 3.2.3 Annexure- 3: Growth Promotion/ Inhibition Test Record for Liquid Media.
    - 3.2.4 Annexure- 4: Acceptance Criteria for the GPT for Culture Media.
- 4. Responsibilities:**
  - 4.1 Microbiologist / Executive - Quality control.**
    - 4.1.1 Responsible for evaluating the growth promotion and Inhibition property of the sterilized media used for microbiological testing.
    - 4.1.2 To maintain all the records as per SOP.
  - 4.2 Quality Control (QC) Head.**
    - 4.2.1 Ensure proper control and compliance of the SOP.
  - 4.3 Quality Assurance (QA) Department:**
    - 4.3.1 To review the SOP.
    - 4.3.2 To ensure the implementation of SOP.
  - 4.4 Regulatory Affairs, Quality Head and Plant Head :**
    - 4.4.1 To review and approve new or revised SOP's.



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### 5. Distribution:

- 5.1 QC
- 5.2 QA

### 6. Abbreviations and Definition of Terms :

#### 6.1 Abbreviations :

- 6.1.1 ATCC : American Type Culture Collection
- 6.1.2 CC : Change Control
- 6.1.3 °C : Degree Celsius
- 6.1.4 cfu : Colony forming unit
- 6.1.5 EHS : Environment, Health and Safety
- 6.1.6 MTCC : Microbial Type Culture Collection
- 6.1.7 NCTC : National Collection of Type Cultures
- 6.1.8 NA : Not Applicable
- 6.1.9 QA : Quality Assurance
- 6.1.10 QC : Quality Control
- 6.1.11 SOP : Standard Operating Procedure

#### 6.2 Definition of Terms :

- 6.2.1 **Standard Operating Procedure (SOP):** A written authorized procedure, which gives instructions for performing operations.
- 6.2.2 **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.
- 6.2.3 **Colony forming unit (CFU):** Visible outcome of growth of micro-organisms arising from a single or multiple cells.



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### 7. Procedure:

- 7.1 Growth promotion test shall be performed whenever new medium is received / Batch No. or lot No. is changed / Make or Vendor is changed.
- 7.2 After procuring the Media, check the Media container for the following details i.e. manufacturing date, Expiry date, Code No and lot number.
- 7.3 Store all the Media in a cool dark place at NMT  $25 \pm 5^{\circ}\text{C}$ .
- 7.4 Before taking the media for use, perform the growth promotion (i.e. nutritive properties) or growth inhibition studies specific to the media as per Annexure-4.
- 7.5 Whenever there is an approved and "in use" medium available, inoculate it with the same organism.
- 7.6 If the growth promotion qualities of the media is not the same as compared to the previously approved lot then discard that media.
- 7.7 Check the growth promotion qualities of the media (Using the organisms as mentioned in Annexure-4) by any one of the following methods.
  - 7.7.1 **For newly received agar media** containers transfer 1.0 ml of culture having a cell count of 10 to 100 cells in the dilution into two sterile petriplates and pour the media of New Container [B]. Swirl the plates and allow solidifying.
  - 7.7.2 Similarly transfer 1.0 ml of culture of having a cell count of 10 to 100 cells in the dilution into two sterile petriplates and pour the prepared Previous Container media [A]. Swirl the plates and allow solidifying.
  - 7.7.3 Incubate the plates at respective temperature, after incubation count the colonies and compare the count with the previous container results. After incubation record the results as per Annexure-2
  - 7.7.4 The recovery in the growth promotion test for the new container must be within factor 2 of the actual inoculum concentration obtained for the previous container.  
For Example: If the standard culture inoculum used has count 100 cfu/ml then the recovery should be within  $100/2= 50$  cfu/ml and  $100*2= 200$  cfu/ml.
  - 7.7.5 **For agar media** prepare inoculum of known concentration (approximately 10 to 100 cells/ml) of the organism and then transfer it to the plate followed by pouring with the agar media. After incubation record the results as per Annexure-1.
  - 7.7.6 **For selective agar media** carry out growth promotion test by streaking specified organism on the plate and observe for the characteristics of colonies and record the result in Annexure-1.



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7.7.7 **In case of liquid media** or broth inoculate 1.0 ml of inoculum, in 100 mL of media or 0.1 mL of inoculum, in 10 mL of media by using the organisms as mentioned in Annexure-4 incubate the media, observe the growth and record the result in Annexure-3.

7.7.8 Liquid media are suitable, if should show clear visible growth in media.

7.7.9 Put an uninoculated Bottle/Tube/plate of media as a negative control to confirm the sterility of the media.

7.8 Label or mark using by marker the name of media, batch/lot no., organism used tested by and date on the Bottle/Tube/plate of media. (For GPT of daily prepared media, if more organisms are prescribed for testing, in that case, minimum two bacteria and one yeast/mould shall be used for testing on daily rotation basis.)

7.9 For solid media, the recovery of inoculated organisms should not be factor 2 from the calculated value of inoculums added.

**Note:** *Before use of any batch of prepared media for testing if GPT is not possible to perform before testing, it can be performed simultaneously with testing.*

7.10 The shelf life of opened media bottle shall not be more than 12 months and for unopened bottle it is till the shelf life of the container.

7.11 Assign the batch Number to prepared media with respect to Growth Promotion Test as

GPT/MMXXX/YY, Where

GPT - For Growth Promotion Test

/ - Slash

MM - Microbiological Media

XXX - 001, 002, 003, ----- to 999

/ - Slash

YY - Last 2 digits of the calendar year.

For example, first batch number for year 2022 shall stand as GPT/MM001/22.

**Note:** Growth promotion test shall be performed every finished (Prepared) lots of dehydrated medium.



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### Annexure -1

### MEDIA GROWTH PROMOTION AND INHIBITION TEST FOR AGAR MEDIA

S.No.	Date	Name of Media	Media Lot No.	Sterilization Cycle No.	GPT Ref. No.	Test for growth promoting and inhibitory property										Checked By		
						Test Organism	Temp. of Incubation	Incubator No.	No. of Cells Inoculated (10-100 cfu)	Observation			% Recovery	Test Performed (Pr / In)	Incubation Completed		Recorded by	
										Plate I	Plate II	Avg.						

Pr: Promoting, In: Inhibitory



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**Annexure -2**

**NEWLY RECEIVED AGAR MEDIA-COMPARATIVE TEST REPORT**

Name of the Media :	Date of Inoculation :
Batch No / Lot No :	Date of Report :
Batch No/ Lot No (Previous Container):	Expiry date :
Incubator ID :	Acceptance Criteria :
GPT Ref. No. :	Recovery :

S.No.	Test Organism	Method of Inoculation	No. of Cells Inoculated (10-100 cfu)	Previous Container [A]			New Container [B]			% Recovery (Bx100/A)
				I	II	Avg.	I	II	Avg.	

Remarks.

Recorded by:  
(Sign/ Date)

Checked by:  
(Sign/ Date)



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**Annexure -3**

**GROWTH PROMOTION/ INHIBITION TEST RECORD FOR LIQUID MEDIA**

Name of Medium : Date of Inoculation :  
Lot No. : Date of Incubation :  
Date of Manufacturing : Incubator ID :  
Date of Expiry : Date of Observation :  
Date of Receiving : Date of Report :  
GPT Ref. No. :

Name of Organism (s)	No. of Cells Inoculated (10-100 cfu)	Method of Inoculation	Test Performed (Pr / In)	Observation	Results Satisfactory/Not Satisfactory

Pr: Promoting, In: Inhibitory

**Remarks:**

Recorded by:  
(Sign/Date)

Checked by:  
(Sign/Date)



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**Annexure -4**

**ACCEPTANCE CRITERIA FOR THE GPT FOR CULTURE MEDIA**

Name of Media	Microorganism Name	Incubation Conditions		Method of inoculation	Growth characteristics on media/ Microscopic examination	Organisms to be used for Growth Inhibitory Test
		Temp. (°C)	Time (Hrs.)			
Soyabean Casein Digest Broth	<i>Escherichia coli</i>	30-35	24-48	Direct	Turbidity, -Ve rods.	NA
	<i>Pseudomonas aeruginosa</i>	30-35	24-48	Direct	Turbidity, -Ve rods.	
	<i>Staphylococcus aureus</i>	30-35	24-48	Direct	Turbidity, +Ve Cocci.	
	<i>Bacillus subtilis</i>	30-35	24-48	Direct	Turbidity, +Ve rods.	
	<i>Micrococcus luteus</i>	30-35	24-48	Direct	Turbidity, +Ve Cocci.	
	<i>Salmonella</i>	30-35	24-48	Direct	Turbidity, -Ve rods.	
	<i>Candida albicans</i>	20-25	48-72	Direct	Turbidity.	
	<i>Aspergillus brasiliensis</i>	20-25	48-72	Direct	Turbidity.	
Soyabean Casein Digest Agar	<i>Pseudomonas aeruginosa</i>	30-35	24-48	Pour Plate	Colourless to yellowish colonies, -Ve rods.	NA
	<i>Staphylococcus. aureus</i>	30-35	24-48	Pour Plate	Yellow shining colonies, +Ve Cocci.	
	<i>Bacillus subtilis</i>	30-35	24-48	Pour Plate	Irregular whitish colonies, +Ve rods.	
	<i>Micrococcus luteus</i>	30-35	24-48	Pour Plate	Small yellowish colonies, +Ve Cocci.	
	EM Isolate	30-35	24-48	Pour plate	Suitable growth observed.	





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Name of Media	Microorganism Name	Incubation Conditions		Method of inoculation	Growth characteristics on media/ Microscopic examination	Organisms to be used for Growth Inhibitory Test
		Temp. (°C)	Time (Hrs.)			
	<i>Candida albicans</i>	20-25	48-72	Pour Plate	Cream/white coloured small colonies.	
	<i>Aspergillus brasiliensis</i>	20-25	48-72	Pour Plate	Formation of mycelia.	
Mac Conkey broth	<i>Escherichia coli</i> / <i>E. coli</i> Mutant	30-35	24-48	Direct	Gas formation in Durham tube, - Ve rods.	<i>Staphylococcus aureus</i>
Sabouraud. Dextrose agar	<i>Candida albicans</i>	20-25	48-72	Pour Plate	Rounded shining whitish colonies.	NA
	<i>Aspergillus brasiliensis</i>	20-25	48-72	Pour Plate	Cottony growth with mycelia, Spores appear as pale to bright greenish/black.	
Sabouraud. broth	<i>Candida albicans</i>	30-35	24-48			
EE broth	<i>Enterobacteria</i>	35-37	18-24	Direct	Turbidity, -Ve rods	<i>Staphylococcus aureus</i>
VRBGA	<i>Enterobacteria</i>	35-37	18-24	Streaking	Red/reddish colonies, -Ve rods.	<i>Staphylococcus aureus</i>
MacConkey. Agar	<i>Escherichia coli</i>	30-35	24	Streaking	Brick red colonies, -Ve rods.	<i>Staphylococcus aureus</i>
Mannitol salt agar	<i>Staphylococcus aureus</i>	30-35	24	Streaking	Yellow colonies with yellow zones, +Ve Cocci.	<i>Escherichia coli</i>



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Name of Media	Microorganism Name	Incubation Conditions		Method of inoculation	Growth characteristics on media/ Microscopic examination	Organisms to be used for Growth Inhibitory Test
		Temp. (°C)	Time (Hrs.)			
Xylose-Lysine-Deoxycholate Agar medium	<i>Salmonella</i>	30-35	24	Streaking	Red colonies with or without black centers, -ve rods.	<i>Staphylococcus aureus</i>
Xylose-Lysine-Deoxycholate Agar medium	<i>Shigella</i>	30-35	24-48	Streaking	Red colored translucent colony without black centers, -ve rods.	<i>Staphylococcus aureus</i>
Cetrimide Agar Medium	<i>Pseudomonas aeruginosa</i>	30-35	24	Streaking	Generally Greenish -Ve rods	<i>Escherichia coli</i>
GN Broth	<i>Shigella</i>	30-35	24-48	Direct	Turbidity, -Ve rods.	<i>Staphylococcus aureus</i>
R2A	<i>Pseudomonas aeruginosa</i>	30-35	24-48	Pour Plate	Colourless to yellowish colonies, -Ve rods.	NA
	<i>Staphylococcus aureus</i>	30-35	24-48	Pour Plate	Yellow shining colonies, +Ve Cocci.	
	<i>Bacillus subtilis</i>	30-35	24-48	Pour Plate	Irregular whitish colonies, +Ve Cocci.	
	<i>Micrococcus luteus</i>	30-35	24-48	Pour Plate	Small yellowish colonies, +Ve Cocci.	
	<i>Candida albicans</i>	20-25	48-72	Pour Plate	Small colonies, Spores appear as pale to bright red.	



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Name of Media	Microorganism Name	Incubation Conditions		Method of inoculation	Growth characteristics on media/ Microscopic examination	Organisms to be used for Growth Inhibitory Test
		Temp. (°C)	Time (Hrs.)			
	<i>Aspergillus niger</i>	20-25	48-72	Pour Plate	Formation of mycelia, Spores appear as pale to bright red.	
Rappaport Vassiliadis Salmonella Enrichment Broth	<i>Salmonella abony</i>	30-35	24-48	Direct	Turbidity, -Ve rods.	<i>Staphylococcus aureus</i>
Reinforced Medium for Clostridia	Clostridia	30-35	24-48	Direct	Good-Luxuriant growth under Anaerobic Condition.	
Columbia Agar	Clostridia	30-35	24-48	Pour Plate	Colony are slightly yellowish.	<i>Staphylococcus aureus</i>



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**8. History:**

<b>Revision No.</b>	<b>Effective Date</b>	<b>Revision Details</b>	<b>CC No.</b>
00		New SOP.	NA