

STANDARD OPERATING PROCEDURE		
Department: MicrobiologySOP No.:		
Title: Growth Promotion, Inhibitory and Indicative Properties of Culture Media	Effective Date:	
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1.0 OBJECTIVE:

To lay down a procedure for Growth Promotion, Inhibitory and Indicative Properties of Culture Media.

2.0 SCOPE:

This SOP is applicable for Growth Promotion, Inhibitory and Indicative Properties of Culture Media at Microbiology Lab of Quality Control Department.

3.0 RESPONSIBILITY:

Operating Person – Microbiology

4.0 ACCOUNTABILITY:

Head - QC

5.0 ABBREVIATIONS:

COA	Certificate of Analysis
Cfu	Colony Forming Unit
°C	Degree Centigrade
GPT	Growth Promotion Test
Hrs.	Hours
LAF	Laminar Air Flow
Ltd.	Limited
No.	Number
ml	Milliliter
QA	Quality Assurance
QC	Quality Control
SDA	Sabouraud Chloroamphenicol Agar
SOP	Standard Operating Procedure

6.0 **PROCEDURE**:

6.1 Perform growth promotion test of one container of single lot of agar media and broth medium along with previously approved culture medium and broth medium.



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6.2 Growth Promotion Test & Indicative Properties of Agar Media or Selective Agar Media:

- **6.2.1** Prepare the required quantity of dehydrated media as per SOP. Sterilize require quantity of culture media and accessories as per SOP.
- **6.2.2** Prepared the required quantity of 10ml broth medium tubes and media plates for pH and growth promotion test.
- **6.2.3** Transfer the sterilized culture medium & pre- sterilized Petri plates/tubes into the Biosafety cabinet.
- **6.2.4** Transfer the quantified microbial inoculum into the Biosafety cabinet from refrigerator through dynamic pass box and kept at room temperature.
- **6.2.5** After thaw use the inoculums having microbial population of not more than 100 cfu of listed microorganism in Table-1. For recovery of microorganism; Spread Plate Method shall be used.
- **6.2.6** By Spread Plate Method: Take prepared agar media Petri plate in duplicate (for general purpose media) & single plate (for selective media) for each microorganism and inoculate having not more than 100 cfu of listed microorganism in table-1; and spread on agar surface with help of sterile spreader aseptically.
- **6.2.7** Incubate all the inoculated petriplate at specified temperature for specified incubation period as per Table-1.
- **6.2.8** Also carry out the negative control for each of culture media with GPT and incubate the plates as per test.
- **6.2.9** After completion of incubation period, observe and count the number of microorganism on general purpose media plates or characteristic Growth of microorganism on the selective agar media as per given below table.

Specified Microorganism	Media Name	Positive Growth Characteristics
Escherichia, coli		Pink/red coloured non-mucoid colonies.
Escherichia. coli	MacConkey Broth	Medium colour turns to yellow.
Salmonella	Rappaport Vasilliadis Salmonella Broth	Medium colour turns to light green.
Sumonena	Xylose lysine Deoxycholate Agar	Red colonies with or without black centers.
Shigella boydii	Xylose lysine Deoxycholate Agar	Red colored translucent colony without black center



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Specified Microorganism	Media Name	Positive Growth Characteristics
Pseudomonas aeruginosa	Cetrimide Agar	Greenish yellow colonies.
Staphylococcus aureus	Mannitol Salt Agar	Yellow colonies surrounded by yellow zones.
Escherichia. coli	Enterobacteria Enrichment Broth, Mossel	Medium colour turns to yellow.
Pseudomonas aeruginosa	Enterobacteria Enrichment Broth, Mossel	Luxuriant growth observed
Escherichia. coli	Violet Red Bile glucose Agar	Pink/red colonies
Pseudomonas aeruginosa	Violet Red Bile glucose Agar	Pink/red colonies

6.3 Growth Promotion Test & Indicative Properties of Broth Medium:

- **6.3.1** Prepare the required quantity of dehydrated broth media as per SOP. Sterilize require quantity of culture broth and other accessories as per SOP.
- **6.3.2** Transfer the sterilized culture medium tubes into the Biosafety cabinet through dynamic pass box.
- **6.3.3** Take sterilized Broth Media and inoculate the inoculums having not more than 100 cfu of listed microorganism in table-1. Incubate all the inoculated Broth media tubes at specified temperature for specified incubation period as per Table-1.
- **6.3.4** Also carry out the negative control for each of culture medium tube (keep the one tubes without inoculation of culture for negative control) with GPT and incubate the tubes as per test.

6.4 Test for Inhibitory Properties of Agar Media:

- **6.4.1** This test shall be performed for agar media, planned for the detection of specified microorganisms.
- **6.4.2** By Spread Plate Method: Take prepared agar media petri plate for each microorganism and inoculate having at least 100 cfu of appropriate listed microorganism as per table 1 and spread the inoculums on agar surface with help of sterile spreader aseptically.
- **6.4.3** Incubate all the inoculated petriplate at specified temperature for specified Incubation period as per Table-1.
- **6.4.4** After completion of incubation period, observe characteristic growth/ No growth of microorganism on the selective agar media plates.



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6.5 Test for Inhibitory Properties of Broth Medium:

- **6.5.1** Take sterilized Broth Media and inoculate the inoculums at least 100 cfu of appropriate microorganism and Incubate all the inoculated Broth media tubes at specified temperature for the specified Incubation period as per Table-1.
- **6.5.2** After completion of incubation period, observe characteristic growth/No growth of microorganism in the Medium.

6.6 Acceptance Criteria:

- **6.6.1** In the test for Growth Promoting Properties; microbial recovery for agar media growth obtained must not differ by a factor greater then 2 (divided by 2 and multiplied by 2) from the calculated value for a standardized Inoculum.
- **6.6.2** For broth culture media; clearly visible growth/luxurious growth of microorganism should be observed, comparable to the previously obtained with previously tested and approved batch of medium occurs.
- **6.6.3** In the test for Inhibitory Properties; there should be no growth of the test microorganisms occurs.
- **6.6.4** Performed the GPT (Growth promoting inhibitory and indicative) of newly received media and results shall be comparable with previous approved media.
- **6.6.5** If previous approved media is not available then Media can be used in routine testing after complies the GPT results on the behalf of Vendor COA.
- **6.6.6** If GPT Failure; repeat the test and investigate through the Incidence and media batch should be hold till the completion of investigation.
- **6.6.7** If the approved culture media not use within one year then repeat the Growth Promotion test before use.
- **6.6.8** Observe the general purpose media plates of GPT with the help of colony counter and observe the characteristic Growth of microorganism on the selective agar media & observe the tubes and record the observations in **Annexure-I**, Titled **"Growth Promotion Test of Culture Media"**.
- **6.6.9** If there is a holiday on the day of release media plates/tubes, take the observation of media plates/tubes on next working day.

6.7 STORAGE:



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6.7.1 Store the approved prepared agar media plates and prepared broth media tubes at 20-25°C and agar media plates can be used upto 14 days and prepared broth media tubes can be used upto 30 days from date of media preparation.

GRO	GROWTH PROMOTING, INHIBITORY AND INDICATIVE PROPERTIES OF CULTURE MEDIA					
Growth Incubation Tem			on Temperatu	ire/Time		
S. No.	Media Name	Growth Promoting / Indicative Microorganism	Inhibitory Microorgan ism	Growth promotion Properties	Inhibitory Properties	Indicative Properties
1.	Antibiotic Assay Medium No. 11 (AAM)	S. aureus ATCC 6538/NCIM 5345 S. epidermidis ATCC 12228/ NCIM 2493 B. subtilis ATCC 6633/NCIM 2063	NA	30-35 ⁰ C 18 -48 hrs.	NA	NA
2.	Antibiotic Assay Medium No. 01 (AA1)	<i>B. subtilis</i> ATCC 6633/NCIM 2063	NA	30-35 ⁰ C 18 -48 hrs.	NA	NA
3.	Antibiotic Assay Medium H (AAH)	<i>B. subtilis</i> ATCC 6633/NCIM 2063	NA	30-35 ⁰ C 18 -48 hrs.	NA	NA
4.	Antibiotic Assay Medium No. 03 (AA3)	<i>S. aureus</i> ATCC 6538/NCIM 5345	NA	30-35 ⁰ C 18 -48 hrs.	NA	NA
5.	B12 Culture Agar(BCA)	<i>E. coli</i> mutant ATCC 11105/NCIM 2068 or ATCC 14169/NCIM2567	NA	30-35 ⁰ C 18 -48 hrs.	NA	NA
6.	Cetrimide Agar (CTA)	P. aeruginosa ATCC 9027/NCIM 2200	<i>E. coli</i> ATCC 8739/NCI M 2065	30-35 ⁰ C 18 -72 hrs.	$30-35^{0}C \ge 72 \text{ hrs.}$	30-35 ⁰ C 18 -72 hrs.
7.	Columbia Agar (CLA)	<i>Cl. sporogenes</i> ATCC 19404/ NCIM 5125	NA	30-35 ⁰ C 48 -72 hrs.	NA	NA
8.	Cooked Meat Medium(CMM)	Cl. sporogenes ATCC 19404/ NCIM 5125	NA	30-35 [°] C 24 -72 hrs.	NA	NA

TABLE-1 OMOTING, INHIBITORY AND INDICATIVE PROPERTIES OF CULTURE



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			Growth	Incubation Temperature/Time		
S. No.	Media Name	Growth Promoting / Indicative Microorganism	Inhibitory Microorgan ism	Growth promotion Properties	Inhibitory Properties	Indicative Properties
9.	De-Engley's Neutralizing Agar (DNA)	<i>E. coli</i> ATCC 8739/ NCIM 2065, <i>S. aureus</i> ATCC 6538/NCIM 5345, <i>A. brasilliensis</i> ATCC 16404/NCIM 1196 <i>C. albicans</i> ATCC 10231/NCIM 3471, Use one strain on Rotation basis in Daily GPT and Use all when New media received, <i>Environment Isolates</i> (In house culture) Use one strain on Rotation basis	NA	$30-35^{0}C$ ≤ 3 days. for Bacteria $20-25^{0}C$ ≤ 5 days for fungus	NA	NA
10.	Enterobacteria Enrichment Broth Mossel(EEM)	<i>E. coli</i> ATCC 8739/ NCIM 2065, <i>P. aeruginosa</i> ATCC 9027/NCIM 2200	<i>S. aureus</i> ATCC 6538/NCI M 5345	30-35 ⁰ C 24 -48 hrs.	$30-35^{\circ}C \ge 48$ hrs.	NA
11.	Eosine Methylene Blue Agar(EMB)	<i>E. coli</i> ATCC 8739/ NCIM 2065,	<i>S. aureus</i> ATCC 6538/NCI M 5345	30-35 ⁰ C 24 -72 hrs.	$30-35^{0}C \ge 72 \text{ hrs.}$	30-35 ⁰ C for 24-72 hrs.
12.	Fluid Thioglycolate Medium(FTM)	<i>Cl. sporogenes</i> ATCC 19404/ NCIM 5125 <i>P. aeruginosa</i> ATCC 9027/NCIM 2200 <i>S. aureus</i> ATCC 6538/NCIM 5345 Use one strain on Rotation basis in Daily GPT and Use all when New media received	NA	30-35 ⁰ C ≤ 3 days	NA	NA
13.	GN Broth(GNB)	<i>Shigella boydii</i> ATCC9207/NCIM5644 or ATCC8700/NCIM5709	<i>S. aureus</i> ATCC 6538/NCI M 5345	30-35 ⁰ C 24 -48 hrs.	$30-35^{0}C \\ \ge 48 \text{ hrs.}$	NA



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			Growth	Incubati	on Temperatu	ure/Time
S. No.	Media Name	Growth Promoting / Indicative Microorganism	Inhibitory Microorgan ism	Growth promotion Properties	Inhibitory Properties	Indicative Properties
14.	Glucose Yeast Extract Agar (GYA)	<i>B. subtilis</i> ATCC 6633/NCIM 2063	NA	35-39 ⁰ C 24 -48 hrs.	NA	NA
15.	Lactobacillus MRS agar (MRS)	S. Faecalis ATCC/NCIM 8043/2080	NA	35-39 ⁰ C 24 -48 hrs.	NA	NA
16.	Lactobacillus MRS Broth (MRB)	<i>B. subtilis</i> ATCC 6633/NCIM 2063	NA	35-39 ⁰ C 24 -48 hrs.	NA	NA
17.	MacConkey Agar(MCA)	<i>E. coli</i> ATCC 8739/NCIM 2065	NA	30-35 ⁰ C 18 -72 hrs.	NA	30-35 ^o C for 18 to 72 hrs.
18.	MacConkey Broth(MCB)	<i>E. coli</i> ATCC 8739/NCIM 2065	<i>S. aureus</i> ATCC 6538/NCI M 5345	42-44 ⁰ C 24 -48 hrs.	$42-44^{0}C \ge 48 \text{ hrs.}$	42-44 ⁰ C for 24 to 48 hrs.
19.	Mannitol Salt Agar Medium(MSA)	<i>S. aureus</i> ATCC 6538/NCIM 5345	<i>E. coli</i> ATCC 8739/NCI M 2065	30-35 ⁰ C 48 -72 hrs.	30-35 ⁰ C ≥ 72 hrs.	30-35 ^o C 48 -72 hrs
20.	Mueller Hinton Agar(MHA)	<i>E. coli</i> ATCC 8739/NCIM 2065	NA	30-35 ^o C 24 -48 hrs.	NA	NA
21.	Mitis Salviris(MTS) Agar	<i>B. subtilis</i> ATCC 6633/NCIM 2063	NA	30-35 ⁰ C 24 -48 hrs.	NA	NA
22.	Nutrient Agar(NAM)	<i>S. aureus</i> ATCC 6538/NCIM 5345	NA	30-35 ^o C 24 -48 hrs.	NA	NA
23.	Pseudomonas Agar Medium (Pyocyanin) (PAP)	P. aeruginosa ATCC 9027/NCIM 2200	NA	30-35 ⁰ C 24 -48 hrs.	NA	30-35 ^o C for 24 to 48 hrs.
24.	Pseudomonas Agar Medium (Fluorescein) (PAF)	P. aeruginosa ATCC 9027/NCIM 2200	NA	30-35 ⁰ C 24 -48 hrs.	NA	30-35 ⁰ C for 24 to 48 hrs.
25.	PNY Agar (PNY)	<i>B. subtilis</i> ATCC 6633/NCIM 2063	NA	35-39 ⁰ C 24 -72 hrs.	NA	NA



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			Growth	Incubation Temperature/Time		
S. No.	Media Name	Growth Promoting / Indicative Microorganism	Inhibitory Microorgan ism	Growth promotion Properties	Inhibitory Properties	Indicative Properties
26.	Rappaport Vassiliadis Salmonella enrichment Broth. (RVS)	Salmonella NCTC 6017/ NCIM 2257 or ATCC14028/NCIM5643	<i>S. aureus</i> ATCC 6538/NCI M 5345	30-35 ⁰ C 18-24 hrs.	30-35 ⁰ C ≥ 24 hrs.	NA
27.	Reinforced medium for clostridia(RMC)	<i>Cl. sporogenes</i> ATCC 19404/ NCIM 5125	NA	30-35 ⁰ C For 48 hrs.	NA	NA
28.	R2A Agar (R2A)	<i>B. subtilis</i> ATCC 6633/NCIM 2063, <i>P.</i> <i>aeruginosa</i> ATCC 9027/NCIM 2200 Use one strain on Rotation basis in Daily GPT and Use both when New media received. Use water <i>Isolate</i> (In house culture) on daily and initial GPT	NA	30-35 ⁰ C 24 -72 hrs.	NA	NA
29.	Sabouraud Chloramphenicol Agar(SDA)	A. brasilliensis ATCC 16404/NCIM 1196 C. albicans ATCC 10231/NCIM 3471 Use one strain on Rotation basis in Daily GPT and Use both when New media received	NA	20-25 ⁰ C ≤ 5 days	NA	NA
30.	Sabouraud Dexrose Broth(SDB)	A. brasilliensis ATCC 16404/ NCIM 1196, C. albicans ATCC 10231/NCIM 3471 Use one strain on Rotation basis in Daily GPT and Use both when New media received	NA	$20-25^{0}C \le 5 \text{ days}$	NA	NA



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			Growth	Incubation Temperature/Time		
S. No.	Media Name	Growth Promoting / Indicative Microorganism	Inhibitory Microorgan ism	Growth promotion Properties	Inhibitory Properties	Indicative Properties
31.	Soybean Casein Digest Agar(SCA)	S. aureus ATCC 6538/NCIM 5345 P. aeruginosa ATCC 9027/NCIM 2200 B. subtilis ATCC 6633/NCIM 2063 A. brasilliensis ATCC 16404/NCIM 1196 C. albicans ATCC 10231/NCIM 3471 Use one strain on Rotation basis in Daily GPT and Use all when New media received, Environment Isolates (In house culture) Use one strain on Rotation basis	NA	30-35 ⁰ C ≤ 3 days for Bacteria 30-35 ⁰ C ≤ 5 days for fungus	NA	NA
32.	Soybean Casein Digest Medium(SCM)	S. aureus ATCC 6538/ NCIM 5345, P. aeruginosa ATCC 9027/NCIM 2200, B. subtilis ATCC 6633/NCIM 2063, A. brasilliensis ATCC 16404/NCIM 1196 C. albicans ATCC 10231/NCIM 3471 Use one strain on Rotation basis in Daily GPT and Use all when New media received, Environment Isolates (In house culture) Use one strain on Rotation basis	NA	$30-35^{0}C$ $\leq 3 \text{ days}$ for Bacteria, $20-25^{0}C$ $\leq 5 \text{ days}$ for fungus	NA	NA
33.	Triple Sugar Iron Agar(TSA)	Salmonella spp. NCTC 6017/ NCIM 2257 or ATCC14028/NCIM5643	NA	30-35 [°] C 24 -48 hrs.	NA	30-35 ^o C for 24 to 48 hrs.



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			Growth	Incubation Temperature/Time		
S. No.	Media Name	Growth Promoting / Indicative Microorganism	Inhibitory Microorgan ism	Growth promotion Properties	Inhibitory Properties	Indicative Properties
34.	Violet Red bile Glucose agar(VBA)	<i>E. coli</i> ATCC 8739/ NCIM 2065 <i>P. aeruginosa</i> ATCC 9027/NCIM 2200 Use on Rotation basis in Routine GPT and Use both when New media received	NA	30-35 ⁰ C 18 -24 hrs.	NA	30-35 ⁰ C for 18 to 24 hrs.
35.	Xylose Lysine Deoxycholate Agar(XLD)	Salmonella spp. NCTC6017/NCIM 2257 or ATCC14028/NCIM 5643 and Shigella boydii ATCC9207/NCIM5644 or ATCC8700/NCIM5709 Use one strain on Rotation basis in Routine GPT and Use both when New media received	NA	30-35 ⁰ C 24-48 hrs.	NA	30-35 ⁰ C for 24-48 hrs.
36.	Sabouraud Dexrose Agar (SBD)	A. brasilliensis ATCC 16404/NCIM 1196 C. albicans ATCC 10231/NCIM 3471 Use on Rotation basis in Routine GPT and Use both when New media received	NA	$20-25^{0}C \le 5 \text{ days}$	NA	NA
37.	Soya Lecithin Broth (SLB)	<i>E. coli</i> ATCC 8739/ NCIM 2065	NA	30-35 [°] C 24 -48 hrs.	NA	NA
38.	Folic Acid Assay Medium (FAA) Broth	S. faecalis ATCC/NCIM 8043/2080	NA	35-39°C 24-48 hrs.	NA	NA
39.	Folic Acid Assay Agar (FCA)	<i>S. faecalis</i> ATCC/NCIM 8043/2080	NA	35-39 ^o C 24-48 hrs.	NA	NA
40.	Glucose Yeast Extract Acetate Broth (GYB)	B. subtilis ATCC 6633/NCIM 2063	NA	35-39 ⁰ C 24 -48 hrs.	NA	NA
41	Burkholderia Cepacia Selective Agar (BCS)	<i>Burkholderia cepacia</i> ATCC 25416, NCTC 10743 or CIP 80.24,	S. aureus ATCC 6538/	30-35 ^o C 48-72 hrs.	$30-35^{0}C \ge 72 \text{ hrs.}$	30-35 ^o C for 48-72 hrs.



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S. No.	Media Name	Growth Promoting / Indicative Microorganism	Inhibitory Microorgan ism	Growth promotion Properties	Inhibitory Properties	Indicative Properties
No.		Indicative Microorganism Burkholderia cenocepacia ATCC BAA-245 or LMG 16656 and Burkholderia multivorans ATCC BAA- 247, LMG 13010, CCUG 34080, CIP 105495, DSM 13243 or NCTC 13007 Use one strain on Rotation basis in Routine GPT and Use all when new media received.	0	promotion Properties		
			new media received.			

7.0 ANNEXURES:

ANNEXURE No.	TITLE OF ANNEXURE	FORMAT No.		
Annexure – I	Growth Promotion and Inhibitory Test Report of Culture			
	Media			
ENCLOSUDES, SOD Training Decord				

ENCLOSURES: SOP Training Record.

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9.0 **REFERENCES**:

- United State Pharmacopoeia 43 Chapter# 60, 61, 62
- Indian Pharmacopoeia 2018, Chapter# 2.2.9

10.0 REVISION HISTORY:

CHANGE HISTORY LOG

Revision	Change	Details of Changes	Reason for	Effective	Updated
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	of Media								r Lot No.				
Media Preparation Date						-			Ref No.				
Date of Tested	of Testing					Incuba Temp			For Bacteria For Fungus				
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5.110	Organism	Used	Added	,	criteria	Promoting	Indicative	Inhibitory	I		II Ave	erage	sign & date
1						Promoting	Indicative	Inhibitory			II Ave	erage	sign & date
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