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Title: Procedure for Growth Promotion Test	**Effective Date:**
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1.0 OBJECTIVE:

To lay down the procedure for growth promotion test of solid media (plate, slant and stab) and liquid media (broth) which are to be used for microbiological analysis.

2.0 SCOPE:

This procedure is applicable for growth promotion test of microbiological media used for analysis in microbiology laboratory.

3.0 RESPONSIBILITY:

QC – Microbiologist

Head – Quality Control

4.0 PROCEDURE:

4.1 Liquid broth medium (Growth Promotion / Positive Control/inhibition):

4.1.1 Prepare culture dilution as per reference SOP.

4.1.2 Inoculate appropriate volume of culture containing NMT 100 cfu / ml to the sterile culture media and incubate at recommended temperature specified for the organism as per Table -I.

4.1.3 The media complies the growth promotion test if luxuriant growth (turbidity) is observed in the inoculated tubes of liquid media or distinct colony formation on the agar media within specified period of incubation (refer Table-I)

4.1.4 If Growth promotion test results does not complies as per acceptance criteria than follow investigation procedure as per SOP.

4.1.5 Test each batch of the media prepared either from dehydrated medium or from the ingredient for growth promotion test. Use the already approve medium prepare from dehydrated medium or from the ingredient as a control. For different types of media procedure mention below:

4.1.5.1 **For liquid medium:** Add appropriate volume of the test suspension containing NMT 100 cfu / ml of the specific test organisms to each tube. For selective media specific organisms are chosen so as to verify the selective nature of the medium under examination.

4.1.5.2 **For Solid agar medium:** Add appropriate volume of test suspension using NMT 100 cfu/ml of the specific test organisms in a sterile petriplate. Add required medium, which has been melted

and cooled to about 45°C by checking with IR thermometer and mix by rotating the plate. Allow solidifying and inverting.

4.1.5.3 For the growth promotion test of solid media (Except Selective media) by pour plate method the recovery of the organisms against added should be verified. The results of the media under test should be compared with the previous pass lot of the same media.

4.1.5.4 For selective media spread specific test organisms to verify the selective nature of the medium and inhibition with respective organism given in table no.1 for media under test. Incubate at appropriate temperature as mentioned in table No.1. Check for the growth and inhibition of the organism.

4.2 Interpretation:

4.2.1 **Test for growth promoting properties, Liquid Media –**

Clearly visible growth of microorganism at the specified temperature for not more than shortest period of time specified in the test and comparable with approved batch of medium occurs.

4.2.2 **Test for growth promoting properties, Solid Media –**

Clearly visible growth of microorganism at the specified temperature for not more than shortest period of time specified in the test and comparable with approved batch of medium occurs.

4.2.3 **Test for Inhibitory Properties, Liquid or Solid Media –**

No growth of microorganism occurs at the specified temperature for not less than longest period of time specified in the test and comparable with approved batch of medium occurs. Negative control should not show any growth after longest incubation time of respective media (refer table-I).

4.2.4 **Test for Indicative Properties, Liquid or Solid Media –**

Clearly visible growth of microorganism at the specified temperature for period of time within the range specified in the test. Colonies are comparable in appearance and indication reactions to those previously obtained with a previously tested and approved batch of medium.

4.3 Acceptance criteria:

4.3.1 Microorganism must show turbidity in the inoculated broth media and distinct colony formation on the solid agar media within shortest period of incubation time as per table-I.

4.3.2 Negative control should not show any growth after longest incubation time of respective media

(refer table-I).

4.3.3 The Pre incubation, inhibition and Growth Promotion Test can be run simultaneously along with the sample or test under examination except for the media used for the environmental monitoring where pre incubation completed plates are used with growth Promotion Test concurrently.

4.3.4 If the pre incubation or Growth Promotion or inhibition Test found failing in any results, the sample analysis carried out with these lot of media stand invalid.

4.3.5 There should be at least by factor of 2 (50 – 200 %) recoveries of the organisms with respect to the No. of organisms added in case of growth promotion test as per pour plate technique for solid media except selective media.

4.3.6 For initial growth promotion test after receiving new lot /batch of generalized media such as SCDA,SCDM and NA shall be carried out using the organism mentioned in table-I and after completion of incubation period note down the results as per annexure-I of SOP while for routine growth promotion test the organism used shall be one Gram negative ,one Gram positive and one yeast/mould as mentioned in the table-I and after completion of incubation period note down the results as per annexure-I of SOP.

4.3.7 Inhibition test are carried out for some of the selective media like RVSM, EBEB, CETA, MASA, MACB etc.

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Table – I

S.No.	Name of Media	Name of Organism	Incubation Temp. & Time	Condition
1.	Fluid Thioglycolate Medium (FLTM)	*Cl. Sporogenes* ATCC 19404 / ATCC 11437	30–35°C ≤ 3 Days	Anaerobic
		Common Isolate-I & II	30–35°C ≤ 3 Days	Aerobic
		B. subtilis ATCC 6633 / NCIM 8054		
		P. aeruginosa ATCC 9027/ NCIM 2200		
		S.aureus ATCC 6538		
2.	Sabouraud dextrose agar (SBDA)and broth (SBDB)	*C.albicans* ATCC 10231	20–25°C ≤ 5 Days	Aerobic
		A. brasiliensis ATCC 16404		
3.	R2A agar(R2AA) (For initial GPT)	*P. aeruginosa ATCC 9027/ NCIM 2200*	30–35°C ≤ 3 Days	Aerobic
		B. subtilis ATCC 6633 / NCIM 8054		
		S.aureus ATCC 6538		
		E. coli ATCC 8739 / NCIM 2065		
		Salmonella Spp. NCTC 6017		
		Common Isolate-I & II		
		C.albicans ATCC 10231	30–35°C ≤ 5 Days	
		A. brasiliensis ATCC 16404		
4.	R2A agar (R2AA) (For routine GPT)	*P. aeruginosa ATCC 9027/ NCIM 2200*	30–35°C ≤ 3 Days	Aerobic
		B. subtilis ATCC 6633 / NCIM 8054		
5.	*Soyabean casein digest medium (SCDM) , Soyabean casein digest agar (SCDA) and nutrient agar(NUTA) (for initial GPT)	P. aeruginosa ATCC 9027/ NCIM 2200	30–35°C ≤ 3 Days	Aerobic
		B. subtilis ATCC 6633 / NCIM 8054		
		S.aureus ATCC 6538		
		E. coli ATCC 8739/NCIM 2065		
		Salmonella Spp. NCTC 6017		
		Common Isolate -I & II		
		C. albicans ATCC 10231	30–35°C ≤ 5 Days	
		A.brasiliensis ATCC 16404		

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		C. albicans ATCC 10231	20–25°C ≤ 5 Days	
		A.brasiliensis ATCC 16404		
		Common Isolate -I & II		
6.	**Soyabean casein digest medium (SCDM), Soyabean casein digest agar(SCDA) & Nutrient agar(NUTA) (used for MLT and other tests except sterility test) (organism use for routine GPT on daily rotation basis)	***__Day-1:__ C. albicans ATCC 10231, P. aeruginosa ATCC 9027/ NCIM 2200, B. subtilis ATCC 6633 / NCIM 8054, Common Isolate -I	As per respective temperature required for growth of bacteria and fungi mention in same table.	Aerobic
		Day-2: S.aureus ATCC 6538, E. coli ATCC 8739/ NCIM 2065, Common Isolate –II, A. brasiliensis ATCC 16404		
		Day-3: C. albicans ATCC 10231, Salmonella Spp. NCTC 6017,B. subtilis ATCC 6633 / NCIM 8054, Common Isolate -I		
7.	Soyabean casein digest medium (SCDM-S) (used for sterility test)	B. subtilis ATCC 6633 / NCIM 8054	20–25°C ≤ 3Days	Aerobic
		Common Isolate -I & II		
		C. albicans ATCC 10231	30–35°C ≤ 5 Days	Aerobic
		A.brasiliensis ATCC 16404		
		C. albicans ATCC 10231	20–25°C ≤ 5 Days	Aerobic
		A.brassilensis ATCC 16404		

*Initial micro organism requirement same GPT of Soyabean casein digest medium used for MLT, Sterilty, media fill, Water testing etc.
**Not applicable for SCDM used for sterility testing
****Day 1 consider as first working day*

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S.No.	Name of Media	Name of Organism		Incubation Temp. & Time	Condition
8.	Dey engley agar (DENA)and broth(DENB)	*P. aeruginosa ATCC 9027 / NCIM 2200*		30–35°C ≤ 5Days	Aerobic
		B. subtilis ATCC 6633 / NCIM 8054			
9.	Potato dextrose agar(PODA)	*C.albicans ATCC 10231*		20–25°C ≤ 5 Days	Aerobic
		A. brasiliensis ATCC 16404			
10.	Sabouraud chloramphenicol agar (SACA)	*C.albicans ATCC 10231*		20–25°C ≤ 5 Days	Aerobic
		A. brasiliensis ATCC 16404			
11.	Antibiotic assay medium No.03(AA03)	*Klebsiella pnemonae ATCC10031*		30–35°C 24 hours	Aerobic
12.	Enterobacteria enrichment broth–mossel(EBEB)	Growth promoting	P. aeruginosa ATCC 9027/NCIM 2200	30–35°C 18– 24 hours	Aerobic
			E. coli ATCC 8739		
		Inhibitory	S.aureus ATCC 6538.	30–35°C 18–72 hours	
13.	Violet red bile glucose agar(VRGA)	Growth promoting + Indicative	*E. coli ATCC 8739/ NCIM 2065*	30–35°C 18–24 hours	Aerobic
			P.aeruginosa ATCC 9027		

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S.No.	Name of Media	Name of Organism		Incubation Temp. & Time	Condition
14	MacConkey's agar(MACA), EMB agar (EMBA)	Growth promoting + Indicative	*E. coli ATCC 8739 / NCIM 2065*	30–35°C 18–24 hours	Aerobic
15	MacConkey's broth(MACB)	Growth promoting	*E. coli ATCC 8739 / NCIM 2065*	42–44°C 18–24 hours	Aerobic
		Inhibitory	*S.aureus ATCC 6538.*	30–35°C 18–72 hours	
16	Xylose lysine Deoxycholate citrate agar(XLDA)	Growth promoting + Indicative	*Salmonella Spp. NCTC 6017*	30–35°C 18–24 hours	Aerobic
17	Mannitol salt agar(MASA)	Growth promoting + Indicative	*S.aureus ATCC 6538*	30–35°C 18–24 hours	Aerobic
		Inhibitory	*E. coli ATCC 8739 / NCIM 2065*	30–35°C 18–72 hours	
18	Cetrimide agar(CETA)	Growth promoting	*P. aeruginosa ATCC 9027 / NCIM 2200*	30–35°C 18–24 hours	Aerobic
		Inhibitory	*E. coli ATCC 8739 / NCIM 2065*	30–35°C 18–72 hours	
19	Rappaport Vassiliadis salmonella(enrichment broth(RVSM)	Growth promoting	*Salmonella Spp. NCTC 6017*	30–35°C 18–24 hours	Aerobic
		Inhibitory	*S.aureus ATCC 6538*	30–35°C 18–72 hours	

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S.No.	Name of Media	Name of Organism		Incubation Temp. & Time	Condition
20.	Clostridia agar(CLOA)	Growth promoting	*Cl. sporogenes ATCC 19404 or ATCC 11437*	30–35°C 24–72 hours	Anaerobic
21.	Pseudomonas agar(Pyocyanin) (PSAP)	Growth promoting	*P. aeruginosa ATCC 9027/NCIM 2200*	30–35°C 18–24 hours	Aerobic
22.	Pseudomonas agar (Fluoresces) (PSAF)	Growth promoting	*P. aeruginosa ATCC9027/NCIM 2200*	30–35°C 18–24 hours	Aerobic
23.	Triple sugar iron agar(TSIA)	Growth promoting	*Salmonella Spp. NCTC 6017*	30–35°C 18–24 hours	Aerobic
24.	Urea Broth(UREB)	Growth promoting	*Salmonella Spp. NCTC 6017*	30–35°C 18–24 hours	Aerobic
25.	Antibiotic assay medium No.01(AA01)	Growth promoting	*B. subtilis ATCC 6633 / NCIM 8054* *Bacillus pumilus ATCC 14884 /NCIM 2327*	30–35°C 24 hours	Aerobic
26.	Antibiotic assay medium No.11(AA11)	Growth promoting	*Bacillus pumilus ATCC 14884 / NCIM 2327*	30-35°c 24 hours	Aerobic
27.	Arret and Krisbaum Medium(AKBM)	Growth promoting	*B. subtilis ATCC 6633/ NCIM 8054*	30–35°C 24 hours	Aerobic
2.8	Cooked Meat Medium(COMM)	Growth promoting	*Cl. sporogenes ATCC 19404 or ATCC 11437*	30–35°C 72 hours	Aerobic

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S.No.	Name of Media	Name of Organism		Incubation Temp. & Time	Condition
29.	GN Medium(GNME)	Growth promoting	*S. boydii ATCC 9207*	30–35°C 24 hours	Aerobic
		Inhibitory	*E.coli ATCC 8739 / NCIM 2065*	30–35°C 72 hours	
30.	Reinforced Medium for Clostridia (RFMC)	Growth promoting	*Clostridium sporogenes ATCC 19404 or ATCC 11437*	30-35°C 48 hours	Anaerobic
31.	Columbia Agar (CLMA)	Growth promoting	*Clostridium sporogenes ATCC 19404 or ATCC 11437*	30-35°C 48-72 hours	Anaerobic

5.0 ANNEXURE (S):

Annexure-I: Growth Promotion and inhibition Test report.

6.0 REFERENCE (S):

SOP: Procedure for preparation of Media

SOP: Disposal of microbial culture media and cleaning of glassware used for culture media.

SOP: Maintenance of microbial cultures & microbial culture dilution

SOP: Investigation of out of specification of test results in microbiology

As per current versions of IP, BP, USP, and European pharmacopoeia.

7.0 ABBREVIATION (S) / DEFINATION (S):

ATCC: American Type Culture Collection.

NCIM: National Institute of Industrial Microorganism.

NMT : Not more than

GPT.: Growth promotion test

IP. Indian Pharmacopoeia

BP.: British Pharmacopoeia

USP: United Pharmacopoeia

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SOP: Standard Operating Procedure

QCM : Quality Control Microbiology

No. : Number

Cfu: Colony Forming Units

I.R : Infra Red

REVISION CARD

S.No.	REVISION No.	REVISION DATE	DETAILS OF REVISION	REASON (S) FOR REVISION	REFERENCE CHANGE CONTROL No.
1	00	---	---	New SOP	---

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Annexure 1
GROWTH PROMOTION & INHIBITION TEST REPORT

Prepared media lot No.		Date of GPT		LAF ID No	

GPT Detail:

Name of organism	Initial CFU	Plate I	Plate II	Average CFU	% Recovery	Observed By	Checked By

GPT Status (limit for % recovery: should be 50% to 200%): complies/Not complies

Inhibition Detail:

Name of organism	Initial CFU	Plate I	Plate II	Average CFU	Observed By	Checked By

Media uses detail:

No of plates/tubes prepared	No. of plates/tubes used	Used on	Used for	Remaining plates/tubes	Used by

Note: Physical observation after 15 days±2 days of preparation

Observation of microbial growth and physical parameter.

Observation date	Microbial growth	Cracked or dimpled surface	Excessive No. of bubbles	Medium colour darkening/changed	Filled volume	Observed by	Checked by

Observed By	**Date of Report**	**Checked By**
(Sign. & Date)		**(Sign. & Date)**