1.0 OBJECTIVE

To lay down a procedure for growth promotion, indicative and inhibition test of Microbiological media.

2.0 SCOPE

This procedure applies for growth promotion, indicative and inhibition test of Microbiological Media used in Microbiology laboratory of Pharma Micro Hub Pvt Ltd.

3.0 RESPONSIBILITY

3.1 Microbiologist is responsible for performing growth promotion, indicative and inhibition test of Microbiological Media used in Microbiology laboratory.

4.0 ACCOUNTABILITY

4.1 Head-Microbiology or designee is accountable for the overall compliance of this SOP.

5.0 PROCEDURE

5.1 **Precautions:**

5.1.1 Wear the PPE equipment's like nose mask, goggles and hand gloves during handling of cultures.

5.1.2 Always use inoculum concentration of not more than 100 CFU for growth promotion and not less than 100cfu for growth inhibition.

5.1.3 Handle cultures in biosafety cabinet.

5.1.4 Wipe the spillage area under LAF with a sterile lint free mop soaked in disinfectant solution to remove traces of medium / culture.

5.1.5 Alternative rehydration fluid can also be used for rehydration of bio ball.

5.1.6 Rehydrate the bio ball cultures soon after taking them out from the deep freezer.

5.2 **Growth Promotion, Indicative Properties and Inhibitory Properties of Media**

5.2.1 Perform the Growth Promotion, indicative and Inhibitory Test for each batch of ready-to use media and each lot/batch of prepared media from dehydrated media.

5.2.2 Carryout the test in culture handling room under biosafety cabinet.

5.2.3 Use culture suspension / Ready to use culture (Bioballs) containing not more than 100 CFU for growth promotion, indicative properties.

5.2.4 Use the culture suspension containing not less than 100 cfu for inhibition test.

5.2.5 Transfer the required quantity of media (tubes/plates/bottles) into the culture handling room

5.2.6 Perform the growth promotion, indicative and inhibitory test of media using the challenge strains specified against each media as per Annexure -III .

Note: In case of pour plate media used for Microbial enumeration test / Preservative efficacy test / culture suspension test etc., parallel growth promotion test shall be performed. However the results for the microbial enumeration test shall be released only after the satisfactory growth promotion test result.

5.2.7 **Solid Media**:

5.2.7.1 Perform the growth promotion test in duplicate plates for all the media that are expected to recover the growth on agar surfaces by pour plate, spread plate method which is suitable.

5.2.7.2 In case of Environmental monitoring media, perform the GPT after pre- incubation of in-house prepared media using spread plate technique.

5.2.7.3 Vortex the suspension to get homogenous culture suspension.

5.2.7.4 **Pour plate method:** Add 0.1mL of test organism suspension containing not more than 100 CFU for growth promotion, indicative and add the suspension containing not less than 100 CFU in to each of the sterile Petri dishes (duplicate)

5.2.7.5 Pour about 15-20 mL of sterilized agar media to each plate at a temperature about 45-50°C. Mix well by rotating the plate clockwise and anticlockwise direction for homogenous mixing and allow the plates to solidify.

5.2.7.6 **Spread plate method**: Add 0.1mL of test organism suspension containing not more than 100 CFU for growth promotion, indicative and add the suspension containing not less than 100 CFU. In case of inhibitory test on to the surface of media which is already poured in plate and spread evenly with the help of sterile spreader.

5.2.7.7 Incubate the plates at specified conditions as given in Annexure-III.

5.2.7.8 Note: If the Growth promoting and indicative organism is same, then same portion / plate shall be used for both the tests.

5.2.7.9 After incubation, count the number of CFU and record the observations in Annexure-I (Growth promotion, indicative properties and Inhibition record for agar media).

5.2.7.10 Incubate one un-inoculated plate for each temperature as negative control from each lot of media and enter the details in Annexure-I.

5.2.8 **Interpretation of results for solid media:**

5.2.8.1 Growth obtained in the tested solid media should not differ by a factor 2 from the calculated value of standardized inoculum. For a freshly prepared inoculum, growth of the microorganisms comparable with a previously tested and approved batch of media.

5.2.8.2 There should not be any growth in the media for inhibitory test.

5.2.8.3 There should not be any growth in the negative controls.

5.2.8.4 There should be defined characteristic growth for differential media.

5.2.9 **For Liquid Media:**

5.2.9.1 Take the required number of sterilized media containers for the growth promotion / inhibitory test. In case if the media volumes are less, the media can be dispensed aseptically in to sterile tubes in portions of 10mL each and these tubes can be used for the test.

5.2.9.2 Vortex the suspension to get homogenous culture suspension.

5.2.9.3 Add test organism suspension containing not more than 100 CFU in to the media containers for each of challenge organism's for growth promotion. In case of inhibitory test, add the suspension containing not less than 100 CFU.

5.2.9.4 Incubate the tubes at specified conditions as given in Annexure-III

5.2.9.5 Incubate one un-inoculated tube for each temperature as negative control from each lot of media.

5.2.9.6 After incubation, record the observations as per Annexure -I (Growth promotion, indicative properties and Inhibition record for agar media).

5.2.9.7 For diluents, solvents and rinsing solutions test for growth promotion properties shall not be performed.

5.2.9.8 In case any media contain neutralizer (liquid media) the growth were unable to distinguish, the media to be subcultured on SCDA agar plate for growth evidence and the results shall be noted based upon the test results.

5.2.10 **In case of media fill GPT samples:**

5.2.10.1 **For Bulk media GPT:**

5.2.10.1.1 Dispense the bulk media aseptically into the sterile tubes in portions of 10 mL each and perform the growth promotion test. Use all the challenge strains mentioned against the type of media as per the Annexure-III.

5.2.10.1.2 Vortex the suspension to get homogenous culture suspension.

5.2.10.1.3 Add 0.1 mL test organism suspension containing not more than 100 CFU to each of container.

5.2.10.1.4 Incubate the tubes at specified conditions as given in Annexure-III.

5.2.10.1.5 Incubate one un-inoculated tube for each temperature as negative control from each lot of media.

5.2.10.2 **For Post GPT:**

5.2.10.2.1 Collect randomly the required number of containers as per the table-I for growth promotion test after completion of 14 days incubation.

5.2.10.2.2 Use all the challenge strains specified against the SCDM media as per Annexure-III

5.2.10.2.3 Vortex the suspension to get homogenous culture suspension.

5.2.10.2.4 Add 0.1 mL test organism suspension containing not more than 100 CFU to the container and incubate the containers as per the Table-I.

5.2.10.2.5 On the day of inoculation into the media filled containers plate out the preserved suspension by pour plate method to confirm the actual count of suspension of each organism used for inoculating into containers and incubate the plates at 30 – 35°C for bacteria and 20 – 25°C for fungi.

5.2.10.2.6 Incubate one un-inoculated container for each temperature as negative control from each lot of media.

Table-I:

Container Type	Number of containers to be tested for each organism	Incubation Conditions
Prefilled syringes(PFS),ampoules or Vials	Perform the test for 8 individual prefilled syringes (PFS),ampoules or vials for each organism and incubate.	Incubate one half of the inoculated (4) containers of each organism at 20-25°C and the other half (4) at 30-35°C.

5.2.10.2.7 Observe for the growth in the containers daily. The bacterial cultures should show growth within 3 days and the fungal cultures should show growth within 5 days.

5.2.11 **Interpretation of results for liquid media:**

5.2.11.1 The media passes growth promotion test, if visible growth appears within the specified incubation time and temperature. There should not be any growth in the media in case of inhibitory test.

5.2.11.2 There should not be any growth in the negative controls.

5.2.11.3 Note: A negative control should be performed for each medium during growth promotion test and it should show no growth until the maximum period of incubation.

5.2.12 **Sterility Check**

5.2.12.1 A negative control shall be performed for each medium during Growth Promotion Test and it should show no growth until the maximum period of incubation of GPT test. If media used for testing at multiple incubation, keep the media at different incubation conditions

5.3 **Investigation for failure in growth promotion and inhibitory test results:**

5.3.1 If two or fewer of the test organisms fail to meet the required limits, inform immediately to the Head Microbiology / Designee and perform the laboratory investigation as per the Annexure-II (investigation report for failure in growth promotion and inhibitory test results).

5.3.2 As part of the investigation, re-test the media with those strains which have failed using the fresh cultures.

Note 1: In case the prepared media was not available (pour plate / spread plate media) Prepare fresh media and perform the growth promotion test as a part of investigation with those organisms (fresh culture) which was failed.

Note 2: In case the prepared media was available (pour plate / spread plate media) use the same media and perform the growth promotion test as a part of investigation with those organisms (fresh culture) which was failed.

5.3.3 If the results of the re-test comply with the test requirements, consider the media lot passing the test.

5.3.3.1 If the results of the original test and the re-test don't comply with the test requirements, raise incident for investigation.

5.3.3.2 No re-testing is permitted if three or more cultures fail to meet the required limits. Raise incident for investigation.

5.3.4 Investigate if the following conditions are observed in growth promotion and inhibitory test as per following procedure and record as per Annexure -II (Investigation report for failure in growth promotion, indicative and inhibitory test results).

5.3.4.1 Growth observed in negative control.

5.3.4.2 Growth observed in inhibitory test.

5.3.4.3 No growth in liquid media or in case solid media recovery study growth differs by factor 2 from initial count or there is no characteristic growth in case of differential media.

5.3.5 As part of the investigation, re-test the media with those strains which have failed using the fresh cultures.

5.3.6 Whenever out of specification results are obtained in GPT / Inhibitory test microbiologist will immediately inform to supervisor and head microbiology.

5.3.7 Head microbiology/his designee will review the method followed by the analyst.

5.3.8 Review whether the analyst has under gone training for the related procedure to perform the test correctly.

5.3.9 If growth observed in negative control.

5.3.9.1 Review the sterilization records, Temperature and RH of testing area, cleaning records of incubator and pass boxes, disinfectant preparation record, Verify the sterilization status of articles used for test but not limited to.

5.3.10 If growth observed in inhibitory test.

5.3.10.1 Culture used for the test, Expiry of the media, reports of media suitability test, verify the addition of supplements as per manufacturer recommendation, Verification of negative control, and review the sterilization records but not limited to.

5.3.11 If no growth in liquid media or in case solid media recovery study growth differ by factor 2 from initial count there is no characteristic growth in case of differential media.

5.3.11.1 Investigate whether any abnormal incidence was occurred during test was carried out e.g. Temperature of media during inoculation / incubation, amount of inoculum added to the media, type of culture and media used for test, vortexing of culture suspension, culture validity period, Validity of media, PH of the media after sterilization, reports of media suitability test, In case of agar media check the calculations to verify if any error has been observed, check the calibration status of micropipettes, Verify the sterilization status of articles used for test, Incubation conditions of the test as per procedure but not limited to.

5.3.12 If any abnormalities observed during investigation further investigation and action to be taken with coordination of Head Microbiology/ his designee.

6.0 ABBREVIATIONS

6.1 ATCC : American type culture collection.

7.0 ANNEXURES

7.1 Annexure-I: Growth Promotion, Indicative Properties and Inhibition Record For Media.

7.2 Annexure-II: Investigation report for the failure in growth promotion, indicative and inhibitory test results.

7.3 Annexure-III: list of cultures used for growth promotion, indicative and inhibitory test of media and incubation conditions.

8.0 REFERENCES

8.1 USP<1117> Good Laboratory Practices in microbiology Laboratory.

8.2 USP<61> Microbiological Examination of Nonsterile Products:Microbial Enumeration Tests

8.3 USP<62> Microbiological Examination of Non Sterile Products: Tests for Specified Microorganisms.

9.0 CHANGE HISTORY

9.1 New SOP

END OF DOCUMENT

Annexure-1

Growth Promotion, Indicative Properties and Inhibition Record For Media

Name of the Media		Media Ref. No.	
Date of Preparation		Valid Up to	

Inoculum Details and Microbial Recovery:

Incubation Conditions	Incubator Id.	Incubation Start Date/Time	Incubated by	Incubation End Date/Time
20-25°C				
30-35°C				
42-44°C				
Anaerobic Jar ID No.		Colony counter ID No.		

Inoculated the Media Petri plates with _____ mL of each of below cultures.

Name of Test Strains	Standard Cultures/Bioball details			Observed Results			Acceptance limit as per Factor 2 of standard inoculum	Characteristic growth/Turbidity	Observered by	Complies/ does not comply
	Culture Ref. No.	Valid Upto	Viable Count (Standard inoculum)	Plate I	Plate II	Average				

Observations:

☐ For growth promotion/ growth Indicative/ growth Inhibitory, growth obtained within/Not within the factor 2 calculated value for a standardized inoculum and Complies/Does Not Comply with the growth promotion results of previously tested and approved Lot ------ of Media.

Negative Control: Complies / does not comply

Remarks: Media Passes / Fails for Growth Promotion & Indicative Properties / Inhibition Test.

Checked by(Sign & Date):

ANNEXURE-II
INVESTIGATION REPORT FOR THE FAILURE IN GROWTH PROMOTION, INDICATIVE AND INHIBITORY TEST RESULTS.

Investigation Report Number:

MEDIA INFORMATION

Name of the media	
Dehydrated media lot Number	
Prepared /ready to use media ref. Number	
Date Tested	
Analyst Name	

Acceptance Criteria:

Test Results:

PRELIMINARY INVESTIGATION

1) Did the test method followed correctly and correct method used?
2) Were the suspensions labelled?
3) Were the suspensions within the expiration dates?
4) Were the suspensions properly stored?
5) Were the suspensions properly standardized?
6) Were the added inoculum size correct?
7) Was the dehydrated media within the expiry date?
8) Was the dehydrated media labelled?
9) Was the dehydrated media stored at recommended storage conditions?
10) Was the water used for the media preparation meets the requirement?
11) Media weighing details:
12) Media reconstitution details:
13) Media preparation details:
14) Was the appropriate volume of dehydrated media dispensed into containers?
15) Were the appropriate containers used?
16) Was the correct load pattern followed?
17) Was the media sterilized using a validated cycle?
18) Whether any alarms or deviations observed during the sterilization cycle?
19) Any charring, dehydration or precipitation observed after sterilization?
20) Was the challenged media incubated as per the test procedure?
21) Was the pH of prepared media within the limit?
22) Were the equipments functioning properly?
23) Were the equipments calibrated properly and within the calibration period?
24) Was the analyst trained in the procedure?
25) Was the negative control shown growth?

Date Performed: _____ Cause: Assignable/Non-assignable

Conclusion from preliminary investigation:

_____

_____

_____

Analyst Supervisor QA Representative

Summary

Root cause and impact analysis assessment:

Conclusion:

Prepared by: **Approved by:**

QA Comments:

Approved by (QA):

Investigation ended on:

ANNEXURE- III
LIST OF CULTURES USED FOR GROWTH PROMOTION, INDICATIVE AND INHIBITORY TEST OF MEDIA AND INCUBATION CONDITIONS

S.No	Media	Test Microbial Culture	Method of control	Property	Incubation time and temperature	Acceptance criteria
01	Soyabean-Casein Digest Agar For routine Microbial analysis	*P.aeruginosa* ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275	Quantitative	Growth promoting	≤ 3 days at 30-35°C	Growth obtained must not differ by a factor two from the calculated value for standardized inoculums
		S.aureus ATCC6538, NCIMB 9518, CIP 4.83 or NBRC 13276			≤ 3 days at 30-35°C	
		B.subtilis ATCC6633, NCIMB 8054, CIP 52.62 or NBRC 3134			≤ 3 days at 30-35°C	
		A. brasiliensis ATCC 16404, NCIMB 149007, IP 1431.83 or NBRC 9455			≤ 5 days at 30-35°C	
		C. albicans ATCC 10231, NCPF3179, IP 48.72 or NBRC 1594			≤ 5 days at 30-35°C	
		2 Environmental Isolates			≤ 3 days at 30-35°C	
		A. brasiliensis ATCC 16404, NCIMBIMI 149007, IP 1431.83 or NBRC 9455			≤ 5 days at 20-25°C	
		C. albicans ATCC 10231, NCPF3179, IP 48.72 or NBRC 1594			≤ 5 days at 20-25°C	
02	Sabouraud Dextrose Agar	*A. brasiliensis* ATCC 16404, NCIMBIMI 149007, IP	Quantitative	Growth promoting	≤ 5 days at 20-25°C	Growth obtained must not differ by

	(With / Without supplements)	1431.83 or NBRC 9455 *C. albicans* ATCC 10231, NCPF3179, IP 48.72 or NBRC 1594				a factor two from the calculated value for standardized inoculums.
03	MacConkey Agar	*E.coli* ATCC 8739, NCIMB 8545, CIP 53.126 OR NBRC 3972	Qualitative	Growth promoting	≤ 18 hours at 30-35°C	Visible growth should be observed
				Indicative Properties	≤ 72 hours at 30-35°C	Brick red colonies; may have surrounding zone of precipitated bile
04	Soyabean-Casein Digest Medium	*P.aeruginosa* ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275	Qualitative	Growth Promoting	≤ 3 days at 30-35°C	Visible growth should be observed
		S.aureus ATCC6538, NCIMB 9518, CIP 4.83 or NBRC 13276			≤ 3 days at 30-35°C	
		B.subtilis ATCC6633, NCIMB 8054, CIP 52.62 or NBRC 3134			≤ 3 days at 30-35°C	
		A. brasiliensis ATCC 16404, NCIMBIMI 149007, IP 1431.83 or NBRC 9455			≤ 5 days at 20-25°C	
		C. albicans ATCC 10231, NCPF3179, IP 48.72 or NBRC 1594			≤ 5 days at 20-25°C	
		2 Environmental Isolates			≤ 3 days at 30-35°C	

05	Fluid Thioglycollate Medium	*P.aeruginosa* ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275	Qualitative	Growth promoting	≤ 3 days at 30-35°C	Visible growth should be observed
		S.aureus ATCC6538, NCIMB 9518, CIP 4.83 or NBRC 13276			≤ 3 days at 30-35°C	
		Cl.sporogenes ATCC 11437, NBRC 14293, NCIMB 12343, CIP 100651) OR ATCC 19404 (NCTC 532 or CIP 79.3)			≤ 3 days at 30-35°C	
		2 Environmental Isolates			≤ 3 days at 30-35°C	
06	Columbia agar	*Cl.sporogenes* ATCC 11437, NBRC 14293, NCIMB 12343, CIP 100651) OR ATCC 19404 (NCTC 532 or CIP 79.3)	Quantitative	Growth promoting	Incubate under anaerobic conditions for ≤ 48 hours at 30-35°C	Visible growth should be observed.
07	R2A Agar	*P.aeruginosa* ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275	Quantitative	Growth promoting	≤ 3 days at 30-35°C	Growth obtained must not differ by a factor two from the calculated value for standardized inoculums
		S.aureus ATCC6538, NCIMB 9518, CIP 4.83 or NBRC 13276			≤ 3 days at 30-35°C	
		B.subtilis ATCC6633, NCIMB 8054, CIP 52.62 or NBRC 3134			≤ 3 days at 30-35°C	
		C. albicans ATCC 10231, NCPF3179, IP 48.72 or NBRC 1594			≤ 5 days at 30-35°C	
		2 Environmental Isolates			≤ 3 days at 30-35°C	

		A. brasiliensis ATCC 16404, NCIMBIMI 149007, IP 1431.83 or NBRC 9455			≤ 5 days at 30-35°C	
08	Soyabean-Casein Digest Medium (Used for sterility test)	*A. brasiliensis* ATCC 16404, NCIMBIMI 149007, IP 1431.83 or NBRC 9455	Qualitative	Growth promoting	≤ 5 days at 20-25°C	Visible growth should be observed.
		C. albicans ATCC 10231, NCPF3179, IP 48.72 or NBRC 1594			≤ 5 days at 20-25°C	
		B.subtilis ATCC6633, NCIMB 8054, CIP 52.62 or NBRC 3134			≤ 3 days at 20-25°C	
		2 Environmental Isolates			≤ 3 days at 20-25°C	
09	Soyabean-Casein Digest Medium (Used for Media fill study)	*P.aeruginosa* ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275	Qualitative	Growth promoting	≤ 3 days at 30-35°C	Visible growth should be observed
		S.aureus ATCC6538, NCIMB 9518, CIP 4.83 or NBRC 13276			≤ 3 days at 30-35°C	
		B.subtilis ATCC6633, NCIMB 8054, CIP 52.62 or NBRC 3134			≤ 3 days at 30-35°C	
		2 Environmental Isolates			≤ 3 days at 30-35°C	
		A. brasiliensis ATCC 16404, NCIMBIMI 149007, IP 1431.83 or NBRC 9455			≤ 5 days at 20-25°C	
		C. albicans ATCC 10231, NCPF3179, IP 48.72 or NBRC 1594			≤ 5 days at 20-25°C	
10	Rappaport vasiliadis salmonella	*Salmonella enterica subsp. Enterica* serovar	Qualitative	Growth promoting	≤ 18 hours at 30-35°C	Visible growth should be

	Enrichment broth (RVSEB)*	*Typhimurium* OR *as an alternative Salmonella enterica subsp. Enterica* serovar Abony ATCC14028, NBRC 100797, NCTC 6017 or CIP 80.39				observed.
		S.aureus ATCC6538, NCIMB 9518, CIP 4.83 or NBRC 13276		Inhibitory	≥ 24 hours at 30-35°C	No growth

*After completion of incubation, streak a loop full from RVSEB on to XLDA and incubate at 30-35°C for ≤ 18 hours.

11	Enterobacteria Enrichment broth (Mossel)	*E.coli* ATCC 8739, NCIMB 8545, CIP 53.126 OR NBRC 3972	Qualitative	Growth promoting	≤ 24 hours at 30-35°C	Visible growth should be observed
		P.aeruginosa ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275				
		S.aureus ATCC6538, NCIMB 9518, CIP 4.83 or NBRC 13276		Inhibitory	≥ 48 hours at 30-35°C	No growth should be observed
12	Violet Red bile glucose agar	*P.aeruginosa* ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275	Qualitative	Growth promoting	≤ 18 hours at 30-35°C	Visible growth should be observed
		E.coli ATCC 8739, NCIMB 8545, CIP 53.126 OR NBRC 3972				
		P.aeruginosa ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275		Indicative Properties	≤ 24 hours at 30-35°C	Pinkish red color colonies should be observed.
		E.coli ATCC 8739, NCIMB 8545, CIP 53.126 OR NBRC 3972				
13	Sabouraud Dextrose Broth	*C. albicans* ATCC 10231, NCPF3179, IP 48.72 or NBRC	Qualitative	Growth promoting	≤ 3 days at 30-35°C	Visible growth should be observed

		1594				
14	Reinforced medium	*Cl.sporogenes* ATCC 11437, NBRC 14293, NCIMB 12343, CIP 100651) OR ATCC 19404 (NCTC 532 or CIP 79.3)	Qualitative	Growth promoting	Incubate under anaerobic conditions for ≤ 48 hours at 30-35°C	Visible growth should be observed.
15	Cetrimide Agar	*P.aeruginosa* ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275	Qualitative	Growth promoting	≤ 18 hours at 30-35°C	Visible growth should be observed
				Indicative Properties Properties	≤ 72 hours at 30-35°C	Greenish, Colored colonies should be observed.
		E.coli ATCC 8739, NCIMB 8545, CIP 53.126 OR NBRC 3972	Qualitative	Inhibitory	≥72 hours at 30-35°C	No growth should be observed
16	Mannitol-Salt Agar	*S.aureus* ATCC6538, NCIMB 9518, CIP 4.83 or NBRC 13276	Qualitative	Growth promoting	≤ 18 hours at 30-35°C	Visible growth should be observed
				Indicative Properties	≤ 72 hours at 30-35°C	White or Yellow colonies surrounded by yellow zones
		E.coli ATCC 8739, NCIMB 8545, CIP 53.126 OR NBRC 3972		Inhibitory	≥ 72 hours at 30-35°C	No growth
17	Sabouraud Dextrose Agar(Used for testing Candida albicans as specified organism)	*C. albicans* ATCC 10231, NCPF3179, IP 48.72 or NBRC 1594	Qualitative	Growth promoting	≤ 24 hours at 30-35°C	Visible growth should be observed.
				Indicative Properties	≤ 48 hours at 30-35°C	White color colonies should be observed.

18	Xylose-Lysine-Deoxycholate Agar Medium	*Salmonella enterica subsp. Enterica* serovar *Typhimurium* OR *as* *An alternative Salmonella enterica subsp. Enterica* serovar Abony	Qualitative	Growth promoting	≤ 18 hours at 30-35°C	Visible growth should be observed.
				Indicative Properties	≤ 18 hours at 30-35°C	Red color colonies with or without black centers
19	MacConkey's Broth	*E.coli* ATCC 8739, NCIMB 8545, CIP 53.126 OR NBRC 3972	Qualitative	Growth promoting	≤ 24 hours at 42-44°C	Visible growth should be observed.
		S.aureus ATCC6538, NCIMB 9518, CIP 4.83 or NBRC 13276		Inhibitory	≥ 48 hours at 42-44°C	No growth
		S.aureus ATCC6538, NCIMB 9518, CIP 4.83 or NBRC 13276				
		B.subtilis ATCC6633, NCIMB 8054, CIP 52.62 or NBRC 3134				

Note: 1.In case of any other media used for special purpose (such as confirmatory test of specified microorganisms, disinfectant validation, etc.), growth promotion test shall be performed using the microorganisms based on the test requirement and manufacturer's recommendations applying appropriate incubation conditions and shortest incubation period.

Note: 2. Initially GPT to be done except environmental isolate.