



Title: Validation of Extended Settle Plate Exposure Time and Extended Incubation of Microbiological Culture Media

PROTOCOL NUMBER	VP/MS/002-00
EFFECTIVE DATE :	19/04/2026
DEPARTMENT:	Microbiology
PAGE NUMBER:	1 of 17

**PROTOCOL FOR
EXTENDED SETTLE PLATE EXPOSURE TIME AND EXTENDED INCUBATION OF
MICROBIOLOGICAL CULTURE MEDIA**



Title: Validation of Extended Settle Plate Exposure Time and Extended Incubation of Microbiological Culture Media

PROTOCOL NUMBER	VP/MS/002-00
EFFECTIVE DATE :	19/04/2026
DEPARTMENT:	Microbiology
PAGE NUMBER:	2 of 17

1.0 PRE-APPROVAL

Signing of this approval page of protocol indicates agreement with the qualification approach described in this document.

Name	Department/Designation	Signature	Date
Prepared By:			
Reviewed By:			
Approved By:			



Title: Validation of Extended Settle Plate Exposure Time and Extended Incubation of Microbiological Culture Media

PROTOCOL NUMBER	VP/MS/002-00
EFFECTIVE DATE :	19/04/2026
DEPARTMENT:	Microbiology
PAGE NUMBER:	3 of 17

2.0 PURPOSE

The purpose of this protocol is to assess the impact of growth promoting properties of the media after extended exposure and extended incubation.

3.0 SCOPE

The scope of this protocol is applicable to assess the impact of growth promoting properties of the media after extended exposure and extended incubation at Pharma Micro Hub Pvt Ltd.

4.0 ABBREVIATIONS

Abbreviations	Details
CFU	Colony Forming Unit
LAF	Laminar Air Flow
NMT	Not More Than
ATCC	American Type Culture Collection
NMT	Not More Than
GPT	Growth Promotion Test

5.0 RESPONSIBILITY

5.1 Microbiology:

- Preparation of miscellaneous protocol and report.
- Execution of Study

5.2 Quality Assurance:

- Review and Approval of miscellaneous protocol/report.

6.0 OVERVIEW

- To assess the impact of growth promoting properties of media during extended exposure and extended incubation.
- To establish the maximum settle plate exposure and incubation time.

7.0 STUDY DESIGN

Media plates shall be exposed under the Grade-A LAF. LAF locations shall be considered as worst-case based on the continuous airflow, expected no recoveries and further it can be subjected for growth promotion test. Plates shall be exposed at different time intervals and incubated as defined in the procedure.

Post completion of incubation, the plates shall be physically verified for dryness, cracks, shrinkage and recoveries prior to subjecting for growth promotion test.



Title: Validation of Extended Settle Plate Exposure Time and Extended Incubation of Microbiological Culture Media

PROTOCOL NUMBER	VP/MS/002-00
EFFECTIVE DATE :	19/04/2026
DEPARTMENT:	Microbiology
PAGE NUMBER:	4 of 17

Perform GPT each media and incubated according to the routine incubation period specified in the table-2 the media were examined for physical characteristics, including dryness, shrinkage, cracks, discoloration, dehydration, contamination, and overall media integrity the results were record in respective annexure.

After recording the routine incubation observations, the same media shall be return to respective incubator and incubation shall be continued under the same incubation conditions until a total incubation period of seven 7 days.

At the end of the seven-day incubation period, the media were examined for physical characteristics, including dryness, shrinkage, cracks, discoloration, dehydration, contamination, and overall media integrity. Growth Promotion Test results were then recorded and compared with those obtained for regular incubation period.

8.0 PROCEDURE

8.1 Required Materials/Equipments:

- Refer Table-I for the details of the test strains required for the study.

Table-I

Name of the Strains	ATCC No. (or) Equivalent Numbers
<i>Pseudomonas aeruginosa</i>	ATCC 9027
<i>Staphylococcus aureus</i>	ATCC 6538
<i>Bacillus subtilis</i>	ATCC6633
<i>Aspergillus brasiliensis</i>	ATCC 16404
<i>Candida albicans</i>	ATCC 10231
Environmental isolate	NA

- Use in-house culture suspension or Ready to use Standardized culture suspension (NMT 100 CFU/0.1mL) for the growth promotion test. ATCC cultures or ATCC equivalent cultures can be used.

Note: Ensure the culture suspensions used in the test are within the 5 passages derived from the original culture.

- Media
- Spreaders.
- Vortex mixture.
- Incubators.
- Colony counter.
- Laminar air flow or Biological safety cabinet.
- Micropipettes and tips.



Title: Validation of Extended Settle Plate Exposure Time and Extended Incubation of Microbiological Culture Media

PROTOCOL NUMBER	VP/MS/002-00
EFFECTIVE DATE :	19/04/2026
DEPARTMENT:	Microbiology
PAGE NUMBER:	5 of 17

8.2 Procedure:

8.2.1 Procedure for environmental monitoring Testing Media:

- 8.2.1.1 Take the required number of ready to use Gamma Irradiated media plates (SCDA) or In house prepared media plates and transfer the plates to the sterility testing area.
- 8.2.1.2 Take required number of plates by considering the growth promotion test, mark each plate with serial number and consider it as Set-I and expose them for 4 hours 15 minutes under the sterility testing/cool zone suspended LAF.
- 8.2.1.3 Collect the plates after 4 hours 15 minutes and transfer them to the incubator room..
- 8.2.1.4 Plates shall be incubated at 20-25°C for about 120 hours i.e. 5 days followed by 30-35°C for about 96 hours i.e. 4 days.
- 8.2.1.5 Repeat the above procedure from point number 10.2.2 to 10.2.4 for Set-II (exposure time 4 hours 30 minutes), Set-III (exposure time 4 hours 45 minutes) and Set-IV (exposure time 5 hours) on different days.
- 8.2.1.6 Record the exposure and incubation details in the Annexure-I.
- 8.2.1.7 Post completion of incubation period, the plates shall be physically verified for dryness, cracks, shrinkage and recoveries prior to subjecting for growth promotion test.
- 8.2.1.8 Perform the growth promotion test with the standard cultures and environmental isolates as mentioned in the Table-I.
- 8.2.1.9 Record the GPT test details and results in the Annexure-II.

8.2.2 Procedure for Water Testing, Product Testing and Sterility Testing Media:

- 8.2.2.1 Perform GPT each media as per table-2 and incubated according to the routine incubation period specified in the table-2 the media were examined for physical characteristics, including dryness, shrinkage, cracks, discoloration, dehydration, contamination, and overall media integrity the results were record in respective annexure.
- 8.2.2.2 After recording the routine incubation observations, the same media shall be return to respective incubator and incubation shall be continued under the same incubation conditions until a total incubation period of seven 7 days.
- 8.2.2.3 At the end of the seven-day incubation period, the media were examined for physical characteristics, including dryness, shrinkage, cracks, discoloration, dehydration, contamination, and overall media integrity. Growth Promotion Test results were then recorded and compared with those obtained for regular incubation period.

	Title: Validation of Extended Settle Plate Exposure Time and Extended Incubation of Microbiological Culture Media	PROTOCOL NUMBER	VP/MS/002-00
		EFFECTIVE DATE :	19/04/2026
		DEPARTMENT:	Microbiology
		PAGE NUMBER:	6 of 17

Table -2

Media	Test Microbial Culture	Incubation time and temperature	Acceptance criteria
Sabouraud Dextrose Agar (With / Without supplements)	<i>A. brasiliensis</i> ATCC 16404, NCIMBIMI 149007, IP 1431.83 or NBRC 9455	≤ 5 days at 20-25°C	Growth obtained must not differ by a factor two from the calculated value for standardized inoculums.
	<i>C. albicans</i> ATCC 10231, NCPF3179, IP 48.72 or NBRC 1594		
MacConkey Agar	<i>E.coli</i> ATCC 8739, NCIMB 8545, CIP 53.126 OR NBRC 3972	≤ 18 hours at 30-35°C	Visible growth should be
		≤ 72 hours at 30-35°C	Brick red colonies; may have surrounding zone of precipitated bile
Soyabean-Casein Digest Medium	<i>P.aeruginosa</i> ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275	≤ 3 days at 30-35°C	Visible growth should be observed
	<i>S.aureus</i> ATCC6538, NCIMB 9518, CIP 4.83 or NBRC 13276	≤ 3 days at 30-35°C	
	<i>B.subtilis</i> ATCC6633, NCIMB 8054, CIP 52.62 or NBRC 3134	≤ 3 days at 30-35°C	
	<i>A. brasiliensis</i> ATCC 16404, NCIMBIMI 149007, IP 1431.83 or NBRC 9455	≤ 5 days at 20-25°C	
	<i>C. albicans</i> ATCC 10231, NCPF3179, IP 48.72 or NBRC 1594	≤ 5 days at 20-25°C	
	2 Environmental Isolates	≤ 3 days at 30-35°C	
Fluid Thioglycollate Medium	<i>P.aeruginosa</i> ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275	≤ 3 days at 30-35°C	Visible growth should be observed
	<i>S.aureus</i> ATCC6538, NCIMB 9518, CIP 4.83 or NBRC 13276	≤ 3 days at 30-35°C	
	<i>Cl.sporogenes</i> 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-	



Title: Validation of Extended Settle Plate Exposure Time and Extended Incubation of Microbiological Culture Media

PROTOCOL NUMBER	VP/MS/002-00
EFFECTIVE DATE :	19/04/2026
DEPARTMENT:	Microbiology
PAGE NUMBER:	7 of 17

		35°C	
Columbia agar	<i>Cl.sporogenes</i> ATCC 11437, NBRC 14293, NCIMB 12343, CIP 100651) OR ATCC 19404 (NCTC 532 or CIP 79.3)	Incubate under anaerobic conditions for ≤ 48 hours at 30-35°C	Visible growth should be observed.
R2A Agar	<i>P.aeruginosa</i> ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275	≤ 3 days at 30-35°C	Growth obtained must not differ by a factor two from the calculated value for standardized Inoculums
	<i>S.aureus</i> ATCC6538, NCIMB 9518, CIP 4.83 or NBRC 13276	≤ 3 days at 30-35°C	
	<i>B.subtilis</i> ATCC6633, NCIMB 8054, CIP 52.62 or NBRC 3134	≤ 3 days at 30-35°C	
	<i>C. albicans</i> ATCC 10231, NCPF3179, IP 48.72 or NBRC 1594	≤ 5 days at 30-35°C	
	2 Environmental Isolates	≤ 3 days at 30-35°C	
	<i>A. brasiliensis</i> ATCC 16404, NCIMBIMI 149007, IP 1431.83 or NBRC 9455	≤ 5 days at 30-35°C	
Soyabean-Casein Digest Medium (Used for sterility test)	<i>A. brasiliensis</i> ATCC 16404, NCIMBIMI 149007, IP 1431.83 or NBRC 9455	≤ 5 days at 20-25°C	Visible growth should be observed.
	<i>C. albicans</i> ATCC 10231, NCPF3179, IP 48.72 or NBRC 1594	≤ 5 days at 20-25°C	
	<i>B.subtilis</i> ATCC6633, NCIMB 8054, CIP 52.62 or NBRC 3134	≤ 3 days at 20-25°C	
	2 Environmental Isolates	≤ 3 days at 20-25°C	
Rappaport vasiladis salmonella Enrichment broth (RVSEB)*	Salmonella enterica subsp. Enterica serovar Typhimurium OR as an alternative Salmonella enterica subsp. Enterica serovar Abony ATCC14028, NBRC 100797, NCTC 6017 or CIP 80.39	≤ 18 hours at 30-35°C	Visible growth should be observed.
	<i>S.aureus</i> ATCC6538, NCIMB 9518, CIP 4.83 or NBRC 13276	≥ 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.

	Title: Validation of Extended Settle Plate Exposure Time and Extended Incubation of Microbiological Culture Media	PROTOCOL NUMBER	VP/MS/002-00
		EFFECTIVE DATE :	19/04/2026
		DEPARTMENT:	Microbiology
		PAGE NUMBER:	8 of 17

Enterobacteria Enrichment broth (Mossel)	<i>E.coli</i> ATCC 8739, NCIMB 8545, CIP 53.126 OR NBRC 3972	≤ 24 hours at 30-35°C	Visible growth should be observed
	<i>P.aeruginosa</i> ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275		
	<i>S.aureus</i> ATCC6538, NCIMB 9518, CIP 4.83 or NBRC 13276	≥ 48 hours at 30-35°C	No growth should be observed
Violet Red bile glucose agar	<i>P.aeruginosa</i> ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275	≤ 18 hours at 30-35°C	Visible growth should be observed
	<i>E.coli</i> ATCC 8739, NCIMB 8545, CIP 53.126 OR NBRC 3972	≤ 24 hours at 30-35°C	Pinkish red color colonies should be observed.
	<i>P.aeruginosa</i> ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275		
	<i>E.coli</i> ATCC 8739, NCIMB 8545, CIP 53.126 OR NBRC 3972		
Sabouraud Dextrose Broth	<i>C. albicans</i> ATCC 10231, NCPF3179, IP 48.72 or NBRC 1594	≤ 3 days at 30-35°C	Visible growth should be observed
Reinforced medium	<i>Cl.sporogenes</i> ATCC 11437, NBRC 14293, NCIMB 12343, CIP 100651) OR ATCC 19404 (NCTC 532 or CIP 79.3)	Incubate under anaerobic conditions for ≤ 48 hours at 30-35°C	Visible growth should be observed.
Cetrimide Agar	<i>P.aeruginosa</i> ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275	≤ 18 hours at 30-35°C	Visible growth should be observed
		≤ 72 hours at 30-35°C	Greenish, Colored colonies should be observed.
	<i>E.coli</i> ATCC 8739, NCIMB 8545, CIP 53.126 OR NBRC 3972	≥ 72 hours at 30-35°C	No growth should be observed
Mannitol-Salt Agar	<i>S.aureus</i> ATCC6538, NCIMB 9518, CIP 4.83 or NBRC 13276	≤ 18 hours at 30-35°C	Visible growth should be observed
		≤ 72 hours at 30-35°C	White or Yellow colonies surrounded by yellow zones
	<i>E.coli</i> ATCC 8739, NCIMB 8545, CIP 53.126 OR NBRC 3972	≥ 72 hours at 30-35°C	No growth
Sabouraud Dextrose Agar(Used for testing <i>Candida albicans</i> as specified organism)	<i>C. albicans</i> ATCC 10231, NCPF3179, IP 48.72 or NBRC 1594	≤ 24 hours at 30-35°C	Visible growth should be observed.
		≤ 48 hours at 30-35°C	White color colonies should be observed.



Title: Validation of Extended Settle Plate Exposure Time and Extended Incubation of Microbiological Culture Media

PROTOCOL NUMBER	VP/MS/002-00
EFFECTIVE DATE :	19/04/2026
DEPARTMENT:	Microbiology
PAGE NUMBER:	9 of 17

Xylose-Lysine-Deoxycholate Agar Medium	Salmonella enterica subsp. Enterica serovar Typhimurium OR as An alternative Salmonella enterica subsp. Enterica serovar Abony ATCC14028, NBRC 100797, NCTC 6017 or CIP 80.39	≤ 18 hours at 30-35°C	Visible growth should be observed.
		≤ 18 hours at 30-35°C	Red color colonies with or without black centers
MacConkey's Broth	<i>E.coli</i> ATCC 8739, NCIMB 8545, CIP 53.126 OR NBRC 3972	≤ 24 hours at 42-44°C	Visible growth should be observed.
	<i>S.aureus</i> ATCC6538, NCIMB 9518, CIP 4.83 or NBRC 13276	≥ 48 hours at 42-44°C	No growth
	<i>S.aureus</i> ATCC6538, NCIMB 9518, CIP 4.83 or NBRC 13276		
	<i>B.subtilis</i> ATCC6633, NCIMB 8054, CIP 52.62 or NBRC 3134		

9.0 ACCEPTANCE CRITERIA:

- 9.1 For Environmental monitoring study on SCDA Media, Growth obtained should not differ by a Factor 2 from the calculated value of standardized inoculum.
- 9.2 Growth obtained after the routine incubation period shall comply with the approved Growth Promotion Test acceptance criteria as per table-2.
- 9.3 Growth obtained after the extended incubation period shall continue to comply with the applicable Growth Promotion Test acceptance criteria as per table-2.
- 9.4 No physical deterioration such as excessive drying, shrinkage, cracking, dehydration, contamination, or discoloration shall be observed in any media after incubation period.
- 9.5 Selective media shall retain their expected growth-promoting and selective characteristics.
- 9.6 Broth media shall demonstrate visible growth of the challenge organisms after the extended incubation period.
- 9.7 Negative controls shall no microbial growth.

10.0 REVALIDATION CRITERIA:

Re-validation shall be performed in case of below reasons.

- Change in the incubation period and conditions.
- Change in the settle plate exposure timings.
- If the GPT results not met the criteria.

	Title: Validation of Extended Settle Plate Exposure Time and Extended Incubation of Microbiological Culture Media	PROTOCOL NUMBER	VP/MS/002-00
		EFFECTIVE DATE :	19/04/2026
		DEPARTMENT:	Microbiology
		PAGE NUMBER:	10 of 17

11.0 ANNEXURES

Annexure-I : Plate Exposure Report.

Date of Monitoring		Date of report	
Time of Exposure	From: To:	Exposed Set number	
Media used	SCDA with lecithin and Polysorbate-80	Medium Lot No & Expiry date	
Done by			

Incubation Details:

Incubator ID	Temperature	Start date & Time	End date & Time
	20 to 25°C		
	30 to 35°C		

Colony counter ID:

S. No. (Plate)	Viable Count (cfu/plate)
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
Negative control	
Observed By (Sign & Date)	Checked By (Sign & Date)



Title: Validation of Extended Settle Plate Exposure Time and Extended Incubation of Microbiological Culture Media

PROTOCOL NUMBER	VP/MS/002-00
EFFECTIVE DATE :	19/04/2026
DEPARTMENT:	Microbiology
PAGE NUMBER:	11 of 17

Annexure-II : Growth Promotion Test Report.

Name of the Media		Media Lot No.		Incubator ID	
Exposed set number		Date of test		Incubator ID	

Test Details:

Name of Test Strains	Standard Cultures/Bio ball lot number	Incubation Details		Observed counts		
		Start date & time	End date & time	Plate-1	Plate-2	Average
<i>P.aeruginosa</i> ATCC 9027						
<i>S.aureus</i> ATCC6538						
<i>B.subtilis</i> ATCC6633						
<i>A. brasiliensis</i> ATCC 16404						
<i>C. albicans</i> ATCC 10231						
Environmental isolate-1						

Negative Control: Complies / does not comply

Results: Growth obtained in the media should not differ by a Factor 2 from the calculated value of standardized inoculum.

Observed By
Sign & Date:

Reviewed by:
Sign & Date:



Title: Validation of Extended Settle Plate Exposure Time and Extended Incubation of Microbiological Culture Media

PROTOCOL NUMBER

VP/MS/002-00

EFFECTIVE DATE :

19/04/2026

DEPARTMENT:

Microbiology

PAGE NUMBER:

12 of 17

12.0 SUMMARY AND CONCLUSION

SET-1

Date of Monitoring	09/06/26	Date of report	18/06/26
Time of Exposure	From: 04:00 To:08:15	Exposed Set number	I
Media used	SCDA with lecithin and Polysorbate-80	Medium Lot No & Expiry date	SCDA26001/ 10/08/26

Incubation Details:

Temperature	Start date & Time	End date & Time
20 to 25°C	09/06/26&08:30	14/06/26&08:35
30 to 35°C	14/06/26&08:40	18/06/26&08:45

Colony counter ID:

S. No. (Plate)	Viable Count (cfu/plate)
1	Nil
2	Nil
3	Nil
4	Nil
5	Nil
6	Nil
7	Nil
8	Nil
9	Nil
10	Nil
11	Nil
12	Nil
13	Nil
Negative control	No Growth

Name of Test Strains	Standard Cultures/Bio ball lot number	Observed counts		
		Plate-1	Plate-2	Avg
<i>P.aeruginosa</i> ATCC 9027	BB240701(51cfu)	48	50	49
<i>S.aureus</i> ATCC6538	BB240702(53cfu)	47	49	48
<i>B.subtilis</i> ATCC6633	BB240703(50cfu)	47	49	48
<i>A. brasiliensis</i> ATCC 16404	BB240704(49cfu)	44	46	45
<i>C. albicans</i> ATCC 10231	BB240705(54cfu)	49	51	50
Environmental isolate-1	BB240706(55cfu)	49	51	50



Title: Validation of Extended Settle Plate Exposure Time and Extended Incubation of Microbiological Culture Media

PROTOCOL NUMBER	VP/MS/002-00
EFFECTIVE DATE :	19/04/2026
DEPARTMENT:	Microbiology
PAGE NUMBER:	13 of 17

SET-11

Date of Monitoring	09/06/26	Date of report	18/06/26
Time of Exposure	From: 08:40 To:12:45	Exposed Set number	II
Media used	SCDA with lecithin and Polysorbate-80	Medium Lot No & Expiry date	SCDA26001/ 10/08/26

Incubation Details:

Temperature	Start date & Time	End date & Time
20 to 25°C	09/06/26&12:50	14/06/26&12:55
30 to 35°C	14/06/26&12:56	18/06/26&12:58

Colony counter ID:

S. No. (Plate)	Viable Count (cfu/plate)
1	Nil
2	Nil
3	Nil
4	Nil
5	Nil
6	Nil
7	Nil
8	Nil
9	Nil
10	Nil
11	Nil
12	Nil
13	Nil
Negative control	No Growth

Name of Test Strains	Standard Cultures/Bio ball lot number	Observed counts		
		Plate-1	Plate-2	Avg
<i>P.aeruginosa</i> ATCC 9027	BB240701(51cfu)	48	49	49
<i>S.aureus</i> ATCC6538	BB240702(53cfu)	48	49	49
<i>B.subtilis</i> ATCC6633	BB240703(50cfu)	47	49	48
<i>A. brasiliensis</i> ATCC 16404	BB240704(49cfu)	44	46	45
<i>C. albicans</i> ATCC 10231	BB240705(54cfu)	49	51	50
Environmental isolate-1	BB240706(55cfu)	50	51	51



Title: Validation of Extended Settle Plate Exposure Time and Extended Incubation of Microbiological Culture Media

PROTOCOL NUMBER

VP/MS/002-00

EFFECTIVE DATE :

19/04/2026

DEPARTMENT:

Microbiology

PAGE NUMBER:

14 of 17

SET-III

Date of Monitoring	09/06/26	Date of report	18/06/26
Time of Exposure	From: 13:00 To:17:05	Exposed Set number	III
Media used	SCDA with lecithin and Polysorbate-80	Medium Lot No & Expiry date	SCDA26001/ 10/08/26

Incubation Details:

Temperature	Start date & Time	End date & Time
20 to 25°C	09/06/26&17:10	14/06/26&17:12
30 to 35°C	14/06/26&17:13	18/06/26&17:15

Colony counter ID:

S. No. (Plate)	Viable Count (cfu/plate)
1	Nil
2	Nil
3	Nil
4	Nil
5	Nil
6	Nil
7	Nil
8	Nil
9	Nil
10	Nil
11	Nil
12	Nil
13	Nil
Negative control	No Growth

Name of Test Strains	Standard Cultures/Bio ball lot number	Observed counts		
		Plate-1	Plate-2	Avg
<i>P.aeruginosa</i> ATCC 9027	BB240701(51cfu)	47	46	47
<i>S.aureus</i> ATCC6538	BB240702(53cfu)	47	49	49
<i>B.subtilis</i> ATCC6633	BB240703(50cfu)	47	48	48
<i>A. brasiliensis</i> ATCC 16404	BB240704(49cfu)	44	46	45
<i>C. albicans</i> ATCC 10231	BB240705(54cfu)	49	51	50
Environmental isolate-1	BB240706(55cfu)	51	53	52



Title: Validation of Extended Settle Plate Exposure Time and Extended Incubation of Microbiological Culture Media

PROTOCOL NUMBER	VP/MS/002-00
EFFECTIVE DATE :	19/04/2026
DEPARTMENT:	Microbiology
PAGE NUMBER:	15 of 17

SET-IV

Date of Monitoring	09/06/26	Date of report	18/06/26
Time of Exposure	From: 17:20 To:21:25	Exposed Set number	IV
Media used	SCDA with lecithin and Polysorbate-80	Medium Lot No & Expiry date	SCDA26001/ 10/08/26

Incubation Details:

Temperature	Start date & Time	End date & Time
20 to 25°C	09/06/26&21:35	14/06/26&21:40
30 to 35°C	14/06/26&21:45	18/06/26&21:50

Colony counter ID:

S. No. (Plate)	Viable Count (cfu/plate)
1	Nil
2	Nil
3	Nil
4	Nil
5	Nil
6	Nil
7	Nil
8	Nil
9	Nil
10	Nil
11	Nil
12	Nil
13	Nil
Negative control	No Growth

Name of Test Strains	Standard Cultures/Bio ball lot number	Observed counts		
		Plate-1	Plate-2	Avg
<i>P.aeruginosa</i> ATCC 9027	BB240701(51cfu)	48	50	49
<i>S.aureus</i> ATCC6538	BB240702(53cfu)	47	49	49
<i>B.subtilis</i> ATCC6633	BB240703(50cfu)	46	47	47
<i>A. brasiliensis</i> ATCC 16404	BB240704(49cfu)	44	46	45
<i>C. albicans</i> ATCC 10231	BB240705(54cfu)	49	51	50
Environmental isolate-1	BB240706(55cfu)	47	48	48

	Title: Validation of Extended Settle Plate Exposure Time and Extended Incubation of Microbiological Culture Media	PROTOCOL NUMBER	VP/MS/002-00
		EFFECTIVE DATE :	19/04/2026
		DEPARTMENT:	Microbiology
		PAGE NUMBER:	16 of 17

- Based on the results obtained during the study it is concluded that extended exposure of SCDA plates under Grade A Laminar Air Flow for up to 5 hours followed by incubation at 20–25°C for 5 days and 30–35°C for 4 days and all media incubation extended time does not adversely affect the physical characteristics and the growth promoting properties of the media.
- All test organisms demonstrated satisfactory growth and the recovered counts complied with the specified acceptance criteria.
- The negative control showed No growth.
- Hence, the study meets the acceptance criteria and the validated exposure time of 5 hours with the specified incubation conditions is considered acceptable for routine environmental monitoring using SCDA settle plates and all other microbiological culture media are suitable for extended incubation up to 7 days under the validated incubation conditions without adversely affecting their intended performance.



Title: Validation of Extended Settle Plate
Exposure Time and Extended Incubation of
Microbiological Culture Media

PROTOCOL NUMBER	VP/MS/002-00
EFFECTIVE DATE :	19/04/2026
DEPARTMENT:	Microbiology
PAGE NUMBER:	17 of 17

13.0 POST-APPROVAL

The signatures below indicate approval of this report.

Name	Department/Designation	Signature	Date
Compiled By:			
Reviewed By:			
Approved By:			

END OF DOCUMENT