



STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Environmental Monitoring of Microbiology Testing Area	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

1.0 OBJECTIVE:

To lay down a procedure for Environmental Monitoring of Microbiology testing area.

2.0 SCOPE:

This SOP is applicable for Environmental Monitoring in Microbiology testing area.

3.0 RESPONSIBILITY:

Microbiologist -Quality Control
Head-Quality Control

4.0 PROCEDURE:

4.1 Passive Air Sampling by Settle Plate Method:

4.1.1 Take 70% v/v IPA and lint free sterile mopping cloth.

4.1.2 Prepare Soybean casein digest agar medium as per SOP and mark the plates with Name of Medium, Lot No., Date of Preparation, Use before and Sign and date on bottom of plates and proceed for pre incubation not less than 48 hours at 20°–25°C.

4.2 Procedure for Plates Exposing:

4.2.1 Take required number of preincubated plates Keep the plates in petricanes, previously disinfect the petricanes with 70 % v/v IPA and keep in the dynamic pass box.

4.2.2 Follow the entry/exit procedure as per reference SOP for Microbiology testing area.

4.2.3 Write down the location number, location name, and initial time of exposure on SCDA plates and put the sterile SCDA plate on S. S. plate exposure stand of respective location.

4.2.4 Expose the petriplate at designated position by opening the lid of pre incubated SCDA plate.

4.2.5 Exposed the pre incubated SCDA plate for 04 hours + 15 minutes.

4.2.6 After completion of exposure period close the lid carefully to avoid the external contamination by hand. Write down the completion time of exposure.

4.2.7 Collect the SCDA plate in S.S. petricane and keep the petricane in dynamic Pass box of Microbiological testing area and follow the procedure for incubation.

4.3 Procedure for Incubation:



STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Environmental Monitoring of Microbiology Testing Area	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

4.3.1 Incubate SCDA plates in inverted position along with control plate (unexposed) at 20°–25°C for 72 hours.

4.3.2 Control plate represents the medium sterility.

4.3.3 On completion of incubation transfer the same plates including control plate for further incubation at 30°–35°C for 48 hours.

4.3.4 After the completion of 05 days incubation period, observe the SCDA plates and count the bacterial and fungal colonies. Record the result in respective Annexure-I.

4.3.5 Bacterial and fungal count should be within limit as per point No.: 4.10

4.4 Precaution:

4.4.1 Ensure plates are pre-incubated having no any growth and media having no crack.

4.5 Active Air sampling :

4.5.1 Take filtered 70% v/v IPA and lint free sterile mopping cloth.

4.5.2 Prepare Soybean casein digest agar medium as per SOP and mark the plates with Name of Medium, Lot No., Date of Preparation, Use before and Sign and date on bottom of plates and proceed for pre incubation not less than 48 hours at 20°–25°.

4.5.3 Take required number of preincubated plates

4.5.4 Keep the plates in petricanes, previously disinfect the petricans with 70 % v/v IPA and keep in the dynamic pass box.

4.5.5 Follow the entry/exit procedure as per reference SOP for Microbiology testing area.

4.5.6 Disinfect the outer surface of air sampler with sterile mop, using filtered 70% v/v IPA.

4.5.7 Sterilise the S.S. perforated lid of air sampler by autoclaving and transfer to microbiology testing area through dynamic pass box.

4.5.8 Before sampling, mark the sterile SCDA plate with location and date of sampling with marker pen on the bottom of plate.

4.5.9 Open the lid of air sampler and put near the site of sampling. Place the sterile SCDA plate in to the sampling head of the instrument and close with perforated lid.

4.5.10 Operate the Air Sampler as per SOP” Operation and Cleaning of Air Sampler”.

4.5.11 Sample 1m³ (1000 litre) of air from each assigned location as per respective diagram.

4.5.12 Ensure that the sample quantity is displayed on the screen of the instrument after each



STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Environmental Monitoring of Microbiology Testing Area	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

sampling operation.

- 4.5.13 After Air sampling, open the perforated lid of Air sampler and take out the SCDA plate, close the lid and put in to the S. S. Petri canes/containers
- 4.5.14 Disinfect the perforated lid by mopping with sterile mop using 70% v/v IPA before taking another sample.
- 4.5.15 Collect the SCDA plate in S.S. petricane and keep the petricane in dynamic Pass box of microbiology testing area and follow the procedure for incubation.

4.6 Procedure for Incubation

- 4.6.1 Incubate SCDA plates in inverted position along with control plate (unexposed) at 20°–25°C for 72 hours.
- 4.6.2 Control plate represents the medium sterility.
- 4.6.3 On completion of above incubation transfer the same plates including control plate for further incubation at 30°–35°C for 48hours.
- 4.6.4 After the completion of incubation period, observe the SCDA plates and count the bacterial and fungal colonies. Record the result in respective Annexure-II.
- 4.6.5 Bacterial and fungal count should be within limit as per point No.: 4.10

4.7 Surface monitoring:

- 4.7.1 Perform the surface monitoring in the locations according to the pre-defined locations and surface monitoring schematic diagrams as per Annexure-IV
- 4.7.2 Perform the surface monitoring by following methods.

4.8 55 mm surface contact (RODAC) plates :

- 4.8.1 Carefully open the RODAC plate, invert and contact the surface of agar to the surface to be monitored. Press the plate firmly to expose the whole surface of agar to be sampled surface.
- 4.8.2 Slowly take back the plate and close the plate with lid. Take care to not to leave any traces of agar medium to the surface monitored
- 4.8.3 Disinfect the sampled area and clean with sterile moping pad
- 4.8.4 After completion of test label each plate on bottom side with location code, shift, sign (initial) and date.
- 4.8.5 Perform the sampling in all the locations as per the sampling plan using same procedure.



STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Environmental Monitoring of Microbiology Testing Area	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

- 4.8.6 Place all the plates in SS carriers
- 4.8.7 Perform the monitoring at the end of activity (before cleaning).
- 4.8.8 Record the

4.9 Procedure for Incubation

- 4.9.1 Incubate SCDA plates in inverted position along with control plate (unexposed) at 20°–25°C for 72 hours.
- 4.9.2 Control plate represents the medium sterility.
- 4.9.3 On completion of above incubation transfer the same plates including control plate for further incubation at 30°–35°C for 48 hours.
- 4.9.4 After the completion of incubation period, observe the SCDA plates and count the bacterial and fungal colonies. Record the result in respective Annexure-III
- 4.9.5 Bacterial and fungal count should be within limit as per point No.: 4.10
- 4.9.6 **Trend Preparation:** Prepare the trend for the result on monthly basis of Passive air sampling test result and Quarterly for Active air sampling test result.

4.10

Active Air sampling (cfu/m ³)						
Grade	Bacteria			Fungus		
	Alert Limit	Action Limit	Specification	Alert Limit	Action Limit	Specification
Grade A #	---	---	<1	---	---	Nil
Grade B	---	---	10	---	---	Nil
Grade C	---	---	100	---	---	Nil
Grade D	---	---	200	---	---	Nil

Settle Plate (cfu/Plate)						
Grade	Bacteria			Fungus		
	Alert Limit	Action Limit	Specification	Alert Limit	Action Limit	Specification
Grade A #	---	---	<1	---	---	Nil
Grade B	---	---	5	---	---	Nil
Grade C	---	---	50	---	---	Nil
Grade D	---	---	100	---	---	Nil



STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Environmental Monitoring of Microbiology Testing Area	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

4.11

cfu/RODAC Plate							
Grade	Bacteria						Fungus
	Alert Limit		Action Limit		Specification		Should be Absent.
	Wall	Floor	Wall	Floor	Wall	Floor	
Grade A #	---	---	---	---	< 1	< 1	
Grade B	---	---	---	---	05	05	
Grade C	---	---	---	---	25	25	
Grade D	---	---	---	---	50	50	

: Any count observed in any location in Grade A shall trigger investigation.

Note: Action and alert limit shall be specified after generating the graphical data for a period of one year for microbial test result by passive air sampling, active air sampling and surface monitoring.

4.12 Frequency:

- 4.12.1 Passive Air Sampling: Daily during operation
- 4.12.2 Active Air Sampling: Weekly during operation
- 4.12.3 Surface Monitoring: Once in Week at end of the operation.

5.0 ANNEXURE (S):

Annexure-I: Environmental Monitoring of Microbiology testing area report by Passive Air Sampling testing report.

Annexure-II: Environmental Monitoring of Microbiology testing area report by active air sampling.

Annexure-III: Environmental Monitoring of Microbiology testing area by surface monitoring.

Annexure-IV: Surface monitoring schematic diagrams for Environmental Monitoring of Microbiology testing area (Passive/Active/RODAC).

6.0 REFERENCE (S):

- SOP: Entry and exit to the Microbiology Testing Area
- SOP: Procedure for preparation of media.



STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Environmental Monitoring of Microbiology Testing Area	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

SOP: Investigation of out of specification of test result
SOP: Operation and cleaning of Air Sampler.
SOP: Preparation, Approval, Distribution control, revision and Destruction of Standard operating Procedure (SOP).

7.0 ABBREVIATION (S) / DEFINITION (S):

LAF : Laminar Air Flow
S.S. : Stainless Steel
BOD : Bacteriological Oxygen Demand.
Cfu/m³: colony forming unit per cubic meter.
SCDA : Soyabean casein digest agar
v/v : volume/volume
IPA : Iso Propyl Alcohol
QC : Quality Control
QCM : Quality Control Microbiology
SOP : Standard Operating Procedure

REVISION CARD

S.No.	REVISION No.	REVISION DATE	DETAILS OF REVISION	REASON (S) FOR REVISION	REFERENCE CHANGE CONTROL No.
01	00	---	---	New SOP	---



STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Environmental Monitoring of Microbiology Testing Area	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

Annexure 1

ENVIRONMENTAL MONITORING OF MICROBIOLOGICAL TESTING AREA BY PASSIVE AIR SAMPLING (SETTLE PLATE METHOD)

SOP Reference No.:

Date of sampling	:	Date of Report	:
Sampling time	:	Sampled By (Sign. &Date)	:
Area status	: In operation /At rest condition		

Media Details	Incubation Temperature	Incubation Time	Incubator ID No.
Soyabean Casein Digest Agar	20°–25°C	72 hours	
Lot No.:	30°–35°C	48 hours	

S.No.	Location ID. No.	Sampling Location Description	Grade	Count (Cfus / plate)	
				Bacteria	Fungi
1.	S-01	Change room – I	D		
2.	S-02	Change room – I	D		
3.	S-03	Change room – II	C		
4.	S-04	Change Room – III	B		
5.	S-05	MLT Room	B		
6.	S-06	MLT Room	B		
7.	S-07	MLT Room	B		
8.	S-08	MLT Room	B		
9.	S-09	MLT Room	B		
10.	S-10	Change Room – IV	B		
11.	S-11	Upper side Laminar air flow	B		
12.	S-12	Right side under Laminar air flow	A		
13.	S-13	Left side under Laminar air flow	A		
14.	S-14	Dynamic pass box(media preparation room)	A		
15.	S-15	Dynamic pass box(Incubator room)	A		
16.	S-16	Garment Cubical	A		



PHARMA DEVILS
MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Environmental Monitoring of Microbiology Testing Area	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

Frequency: Daily in operation	Negative control:
-------------------------------	-------------------

Limit: Count (Cfu / plate)			
Grade	Alert limit	Action Limit	Specification limit
Grade A			<01CFU
Grade B			<05 CFU
Grade C			<50 CFU
Grade D			<100 CFU

Remark: Area Complies / Does not Complies with specified limit.

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



PHARMA DEVILS
MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Environmental Monitoring of Microbiology Testing Area	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

Annexure 2
ENVIRONMENTAL MONITORING OF MICROBIOLOGICAL TESTING AREA
BY ACTIVE AIR SAMPLING

SOP Reference No.:

Date of sampling	:		Date of Report	:	
Sampling time	:		Sampled By (Sign. & Date)	:	
Area status	:	In operation /At rest condition			

Media Details	Incubation Temperature	Incubation Time	Incubator ID No.
Soyabean Casein Digest Agar	20°–25°C	72 hours	
Lot No.:	30°–35°C	48 hours	

S.No.	Location ID. No.	Sampling Location Description	Grade	Count (Cfu / plate)	
				Bacteria	Fungi
1.	A-01	Change room – I	D		
2.	A-02	Change room – II	C		
3.	A-03	Change Room – III	B		
4.	A-04	Change Room – IV	B		
5.	A-05	MLT Room Left side of LAF	B		
6.	A-06	MLT Room in front of change room IV	B		
7.	A-07	Right side under Laminar air flow	A		
8.	A-08	Left side under Laminar air flow	A		
9.	A-09	Dynamic pass box(media preparation room)	A		
10.	A-10	Dynamic pass box(Incubator room)	A		
11.	A-11	Garment Cubical	A		

Frequency: Daily in operation	Negative control:
--------------------------------------	--------------------------

Limit: Count (Cfu / plate)			
Grade	Alert limit	Action Limit	Specification limit
Grade A			<01CFU
Grade B			<10 CFU
Grade C			<100CFU
Grade D			<200 CFU

Remark: Area Complies / Does not Complies with specified limit.

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



STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Environmental Monitoring of Microbiology Testing Area	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

Annexure 3

ENVIRONMENTAL MONITORING OF MICROBIOLOGICAL TESTING AREA BY SURFACE MONITORING (RODAC PLATE METHOD)

SOP Reference No.:

Date of sampling	:	Date of Report	:
Sampling time	:	Sampled By (Sign. & Date)	:
Area status	: In operation /At rest condition		

Media Details	Incubation Temperature	Incubation Time	Incubator ID No.
Soyabean Casein Digest Agar	20°-25°C	72 hours	
Lot No.:	30°-35°C	48 hours	

S.No.	Location ID. No.	Sampling Location Description	Grade	Count (Cfu / plate)	
				Bacteria	Fungi
1.	R-01	Change room – I	D		
2.	R-02	Change room – II	C		
3.	R-03	Change Room – III	B		
4.	R-04	Change Room – IV	B		
5.	R-05	MLT Room in front of change –III	B		
6.	R-06	MLT Room in front of change –IV	B		
7.	R-07	MLT Room (middle of the room)	B		

Frequency: Weekly	Negative control:
-------------------	-------------------

Limit: Count (Cfu/plate)			
Grade	Alert limit	Action Limit	Specification limit
Grade B			<05 CFU
Grade C			<25 CFU
Grade D			<50 CFU

Remark: Area Complies / Does not Complies with specified limit.

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



PHARMA DEVILS
MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

Department: Microbiology	SOP No.:
Title: Environmental Monitoring of Microbiology Testing Area	Effective Date:
Supersedes: Nil	Review Date:
Issue Date:	Page No.:

Annexure 4
MICROBIOLOGY ENVIRONMENT MONITORING LOCATION DIAGRAM