1.0 Objective

1.1 To lay down procedure for environmental monitoring in production and warehouse area.

2.0 Scope

2.1 This SOP is applicable for the routine monitoring of environment using microbiological methods such as passive air sampling, active air sampling in production, microbiology and warehouse area.

3.0 Responsibility

3.1 Microbiologist/Trained personnel shall be responsible to follow the procedure as per the SOP.

3.2 Head production and Head Engineering or designee are responsible to facilitate monitoring.

3.3 HOD Microbiology/designee shall be responsible for compliance of this SOP.

4.0 Accountability

4.1 Head- Quality Control.

5.0 Procedure

5.1 Rationale for selection of sample sites:

5.1.1 Locations for environmental monitoring shall be selected based on them being most likely to indicate any contamination or failure in contamination control systems. Typical examples include:

5.1.2 **Accumulation:** Locations close to return air risers where all microorganisms carried in the air stream may be expected to pass before leaving the room.

5.1.3 **Personnel traffic:** Locations where personnel are known to be required frequently to pass through. Typically, these include doorways.

5.1.4 **Personnel activity:** Locations where personnel are known to be required to work or intervene. Care should be taken to ensure that the value of having data from monitoring these areas of personnel activity is not outweighed by the risk of creating unnecessary contamination as a result of making the work more difficult or by creating additional interventions.

5.2 Definitions

5.2.1 **Air Sampler:** Devices or equipment used to sample a measured amount of air in a specified time to quantitative the microbiological status of air in the controlled environment.

5.2.2 **At Rest:** "At Rest" - Status of a clean room facility with all services functioning and equipment installed and operable, but not in use and no personnel activities being performed other than cleaning and environmental monitoring.

5.2.3 **In-Operation:** A clean room in normal operation, with all services functioning and with equipment and personnel, if applicable, present and performing their normal work functions in the facility.

5.2.4 **Alert limit:** In case of counts over the alert level, information shall be given to the department HOD and distribution quality should be made finely until the count returns under the alert limit. Alert levels are specific for a given facility and are established based on a baseline developed under an environmental monitoring program. These alert levels can be modified depending on the trend analysis done on the monitoring program. Alert levels are always lower than action levels.

5.2.5 **Action limit:** A microbiological limit, when exceeded, indicates that a process drifted from its normal operating condition. A documented investigation program is required to know the roots cause and corrective actions derived based on the investigation.

5.3 Materials required for monitoring:

5.3.1 SS containers

5.3.2 Ready to use or In-house prepared 90 mm SCDA/TSA plates with 0.5% polysorbate 80 and 0.07% Soya lecithin.

5.3.3 70% v/v IPA

5.3.4 Sterile swab sticks

5.3.5 Sterile hand gloves

5.3.6 Sterile Mopping cloth

5.3.7 Nose mask

5.3.8 Settle plate stands

5.3.9 **Equipment/Instrument:**

5.3.9.1 Incubator 20-25°C

5.3.9.2 Incubator 30-35°C

5.3.9.3 Calibrated air sampler

5.3.9.4 Calibrated colony counter

5.4 Procedure for Environmental monitoring:

5.4.1 Prior to perform the environmental monitoring activity, AR number shall be allotted and shall mention the details in Environmental monitoring AR number register – Annexure-03.

5.4.2 AR number shall consist of 10 characters.

5.4.3 First three characters should denote “AR” representing Analytical Report Number.

5.4.4 Fourth and fifth characters shall be numerical that represent the last two digits of year of generation. For example, for the year 2022 it should be 22.

5.4.5 Sixth and seventh characters represent activity code. For example, EM stands for Environmental monitoring.

5.4.6 Eighth, ninth, tenth characters shall be numerical which represent the serial number of the samples starting from 001.

5.4.7 For example, the first environmental monitoring in the year 2022 shall be number as ARN22EM001.

5.5 Sampling Location

5.5.1 For location details label the plates as mentioned below.

5.5.2 First two characters "PR" denotes block name the next two characters "SP" denotes Settle plate and "AS" for Air sampling, "01" denotes serial number of the location.

Activity	Code
Settle Plate	SP
Air sampling	AS

- Example for Settle plate: WHSP-01
- Example for Air Sampling: WHAS-01

5.6 Environmental monitoring frequency.

5.6.1 Perform environmental monitoring as per scheduled.

5.6.2 Update the schedule with activity performed date.

5.6.3 Note: Perform the activity within ± 3 working days from the scheduled date.

5.7 Preparation for Environmental monitoring:

5.7.1 Ready to use media plates or in house prepared plates can be used for environmental monitoring.

5.7.2 Use 90mm SCDA plates with 0.5% Polysorbate 80 and 0.07% soya lecithin for settle plate and air sampling.

5.7.3 Take the required number of ready to use or prepared in-house media for monitoring.

5.7.4 Record the reconciliation details in (Ready to use media reconciliation log book) or (in house prepared media reconciliation log book).

5.7.5 If any abnormalities observed (like plate contamination, cracks, non-uniformity of media, dehydration, sticky in nature due to media residues, condensate and expiry date) discard the plates as per SOP.

5.7.6 Whenever ready to use media plates are used for environmental monitoring, remove the first and second wrap in microbiology laboratory and inspect the pack for any abnormalities. If any abnormalities observed, discard the same as per SOP.

5.7.7 Check the calibration and operation status of all air samplers prior to proceeding for environmental monitoring.

5.7.8 Check the expiry dates of all media and accessories prior to proceeding for environmental monitoring.

5.7.9 Sanitize the external and inner surfaces of SS bin/ carrier with 70% IPA and carefully arrange the plates inside the SS bin/carrier.

5.7.10 Monitoring plates and accessories shall be carried from microbiology lab to the respective sampling area in a closed SS container. Wipe the outer surface of the SS bin/carrier with 70% IPA before entering into the area by following respective entry and exit procedures.

5.7.11 Before sampling, mark the plates with AR No., Date of monitoring and sample location ID.

5.7.12 The sampling shall be done in at rest or in operation conditions depending on the availability of the manufacturing area.

5.7.13 Do not perform monitoring during cleaning of the area (with water).

5.7.14 Microbiology personnel shall ensure the AHU operation condition of respective area and initiate the monitoring activity after attainment of designed environmental conditions of the temperature, humidity and differential pressure of the area, if not inform to respective area in-charge for corrective action.

5.8 Environmental monitoring shall be done by following methods:

5.8.1 Passive Air Sampling (Settle plate exposure method).

5.8.2 Active Air Sampling (Volumetric Air Sampling method).

5.9 Passive Air Sampling (Settle plate exposure):

5.9.1 Perform the settle plate method as per the schedule of respective area.

5.9.2 To perform microbial environmental monitoring, enter in to the production facilities by following respective entry and exit procedures.

5.9.3 Keep the petri plate stands in respective locations.

5.9.4 Label each individual plate at respective location with the details such as location ID, Date of exposure and time of exposure (start time) at the rear side of the Petri plate & end time on the last plate.

Note: Note down the time of exposure in respective format.

5.9.5 Expose the plates on petri-plate exposure stands at working height in upright position (i.e. 90° angle) in the area and near risers the stand should be bent around 45° angle in designated locations as per the drawing . (Location ID is fixed with radium sticker) for NLT 4 hours (first plate collection should start after completion of 4 hours).

5.9.6 While opening the plates for exposing, first keep the plates on the stand then lift and slide open the lid of the plate and keep the lid facing down position to avoid contamination of the media.

5.9.7 Never bend or lean over the plates while exposing or while collecting the plates.

5.9.8 Disinfect the hands with 70% IPA for each activity. During monitoring ensure monitoring not to sanitize the hands near/over to the sampling area/exposure plate.

5.9.9 After completion of exposure, close the lid with utmost care to avoid aerial and fingers does not touch the surface of the media, collect all the plates after exposure and place them in the petri carrier.

5.9.10 Plates shall be collected carefully after completion of exposure time in the same order as it was exposed.

5.10 Active air sampling (Volumetric Air Sampling method):

5.10.1 Collect the plates and transfer to incubator in closed petri-can/ SS box for the incubation.

5.10.1.1 Negative control: Keep one un-exposed plate from the same lot of plates carried into the monitoring area.

5.10.1.2 Incubate the exposed plates and negative control plate in inverted position in respective incubators.

5.10.11 Active Air Sampling (Volumetric Air Sampling method):

5.10.12 Check the calibration and operation status of air sampler prior to use. Perform the activity as per respective SOP for the use of the equipment before performing the activity.

5.10.13 To perform microbial environmental monitoring, enter in to the production facilities by following respective entry and exit procedures.

5.10.14 Label each individual plate at respective location with the details such as AR No., Date of sampling, time of sampling and location (Location ID with fixed radium sticker).

5.10.15 Perform active air sampling at each location by following the area location (Location ID with fixed radium sticker).

5.10.16 Perform active air sampling from higher classification to lower classification in every area.

5.10.17 Sanitize the sampler head with 70% IPA before sampling at each location. After each sampling wipe with 70% IPA.

5.10.18 Whenever monitoring the air sample from lower grade air classification to higher grade air classification disconnect the move and disinfect the outer surface of the sampler with 70% IPA.

5.10.19 Perform the air sampling by performing the air suction by the air sampler as per SOP to sample 1000 litres of (1 m3) air.

5.10.20 Avoid touching inside and outside of the slit/ side of sampler slots and sampling media surface at any time during the sampling.

5.10.21 After completion of the sampling, remove the sampling plate, close the lid with utmost care to avoid aerial contamination and fingers does not touch the surface of the sampler media. (Note: IF Air sampler stops for a particular location during the sampling process, repeat the activity with new media plate at the same location. Record the mentioned details in record sheet.

5.10.22 Collect all the plates and transfer to incubator in closed petri-can / SS box for incubation.

5.10.23 Negative control: Keep one un-exposed plate from the same lot of plates carried into the monitoring area.

5.10.24 Incubate the exposed plates and negative control plates in inverted position as per respective incubation conditions.

5.11 Incubation conditions:

5.11.1 Incubate the monitored plates at 20-25°C for NLT 72 hours. After completion of incubation record the details in respective formats and shift the plates to 30-35°C for NLT 48 hours. (Incubator usage log book from SOP on Operation of B.O.D incubator).

5.11.2 After completion of incubation period, observe the plates under digital colony counter and record the count (cfu/plate) and findings in related format. (Digital colony counter usage log book from SOP on Operation of B.O.D incubator).

5.11.3 During violation, the incubation of plates (Settle plates and Air sampling) will be done in separate incubator and observation of the plates shall be done on daily basis.

5.12 Acceptance Criteria: 5.12.1 After completion of observations, send the plates for decontamination as per the respective SOP No: SOP/MB/GEN/023.

5.12.2 Acceptance criteria:

Clean Room Classification	Settle plate exposure (cfu/4 hours)	Active air sampling (cfu/1 m3)
Grade "D" (ISO 8 / Class 100,000)	100 CFU / 4 hours	200 CFU / m3
Production / Warehouse/Microbiology	100 CFU / 4 hours	200 CFU / m3

5.13 Handling of abnormal situation during monitoring:

5.13.1 If the plates are fallen down during marking/sampling/exposure discard the plates and repeat the activity. Investigation shall be carried out. Take new plates for monitoring.

5.13.2 If any plate lid opens after exposure/sampling or before loading into the incubator mark the plate with details, sign and date/ mention and note the deviation as per SOP.

5.13.3 During exposure of settle plates if any cleaning activity or spillage is noticed in the area, discontinue the sampling, sanitize with 70% IPA and allow it for a settled period, carry out the monitoring by using fresh plates for next 4 hours and discard the previously exposed plates as per SOP.

5.13.4 During the holidays, incident occurs, power failure, or air handling unit's maintenance / breakdown / product change over, manual environmental monitoring shall be done on next working day.

5.14 Environmental monitoring sampling frequency:

5.14.1 Environmental monitoring (Active and Passive) in manufacturing area shall be followed as per schedule for environmental monitoring and perform the activity on scheduled days in month. ± 3 working days from the scheduled date in respective areas.

5.14.2 Perform the activity on next working day if there is any holiday on scheduled date or any other unforeseen events. ± 3 working days from the scheduled date in respective areas.

Area	Activity	Condition	Frequency of Monitoring
Production	Active air sampling	At rest & In-operation	Monthly ± 3 working days
Warehouse	Active air sampling	At rest & In-operation	Monthly ± 3 working days
Microbiology	Active air sampling	At rest & In-operation	Monthly ± 3 working days

5.15 Industrial hygiene material used in environmental monitoring shall be handled by following SOP .

5.16 Safety, health and environment

5.16.1 Check the cleaning status of area where plate has to be exposed.

6.0 Abbreviations

6.1 **MB:** Microbiology

6.2 **QA:** Quality Assurance

6.3 **SCDA:** Soybean Casein Digest Agar

6.4 **m3:** Meter Cube

6.5 **cm2:** Centimeter Square

6.6 **CFU:** Colony Forming Unit

6.7 **SS:** Stainless Steel

6.8 **IPA:** Isopropyl alcohol

6.9 **°C:** Degree centigrade

6.10 **%:** Percentage

6.11 **g:** Grams

6.12 **w/v:** Weight by volume

6.13 **v/v:** Volume by volume

6.14 **mm:** Millimeter

6.15 **HOD:** Head of the department

6.16 **NLT:** Not less than

7.0 Reference

7.1 Title: Decontamination and Disposal of used media in microbiology.

7.2 Title: Handling of out of specification results in microbiology.

7.3 Title: Operation of Colony counter

7.4 ISO 14644 and EU ANNEXURE-1.

7.5 USP<1116> Microbiological control and monitoring of aseptic processing environment.

8.0 Annexures

8.1 Annexure-01: Passive Air Sampling Method

8.2 Annexure-02: Active Air sampling method

8.3 Annexure-03: Environmental monitoring AR number register.

END OF THE DOCUMENT

Annexure -1

Environmental monitoring record

PASSIVE AIR SAMPLING METHOD					
Start Date			A.R No:		
Activity Status			Department		
Media Used				Activity start time	
Colony counter ID				Activity end time	
Sampling details	Plates shell be exposed NLT 4 hrs.				
Incubation conditions	Incubator ID	Incubation Start Date & time	Sign& Date	Incubation end Date & time	Sign& Date
20-25°C NLT 72 hrs					
30-35°C NLT 48 hrs					

Room name /equipment name	Location ID	Sampling location Name	Number of CFU/4 hr
			Total count (After5 days)
Negative control		Limit	100 cfu/4hr
Performed By:		Checked By:	Remarks :

Annexure -2

Environmental monitoring record

ACTIVE AIR SAMPLING METHOD					
Start Date			A.R No:		
Activity Status			Department		
Media Used				Activity start time	
Colony counter ID				Activity end time	
Sampling details	Collect 1000 litters of air.				
Incubation conditions	Incubator ID	Incubation Start Date & time	Sign& Date	Incubation end Date & time	Sign& Date
20-25°C NLT 72 hrs					
30-35°C NLT 48 hrs					

Room name /equipment name	Location ID	Sampling location Name	Number of CFU/m^3 Total count (After5 days)
Negative control		Limit	200 cfu/m^3
Performed By:		Checked By:	Remarks :

Annexure -3

Environmental monitoring record

	AR Number Inward Register				
Date	Department	Method	AR number	Generated by	Remarks
Verified By:					