STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Bio burden Sampling and Testing of Bulk Sample, Process Validation, Hold Time Study, Swab and Rinse Sample	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 OBJECTIVE:

To lay down a procedure for bio-burden testing of bulk, process validation, hold time study, swab and rinse sample.

2.0 SCOPE:

This SOP is applicable for Sampling and Microbiological Analysis of bulk, process validation, hold time study, Swab and Rinse sample.

3.0 RESPONSIBILITY:

Officer / Executive - Microbiology

4.0 ACCOUNTABILITY:

Head-QC

5.0 ABBREVIATIONS:

CFU	Colony Forming Unit
Ltd.	Limited
ml	milliliter
No.	Number
Pvt.	Private
QA	Quality Assurance
QC	Quality Control
SOP	Standard Operating Procedure

6.0 PROCEDURE:

Prerequisite for microbiological analysis of bio-burden testing:

S. No.	Requirements
01.	Swab and rinse sample
02.	Sterile SS filtration Assembly
03.	Sterile Vacuum filtration flask
04.	Sterile forceps
05.	Sterile 0.45 µ Membrane Filter
06.	Sterile SS Manifold filtration assembly
07.	Sterile silicone tube and cork

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Bio burden Sampling and Testing of Bulk Sample, Process Validation, Hold Time Study, Swab and Rinse Sample	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

S. No.	Requirements
08.	Vacuum pump
09.	Glass bid sterilizer
10.	SS Bucket
11.	Sterile water/sterile 0.1%peptone water
12.	Preincubated SCA Media Plate.

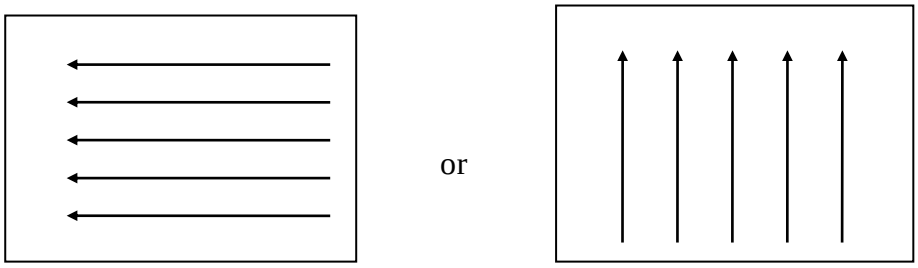
6.1 SAMPLING AND TESTING OF SWAB :

6.1.1.1 Transfer the swabs tubes into microbial limit test area through dynamic pass pox for preparation.

6.1.1.2 Take 10 ml of 0.9% sterile sodium chloride (normal saline) in swab tube under laminar air flow.

6.1.1.3 After preparation transfer the swab to respective area for sampling.

6.1.1.4 Take aseptically swab of 5 X 5cm area



6.1.1.5 After taking the swab, dip swab stick in same 0.9% sodium chloride (normal saline) tube and label the swab tubes.

6.1.1.6 Labeling of swab sample shall be done as follow:

Name of Equipment
Date of sampling
Sampling Location Name
Sampled By Date/ Sign

6.1.1.7 After sampling; take the sampled swab in microbiology laboratory and connect the filtration assembly with vacuum pump, place the 0.45 µm membrane filter aseptically on the support disc of sterile filtration assembly and aseptically fix the sterilized funnel on the membrane filter holder.

6.1.1.8 Pre wet the membrane filter with approximately 10 ml of sterile 0.1% peptone water, or sterile water.

6.1.1.9 Vortex the swab tube properly and filter whole content of 0.9% sodium chloride through sterilized filtration assembly (0.45 µm membrane filter).

6.1.1.10 Rinse the membrane filter with 100 ml of sterilized 0.1% peptone water or sterile water.

6.1.1.11 After filtration; lift the membrane filter aseptically with the help of sterilized forcep and place it on Pre-incubated Soyabean Casein Digest Agar Plate avoiding air bubble entrapped under filter paper.

6.1.1.12 For negative control: Pre wet the membrane filter with approximately 10 ml of sterile 0.1% peptone water or sterile water, transfer 10 ml of 0.9% sterile sodium chloride (normal saline) solution to membrane filtration assembly and filter it. Rinse the membrane filter with 100 ml of suitable solution such as sterile 0.1% peptone water or sterile water through 0.45µ sterile membrane filter.

6.1.1.13 After filtration; remove the membrane filter aseptically with help of sterilized forceps and place it on pre-incubated Soyabean Casein Digest Agar plates (SCA).

6.1.1.14 Incubate the plates in inverted position at 22.5°C ±2.5°C for NLT 72 hours followed by 32.5°C ± 2.5°C for NLT 48 hrs.

6.1.1.15 After completion of incubation; count the number of colonies, both side of the plate with help of colony counter and express the result as CFU/25 cm^2

6.2 SAMPLING AND TESTING OF RINSE WATER:

6.2.1 Take the sterilized bottle, and label the bottle as per Annexure-II.

6.2.2 Microbiologist shall take the 100 ml of rinse water sample in sterilized bottle from specified location in the presence of QA person.

6.2.3 After taking the sample, sample shall be transferred to Microbiology Laboratory in S.S. container sanitized with 0.22 µ filtered 70 % IPA.

6.2.4 Connect filtration assembly with vacuum pump, Place the 0.45 µm membrane filter aseptically on the support disc of sterile filtration assembly and aseptically fix the sterilized funnel on the membrane filter holder, Prewet the membrane filter with approximately 10 ml of sterile water/0.1% peptone water, than filter 100 ml rinse sample through sterile 0.45µ membrane filter.

6.2.5 Rinse the membrane filter with100 ml of the sterile 0.1% peptone water or sterile water.

6.2.6 For negative control: Pre wet the membrane filter with approximately 10 ml of sterile water/0.1% peptone water, than filter the 100 ml of the sterile 0.1% peptone water or sterile water through sterile 0.45µ membrane filter.

6.2.7 After filtration; remove the membrane filter aseptically with the help of sterilized forcep and place it on pre-incubated Soyabean Casein Digest Agar Plates (SCA).

6.2.8 Incubate the plates in inverted position at 22.5°C ±2.5°C for NLT 72 hrs followed by 32.5°C ± 2.5°C for NLT 48 hrs.

6.2.9 Count the numbers of colonies both side of the plate with the help of colony counter and express the result as CFU/100 ml.

NOTE: If there is a holiday on the day of release or Transfer of media plates, take the transfer and observation of media plates on next working day.

7.0 ANNEXURES:

ANNEXURE No.	TITLE OF ANNEXURE	FORMAT No.
Annexure-I	Bioburden Record of Bulk Sample , Process Validation, Hold Time Study, Swab and Rinse Sample	
Annexure-II	Label for bio-burden sample	

ENCLOSURES: SOP Training Record.

8.0 DISTRIBUTION:

- Controlled Copy No.01 — Quality Assurance
- Controlled Copy No.02 — Microbiology
- Master Copy — Quality Assurance

9.0 REFERENCES:

In-House

10.0 REVISION HISTORY:

CHANGE HISTORY LOG

Revision No.	Change Control No.	Details of Changes	Reason for Change	Effective Date	Updated By

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Bio burden Sampling and Testing of Bulk Sample, Process Validation, Hold Time Study, Swab and Rinse Sample	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

ANNEXURE-I
BIOBURDEN RECORD OF BULK SAMPLE , PROCESS VALIDATION, HOLD TIME STUDY, SWAB AND RINSE SAMPLE

Name of Product		Batch No.	
AR No.		Date of Sampling	
Sampled By		Analyzed By	
Date of Testing		Date of Incubation	
Incubator I.D. No.		Date of Release	
		Media Autoclave Reference No.	

S.No.	Name of Rinse sample / Location of Swab sample	No. of CFU observed		Limit (cfu)	
		TFC	TBC	TFC	TBC
1.					
2.					
3.					
4.					
5.					
Negative control =					

Remarks: The Rinse/ Swab sample result is complies/does not comply as per IH specification.

Observed By:
Sign & Date

Checked By:
Sign & Date

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Bio burden Sampling and Testing of Bulk Sample, Process Validation, Hold Time Study, Swab and Rinse Sample	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

ANNEXURE – II
LABEL FOR BIOBURDEN SAMPLE

Name of Sample	
Batch No.	
Sample Quantity	
Sampled On	
Sampled By (Sign & Date)	
QA Reviewer (Sign & Date)	