PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Bioburden of Primary Packaging Material	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 OBJECTIVE:

To lay down procedure for Bioburden of Primary Packaging Material.

2.0 SCOPE:

This SOP is applicable for Bioburden of all primary packaging material (PVC, Plain Aluminum Foil & Blister Aluminum Foil, Printed Foil & Glass Bottle) in Microbiology Section of Quality Control Laboratory.

3.0 RESPONSIBILITY:

Officer / Executive - Microbiology

4.0 ACCOUNTABILITY:

Head – QC

5.0 ABBREVIATION:

CFU	Colony Forming Unit
No.	Number
PVC	Poly Vinyl Chloride
QA	Quality Assurance
QC	Quality Control
SOP	Standard Operating Procedure

6.0 PROCEDURE:

6.1 PROCEDURE FOR ANALYSIS OF PRIMARY PACKAGING MATERIAL :

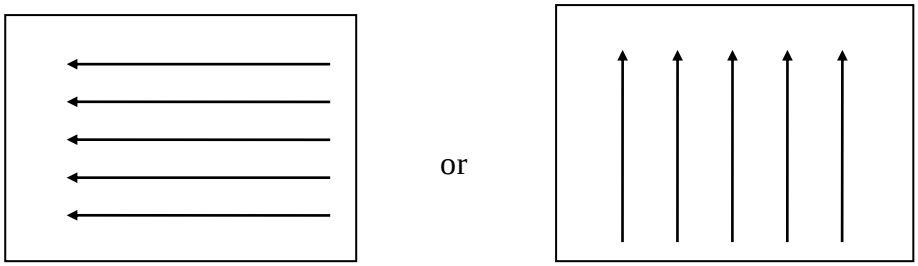
6.1.1 Aluminum Foil/PVC:

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

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

6.1.1.3 Tight the screw of swab tube.

6.1.1.4 At a time of sampling remove the swab stick from tube and take aseptically swab of 5 × 5cm area from the internal surface of aluminum foil and PVC sheet.



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 After sampling; connect the filtration assembly with vacuum pump, place the 0.45 µ sterile membrane filter aseptically on the support disc of sterile filtration assembly and aseptically fix the sterilized funnel on the membrane filter holder.

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

6.1.1.8 Vortex the swab tube properly and filter whole content of 0.9% sodium chloride (normal saline) through 0.45 µ sterile membrane filter.

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

6.1.1.10 After filtrations lift the membrane filter aseptically with the help of sterilized forceps and place it on Pre-incubated Soyabean Casein Digest Agar (SCA) Plate avoiding air bubble entrapped under filter paper.

6.1.1.11 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 hours.

6.1.1.12 After completion of incubation; count the number of colonies, both side of the plates with the help of colony counter and express the result in CFU/ 25 cm^2.

6.1.1.13 **Negative Control:** Pre wet the membrane filter with 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 filters with 100 ml of suitable solution such as sterile 0.1% peptone water or sterile water. After filtration; transfer membrane filter to Pre incubated Soyabean Casein Digest Agar (SCA) Plate. 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 hours.

6.1.1.14 Negative control should not show any growth.

6.1.2 Bottles:

6.1.2.1 Take required/received numbers of bottles and aseptically fill approximately 10 ml of sterile 0.9% sodium chloride (normal saline) solution in each bottle, shake bottles in such a way that internal surface of bottle are to be rinsed properly.

6.1.2.2 Connect filtrations 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.

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

6.1.2.4 Filter aseptically whole content of each bottle through sterilized filtration assembly (0.45 µm membrane filter) and rinse with 100 ml of sterilized 0.1% peptone water or sterile water.

6.1.2.5 After filtrations lift the membrane filter aseptically with the help of sterilized forceps and place it on Pre-incubated Soyabean Casein Digest Agar (SCA) Plate avoiding air bubble entrapped under filter paper.

6.1.2.6 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 hours.

6.1.2.7 After completion of incubation; count the number of colonies both side of the plates with the help of colony counter and express the result as CFU/–Number of Units.

6.1.2.8 **Negative Control:** Pre wet the membrane filter with 10 ml of sterile 0.1% peptone water or sterile water, transfer 100ml of 0.9% sodium chloride (normal saline) solution to membrane filtration assembly and filter it. Rinse the membrane Filter with 100 ml of sterile 0.1% peptone water or sterile water. After filtration; transfer membrane filter to Pre incubated Soyabean Casein Digest Agar (SCA) plate. Incubate the plate 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 hours.

6.1.2.9 Negative control should not show any growth.

6.1.3 Caps:

6.1.3.1 Take required/received numbers of caps and transfer in a sterile container containing 100 ml of 0.9% sodium chloride (normal saline) solution. Keep for 10 minute so that sample flora comes in contact with the 0.9% sodium chloride (normal saline) solution.

6.1.3.2 Connect filtration assembly with vacuum pump, place the 0.45 µ sterile membrane filter aseptically on the support disc of sterile filtration assembly and aseptically fix the sterilized funnel on the membrane filter holder.

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

6.1.3.4 Filter aseptically whole content through sterilized filtration assembly (0.45 µ membrane filter) and rinse with 100 ml of sterilized 0.1% peptone water or sterile water.

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

6.1.3.6 Incubate the plate 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 hours.

6.1.3.7 After completion of incubation; count the number of colonies from both side of the plate with the help of colony counter and express the result as CFU/Number of Units.

6.1.3.8 **Negative Control**: Pre wet the membrane filter with 10 ml of sterile 0.1% peptone water or sterile water, transfer 100 ml of 0.9% sodium chloride (normal saline) solution to membrane filtration assembly and filter it. Rinse the membrane filter with 100 ml of sterile 0.1%

peptone water or sterile water. After filtration transfer membrane filter to Pre incubated Soyabean Casein Digest Agar (SCA) plate. Incubate the plate 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 hours.

6.1.3.9 Negative control should not show any growth.

6.1.3.10 ACCEPTANCE CRITERIA: Results should be obtained with in specific limit.

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

7.0 ANNEXURES:

ANNEXURE No.	TITLE OF ANNEXURE	FORMAT No.
Annexure -I	Total Aerobic Microbial Count Report	

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:

Not Applicable

10.0 REVISION HISTORY:

CHANGE HISTORY LOG

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

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ANNEXURE – I
TOTAL AEROBIC MICROBIAL COUNT REPORT

Product Name		**A.R. No.**	
Batch No.		**Date of Receipt**	
Sampled By		**Date of Test**	
Sampled Qty.		**Date of Release**	
Analyzed By		**Method Used**	Membrane Filtration
Media Reference No.		**Incubator ID No.**	

Preparation of Sample: Filter the of sample and rinse the membrane with 100 ml of sterile 0.1% peptone water or sterile water than transfer the filter paper on Pre-incubated Soyabean Casein Digest Agar and 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 hours.

OBSERVATIONS:

Sample details	No. of cfu observed per plate		Total cfu / 25cm^2/ units	Limit (cfu)	
	TFC	TBC		TFC	TBC
Negative Control					

Remarks: The above sample is complies/does not comply as per IP/BP/USP/IH specification.

Observed By: **Checked By:**
Date: **Date:**