1.0 OBJECTIVE

To lay down the procedure for testing of bioburden test of packaging Materials.

2.0 SCOPE

This procedure is applicable for testing of Bioburden test of Packaging Material at Pharma Micro Hub Pvt Ltd.

3.0 RESPONSIBILITY

3.1 Microbiologist is responsible to test the sample for Bioburden in Packaging materials.

4.0 ACCOUNTABILITY

4.1 Head–Microbiology / his designee is accountable for overall compliance of this SOP.

5.0 PROCEDURE

5.1 **Precautions:**

5.1.1 Prior to transfer of sterilized sampling bottles to the production block, check the bottles for absence of the moisture, if any bottle contains moisture, dry the bottles in oven at about 60°C.

5.1.2 Use suitable membrane filters having a nominal pore size not greater than 0.45µm.

5.2 **Media/Accessories Requirement:**

5.2.1 Receive and store the Bioburden sample as per the SOP BEL-MB059 procedure for sample management in Microbiology laboratory.

5.2.2 Sterilized glass containers (Tubes/Bottles/Flasks), Burner, Laminar air flow, Incubators, Sterile Filtration Cups, Sentino Pump, Sterile Forceps, Sterile 0.45µm filter with 47mm edge, sterile 0.9% NaCl with 0.5% of tween 80.

5.2.3 Soyabean Casein Digest Agar (Ready to use or in house prepared) ,BSCP.

5.3 **Test Procedure**

5.3.1 **Bioburden analysis for rubber stoppers:**

5.3.1.1 **Sample Preparation:**

5.3.1.1.1 Collect required quantity of rubber stoppers.

5.3.1.1.2 Aseptically transfer the estimated number of rubber stoppers into a glass container/bottle containing about 400 ml of sterile 0.9% NaCl with 0.5% of tween 80 for each sample to estimate bio load.

5.3.1.1.3 Vortex/swirl the containers/bottles for not less than 5 minute.

5.3.1.1.4 Allow to stand for 15 minutes.

5.3.1.1.5 Again vortex/swirl the containers/bottles for not less than 2 minute just before filtration.

5.3.1.2 **Test for Bioburden:**

5.3.1.2.1 Assemble the membrane filtration kit with a sterile membrane filter of pore size 0.45µm under the laminar Air Flow.

5.3.1.2.2 Pre-wet the membrane with 50 mL of the BSCP/ suitable Rinsing fluid.

5.3.1.2.3 Filter the entire quantity of the above solution (Sample preparation) through the membrane filter by applying vacuum.

5.3.1.2.4 Rinse the membrane with 3 X 100 ml of the BSCP.

5.3.1.2.5 Transfer the membrane on to the pre-incubated SCDA plate.

5.3.1.2.6 Incubate the SCDA plate at 20-25°C for 72 hours followed by 30-35°C for the next 48 hours.

5.3.1.3 **Negative Control:**

5.3.1.3.1 Keep a glass container/bottle containing 400mL of sterile 0.9% NaCl with 0.5% of tween 80 for negative control.

5.3.1.3.2 Pre-wet the membrane with 50 mL of the BSCP / suitable Rinsing fluid.

5.3.1.3.3 Filter the entire quantity of above solution through the membrane filter by applying vacuum.

5.3.1.3.4 Rinse the membrane with 3 X 100 mL of the BSCP.

5.3.1.3.5 Transfer the membrane on to the pre-incubated SCDA plate.

5.3.1.3.6 Incubate the SCDA plate at 20-25°C for 72 hours followed by 30-35°C for the next 48 hours.

5.3.1.4 **Interpretation of results:**

5.3.1.4.1 **Calculations:**

5.3.1.4.1.1 Count entire membrane with the help of colony counters.

5.3.1.4.1.2 Results are expressed as follows.

5.3.1.4.1.3 Results expressed as CFU/Cm^2 and CFU/Rubber stopper.

For Example: If 10 CFU observed for 200 Cm^2 surface area of 20mm of rubber stoppers (20 numbers).

CFU/Cm^2 = Observed CFU/Total surface area (200 Cm^2)

= 10/200 Cm^2

= 0.05 CFU/Cm^2

CFU/Rubber stopper = Observed CFU/Number of rubber stoppers

= 10/20

= 0.5 CFU/Rubber Stopper.

6.0 ABBREVIATIONS

6.1 CFU : Colony Forming Unit

6.2 Cm^2 : Centimeter square

6.3 NaCl : Sodium chloride

6.4 SCDA : Soyabean Casein Digest Agar

6.5 BSCP : Buffered sodium chloride peptone

7.0 ANNEXURES

7.1 Annexure-I: Bioburden test report for Packaging Materials

8.0 REFERENCES

8.1 Nil

9.0 CHANGE HISTORY

9.1 New SOP

END OF DOCUMENT

ANNEXURE-I
BIOBURDEN TEST REPORT FOR PACKAGING MATERIALS

Sample Name:		**Specification No:**	
Batch Number		AR No.	
Sampling date		Method	
Required Qty for testing		Sampled by	
Date of analysis		Test completed on	
Media and Equipment Details:			
1) SCDA Medium		**2) 0.9% NaCl**	
Lot No:		Lot No:	
Prepared on :		Prepared on :	
Use Before:		Use Before:	
3) Rinse fluid/Prewetting: BSCP		**4) Membrane details**	
Lot No:		Type of Membrane used:	
Prepared on:		Membrane filter Lot No:	
Use Before:		Expiry Date:	
Incubation Details:			
Incubation Condition:20-25°C for 72 hours followed by 30-35°C for 48 hours.			
Incubator I.D No. (20-25°C):			
Calibrated on:		Calibration Due on:	
Validated on:		Validation Due on:	
Incubation Start date & Time:		Incubation End date & time:	
Incubator I.D No.(30-35°C):			
Validated on:		Validation Due on:	
Incubation start date & time:		Incubation End date & time:	
Observation & Calculation:			
Limit			
Results			
Negative Control			
Remarks: Complies/Does not comply with specification.			
Analysed by: Sign/Date:		Reviewed by: Sign/Date:	