PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

Title: Product Bioburden Testing				
SOP No.:		**Department:**	Microbiology	
		Effective Date:		
Revision No.:	00	**Revision Date:**		
Supersede Revision No.:	Nil	**Page No.:**	1 of 5	

1.0 OBJECTIVE

To lay down procedure for testing of product bioburden.

2.0 SCOPE

This SOP is applicable for testing of non-sterile bulk solution and non-sterile finished product in manufacturing.

3.0 RESPONSIBILITY

Prepared by - Executive Microbiology

Checked by - Assistant Manager Microbiology / QC

Approved by - Head QA, QC

4.0 PROCEDURE

Note: Use Membrane Filtration method if estimated CFU is low and Pour plate method if estimated CFU is high.

4.1 Membrane Filtration Method

4.1.1 Prepare and sterilize the media as per SOP.

4.1.2 Collect the samples for microbiological examination in pre sterilized glass bottles.

4.1.3 Testing to be performed under the LAF of specified area

4.1.4 Sterilize the required articles and keep it in the dedicated area for analysis.

4.1.5 Arrange all the required material on the LAF before starting the test.

4.1.6 Place the Manifold on the LAF bench and fix sterilized Filter cups.

4.1.7 Place the sterile 47 mm, 0.45-µ membrane filters in the filtration cups.

4.1.8 Filter not less than 100 mL volume of sample through a sterile 47 mm, 0.45-µ membrane filters, by applying vacuum.

4.1.9 For the solid products dissolve approximately 1g of the sample in 100 mL of suitable sterile diluents and filter through a sterile 47 mm, 0.45-µ membrane filters, by applying vacuum.

4.1.10 For the samples having anti microbial activity rinse the membrane with 3 X 100 mL of sterile 0.1% peptone water.

4.1.11 Aseptically place the filtered membrane on the surface of Soyabean Casein Digest Agar plate.

4.1.12 Prepare one -ve control plate by filtering not less than 100 mL sterile water through the membrane and put the membrane on the surface of Soyabean Casein Digest Agar and incubate along with test samples.

4.1.13 Incubate the plates at 30 - 35°C for 48 hours followed by 20 - 25°C for 72 hrs.

4.1.14 Observe the plates for CFU counts after incubation period and interpret the result as number of CFU per 100 ml / gram as applicable.

4.1.15 Record the results in Annexure - I.

4.2 Pour plate Method

4.2.1 Prepare and sterilize the media as per SOP.

4.2.2 Collect the samples for microbiological examination in pre sterilized glass bottles.

4.2.3 Testing to be performed under the LAF of specified area.

4.2.4 Arrange all the required material on the LAF before starting the test.

4.2.5 Prepare single plates for each sample to be tested.

4.2.6 Use micropipette and sterile tips for transfer of sample in each plate.

4.2.7 For the solid products dissolve or suspend approximately 10g of the sample in 100 mL of suitable sterile diluents mark the dilution as 1:10.

4.2.8 For the samples estimated to be having more microbial load (More than 300CFU/ml), do the required dilution.

4.2.9 Pipette out 1.0 ml of sample into sterilized Petri plate before adding molten medium.

4.2.10 Maintain molten medium at about 55°C and cool to about 40 to 45°C just before use.

4.2.11 Pour approximately 20 mL sterilized molten Soyabean Casein digest agar medium into each plate.

4.2.12 Smoothly rotate the petridish clock wise and anti clock wise so as to mix the sample evenly with the molten agar. Allow the medium to solidify.

4.2.13 After medium is solidified, incubate the plates in an inverted position at 30 to 35°C for about 48 hours followed by 20 - 25°C for 72hrs.

4.2.14 Count all the colonies on the plates after incubation period with the help of a colony counter and record the counts as number of CFU per gm or mL for each sample.

4.2.15 Record the results in Annexure - I.

CALCULATION: Count per gm/ml = Dilution Factor X Number of CFU

5.0 SAFETY & PRECAUTIONS

5.1 Follow the entry, exit procedure of respective areas for sampling.

5.2 Wear Sterile gloves, cap, facemask while collecting the sample.

6.0 REVISION HISTORY

Revision No.	Reason for Revision	Superseded from & Date
00	First Issue	--------

7.0 REFERENCES

SOP.

8.0 ABBREVIATIONS

SCDA	:	Soyabean Casein Digest Agar
SOP	:	Standard Operating Procedure
QC	:	Quality Control
QA	:	Quality Assurance
CFU	:	Colony Forming Unit
No.	:	Number
LAF	:	Laminar Air Flow
Mm	:	Millimeter
µ	:	Micron
g	:	Gram
mL	:	Milliliter
%	:	Percentage
°C	:	Degree Centigrade

9.0 ANNEXURES

Annexure - I : Bioburden Test Report

ANNEXURE - I

BIOBURDEN TEST REPORT

Name of the Product:_____

Batch No: _____ A.R.No:_____

Bulk Solution prepared on: _____ Sampled on: _____

Date of Testing: _____ Report date: _____

Method Used: Membrane Filtration method / Pour plate method.

Media used: _____ Sterilized medium lot no.: _____

Incubation Conditions:

Incubate the plates at 30 - 35°C for 48 hours followed by 20 - 25°C for 72 hours.

Test Results:

Observed CFU/ : _____

Negative Control : _____

Done by:
Date:

Checked by:
Date: