STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Bio-burden of Primary Packing Material	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

1.0 PURPOSE:

1.1 To lay down a procedure for determining the bio burden (Total bacterial and fungal count) level of Primary packaging materials being used for packing of Tablets, Capsules, Liquids, Ointments and Creams.

2.0 SCOPE:

2.1 This procedure is applicable to Microbiology Laboratory.

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3.0 RESPONSIBILITY:

3.1 Officer/Executive - Quality Control.

4.0 ACCOUNTABILITY:

4.1 Head –QC department.

5.0 **PROCEDURE**: (Frequency: First two consignments of each vendor per year)

5.1 Make entry of packing materials in microbial limit test log book as per SOP in Annexure-II. Carry out the test under LAF for all primary packing materials.

5.2 For Bottles/Caps (method used: membrane filtration method)

5.2.1 Sampling units: 10 bottles/caps

5.2.2 **For bottles:**

5.2.2.1 Pipette 10 ml of sterile 0.1% peptone water from the conical flask and transfer into one bottle and vortex for 05-10 seconds on a vortex mixer.

5.2.2.2 After vortexing transfer the 0.1% peptone water from each bottle into sterile filter assembly fixed on the manifold.

5.2.2.3 Pass 0.1% peptone water through 0.45µ membrane filter by using vacuum pump.

5.2.2.4 Like wise repeat for the other four bottles as mentioned in 5.2.2.1 to 5.2.2.3.

5.2.2.5 Discard the bottles after testing.

5.2.2.6 Carefully open the filter assembly. Ensure that the membrane filter is not sticking to the bottom of the filtration cup.

5.2.2.7 Aseptically remove the membrane filter from the assembly by using sterile forceps.

5.2.2.8 Place the membrane filter on to the surface of SCDA plate in such a way that the filtrate on the filter paper is facing upperside and the bottom is in direct contact with the media.

5.2.2.9 Incubate the above plate at 30°C -35°C for 3 days.

5.2.2.10 On completion of incubation period count the number of colonies observed on the membrane filter and record the total bacterial count as `X' colony forming unit(cfu)/5 units in Annexure -I.

5.2.2.11 Repeat the above steps from 5.2.2.1 to 5.2.2.8 for fungal count by using Sabouraud dextrose agar (SDA) plate in place of SCDA and incubate the plate at 20°C -25°C for 5 days.

5.2.2.12 On completion of incubation period count the number of colonies observed on the membrane filter and record the total fungal count as `Y' cfu/5 units in Annexure-I.

5.2.3 **For caps:**

5.2.3.1 Take two wide mouthed 250ml bottles containing 50 ml of sterile 0.1% peptone water. Place five No. of caps into each bottle.

5.2.3.2 Shake vigorously for one minute.

5.2.3.3 Open the mouth and pour the 0.1% peptone water into sterile filter assembly fixed on the manifold. Care shall be taken for the caps not to fall into the filtration cup.

5.2.3.4 Pass the solution through 0.45µ membrane filter by using vacuum pump.

5.2.3.5 Discard the caps after testing.

5.2.3.6 Follow the steps from 5.2.2.6 to 5.2.2.12.

5.3 For Tubes Used For Ointments/Creams(method used: membrane filtration method)

5.3.1 Sampling Units: 20 Tubes.

5.3.2 Pipette 4 ml of sterile 0.1% peptone water from the conical flask and transfer into one tube from the open end, fold the open end and vortex for 05-10 seconds on a vortex mixer.

5.3.3 After vortexing transfer the 0.1% peptone water from the tube into sterile filter assembly fixed on the manifold. Pass 0.1% peptone water through 0.45µ membrane filter by using vacuum pump.

5.3.4 Like wise repeat for the other tubes as mentioned in 5.3.1 and 5.3.2.

5.3.5 Discard the tubes after testing.

5.3.6 Carefully open the filter assembly. Ensure that the membrane filter is not sticking to the bottom of the filtration cup.

5.3.7 Aseptically remove the membrane filter from the assembly by using sterile forceps. Place the membrane filter on to the surface of SCDA plate in such a way that the filtrate on the filter paper is facing upperside and the bottom is in direct contact with the media.

5.3.8 Incubate the above plate at 30°C -35°C for 3 days.

5.3.9 On completion of incubation period count the number of colonies observed on the membrane filter and record the total bacterial count as `X' colony forming unit(cfu)/5 units in Annexure -I.

5.3.10 Repeat the above steps from 5.3.2 to 5.3.7 for fungal count by using Sabouraud dextrose agar (SDA) plate in place of SCDA and incubate the plate at 20°C -25°C for 5 days.

5.3.11 On completion of incubation period count the number of colonies observed on the membrane filter and record the total fungal count as `Y' cfu/5 units in Annexure-I.

5.4 For PVC Film / Aluminium Foil(method used: contact plate method)

5.4.1 Carefully remove initial 2 to 3 meters of the foil/film from the roll and discard.

5.4.2 Open the lid of the Contact plate containing SCDA medium, bring the open portion of media in contact with the inner surface areas of the foil /film.

5.4.3 Gently press the plate with palm and hold, so that the media come in contact with the foil / film for 5-10 seconds.

5.4.4 Slowly relax the palm and remove the plate from the contact. Take Care that the media should not stick to the foil / film.

5.4.5 Close the lid of the contact plate.

5.4.6 Cut off the tested portion of the foil / film and destroy.

5.4.7 Incubate the above plate at 30°C -35°C for 3 days.

5.4.8 On completion of incubation period count the number of colonies observed on SCDA plate and record the total bacterial count as `X' cfu/plate in Annexure-I.

5.4.9 Repeat the above steps from 5.4.1 to 5.4.6 for fungal count by using Sabouraud dextrose agar (SDA) plate in place of SCDA and incubate the plate at 20°C -25°C for 5 days.

5.4.10 On completion of incubation period count the number of colonies observed on the SDA plate and report the total fungal count as `Y' cfu/plate in Annexure-I.

Limits:

Material	Total Bacterial Count	Total Fungal Count
Bottles	NMT 50 cfu / 5 units	NMT 10 cfu / 5 units
Caps	NMT 50 cfu / 5 units	NMT 5 cfu / 5 units
Tubes for ointments / creams	NMT 50 cfu / 10 units	NMT 10 cfu / 10 units
Aluminium foil / PVC film	NMT 20 cfu / plate	NMT 2 cfu / plate.

ANNEXURE(S):

Annexure -I: Bio burden of primary packaging materials.

REFERENCES:

NIL.