Department	Micro Laboratory		Document no	MICLAB – METHOD 001	
Title	Bioburden Determination				
Prepared by:		Date:		Supersedes:	
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Approved by:		Date:		Review Date:	

1. SCOPE AND APPLICATION

This general test method shall be followed in order to determine total aerobic bioburden. This procedure applies to packaging components such as bottles, tubes, etc., which are used in the manufacture of products at a GMP site. This procedure is also used for determining the bioburden of sampling jars.

2. REAGENTS AND MATERIALS REQUIRED

2.1 0.1% peptone containing 0.1% Tween 80 (Dil + T). (Oxoid L37)

2.2 Blender bag - sterile.

2.3 Filtration assembly - sterile.

2.4 0.45 micron filter membranes (cellulose-nitrate) - sterile hydrophilic edge.

2.5 Sterile forceps.

2.6 Tryptone Soya Agar (Oxoid CM131) plates (TSA).

2.7 Sabouraud Dextrose Agar (Oxoid CM41) plates (SDA).

2.8 Method - Membrane Filtration Technique

2.9 Top Pan Balance

2.10 Form – Microbiological Report for Bioburden Testing.

2.11 Procedure – Identifications of Contaminants.

3. GENERAL TEST METHOD

3.1 For components requiring a bioburden of the entire surface i.e. droppers

3.1.1 Place components inside the blender bag.

3.1.2 Aseptically pour into the bag sufficient Dil + T to submerge components ensuring that the inside cavity of the components make contact with the medium.

3.1.3 Record the volume of Dil + T used.

3.1.4 Shake the bag in order to wet/wash off components thoroughly.

3.1.5 Close and tape the opening of the bag and place bag inside a holding container.

3.1.6 Proceed with testing as detailed in 3.3 below.

3.2 For bottles requiring a bioburden of their inner surface

3.2.1 Place the component on a top pan balance and TARE the balance.

3.2.2 Add the required amount of DIL+T to fill the bottle. Record this volume.

3.2.3 Proceed with testing as detailed in 3.3 below.

3.3 Test Procedure

3.3.1 Allow the component/bag to stand for 15-30 min. This will aid in the resuscitation of micro-organisms.

3.3.2 Shake the bag containing components for approx. 30 sec prior to filtration.

3.3.3 Set up the filtration assembly as per “Membrane Filtration Technique”.

3.3.4 If high bioburden count is expected, plate out 1ml and 0.1ml in duplicate of the Dil + T in bag.

3.3.5 If required, filter 10mL, 50mL or 100mL amounts.

3.3.6 Apply vacuum and draw the liquid through 0.45 micron membrane.

3.3.7 Rinse the inside walls of filter funnel with the rinse solution at least 3 times using a minimum of 100 mL of Dil + T each time.

3.3.8 Remove membrane and plate by rolling it onto TSA plate. Avoid air bubbles under the membrane.

3.3.9 Pour the remaining volume of rinse solution (Dil + T) into filter funnel.

3.3.10 Apply vacuum and draw the liquid through 0.45 micron membrane.

3.3.11 Rinse the inside walls of filter funnel with the rinse solution at least 3 times using a minimum of 100 mL of Dil + T each time. This is referred to as ‘rest’ on SF150124.

3.3.12 Remove membrane and plate by rolling it onto TSA plate.

3.3.13 Incubate plates in an inverted position at 30 ± 1°C for 3 days.

3.3.14 If total yeast and mould count is required proceed as in 3.3.1 to 3.3.10 where SDA plate shall be substituted for TSA plate.

3.3.15 Incubate SDA plate in upright position at 25 ± 1°C for 5 days.

3.3.16 Remove plates from incubator and count colonies.

3.3.17 For Total Plate Count and Yeast & Mould Count, record the number of cfu per number of items tested.

3.3.18 When total viable aerobic count is required, add number of cfu from TPC and Y&M plates. Record the number of cfu per number of items tested.

3.3.19 Record results on "Microbiological Report for Bioburden Testing".

3.3.20 If identification of colonies is required, follow Procedure – "Identification of Contaminants".

3.4 Specification For Packaging Components

	Total viable aerobic count cfu/10 items
Acceptable level	≤ 100
Warning level	> 100 - 500
Action level	> 500

4. REFERENCE DOCUMENTS

4.1 AS 1766.1.3 (1991) – General Procedures and Techniques – Colony Count – Pour Plate Method.
AS 1766.2.2 (1997) – Examination for Specific Organisms – Colony Count of Yeasts and Moulds.
AS 1766.1.5 (1991) – General Procedures and Techniques – Colony Count – Membrane Filtration Method

5. REVIEW HISTORY

Version #	Revision History
MICLAB – METHOD 001	New