STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Microbial Limit Test	**Effective Date:**
Supersedes: Nil	**Review Date:**
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1.0 Objective:

To lay down a procedure for Microbial limit test.

2.0 Scope:

This Standard Operating Procedure is applicable to Quality Control.

3.0 Responsibility

Executive / Sr. Executive-QC : Shall be responsible to follow procedure for microbial limit test.

Head - QC/Designee : Shall be responsible for the compliance of this SOP.

4.0 Abbreviations and Definitions

SOP	**:**	Standard Operating procedure
No.	:	Number
TAMC	:	Total aerobic microbial count
TYMC	:	Total yeast and mold count
%	:	percentage

5.0 Procedure

5.1 Sample Receiving and Preparation

5.1.1 Receive all the Raw materials, in-process materials and Bulk finished samples in a sterile glass container or sterile Polythene bag and make the entry as per Annexure -1.

5.2 **Precautions**

5.2.1 Handle all cytotoxic materials with care using proper personal protective clothing and in case of any accidental spillage follow SOP.

5.2.2 Ensure the Bio-safety cabinet is in operational conditions as per Sop “Standard Operating and cleaning procedure of Biosafety Cabinet”.

5.3 Determination of Total aerobic microbial counts and specified microorganisms

5.3.1 Unless otherwise specified in the product release specification, carry out the following tests in Raw Materials, in-process materials and bulk Finished Products.

5.3.2 Test for Total aerobic bacterial count.

5.3.3 Test for Total yeast and mold count.

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5.3.4 Test for detection of Pseudomonas aeruginosa.

5.3.5 Test for detection of Escherichia coli

5.3.6 Test for detection of Salmonella species

5.3.7 Test for detection of Staphylococcus aureus

5.3.8 If testing of product as per IP, refer to current version of GTP and record the results as per Annexure No.1

5.3.9 If testing of the product as per USP, EP and BP, refer to current version of GTP and record the results as per Annexure No. 01.

5.4 Determination of bio load of empty containers

5.4.1 Collect 10 containers in sterile tray. Use 10 ml of sterile peptone 0.1% as rinse fluid for rinsing. If container is very small (such as with fill volume of 1.0 ml) then collect 20 containers and use 1.0 ml of sterile peptone 0.1% as rinse fluid for rinsing

5.4.2 Take individual container and add aseptically sterile peptone 0.1% and rinse properly. Transfer the same in a sterile test tube aseptically.

5.4.3 Carry out the procedure for all ten/twenty containers, every time transferring the rinse fluid to the same test tube and thus finally a pool sample of ten/twenty containers is obtained.

5.4.4 Transfer 10.0 ml of the pool sample to each of two sterile membrane filters and filter immediately.

5.4.5 Wash each membrane by filtering through it appropriate volume (as per validation data) of a sterile buffered sodium chloride peptone solution pH 7.0 or 0.1% Peptone solution.

5.4.6 Transfer aseptically one of the membrane filters, intended for the enumeration of bacteria, on to the surface of Soya bean Casein Digest Agar plate and the other, intended for the enumeration of fungi, on to the surface of Sabouraud dextrose Agar.

5.4.7 Incubate the plates for 5 days at 30°C to 35°C for bacteria and 20°C to 25°C for yeast and molds.

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5.4.8 Count the number of colonies that are formed on each type of plate. Report the observed count as colony forming units (cfu) per container. Record the results in data sheet as per Annexure No. 1.

6.0 Forms and Records

6.1 Data Sheet for Microbiological Analysis : Annexure-1

6.2 Microbial limit test acceptance criteria : Annexure-2

6.3 Certificate Of Analysis (Microbial limit test) : Annexure-3

6.4 Data Sheet for bio load of empty containers : Annexure-4

7.0 Reference

7.1 Standard Operating and cleaning procedure of Biosafety Cabinet

7.3 GTP.

8.0 Distribution

8.1 Master Copy : Documentation Cell (Quality Assurance)

8.2 Controlled Copies : Quality Control, Quality Assurance

9.0 History

Date	Revision Number	Reason for Revision
	00	New SOP