MICROBIAL LIMIT TEST VALIDATION PROTOCOL

Protocol No.	
Effective Date	

Company Name and Address

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Signature			
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1.0 PRE-APPROVAL SIGNATURES:

This document is prepared by the validation and the GMP compliance team of xxxxxxxxxxxxxxxx under the authority of Quality Control Authority. Hence this document before being effective shall be approved by xxxxxxx QA & CQU team.

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2.0 OBJECTIVE:

To establish the documentary evidence that the method for Microbial limit test for Non-sterile materials is capable of correctly estimating the microbial counts in the Materials. The validity of the test results largely upon the adequacy of a Demonstration that the test specimens to which they are applied do not, of them -selves, inhibit the multiplication, under the test condition, of microorganisms that may be present.

The validation exercise shall demonstrate that the method employed is capable for correct enumeration of microorganisms without adversely effecting the Growth even in case of materials, which have antimicrobial activity.

3.0 SCOPE:

This validation protocol is applicable for validating the Microbial limit test of on-sterile products and raw materials.

The protocol shall be used for validation of the methods applicable for all Dosage forms and materials, which have requirement for Microbial limit test.

Whenever the method is used for Microbial limit test for scale up/ scale down Formulation, the validation shall be done on only one strength of product.

4.0 REFERENCE DOCUMENT:

Following documents are referred during preparation of the protocol

Document Name	Document Number
Microbial limit Test SOP	xxxxxxxxxxxxxxxx

5.0 RESPONSIBILITY:

Trained Microbiologist or a suitable trained authorized person

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6.0 VALIDATION METHODOLOGY

6.1 TOTAL VIABLE AEROBIC COUNT

% of Tween 20 in Buffered Sodium chloride Peptone Solution	%Recovery of Microorganisms
0.025 %	Recovery not obtained
0.050%	Recovery not obtained
0.075%	Recovery less than 70%
0.1 %	Recovery up to 70%

6.1.1 10 gm/ml of sample + 90 ml of Buffered Sodium Chloride Peptone + 0.1% Tween 20 / 80. (Solution A) 1:10.

15 ml solution A + 60 ml of Buffered Sodium Chloride Peptone + 0.1 % Tween 20 1:50.

10 ml of solution A + 90 ml of Buffered Sodium chloride Peptone + 0.1% Tween 20 1:100.

6.1.2 Prepared six test tube containing 10 ml each from Solution A (1:10) and follow the same for the other preparation i.e. Solution B (1:50) and Solution C (1:100) respectively.

6.1.3 Grow the bacterial cultures separately in Soyabean casein digest medium at 30^0C to 35^0C for 24 hours and Fungal cultures separately in Soyabean casein digest medium at 20^0C to 25^0C for 48 hours. The organisms to be used are as mentioned below.

- E.coli
- S. aures
- Salmonella
- Pseudomonas aeruginosa
- C. albicans
- A. niger
- Bile tolerant gram-negative bacteria ***(replaced the EP procedure "Test for Enterobacteria and Certain Other Gram-Negative Bacteria")***

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6.1.4 Prepare reference suspension separately of the above organisms by diluting the broth cultures to get containing about not less than 10^3 viable microorganisms per ml.

6.1.5 Add separately 1 ml of the culture dilutions of S. aureus, E. coli, Salmonella, Pseudomonas aeruginosa, C. albicans, A. niger and other bacterial species prepared above in all the three sets of six test tubes prepared from each solution A,B,C.

6.1.6 From each set pipette out 1 ml separately in two pre- sterilized Petri plate .Pour 20-22 ml of liquefied Soyabean casein digest agar for the cultivation of bacteria and 20-22 ml of liquefied Sabouraud dextrose agar for the cultivation of fungi.

6.1.7 Incubate plates of soyabean casein digest agar at 30^0C to 35^0C for 5 days and plates of Sabouraud dextrose agar at 20^0C to 25^0C for 5 days.

6.1.8 Sample controls:
Inoculate 1.0 ml of solution A, B, and C in duplicate and add 20-22 ml soyabean casein digest agar for bacterial count and Sabouraud dextrose agar for total combined mold and yeasts count respectively. Incubate at 30^0C to 35^0C for bacterial count for 5 days and 20^0C to 25^0C for total combined mold and yeasts count for 5 days.

6.1.9 Positive control
Inoculate1 ml of each of the diluted bacterial and fungal culture into two plates Each and add 20-22 ml of soyabean casein digest agar and Sabouraud dextrose Agar respectively. Incubate at 30^0C to 35^0C for bacterial culture for 5 days And at 20^0C to 25^0C for total combined mold and yeasts count for 5 days.

6.1.10 Negative control for media:
Inoculate 1.0 ml of Buffered Sodium chloride Peptone Solution in four plates And add 20-22 ml of soyabean casein digest agar into 2 plates and Sabouraud dextrose agar into other 2 plates respectively. Incubate at 30^0C to 35^0C for bacterial culture for 5 days and at 20^0C to 25^0C for total combined mold and yeasts count for 5 day.

6.2 TESTS FOR PATHOGENS:

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6.2.1 **Preparation** of sample**:**

1.0 gm sample + 100 ml soyabean casein digest medium + 4% Tween 20

1.0 gm sample + 100 ml Fluid lactose medium + 4% Tween 20

100 ml Fluid lactose medium + 4% Tween 20 (Positive control)

100 ml soyabean casein digest medium + 4% Tween 20 (Positive control)

6.2.2 Mix well to obtain uniform solution or suspension:

6.2.3 Grow the bacterial culture separately in soyabean casein digest medium at 30^0C to 35^0C for 24 hours. The organisms to be used are as mentioned below.

- E.coli
- S. aures
- Salmonella
- Pseudomonas aeruginosa

6.2.4 Prepare reference suspension separately of above organisms by diluting the The broth cultures to get not less than 10^3 viable organisms per ml. Mix equal volume of each suspension.

6.25 Pipette 1.0 ml mix suspension of the microorganism separately in tube of Soyabean casein digest medium and Fluid lactose medium containing the sample to be examined.

6.2.5 Incubate the tube of soyabean casein digest medium and Fluid lactose medium at 30^0C to 35^0C for 24 to 48 hours and proceed for Isolation and Identification of *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella, Escherichia coli* from this broth as per specified SOP.

7.0 ACCEPTANCE CRITERIA

7.1 Recovery of the test organisms should not be less than 70 % of the calculated value of the inoculum suspension is to be obtained.

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7.2 There shall not be failure in isolation and identification of organisms inoculated in the medium along with material.

7.3 The negative control should show no growth.

8.0 REVALIDATION CRITERIA/ DEMONSTRATION OF METHOD SUITABILITY

Change in formulation where in new component has been added.

When concentration of preservative has been changed.

Major change in method for Microbial limit test like method for deactivation of Antimicrobial activity.

Three batches of each product / materials shall be validated for Microbial limit Test.

The "absence of specified organisms" tests to provide procedures for demonstration of the absence of Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella species & Escherichia coli.

9.0 RESULT REPORTING / REVIEWING REQUIREMENTS

Report all results on a method validation report form. If results are unacceptable, the method accordingly to rule out the affecting factor.

10.0 CONCLUSION

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11.0 KEY CONSIDERATIONS:

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1. **The fundamental shortcomings of these tests in regards to the current good manufacturing practice (CGMP) requirements for "absence of objectionable organisms" should be discussed by scientific teams.**

2. **Product risk analysis including product use and route of administration, growth potential, preservation, and other considerations which are recommended in Pharmacopoeia texts must be properly taken into account. The quality group must take a proper and reasonable scientific approach how to handle, validate and test in special cases of product recalls due to presence of objectionable organisms.**

3. **Re-validation of existing tests to align with current harmonized standards and level of detail should properly discussed and made by using a matrix approach.**

4. **A proper reporting format should be developed by both quality and documentation teams.**

5. **Training microbiologists for the revised tests should be considered as a priority by both validation and quality team during transfer of procedures.**

12.0 ABBREVIATIONS

Abbreviations	
QA	Quality Assurance
SOP	Standard Operative Procedure
GMP	Good Manufacturing Practice
CQU	Central Quality Unit

13.0 Flow charts

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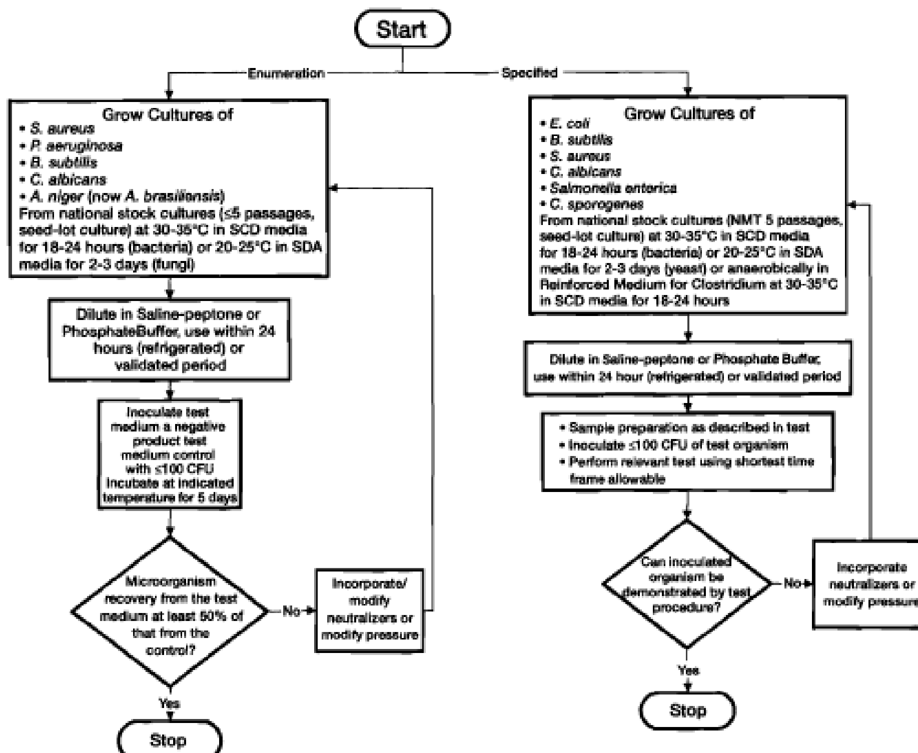

14.0 POST APPROVAL

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