STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Efficacy of Antimicrobial Preservation	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

PURPOSE:

The purpose of this SOP is to check the efficacy of antimicrobial preservatives.

SCOPE:

This procedure describes testing of efficacy of antimicrobial preservatives done in microbiology laboratory.

RESPONSIBILITY:

Quality control officer/Executive whoever is performing the activity.

ACCOUNTABILITY:

Head – QC Department

PROCEDURE: (Frequency: As per product specification)

1.0 Preparation of stock culture suspension for preservative efficacy testing:

1.1 Following microorganisms are used for preservative efficacy testing

- *Candida albicans* ATCC 10231
- *Aspergillus niger* ATCC 16404
- *Pseudomonas aeruginosa* ATCC 9027
- *Staphylococcus aureus* ATCC 6538
- *Escherichia coli* ATCC 8739

1.2 Preparatory to the test, inoculate the surface of Soyabean Casein Digest Agar slant from a recently revived working bacterial culture of *P.aeruginosa*, *E.coli* and *S.aureus* and incubate at 32.5 ± 2.5℃ for 24 to 72 hours.

1.3 Similarly inoculate the surface of Sabouraud Dextrose slant from a recently revived working yeast/mold culture of *Candida albicans* and *Aspergillus niger* and incubate at 22.5 ± 2.5 ℃ for 48 to 72 hours for *Candida albicans* and at 22.5 ± 2.5 ℃ for 5 days or until good sporulation is obtained for *Aspergillus niger* .

1.4 Harvest the bacterial and *C. albicans* cultures by washing the surface growth by using 20 ml of sterile saline TS and *A. niger* culture is harvested by washing the surface growth by using 20 ml of sterile

saline TS having 0.05% of Polysorbate 80.

1.5 To count the stock suspension, prepare dilutions by transferring 1.0 ml aliquot from above suspension to 9.0 ml sterile normal saline solution.

1.6 Make further appropriate serial dilutions in sterile normal saline and mark each tube for its dilution factor and organism.

1.7 Pour 1.0 ml from selected dilutions in each of two Petri dishes and mark appropriate dilutions factor on each Petri dish.

1.8 Pour 15 to 20 ml of SCDA, previously sterilized and cooled to 40°C to 45°C.Swirl to mix the content and allow the agar to solidify.

1.9 Incubate the plates in inverted position at 30°C to 35°C for 48 to 72 hours for bacterial culture and 20°C to 25°C for 48 hours to 72 hours for yeast/mold cultures.

1.10 To count the no. of CFU, use plates with not less than 30 and not more than 300 colonies for bacteria and yeast, 8 to 80 colonies in case of mold to determine the stock suspension count.

1.11 Average the no. of colonies on the duplicate plate and multiply that average by the reciprocal of the dilution factor to obtain the count of the original stock suspension and record the results in the worksheet as per Annexure-II.

2.0 Working Suspension preparation:

2.1 Based on the stock suspension count, on the day of the test, prepare serial dilutions in saline solution from the stock suspension to yield a working suspension of 10^6 to 10^7 CFU/ ml. This suspension serves as an inoculum for preservative efficacy study and record the calculation for working suspension in worksheet as per Annexure-II.

3.0 Test Sample Preparation:

3.1 Arrange five sets, each set comprising of three sterile screw capped vials for bacterial counts and fungal counts.

3.2 Transfer 1 gm of content of the product in each of the vials.

3.3 Inoculate each vial of one set with 0.1 ml of one of the prepared and standardized (working culture) test organism inoculum so that the final concentration of the test organism is between 1 x 10^5 to 1 x 10^6 cfu / gm of the product. Similarly repeat the preparation for other test organisms.

3.4 Heat the content to not more than 40°C to facilitate melting and mix gently by swirling the vials.

3.5 Keep all the vials in incubator at 20 to 25°C except one vial for Zero hour count.

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Efficacy of Antimicrobial Preservation	**Effective Date:**
Supersedes: Nil	**Review Date:**
Issue Date:	**Page No.:**

4.0 Initial Count of Inoculated Culture Suspension.

4.1 Test the concentration of the culture suspension at the initial time (zero hour) by the following procedure from each container.

4.2 To each of the vial containing 1 gm of the product and 0.1 ml of the test organism inoculum , add 10 ml of sterile phosphate buffer solution containing 2% of polysorbate 80 and 0.3% of Lecithin, 4.3 Heat the mixture to not more than 40°C to facilitate dissolution. This is 10^{-1} Dilution.

4.4 Make further serial dilutions in sterile normal saline by transferring 1 ml aliquot of above dilution to 9 ml sterile saline up to 10^{-4} or 10^{-5} based on working suspension count. Mark each tube for its dilution factor and organism.

4.5 Dispense 1 ml from 10^{-4} and 10^{-5} dilution in petriplate in duplicates. Add to petriplate about 15 to 20 ml of liquefied soyabean casein digest agar medium [SCDA] for cultivation of bacteria and incubate at 32.5 ± 2.5 °C for 48 to 72 hours for bacteria.

4.6 Similarly add to other petriplate about 15 to 20 ml of liquefied Sabouraud dextrose agar [SDA] for cultivation of yeast / fungi and incubate *Candida.albicans* at 22.5 ± 2.5 °C for 48-72 hours and *A.niger* at 22.5 ± 2.5 °C for 5 days. Record the initial count in the worksheet as per Annexure-II

5.0 Sample Withdrawal and counting:

5.1 Remove one vial from each set from storage on 14th day, and 28th day for bacterial count and fungal count. Count the no of colony forming units as given in Point no.4.0.

5.2 Prepare dilutions on the basis on expected reduction in count.

5.3 Calculate the concentration of viable microorganism for 14th day and 28th day by multiplying the average count with reciprocal of dilution.

6.0 Calculation :

6.1 Using the calculated concentrations of CFU/ml present at the start of the test (zero hour), calculate the change in log 10 values of the concentration of cfu per ml for each microorganism at the applicable test intervals, and express the change in terms of log reduction as given below:

Log reduction = Log of Initial Concentration of cfu/ml – Log of concentration of cfu/ml at test interval

6.2 Record the log reduction for each withdrawl in the worksheet as per Annexure-II.

7.0 Acceptance Criteria:

Following are the minimum log reduction in counts to be obtained.

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Efficacy of Antimicrobial Preservation	**Effective Date:**
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Organism	**Minimum Log Reduction**	
	14 Day	**28th Day**
Bacteria	2 log	No Increase
Fungi	No Increase	No Increase

ANNEXURE(S):

Annexure I : Antimicrobial Efficacy Test Log Book.

Annexure II : Worksheet for Antimicrobial Efficacy Test.

Annexure III : Analytical Test Report for Antimicrobial Efficacy Test.

REFERENCES:

United States Pharmacopoeia.