



EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

A) Purpose of the experiment:

To validate the test procedure for Effectiveness of Antimicrobial Preservatives of

B) Name of the method or test to be validated

Effectiveness of Antimicrobial Preservatives of _____ as per IP.

C) Experimental design of the study (Perform three independent replicates)

1) Cultures to be used –

Escherichia coli ATCC 8739,

Pseudomonas aeruginosa ATCC 9027,

Staphylococcus aureus ATCC 6538,

Candida albicans ATCC 10231,

Aspergillus niger ATCC 16404,

Zygosaccharomyces rouxii NCYC 381 (for preparations having a high sugar concentration).

The bacterial cultures are inoculated on the surface of Soyabean caesin digest agar and Sabouraud's dextrose agar without antibiotics for fungal cultures for the appropriate period and temperature as specified in the pharmacopoeia.

The bacterial and yeast cultures are suspended in a diluent comprising of 0.9% NaCl and for *A.niger*, 0.9% NaCl and 0.05% Tween 80. Dilute the suspensions cultures with the same diluting fluid and determine the viable count using a 1:10 serial dilution and pour plate method.

2) Preparation of sample:

For each culture 20 g or 20 ml of the product is dispensed into a sterile screw capped tubes.

3) Test group: (Neutralised product with Inoculum)

20 g or 20 ml of the product is inoculated with 0.1ml of each culture suspension containing approximately 1×10^8 cfu/ml, Mix well and dilute 1 ml with 9ml of sterile 0.05 % soyalecithin and 0.9 % NaCl & 0.1 % peptone for bacterial cultures i.e E. Coli, Ps. aeruginosa and S. aureus & yeast cultures i.e C. albicans and Z.rouxii. For *Aspergillus niger* use 9 ml sterile 0.05 % Soyalecithin & 0.9 % NaCl + 0.05 % Tween 80 + 0.1 % peptone . Carry out serial dilutions. (1 :10) upto 10^{-6} and plate out 1ml in duplicate for all the cultures. Add sterile Soyabean casein digest agar for bacterial cultures, Sabourauds dextrose agar with chloramphenicol for fungal cultures. i.e Asp. niger and C. albicans and Sabourauds dextrose agar with Chloramphenicol & 20 % dextrose for *Zygosaccharomyces rouxii*.

Peptone control group (Challenge inoculum control in buffered solution):

20 ml of sterile 0.1% peptone is inoculated with 0.1 ml of each culture suspension containing approximately 1×10^8 cfu/ml, Mix well and dilute 1 ml with 9ml of sterile 0.05% soyalecithin and 0.9 % NaCl & 0.1% peptone. Carry out serial dilutions till 10^{-6} and plate out in duplicate for all the cultures. Add suitable medium as given in point no. 3.



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Viability Group: (Inoculum in absence of product or Neutralizer)

20 ml of sterile 0.1 % peptone is inoculated with 0.1 ml of each culture suspension containing approximately 1×10^8 cfu/ml, Mix well and dilute 1 ml with 9ml of sterile and 0.9 % NaCl & 0.1 % peptone. Carry out serial dilutions till 10^{-6} and plate out in duplicate for all the cultures. Add suitable medium as given in point no. 3

D] Acceptance criteria:

There should be similar recovery between the test group and the peptone group to demonstrate adequate neutralizer efficacy; similar recovery between peptone group and the viability group to demonstrate adequate neutralizer toxicity; and each replicate of the experiment should demonstrate that the average number of cfu recovered from the challenge product is not less than 70% of that recovered from the control inoculum.

E] Finding & Observation of the study:

F] Remarks / Conclusions of the study:

G] Validation Experiments conducted by:

Laboratory Head:

Date _____ :



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OBSERVATION SHEET FOR VALIDATION OF PRESERVATIVE EFFICACY

Product :

Batch No :

A. R. No :

1] **Test Culture:**

Volume of Culture Inoculum:

Quantity of sample inoculated: _____

Quantity of 0.1% of peptone Inoculated (Control): _____

Dilution	No. of Organisms		
	Test Group (cfu/ml)	Peptone Control Group (Cfu/ml)	Viability Group (cfu/ml)
10 ⁻¹			
10 ⁻²			
10 ⁻³			
10 ⁻⁴			
10 ⁻⁵			
10 ⁻⁶			

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$$\begin{aligned} \text{\% Recovery for Peptone} & \quad \text{Peptone control group (cfu/ml)} \\ \text{control group} & = \frac{\text{-----}}{\text{Viability Group (cfu/ml)}} \times 100 \\ \text{(Neutralizer Toxicity)} & \\ & = \text{-----} \times \text{-----} \\ & = \text{-----} \% \end{aligned}$$

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$$\begin{aligned} \text{\% Recovery for Peptone control group} &= \frac{\text{Peptone control group (cfu/ml)}}{\text{Viability Group (cfu/ml)}} \times 100 \\ &= \underline{\hspace{2cm}} \times \underline{\hspace{2cm}} \\ &= \underline{\hspace{2cm}} \% \end{aligned}$$

DETAILS OF MEDIA USED:

- 0.9 % NaCl + 0.1 % peptone (Load No : _____)
- 0.9 % NaCl + 0.05 % Tween 80 (Load No : _____)
- 0.9 % NaCl + 0.05 % Soyalecithin + 0.1% peptone (Load No : _____)
- 0.9 % NaCl + 0.05 % Soyalecithin + 0.05 % Tween 80 + 0.1% peptone (Load No. : _____)
- 0.1 % Peptone (Load No. : _____)
- Soyabean Casein digest agar – slant (Load No : _____)
- Sabouraud dextrose agar (Load No. _____)
- Sabouraud dextrose agar + 20 % Dextrose (Load No. : _____)
- Soyabean Casein digest agar (Load No : _____)
- Sabouraud dextrose agar + chloramphenicol (Load No. : _____)
- Sabouraud dextrose agar +chloramphenicol + 20 % Dextrose (Load No. : _____)

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DETAILS OF MEDIA USED:

- 0.9 % NaCl + 0.1 % peptone (Load No : _____)
- 0.9 % NaCl + 0.05 % Tween 80 (Load No : _____)
- 0.9 % NaCl + 0.05 % Soyalecithin + 0.1% peptone (Load No : _____)
- 0.9 % NaCl + 0.05 % Soyalecithin + 0.05 % Tween 80 + 0.1% peptone (Load No. : _____)
- 0.2 % Peptone (Load No. : _____)
- Soyabean Casein digest agar – slant (Load No : _____)
- Sabouraud dextrose agar (Load No. _____)
- Sabouraud dextrose agar + 20 % Dextrose (Load No. : _____)
- Soyabean Casein digest agar (Load No : _____)
- Sabouraud dextrose agar + chloramphenicol (Load No. : _____)
- Sabouraud dextrose agar +chloramphenicol + 20 % Dextrose (Load No. : _____)

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DETAILS OF MEDIA USED:

- 0.9 % NaCl + 0.1 % peptone (Load No : _____)
- 0.9 % NaCl + 0.05 % Tween 80 (Load No : _____)
- 0.9 % NaCl + 0.05 % Soyalecithin + 0.1% peptone (Load No : _____)
- 0.9 % NaCl + 0.05 % Soyalecithin + 0.05 % Tween 80 + 0.1% peptone (Load No. : _____)
- 0.3 % Peptone (Load No. : _____)
- Soyabean Casein digest agar – slant (Load No : _____)
- Sabouraud dextrose agar (Load No. _____)
- Sabouraud dextrose agar + 20 % Dextrose (Load No. : _____)
- Soyabean Casein digest agar (Load No : _____)
- Sabouraud dextrose agar + chloramphenicol (Load No. : _____)
- Sabouraud dextrose agar +chloramphenicol + 20 % Dextrose (Load No. : _____)

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DETAILS OF MEDIA USED:

- 0.9 % NaCl + 0.1 % peptone (Load No : _____)
- 0.9 % NaCl + 0.05 % Tween 80 (Load No : _____)
- 0.9 % NaCl + 0.05 % Soyalecithin + 0.1% peptone (Load No : _____)
- 0.9 % NaCl + 0.05 % Soyalecithin + 0.05 % Tween 80 + 0.1% peptone (Load No. : _____)
- 0.4 % Peptone (Load No. : _____)
- Soyabean Casein digest agar – slant (Load No : _____)
- Sabouraud dextrose agar (Load No. _____)
- Sabouraud dextrose agar + 20 % Dextrose (Load No. : _____)
- Soyabean Casein digest agar (Load No : _____)
- Sabouraud dextrose agar + chloramphenicol (Load No. : _____)
- Sabouraud dextrose agar +chloramphenicol + 20 % Dextrose (Load No. : _____)

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DETAILS OF MEDIA USED:

- 0.9 % NaCl + 0.1 % peptone (Load No : _____)
- 0.9 % NaCl + 0.05 % Tween 80 (Load No : _____)
- 0.9 % NaCl + 0.05 % Soyalecithin + 0.1% peptone (Load No : _____)
- 0.9 % NaCl + 0.05 % Soyalecithin + 0.05 % Tween 80 + 0.1% peptone (Load No. : _____)
- 0.5 % Peptone (Load No. : _____)
- Soyabean Casein digest agar – slant (Load No : _____)
- Sabouraud dextrose agar (Load No. _____)
- Sabouraud dextrose agar + 20 % Dextrose (Load No. : _____)
- Soyabean Casein digest agar (Load No : _____)
- Sabouraud dextrose agar + chloramphenicol (Load No. : _____)
- Sabouraud dextrose agar +chloramphenicol + 20 % Dextrose (Load No. : _____)

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DETAILS OF MEDIA USED:

- 0.9 % NaCl + 0.1 % peptone (Load No : _____)
- 0.9 % NaCl + 0.05 % Tween 80 (Load No : _____)
- 0.9 % NaCl + 0.05 % Soyalecithin + 0.1% peptone (Load No : _____)
- 0.9 % NaCl + 0.05 % Soyalecithin + 0.05 % Tween 80 + 0.1% peptone (Load No. : _____)
- 0.6 % Peptone (Load No. : _____)
- Soyabean Casein digest agar – slant (Load No : _____)
- Sabouraud dextrose agar (Load No. _____)
- Sabouraud dextrose agar + 20 % Dextrose (Load No. : _____)
- Soyabean Casein digest agar (Load No : _____)
- Sabouraud dextrose agar + chloramphenicol (Load No. : _____)
- Sabouraud dextrose agar +chloramphenicol + 20 % Dextrose (Load No. : _____)

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