

MICROBIOLOGY DEPARTMENT

EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

A)	Purpose of the experiment:	
	To validate the test procedure for Effectiveness of Antimicrobial Preservative	es 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 Sabauraud'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 Inoculumn)

20 g or 20 ml of the product is inoculated with 0.1ml of each culture suspension containing approximately 1 x 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 x 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.



MICRORIOLOGY DEPARTMENT

EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

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

20 ml of sterile 0.1 % peptone is incoulated with 0.1 ml of each culture suspension containing approximately 1 x 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 toll 10^{-6} and plate out in duplicate for all the cultures. Add suitable medium as given in point no. 3

D] Acceptance criteria:

E] Finding & Observation of the study:

There should be similar recovery between the test group and the peptone group ton 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.

F] Remarks / Conclusions of the study:	
G] Validation Experiments conducted by:	
	Laboratory Head: Date :



MICROBIOLOGY DEPARTMENT

EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

Product:							
Batch No	Batch No :						
A. R. No	:						
1] Tes	t Culture:						
Vol	ume of Culture Inoculum:						
Qua	antity of sample inoculated:						
		lated (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							
			CHECKED BY: DATE :				



MICROBIOLOGY DEPARTMENT

EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

Product:		
Batch No :		
A. R. No :		
% Recovery for test group	Test group (cfu/ml) = x 100	
(Neutralizer efficacy)	Peptone control group (cfu/ml)	
	= x =	%
	Peptone control group (cfu/ml)	
C I	= x 100 Viability Group (cfu/ml)	
	=x	
	=%	
	CHI DAT	ECKED BY: EE :



MICROBIOLOGY DEPARTMENT

EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

Product :	
Batch No :	
A. R. No :	
% Recovery for Peptone	Peptone control group (cfu/ml) = x 100
control group	Viability Group (cfu/ml)
	= x
	= %
DETAILS OF MEDIA US	ED:
0.9 % NaCl + 0.05 % Soyale 0.9 % NaCl + 0.05 % Soyale 0.1 % Peptone (Load No. : _ Soyabean Casein digest agar Sabouraud dextrose agar (Lo Sabouraud dextrose agar + 2 Soyabean Casein digest agar Sabouraud dextrose agar + c	(Load No :
	CHECKED BY: DATE :



Product:

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

Batch No			
A. R. No	:		
1] Test	Culture:		
Volu	ume of Culture Inoculum:		
Qua	ntity of sample inoculated:		
Qua	antity of 0.1% of peptone Inocula	ated (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}			
•••••			
•••••			
			CHECKED BY: DATE :



MICROBIOLOGY DEPARTMENT

EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

Product:				
Batch No :				
A. R. No :				
% Recovery for test group (Neutralizer efficacy)		Test group (cfu/ml) Peptone control group (cfu/ml)	v 100	
(Neutranizer criteacy)		Peptone control group (cfu/ml)	· X 100	
	=	x	=	%
% Recovery for Peptone		Peptone control group (cfu/ml)	100	
(Neutralizer Toxicity)	=	Peptone control group (cfu/ml) x 100 Viability Group (cfu/ml)		
	=	x		
	=	%		
	•••••			
	• • • • • • • •			
	• • • • • • •	•••••	•••••	
			CHE(DATE	CKED BY:



MICROBIOLOGY DEPARTMENT

EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

Product :			
Batch No :			
A. R. No :			
% Recovery for Peptone control group	Peptone contro	ol group (cfu/ml)	100
control group	=Viability Grou	p (cfu/ml)	x 100
	=	_ X	
	=	_ %	
DETAILS OF MEDIA US	ED:		
0.9 % NaCl + 0.1 % peptone 0.9 % NaCl + 0.05 % Tween 0.9 % NaCl + 0.05 % Soyale 0.9 % NaCl + 0.05 % Soyale 0.2 % Peptone (Load No. : _ Soyabean Casein digest agar Sabouraud dextrose agar (Lo Sabouraud dextrose agar + 2 Soyabean Casein digest agar Sabouraud dextrose agar + co Sabouraud dextrose agar + co	ecithin + 0.1% peptone (ecithin + 0.05 % Tween) r - slant (Load No :) and No 20 % Dextrose (Load No r (Load No :)	(Load No:) 80 + 0.1% peptone (Loa) No.:)	ad No. :)
			CHECKED BY: DATE :



MICROBIOLOGY DEPARTMENT

EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

Product:			
Batch No			
A. R. No	:		
1] Test	Culture:		
Volu	ame of Culture Inoculum:		
Qua	ntity of sample inoculated:		
	ntity of 0.1% of peptone Inoculat		
Dilution	T C	No. of Organisms	V7 1 194 G
	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			
			CHECKED BY: DATE :



MICROBIOLOGY DEPARTMENT

EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

OBSERVATION SHEET FOR VALIDATION OF PRESERVATIVE EFFICACY

Product:				
Batch No :				
A. R. No :				
		Test group (cfu/ml)	x 100	
(realized efficacy)		Peptone control group (cfu/ml)	A 100	
	=	X	=_	%
% Recovery for Peptone		Peptone control group (cfu/ml)	100	
control group (Neutralizer Toxicity)	=	Viability Group (cfu/ml)	X 100	
	=	x		
	=	%		
			СНЕ	CKED BY:

DATE



MICROBIOLOGY DEPARTMENT

EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

Product :	
Batch No :	
A. R. No :	
% Recovery for Peptone	Peptone control group (cfu/ml) = x 100
control group	= x 100 Viability Group (cfu/ml)
	= x
	= %
DETAILS OF MEDIA US	ED:
0.9 % NaCl + 0.05 % Soyale 0.9 % NaCl + 0.05 % Soyale 0.3 % Peptone (Load No. : _ Soyabean Casein digest agar Sabouraud dextrose agar (Lo Sabouraud dextrose agar + 2 Soyabean Casein digest agar Sabouraud dextrose agar + c	e (Load No :
	CHECKED BY: DATE :



MICROBIOLOGY DEPARTMENT

EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

Product :			
Batch No	:		
A. R. No	:		
1] Te s	st Culture:		
Vo	lume of Culture Inoculum:		
Qu	antity of sample inoculated:		
Qua	antity of 0.1% of peptone Inoculate	ed (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			
			HECKED BY: ATE :



MICROBIOLOGY DEPARTMENT

EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

Product:				
Batch No :				
A. R. No :				
% Recovery for test group		Test group (cfu/ml) =		
(realizable criteties)		Peptone control group (cfu/ml)	A 100	
	=	X	=	%
% Recovery for Peptone				
control group (Neutralizer Toxicity)	=	Viability Group (cfu/ml)	x 100	
	=	x		
	=	%		
	•••••			
			CHEC	CKED BY:



MICROBIOLOGY DEPARTMENT

EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

Product :	
Batch No :	
A. R. No :	
% Recovery for Peptone control group	Peptone control group (cfu/ml) = x 100
0 1	Viability Group (cfu/ml)
	= x
	= %
DETAILS OF MEDIA US	SED:
0.9 % NaCl + 0.05 % Soyal 0.9 % NaCl + 0.05 % Soyal 0.4 % Peptone (Load No. : Soyabean Casein digest aga Sabouraud dextrose agar + Soyabean Casein digest aga Sabouraud dextrose agar +	e (Load No :) n 80 (Load No :) ecithin + 0.1% peptone (Load No :) ecithin + 0.05 % Tween 80 + 0.1% peptone (Load No. :) ar - slant (Load No :) oad No) 20 % Dextrose (Load No. :) ar (Load No :) chloramphenicol (Load No. :) chloramphenicol + 20 % Dextrose (Load No. :)
	CHECKED BY: DATE :



MICROBIOLOGY DEPARTMENT

EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

Product :			
Batch No	:		
A. R. No	:		
1] Te	st Culture :		
Vo	lume of Culture Inoculum:		
Qu	antity of sample inoculated:		
Qua	antity of 0.1% of peptone Inoculat	ed (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			
•••••		•••••	
•••••			
			CHECKED BY: DATE :



MICROBIOLOGY DEPARTMENT

EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

Product:				
Batch No :				
A. R. No :				
% Recovery for test group	=	Test group (cfu/ml)	v 100	
(reutralizer criteacy)	_	Peptone control group (cfu/ml)	X 100	
	=	x	=	%
% Recovery for Peptone		Peptone control group (cfu/ml)	100	
control group (Neutralizer Toxicity)	=	Viability Group (cfu/ml)	oup (cfu/ml)	
	=	X		
	=	%		
			••••••	
	•••••		•••••	
	•••••			
			CHEC DATE	KED BY:



MICROBIOLOGY DEPARTMENT

EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

Product :				
Batch No :				
A. R. No :				
	Pepton	Peptone control group (cfu/ml) = x 100 Viability Group (cfu/ml)		
control group	– Viabilit			
	=	x		
	=	%		
DETAILS OF MEDIA US	SED:			
0.9 % NaCl + 0.1 % pepton 0.9 % NaCl + 0.05 % Twee 0.9 % NaCl + 0.05 % Soyal 0.9 % NaCl + 0.05 % Soyal 0.5 % Peptone (Load No. : Soyabean Casein digest aga Sabouraud dextrose agar + Soyabean Casein digest aga Sabouraud dextrose agar + Soyabean Casein digest aga Sabouraud dextrose agar + Sabouraud dextrose agar +	lecithin + 0.1% polecithin + 0.05 % ar - slant (Load No	eptone (Load No : Tween 80 + 0.1% peptone) No :) Load No. :) (Load No. :)	(Load No. :))	
			CHECKED BY: DATE :	



Product:

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

Batch No	:		
A. R. No	:		
1] Te	st Culture:		
Vo	lume of Culture Inoculum:		
Qu	antity of sample inoculated:		
Qua	antity of 0.1% of peptone Inoculated	(Control):	
		· · · · · · · · · · · · · · · · · · ·	
Dilution		No. of Organisms	
2	Test Group	Peptone Control	Viability Group
10-1	(cfu/ml)	Group (Cfu/ml)	(cfu/ml)
10-2			
10 ⁻³			
10 ⁻⁴			
10 ⁻⁵			
10 ⁻⁶			
•••••			
•••••			•••••
			HECKED BY: ATE :



MICROBIOLOGY DEPARTMENT

EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

Product :				
Batch No :				
A. R. No :				
% Recovery for test group (Neutralizer efficacy)	=	Test group (cfu/ml)	x 100	
(Peptone control group (cfu/ml)		
	=	X	=	%
% Recovery for Peptone		Peptone control group (cfu/ml)	group (cfu/ml)	
control group (Neutralizer Toxicity)	=	Viability Group (cfu/ml)	x 100	
	=	X		
	=	%		
			CHEC DATE	CKED BY:



MICROBIOLOGY DEPARTMENT

EFFECTIVENESS OF ANTIMICROBIAL PRESERVATIVES

Product :	
Batch No :	
A. R. No :	
% Recovery for Peptone control group	Peptone control group (cfu/ml) = x 100
	Viability Group (cfu/ml)
	= x
	= %
DETAILS OF MEDIA US	SED:
0.9 % NaCl + 0.05 % Soya 0.9 % NaCl + 0.05 % Soya 0.6 % Peptone (Load No. : Soyabean Casein digest aga Sabouraud dextrose agar + Soyabean Casein digest aga Sabouraud dextrose agar +	ne (Load No :) en 80 (Load No :) lecithin + 0.1% peptone (Load No :) lecithin + 0.05 % Tween 80 + 0.1% peptone (Load No. :) ar - slant (Load No :) coad No) 20 % Dextrose (Load No. :) ar (Load No :) chloramphenicol (Load No. :) chloramphenicol + 20 % Dextrose (Load No. :)
	CHECKED BY: DATE :