PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

Title: Antimicrobial Effective Testing

SOP No.:		**Department:**	Microbiology
		Effective Date:	
Revision No.:	00	**Revision Date:**	
Supersede Revision No.:	Nil	**Page No.:**	1 of 5

1.0 Objective

To lay down a procedure for Antimicrobial Effectiveness Testing.

2.0 Scope

This Standard Operating Procedure is applicable for formulation plant.

3.0 Responsibility

Executive/ Officer-Microbiology : Shall be responsible for following the procedure for Antimicrobial Effectiveness Testing.

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

4.0 Abbreviations And Definitions

QC	:	Quality Control
SOP	:	Standard Operating Procedure
CFU	:	Colony Forming Units
ATCC	:	American Type Culture Collection

5.0 Procedure

5.1 Test Organisms

5.1.1 The following test organisms shall be used in the test.

5.1.1.1 *Escherichia coli* (ATCC No.8739)

5.1.1.2 *Pseudomonas aeruginosa* (ATCC No.9027)

5.1.1.3 *Staphylococcus aureus* (ATCC No.6538)

5.1.1.4 *Candida albicans* (ATCC No.10231)

5.1.1.5 *Aspergillus niger* (ATCC No.16404)

5.2 Preparation of Inoculum

5.2.1 From a recently grown stock culture of each of the test organism, subculture on the surface of respective media slants (2 each) as given in Table-1.

Table-1 Culture Conditions for Inoculum Preparation

Organism	Medium	Incubation Temperature	Inoculum Incubation Time	Microbial Recovery Incubation Time
Escherichia coli (ATCC No.8739)	Soyabean-Casein Digest Broth, Soyabean-Casein Digest Agar	32.5 ± 2.5°C	18 to 24 hours	3 to 5 days
Pseudomonas aeruginosa (ATCC No.9027)	Soyabean-Casein Digest Broth, Soyabean-Casein Digest Agar	32.5 ± 2.5°C	18 to 24 hours	3 to 5 days
Staphylococcus aureus (ATCC No.6538)	Soyabean-Casein Digest Broth, Soyabean-Casein Digest Agar	32.5 ± 2.5°C	18 to 24 hours	3 to 5 days
Candida albicans (ATCC No.10231)	Sabouraud Dextrose Agar, Sabouraud Dextrose Broth,	22.5 ± 2.5°C	44 to 52 hours	3 to 5 days
Aspergillus niger (ATCC No.16404)	Sabouraud Dextrose Agar, Sabouraud Dextrose Broth,	22.5 ± 2.5°C	6 to 10 days	3 to 7 days

5.2.2 Use about 9-10 ml of sterile normal saline (0.9 % w/v) for harvesting bacterial cultures and yeast cultures, use about 9-10 ml of sterile normal saline (0.9 % w/v) with 0.05 % w/v polysorbate-80 for harvesting *Aspergillus niger culture.*

5.2.3 Prepare inoculum of about $1x10^{8}$ CFU/ml as per 'Preparation of Standardised Cell Suspension'.

5.2.4 Prepare ten fold serial dilutions of each of the cultures using sterile normal saline (0.9 % w/v) up to 10^{-8}.

5.2.5 Determine the number of cfu / ml in each suspension by carrying out the viable count in duplicate for final inoculum, to confirm the initial cfu per ml.

5.2.6 Record all the observations in 'Inoculum Preparation Record' as Annexure-1.

5.2.7 This value serves to calibrate the size of inoculum used in the test.

5.2.8 Use the bacterial and yeast suspensions within 24 hours of harvest and fungal suspension within 7 days. Store in the refrigerator if not used within 2 hours.

5.3 **Test Procedure**

5.3.1 For testing purpose the products are divided into four categories as givn in Table-2.

Table -2 Compendial Product Categories.

Category	Product Description
1	Injections, other parenterals including emulsions, otic, sterile nasal products, and ophthalmic products made with aqueous bases or vehicles.
2	Topically used products made with aqueous bases or vehicles, nonsterile nasal products, and emulsions, including those applied to mucous membranes.
3	Oral products other than antacids made with aqueous bases or vehicles.
4	Antacids made with an aqueous base

5.3.2 The test shall be carried out either in the original container, if sufficient volume of product is available in each container and the product container can be entered aseptically (i.e. using a needle and syringe through an elastomeric rubber stopper), or in sterile, capped bacteriological containers of suitable size into which a sufficient volume (10 -20 ml) of product has been transferred.

5.3.3 Inoculate each original product container or sterile capped bacteriological tube with one of the standardised microbial suspensions using a ratio equivalent to 0.1 ml to 0.2 ml of inoculum suspension to 10-20 ml of product and mix thoroughly.

5.3.4 The final concentration of test organisms shall be between 1×10^5 to 1×10^6 micro-organisms per ml of product for products of category 1, 2, and 3.

5.3.5 For category 4 products the final concentration of the test preparation after inoculation is between 1×10^3 to 1×10^4 micro-organisms per ml of product

5.3.6 The initial concentration of viable micro-organisms in each test preparation is calculated on the basis of the count of micro-organisms determined in the inoculum.

5.3.7 Incubate the inoculated containers at 22.5 ± 2.5°C.

5.3.8 Withdraw the sample to determine the viable count at applicable intervals from the day of inoculation.

5.3.9 Prepare ten fold serial dilutions and determine the viable count by plate count method.

5.3.10 Transfer 1 ml of the selected dilution to plate and pour 15-20 ml of the respective medium as per Table-1. Incorporate suitable inactivator in the medium used for the plate count.

5.3.11 Incubate the plates for bacterial count at 32.5 ± 2.5°C for 3-5 days. Incubate the plates for yeast and fungal count at 22.5 ± 2.5°C for 3-5 days and for 3 to 7 days respectively as given in table-1.

5.3.12 Count the numbers of organisms per ml after completion of incubation period.

5.3.13 Record the initial count and counts obtained at different intervals to assess the efficacy of the antimicrobial preservatives. Using the calculated concentrations of cfu per ml present at the start of the test.

5.3.14 Calculate the change in $\log_{10}$ values of the concentration of cfu per ml for each micro-organism at the applicable test intervals, and express the changes in terms of log reduction.

5.3.15 Record all the observations in 'Data Sheet for Antimicrobial Effectiveness Test' as Annexure-2.

5.4 **Interpretation of Results**

5.4.1 The product meets with the requirement of Antimicrobial Effectiveness Test if the results meet the criteria given in Table 3.

Table – 3 Criteria for Tested Microorganisms

For Category 1 Products	
Bacteria :	Not less than 1.0 log reduction form the initial calculated count at 7 days, not less than 3.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days.
Yeast and Molds :	No increase from the initial calculated count at 7, 14, and 28 days.
For Category 2 Products	
Bacteria :	Not less than 2.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days.
Yeast and Molds :	No increase from the initial calculated count at 14 and 28 days.
For Category 3 Products	
Bacteria :	Not less than 1.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days.
Yeast and Molds :	No increase from the initial calculated count at 14 and 28 days.

For Category 4 Products	
Bacteria, Yeast, and Molds:	No increase from the initial calculated count at 14 and 28 days.

6.0 Forms and Records

6.1	Inoculum Preparation Record	:	Annexure-1
6.2	Data Sheet for Antimicrobial Effectiveness Test	:	Annexure-2

7.0 Distribution

7.1	Master Copy	:	Documentation Cell (Quality Assurance)
7.2	Controlled Copies	:	Quality Control, Quality Assurance

8.0 History

Date	Revision Number	Reason for Revision