PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Antimicrobial Effectiveness Testing	**Effective Date:**
Supersedes: Nil	**Review Date:**
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1.0 PURPOSE

To lay down the procedure to perform Antimicrobial effectiveness testing to be performed using standard culture.

2.0 SCOPE

It is applicable to the microbiology lab for Antimicrobial effectiveness testing.

3.0 RESPONSIBILITY

Microbiology personnel

4.0 PROCEDURE

4.1 Precaution taken during antimicrobial effectiveness testing.

4.1.1 Glassware to be used shall be sterilized.

4.1.2 Media to be used shall be pre incubated

4.1.3 Standard culture to be used shall not be more then 4 passage

4.2 Test organisms used.

Use cultures of the following microorganisms *Candida albicans* ATCC No. 10231, *Aspergillus niger* ATCC No. 16404, *Escherichia coli* ATCC No. 8739, *Pseudomonas aeruginosa* ATCC No. 9027, and *Staphylococcus aureus* ATCC No. 6538. The viable microorganisms used in the test must not be more than five passages removed from the original ATCC culture or any other equivalent cultures.

4.3 Preparation of innoculums

4.3.1 Prepare the inoculums as per the SOP No. SOP/QCM/016.

4.3.2 To harvest the bacterial and *Candida albicans* cultures, use sterile peptone saline, wash the surface growth, collecting it in a suitable glassware, and adding sufficient sterile peptone saline to obtain a microbial count of about 1×10^8 colony-forming units (CFU) per ml. To harvest the cells of

Aspergillus niger, use sterile peptone saline containing 0.05% of polysorbate 80, and add sufficient sterile peptone saline to obtain a count of about 1×10^8 CFU per ml.

4.3.3 Determine the number of CFU per ml in each suspension, using the conditions of media and microbial recovery incubation times listed in *Table below* to confirm the initial CFU per ml. This value serves to calibrate the size of inoculum used in the test. The bacterial and yeast suspensions are to be used within 24 hours of harvest, but the fungal preparation may be stored under refrigeration for up to 7 days.

4.3.4 Culture Conditions for Inoculum Preparation as below table

Organism	Suitable Medium	Incubation Temperature	Incubation Time	Microbial Recovery Incubation Time
Escherichia coli	SCM /SCA	32.5 ± 2.5° C	18 to 24 hours	3 to 5 days
Pseudomonas aeruginosa	SCM /SCA	32.5 ± 2.5° C	18 to 24 hours	3 to 5 days
Staphylococcus aureus	SCM /SCA	32.5 ± 2.5° C	18 to 24 hours	3 to 5 days
Candida albicans	SCM /SDA	22.5 ± 2.5° C	44 to 52 hours	3 to 5 days
Aspergillus niger	SCM /SDA	22.5 ± 2.5° C	6 to 10 days	3 to 7 days

4.4 Test Procedure

4.4.1 The test can be conducted either in five original containers if sufficient volume of product is available in each container or in five sterile capped bacteriological containers of suitable size into which a sufficient volume (Not less than) of product has been transferred.

4.4.2 Inoculate each container with one of the prepared and standardized inoculum, and mix. The volume of the suspension inoculum used is between 0.5% and 1.0% of the volume of the product.

4.4.3 The concentration of test microorganisms that is added to the product are such that the final concentration of the test preparation after inoculation is between 1×10^5 and 1×10^6 CFU per ml of the product.

4.4.4 The initial concentration of viable microorganisms in each test preparation is estimated based on the concentration of microorganisms in each of the standardized inoculum as determined by the plate-count method.

4.4.5 Incubate the inoculated containers at 22.5 ± 2.5°C. Sample each container at the appropriate intervals specified in point number 4.5. Record any changes observed in appearance at these intervals.

4.4.6 Determine by the plate-count procedure the number of CFU present in each test preparation for the applicable intervals

4.4.7 Incorporate an inactivator (neutralizer) of the specific antimicrobial in the plate count or in the appropriate dilution prepared for plating (if required).

4.4.8 Using the calculated concentrations of CFU per ml present at the start of the test, 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 changes in terms of log reductions.

4.5 Frequency and Acceptance criteria

As per EP:

Category of products	Case	Bacterial log reduction						Fungal log reduction		
		6 hrs	24 hrs	2 days	7 th day	14 th day	28 th day	7 th day	14 th day	28 th day
Parenteral and ophthalmic preparations	A	2	3	-	-	-	NR	2	-	NI
	B	-	1	-	3	-	NI	-	1	NI
Topical preparations	A	-	-	2	3	-	NI	-	2	NI
	B	-	-	-	-	3	NI	-	1	NI
Oral preparations	A	-	-	-	-	3	NI	-	1	NI

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As per USP:

Category	Bacteria	Yeast and Molds
Injections, other parenterals including emulsions, otic products, sterile nasal products, and ophthalmic products made with aqueous bases or vehicles	Not less than 1.0 log reduction from 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.	No increase from the initial calculated count at 7, 14, and 28 days.
Topically used products made with aqueous bases or vehicles, nonsterile nasal products, and emulsions, including those applied to mucous membranes.	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.	No increase from the initial calculated count at 14 and 28 days.
Oral products other than antacids, made with aqueous bases or vehicles.	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.	No increase from the initial calculated count at 14 and 28 days.
Antacids made with an aqueous base	No increase from the initial calculated count at 14 and 28 days.	

As per Harmonized criteria:

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Category of products	Case	Bacterial log reduction						Fungal log reduction		
		6 hrs	24 hrs	2 days	7th day	14th day	28th day	7th day	14th day	28th day
Parenteral and ophthalmic preparations	A	2	3	-	NI	NI	NR	2	NI	NI
	B	-	1	-	3	NI	NI	NI	1	NI
Topical preparations	A	-	-	2	3	NI	NI	-	2	NI
	B	-	-	-	-	3	NI	-	1	NI
Oral preparations	A	-	-	-	-	3	NI	-	1	NI
Antacids	-	-	-	-	-	NI	NI	-	NI	NI

NR: No recovery NI: No increase

A: Recommended efficacy expected

B: In justified cases where the A criteria cannot be attained, the B criteria must be satisfied.

5.0 ABBREVIATIONS AND DEFINITIONS

SOP Standard Operating Procedure
QCM Quality Control Microbiology
QAD Quality assurance Department
Rev. Revision
No. Number
ATCC American Type of Culture Collection
CFU Colony Forming Unit
SCM Soyabean Casein Digest Medium
SCA Soyabean Casein Digest Agar
SDA Sabouraud Dextrose Agar
°C Degree Centigrade
% Percentage

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8.0 REVISION LOG

Revision Number	Effective Date	Reason for Revision

6.0 REFERENCE DOCUMENTS

SOP: Procedure for Storage and Preparation of microbiological Culture media".

7.0 ANNEXURE/ATTACHMENTS

None