SOP: LFA 00071
SOP For Antimicrobial Effectiveness Testing

Alastair Sanderson

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1 Objective

To provide the proper procedure to follow for effective antimicrobial testing using standard culture

2 Scope

Applicable to Microbiology Lab

3 Responsibility

By Microbiologist

4 Accountability

Head of the Department

5 Procedure

5.1. Precautions to be observed during testing

5.1.1. Sterilized glasses should be used during testing.

5.1.2. It is important to pre-incubate any media that will be used.

5.1.3. Standard culture to be used should not be more than four passages.

5.2. Type of Organism to use

5.2.1. Choose the culture from the following microorganisms:

- Candida albicans ATCC No. 10231
- Aspergillus Niger ATCC No. 16404
- Escherichia coli ATCC No. 6538

The microorganism to be used should not be more than five passages from the original ATCC culture of any of the equivalent cultures.

5.3. Preparing the Inoculums

5.3.1. Follow the SOP of Preparation for Culture Suspension to prepare the inoculums.

5.3.2. In order to harvest the bacteria and Candida culture, use a sterile peptone saline. Wash the surface growth and collect in an appropriate glass container. Add enough sterile peptone saline in order to gather a microbial count of approximately 1×10^8 CFU/ml.

To harvest Aspergilus niger cells, use a sterile peptone saline with 0.05% polysorbate 80, adding enough sterile peptone saline to achieve a count of 1×10^8 CFU/ml.

5.3.3. Obtain the total number of CFU/ml for each suspension using media conditions and microbial recovery times indicated in the table below in order to confirm the initial CFU/ml. The value would help in calibrating the inoculum size used in the test. Bacterial and yeast suspensions to be used for testing should be used within 24 hours of the harvest; however fungal preparations can be stored for up to 7 days in the fridge.

5.4. Test Procedures

Table 1: Table for Culture Conditions for Inoculum Preparation:

Organism	Sutable Medium	Incubation Temperature	Incubation Time	Microbial Recovery Incubation Time
Escherichia coli	SCM / SCA	$32.5 \pm 2.5C$	10 to 24 hours	3 to 5 days
Pseudomonas aeruginosa	SCM / SCA	$32.5 \pm 2.5C$	10 to 24 hours	3 to 5 days
Staphylococcus aureus	SCM / SCA	$32.5 \pm 2.5C$	10 to 24 hours	3 to 5 days
Candida albicans	SCM / SCA	$22.5 \pm 2.5C$	44 to 52 hours	3 to 5 days
Aspergillus niger	SCM / SCA	$22.5 \pm 2.5C$	6 to 10 days	3 to 7 days

5.4.1. The testing can be performed in five containers if there is sufficient product volume available; however, if there is insufficient product, one can also use five sterile capped bacteriological containers where suitable volume can be transferred.

5.4.2. Place the prepared and standardized inoculum to each container and mix. The suspension inoculum volume used should be between 0.5% and 1% of the product?s volume.

5.4.3. The microorganism concentration volume is such that the final concentration of the test preparation after the inoculation phase should be between 1×10^5 and 1×10^6 CFU/ml of the product.

5.4.4. The initial concentration of viable microorganism is estimated based on the microorganism concentration volume added to each of the standardized inoculum as determined by the plate-count method.

5.4.5. Incubate the inoculated containers as $22.5 \pm 2.5^\circ$ C. Check the samples at certain intervals as specified in 5.5 and document any changes observed during the intervals.

5.4.6. Determine by using the plate-count method the total number of the CFU present in each of the intervals.

5.4.7. Add an in-activator or neutralizer of the specified antimicrobial to the

plate count or if required, to the appropriate dilution that is prepared for plating.

5.4.8. To calculate the changes, use the calculated concentration of the CFU/ml at present of the test, and calculate the change in log 10 value of the CFU/ml concentration for each of the microorganism at the specified test interval. Document the changes in the log reductions.

Table 2: Criteria for Frequency and Acceptance

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 opthalmic preperations	A	2	3	-	-	-	NR	2	-	NI
	B	-	1	-	3	-	NI	-	-	NI
Topical preperations	A	-	-	2	3	-	NI	-	-	NI
	B	-	-	-	-	3	NI	-	-	NI
Oral preperations	A	-	-	-	-	3	NI	-	-	NI

As Per USP:

CATEGORY	BACTERIA	YEAST/MOLDS
Injections • parenteral injections • emulsions • otic • sterile nasal products • ophthalmic products • made with aqueous bases/vehicles	• Not less than 1.0g reduction from the first calculated count at seven days • Not less than 3.0 log reduction from the first count at 14 days • No increase from the 14 days count at 28 days	• No increase from the first calculated count at 7, 14, and 28 days
• Topical products with aqueous base • Non-sterile nasal products • Emulsions including those applied to the mucous membrane	• Not less than 2.0 log reduction from the first count at 14 days • No increase from the 14 days count at 28 days	• No increase from the first calculated count at 14 and 28 days
• Oral products other than antacids (made with aqueous bases/vehicles)	• Not less than 1.0 log reduction from the first count at 14 days • No increase from the 14 days count at 28 days	• No increase from the first calculated count at 14 and 28 days
• Antacids (made with aqueous base)	• No increase from the first calculated count at 14 and 28 days	• No increase from the first calculated count at 14 and 28 days

As Per Harmonized Criteria

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 opthalmic preperations	A	2	3	-	-	-	NR	2	-	NI
	B	-	1	-	3	-	NI	-	-	NI
Topical preperations	A	-	-	2	3	-	NI	-	-	NI
	B	-	-	-	-	3	NI	-	-	NI
Oral preperations	A	-	-	-	-	3	NI	-	-	NI
Antacids	-	-	-	-	-	NI	NI	-	NI	NI

6 Abbreviations

SOP Standard Operating Procedure

ATCC American Type of Culture Collection

CFU Colony Forming Unit

SCM Soybean Casein Digest Medium

SCA Soybean Casein Digest Agar

SDA Sabouraud Dextrose Agar

$^{\circ}$ Degree Centrigrade

% Percentage

EP European Pharmacopoeia

USP United State Pharmacopoeia