



## STANDARD OPERATING PROCEDURE

<b>Department:</b> Microbiology	<b>SOP No.:</b>
<b>Title:</b> Testing of Disinfectant Solutions	<b>Effective Date:</b>
<b>Supersedes:</b> Nil	<b>Review Date:</b>
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### 1.0 OBJECTIVE:

To lay down the procedure for determination of Antibacterial and Antifungal activity of disinfectant and surface challenge test of disinfectant solutions.

### 2.0 SCOPE:

This SOP is applicable to methods for testing disinfectant solutions used in microbiology laboratory and production area.

### 3.0 RESPONSIBILITY:

Microbiologist – Quality Control

Head – Quality Control

### 4.0 PROCEDURE:

#### 4.1 Antibacterial and Antifungal Efficacy Test:

4.1.1 Use the following cultures: Escherichia coli ATCC 8739, Pseudomonas aeruginosa ATCC 9027, Staphylococcus aureus ATCC 6538, Bacillus subtilis ATCC 6633, Candida albicans ATCC 10231 Aspergillus niger ATCC 16404 and two common isolates of environment. Make serial dilution of above culture and note down in Annexure-I

4.1.2 Prepare stock solution of above-mentioned cultures as per the SOP to obtain a concentration of  $10^7$  to  $10^8$  organisms per ml and note down the results in Annexure-II.

4.1.3 Prepare appropriate quantity of the required concentration of disinfectant solution in sterile water.

4.1.4 All media used shall be prepared from dehydrated media using purified water. These media should be tested for growth promotion test before use.

4.1.5 All experiments to be conducted under laminar air flow bench and media plates are to be incubated in incubators.

4.1.6 For Sample control, prepare a 20 ml of particular concentration of desired disinfectant under examination in sterile water in each test tube for each organism. Add 0.1 ml of each culture having  $10^7$  to  $10^8$ cfu of organism to the tube. Allow it to stand for 5 minutes and immediately



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after completion of 5 minutes, Perform the ten fold serial dilutions in Dey Engleys broth as neutralizer and determine the count by plating out 1 ml of sample in duplicate from each dilution and add 15-20 ml of the Soyabean casein digest agar for bacteria and Sabouraud's dextrose agar for fungi incubate at 30-35°C for bacteria and 20-25°C for fungi for 5 days.

- 4.1.7 Repeat the same procedure described above for the 10 minutes and 15 minutes time interval.
- 4.1.8 Same procedure mentioned above should be followed for remaining disinfectant.
- 4.1.9 In the same way perform the efficacy of hand disinfectant at interval of 30 seconds, 1 minute & 2 minute.
- 4.1.10 For positive control test, prepare the 20ml of sterile saline in sterile test tubes. Add 0.1ml of each culture having  $10^7$  to  $10^8$  cfu of organism to the tube so as to get a final concentration after dilution between  $10^5$  to  $10^6$  and Perform the ten fold serial dilutions in Dey Engleys broth as neutralizer and determine the count by plating out 1 ml of each dilution in duplicate and add 15-20 ml of the Soyabean casein digest agar for bacteria and Sabouraud's dextrose agar for fungi incubate at 30-35°C for bacteria and 20-25°C for fungi for 5 days.
- 4.1.11 Calculate the log of initial count and the final count.  
Subtract the log of final count from initial to get log reduction.
- 4.1.12 For negative control, plate out 1ml sterile saline in duplicate and add 15-20ml of Soyabean casein digest agar for bacterial enumeration (If any) and 15-20ml of Sabouraud's dextrose agar for fungal enumeration (If any). Incubate at 20-25°C for fungi and 30-35°C for bacterial and incubate for 5 days.

**4.2 Frequency:** Yearly

#### **4.3 SURFACE CHALLENGE TEST:**

- 4.3.1 In practice, sufficient organisms need to be inoculated on a 2-inch x 2- inch square of the surface to be decontaminated e.g. a coupon to demonstrate at least 2 log reduction for the bacterial spores and 3-log reduction for the vegetative Bacterial and fungal cells.
- 4.3.2 Use the template of above mentioned size for the different materials that are used in manufacturing areas like Epoxy coated template, template of Glass, S.S. 304 and S.S. 316 etc.



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- 4.3.3 The titer of organisms to be used for the test should be  $10^5$  to  $10^6$  organisms/ ml. Use the following cultures: Escherichia coli ATCC 8739, Pseudomonas aeruginosa ATCC 9027, Staphylococcus aureus ATCC 6538, Bacillus subtilis ATCC 6633, Candida albicans ATCC 10231 Aspergillus niger ATCC 16404 and common isolates of environment.
- 4.3.4 Clean and dry the template. Pour 0.1ml of the culture on the surface of the template and add disinfectant under examination for 5 minutes, 10 minutes and 15 minutes.
- 4.3.5 Allow the template to dry under LAF taking care that culture shouldn't be desiccated. After sufficient contact time swab the surface with cotton swab and dip it into 9ml of Dey Engleys broth and perform the ten fold serial dilution up to  $10^6$  with Dey Engleys broth.
- 4.3.6 Perform the same procedure with respect to all cultures.
- 4.3.7 Plate out 1ml of each dilution in duplicate. For bacterial cultures plate out on 15-20ml of Soyabean casein digest agar and for the fungal cultures on 15-20 ml of Sabouraud's dextrose agar. Incubate the plates of SCDA at 30-35<sup>0</sup>C for 5 days and that of SDA at 20-25<sup>0</sup>C for 5 days.
- 4.3.8 For positive control test, Clean and dry the template. Pour 0.1ml of the culture on the surface of the template and allow reacting with the surface to 5 minutes, 10 minutes and 15 minutes with out disinfectant under examination.
- 4.3.9 Allow the template to dry under LAF taking care that culture shouldn't be desiccated. After contact time as that of the test, swab the surface with cotton swab and dip it into 9 ml of Dey Engleys broth and perform the ten fold serial dilution up to  $10^6$  with Dey Engleys broth.
- 4.3.10 Plate out 1ml of each dilution in duplicate. For bacterial cultures plate out on the 15-20 ml of Soyabean casein digest agar and for the fungal cultures on the 15-20 ml of Sabouraud's dextrose agar. Incubate the plates of SCDA at 30-35<sup>0</sup>C for 5 days and that of SDA at 20-25<sup>0</sup>C for 5 days.
- 4.3.11 Calculate the log reduction and evaluate the effect exerted by the respective surfaces on the particular concentration of cultures.
- 4.4 Frequency:** Yearly
- 4.5** Allocation of protocol of disinfectant efficacy test as per SOP.



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**5.0 ANNEXURE (S):**

Annexure – I: Serial dilution of microorganism.

Annexure–II: Determination of antibacterial and antifungal efficacy of disinfectant solution

Annexure –III: Surface challenge test for Disinfectant solution

**6.0 REFERENCE (S):**

SOP: Maintenance of microbial cultures and Microbial culture dilution.

SOP: Numbering of validation/Qualification documents in Microbiology Section

SOP: Preparation, Approval, Distribution control, revision and Destruction of Standard operating Procedure (SOP).

**7.0 ABBREVIATION (S) / DEFINITION (S):**

LAF : Laminar air flow

AC : Air conditioning

<sup>0</sup> C : Degree centigrade

% : Percentage

SCDA: Soya bean casein digest agar

SDA: Sabouraud dextrose agar.

**REVISION CARD**

S. No.	REVISION No.	REVISION DATE	DETAILS OF REVISION	REASON (S) FOR REVISION	REFERENCE CHANGE CONTROL No.
01	00	--	----	New SOP	----





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**Annexure 2**

**DETERMINATION OF ANTIBACTERIAL AND ANTIFUNGAL EFFICACY OF DISINFECTANT SOLUTION**

**ITEM:** \_\_\_\_\_ **A.R. No. :** \_\_\_\_\_

**BATCH No.:** \_\_\_\_\_

Concentration of the Disinfectant: \_\_\_\_\_

Quantity of Disinfectant inoculated: \_\_\_\_\_ ml

Volume of culture Inoculum: \_\_\_\_\_ ml

Quantity of sterile saline inoculated (control): \_\_\_\_\_ ml

Final culture concentration in the tube: \_\_\_\_\_

Cultures Used	Dilutions / Test Count					
	$10^{-1}$	$10^{-2}$	$10^{-3}$	$10^{-4}$	$10^{-5}$	$10^{-6}$
<b>Escherichia coli ATCC 8739</b>						
Positive Control						
Time (secs/ Mins)						
<b>Pseudomonas aeruginosa ATCC 9027</b>						
Positive Control						
Time (secs/ Mins)						
<b>Staphylococcus aureus ATCC 6538</b>						
Positive Control						
Time (secs/ Mins)						
<b>Bacillus subtilis ATCC 6633</b>						
Positive Control						
Time (secs/ Mins)						

**Tested By:**  
**Date :**

**Checked By:**  
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**ITEM** : \_\_\_\_\_

**REPORT No./A.R. No.:** \_\_\_\_\_

Cultures Used	Dilutions / Test Count					
	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>
Candida albicans ATCC 10231						
Positive Control						
Time (secs/ mins)						
Aspergillus niger ATCC 16404						
Positive Control						
Time (secs/ mins)						
In-house Isolate						
Positive Control						
Time (secs/ mins)						
Inhouse Isolate						
Positive Control						
Time (secs/ mins)						

**Tested By:**  
**Date** :

**Checked By:**  
**Date** :



**PHARMA DEVILS**  
MICROBIOLOGY DEPARTMENT

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**ITEM :**

**REPORT NO./A.R. No.:** \_\_\_\_\_

**Calculations:**

Test Organism	Time (secs / mins)	Observed count	Log of observed count (a)	Control count	Log of control count (b)	Log reduction (b - a)	Sign and date
Escherichia coli ATCC 8739							
Pseudomonas aeruginosa ATCC 9027							
Staphylococcus aureus ATCC 6538							
Bacillus subtilis ATCC 6633							
Candida albicans ATCC 10231							
Aspergillus niger ATCC 16404							
In-house Isolate							
In-house Isolate							

**Tested By :**  
**Date :**

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**Date :**





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**ITEM :**

**REPORT NO./A.R. No.:** \_\_\_\_\_

**Media Details:**

Media Used	Soyabean Casein Digest agar	Sabourauds dextrose agar
Load No.		
Incubator No.		
Incubation Started at Date / Sign		
Incubation Completed at Date / Sign		

Diluent Used	Load No.	Date / Sign
Dey Engley broth		

**ACCEPTANCE CRITERIA:**

The disinfectant solution must demonstrate atleast 2 log reduction (for bacterial spores) and 3 log reduction (for fungi and vegetative bacteria) in the viable count of the test organisms used within 30 seconds for hand disinfectants and within 10 minutes for other disinfectants.

**CONCLUSION:**

**Tested By:**  
**Date :**

**Checked By :**  
**Date :**



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**Annexure 3**

**SURFACE CHALLENGE TEST FOR DISINFECTANT SOLUTION**

**ITEM :**

**BATCH NO. :**

**A.R. No. :**

Description of Surface Material:

Concentration of Disinfectant:

Method of Swab:

Time of testing:

Test Organism	Observed Count	Log of observed count (a)	Control count	Log of control count (b)	Log reduction (b-a)	Sign and Date
E.coli ATCC 8739						
S. aureus ATCC 6538						
P. aeruginosa ATCC 9027						
Bacillus subtilis ATCC 19659						
C. albicans ATCC 10231						
Aspergillus niger ATCC 16404						
In house Isolate						
In house Isolate						

**Tested By:**  
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**ITEM :**

**REPORT NO./A.R. NO.:**

**Media Details:**

<b>Media Used</b>	<b>Soyabean Casein Digest agar</b>	<b>Sabourauds Chloramphenicol agar</b>
Load No.		
Incubator No.		
Incubation Started at Date / Sign		
Incubation Completed at Date / Sign		

<b>Diluent Used</b>	<b>Load No.</b>	<b>Date / Sign</b>
Dey Engley broth		

**ACCEPTANCE CRITERIA:**

The disinfectant solution must demonstrate atleast 2 log reduction (for bacterial spores) and 3 log reduction (for fungi and vegetative bacteria).

**CONCLUSION:**

**Tested By :**  
**Date :**

**Checked By :**  
**Date :**