PROTOCOL

OF

DISINFECTANT VALIDATION

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ACME Formulation Pvt Ltd.,

Village Chaukiwala,

Nalagarh, District-Solan

Himachal Pradesh

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1.0 APPROVAL

Signing this protocol approval section is agreement with the tests, methods, relevant SOPs and documentation defined in this document. During the execution of this protocol changes may be encountered. Changes are allowed only if accompanied by an approved change or protocol amendment. All approved change and protocol amendments shall be included with this protocol prior to the final approval of the report.

Prepared By	**Name**	**Signature**	**Date**
Officer/Executive (Quality Assurance)			

Checked By	**Name**	**Signature**	**Date**
Head (Production)			
Head (Quality Control)			

Approved By	Name	**Signature**	**Date**
Head (Quality Assurance)			

2.0 OVERVIEW:

2.1 OBJECTIVE:

Objective of this study is to perform validation of disinfectant solution to be used for the cleaning of walls, floor, glass and outer surface of equipment to test the microbial residue with effective swabbing procedure, and to set acceptance limit for the routine monitoring. This study is based on evaluation of microbial recovery test and to ensure the effectiveness of various disinfectants used in sanitisation of lab & working area to maintain the better environment thereby the quality of the products can be assured.

2.2 SCOPE & PURPOSE:

The scope of this validation exercise is applicable for testing of disinfectants before releasing it for routine usage and to check the bio-burden level in disinfectant used in General Block, at Acme Formulation Private Limited, Nalagarh.

This validation covers all disinfectants, which are employed in area for sanitization. Validation study includes the evaluation of effectiveness of these disinfectants against the test Micro-organisms as well as their effectiveness in Laboratory and manufacturing area.

Purpose of validation is to provide a data for individual disinfectant for its potency, effective concentration, contact time & usefulness so as to include / continuation of these in routine sanitization practices.

3.0 RESPONSIBILITY:

3.1 Quality Control:

- To verify the growth Promotion properties and sterility of prepared media.
- To prepare the disinfectant and to test the same at different concentrations
- To prepare the culture suspension and enumerate the same.
- To verify recovery results.

3.2 Quality Assurance:

- To plan the validation study in coordination with Microbiology and Production Department.
- To prepare the protocol and report of the study.
- To verify and conclude the results.

3.3 Production Department:

❖ To provide necessary inputs for planning and conducting validation study.

4.0 Validation Matrix:

Sr. No.	Validation Criteria	Re-Validation Frequency
1.	Any disinfectant to be used in the facility of Acme Formulation	Once in a year

2.	Whenever there is change in the composition of disinfectant	
3	If there is change in method of testing	

5.0 REQUIREMENTS :

5.0 Sterile petridish (90 mm)

5.1 Culture Suspension

5.2 Micropipette

5.3 Sterile Micropipette Tips

5.4 Laminar Air Flow

5.5 BOD Incubators (30-35°C & 20-25°C)

5.6 Molten sterile Soyabean casein digest agar and Sabouraud Dextrose Agar

5.7 Sterile cotton swabs

5.8 Sterile normal saline (0.9 % NaCl in distilled water)

5.9 Floor tiles (Cota Stone) of the type similar to that used for manufacturing facility in General Block

5.10 Epoxy and Glass in Microbiology Area.

5.11 List of Disinfectant to be validated as per Annexure-I.

5.12 List of Equipments for sampling as per Annexure-II.

6.0 DETAIL METHODOLOGY (PROCEDURE)

6.1 Suspension Method:

6.1.1 To establish the test concentration and the contact time, suspension test is generally applied the suspension test estimate the in vitro bactericidal activity of the disinfectant Under precise experimental conditions including:

6.1.2 **Surfaces to be tested:**

a) Epoxy

b) Glass

c) Floor Tiles

d) Stainless Steel

e) Walls

• **Microbial strain**

Staphylococcus aureus	*ATCC 6538*
Pseudomonas aeruginosa	*ATCC 9027*
Bacillus subtilis	*ATCC 6633*
Candida albicans	*ATCC 10231*
Aspergillus niger	*ATCC 16404*

6.1.3 Procedure:

6.1.3.1 Prepare the culture suspension from the original culture as per the SOP for preparation of Microbiological culture media (SOP/QC/073)

6.1.3.2 Select the dilution which will yield 10^5 to 10^6 cells per ml.

6.1.3.3 Prepare 10 ml of test dilution to be tested with sterile Peptone water.

6.1.3.4 Vortex the tube for 1 minute atleast.

6.1.3.5 Add 0.1 to 1 ml of any one culture suspension containing 10^5 to 10^6 cells per ml into the test disinfectant with the decided concentration.

6.1.3.6 The final concentration shall be 10^4 to 10^5 cells per the tube. Consider the preparation for 0 Minutes.

6.1.3.7 Prepare like the same above for other 3 different concentrations for different time intervals.

6.1.3.8 Give a contact time of 0 min, 5 min & 10 min for each dilution and each concentration.

6.1.3.9 After the specified contact time, pipette out 1 ml of the specific solution and pour into the Petri plates.

6.1.3.10 Pour the Pre sterilized media SCDA for bacteria and SDA for fungus in particular petri plates.

6.1.4 Recovery:

6.1.4.1 Incubate the bacterial culture at 30-35 °C for 24 to 48 hours and fungal cultures at 20-25 °C for 5 days.

6.1.4.2 Recovery shall be calculated from the Initial population before contact time and counts obtained after specific contact time at a particular concentration.

6.1.4.3 Note down the Number of colonies

6.1.4.4 Select the plates, which have least to Nil counts.

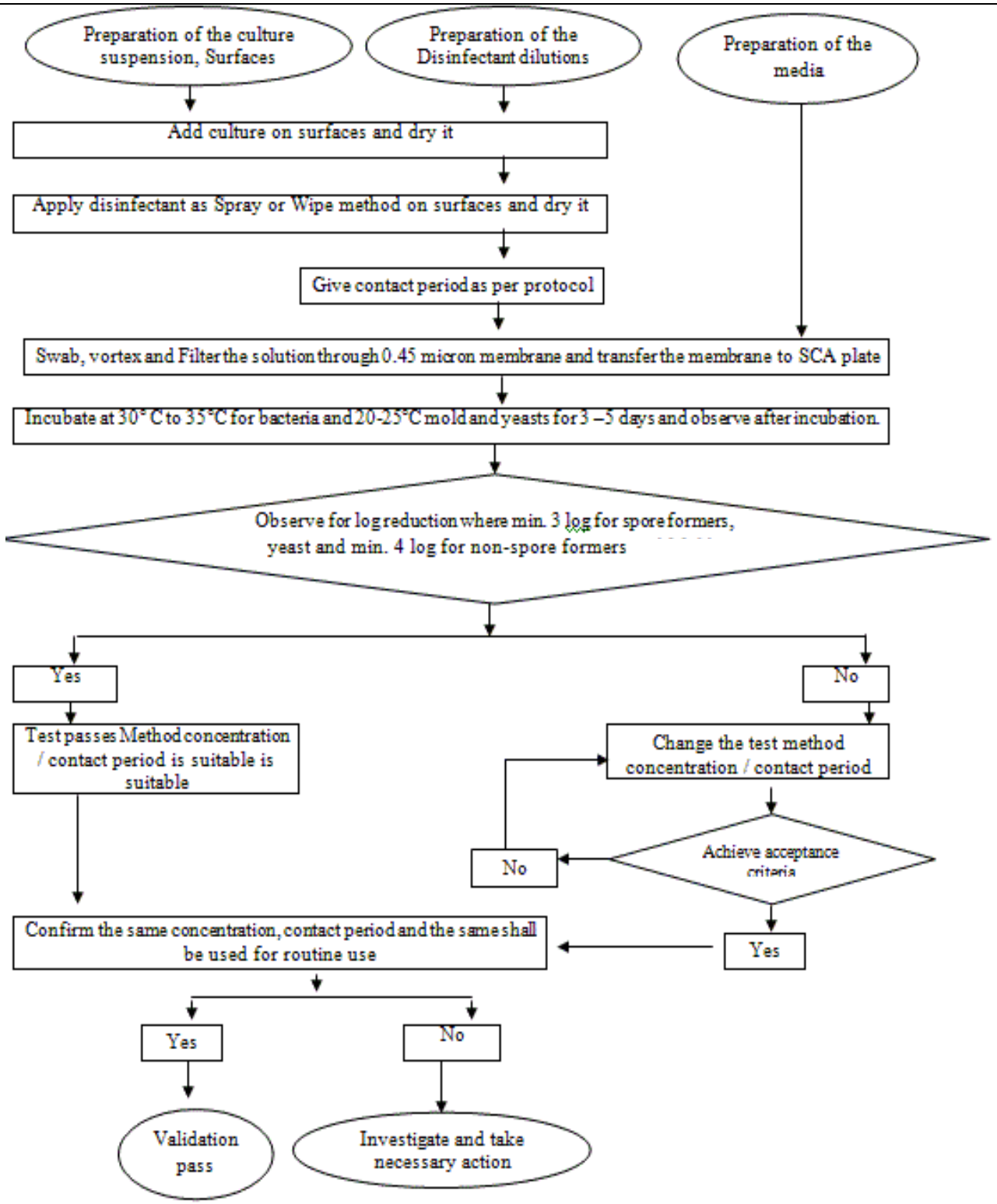
6.1.4.5 Proceed in the same manner taking all the cultures to be tested

6.1.4.6 Contact time for the usage of the disinfectant will be set on the basis of the results, which will have least counts.

6.1.4.7 Now calculate the log reduction of the Microorganism form Initial to Final Count

Log Reduction = Log value of initial challenged Concentration-Log value of Final cfu obtained after specific contact Time

TEST PROCESS FLOW CHART FOR SUSPENSION METHOD



6.1.5 ACCEPTANCE CRITERIA:

6.1.5.1 Surface-swabbing method must recover Not less than 70% of microbial population and should be reflected in forms countable CFU.

6.1.5.2 Decrease in bacterial load to the exposed disinfectant shall indicate that the disinfectant is Capable of reducing the contaminant when used in the area.

6.1.5.3 There shall be minimum 4 log reduction for non spore forming microorganisms, and 3 log reduction for spore forming bacteria yeast and mould. with decided concentration and specified contact time

6.2 Surface spray / Wipe method:

6.2.1 Objective:

To establish the effectiveness of the test concentration and the contact time generally applied. The suspension test estimates the in vitro bactericidal activity of the disinfectant under precise experimental conditions including.

• **Microbial strain:**

Staphylococcus aureus	*ATCC 6538*
Pseudomonas aeruginosa	*ATCC 9027*
Bacillus subtilis	*ATCC 6633*
Candida albicans	*ATCC 10231*
Aspergillus niger	*ATCC 16404*

6.2.2 Surfaces to be tested:

a) Epoxy

b) Glass

c) Floor Tiles

d) Stainless Steel

e) Walls

6.2.3 Procedure:

6.2.3.1 Prepare the culture suspension from the original culture as per the SOP for preparation of Microbiological culture media.

6.2.3.2 Select the dilution which will yield 10^4 to 10^5 cells per ml.

6.2.3.3 Take plate of different surfaces such as SS, Epoxy, Panel, present in the clean room Having a surface area of 25 cm^2.

6.2.3.4 From the previously determined suspension having 10^4 to 10^5 cells per ml inoculate one culture on Different surfaces mentioned above.

6.2.3.5 With the help of a sterile spatula spread the culture on the surface.

6.2.3.6 Keep it on the LAF bench for drying.

6.2.3.7 After the exposed duration for drying (a) spray the disinfectant (b) disinfect the surface by wipe Method.

6.2.3.8 Give a contact time of 0 min, 5 min, 10 min and 15 min.

6.2.3.9 The exact procedure for sanitation followed in the clean room should be followed.

6.2.3.10 With the help of a sterile moistened swab, swab the surface gently covering all the area of the Surface.

6.2.3.11 Place the swab sticks in a test tube having sterile saline solution.

6.2.3.12 Vortex the test tube gently for 1.0 minutes.

6.2.3.13 Now pour the plates for bacteria and fungus respectively with SCDA & SDA agar media.

6.2.3.14 Incubate the bacterial culture at 30-35 °C for 24 to 48 hours and fungal cultures at 20-25 °C for 72 to 120 hours.

6.2.3.15 After incubation count the number of colonies present on the membrane.

6.2.3.16 Proceed in the same manner taking all the cultures to be tested with the all mentioned Disinfectants.

6.2.4 Recovery:

6.2.4.1 Incubate the bacterial culture at 30-35 °C for 24 to 48 hours and fungal cultures at 20-25 °C for 5 days.

6.2.4.2 Recovery shall be calculated from the Initial population before contact time and counts obtained after specific contact time at a particular concentration.

6.2.4.3 Note down the Number of colonies

6.2.4.4 Select the plates, which have least to Nil counts.

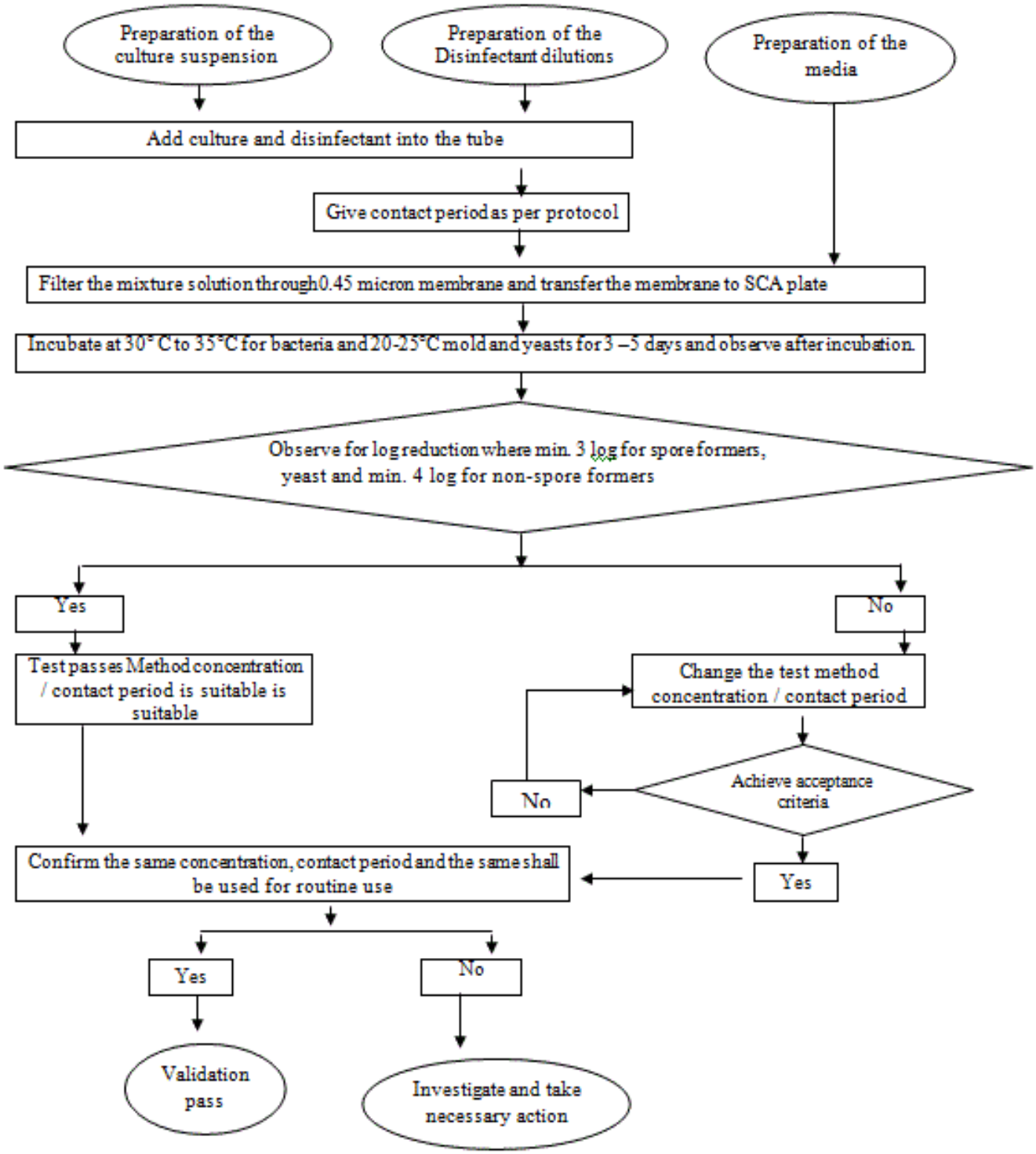
6.2.4.5 Proceed in the same manner taking all the cultures to be tested

6.2.4.6 Contact time for the usage of the disinfectant will be set on the basis of the results, which will have least counts.

6.2.4.7 Now calculate the log reduction of the Microorganism form Initial to Final Count

Log Reduction = Log value of initial challenged Concentration-Log value of Final cfu obtained after specific contact Time

TEST PROCESS FLOW CHART FOR SURFACE SPRAY/WIPE METHOD



6.2.5 Acceptance criteria:

6.2.5.1 The decrease in the bacterial load to the exposed disinfectant indicates that the disinfectant is capable of reducing the contaminant when used in the area. That shall be minimum of 4-log reduction for non-spore forming microorganisms, Yeast and minimum of 3-log reduction shall achieve for Spore forming organisms, molds with the decided concentration.

6.2.5.2 Determine the contact period where the above said population log reduction of microorganisms achieved.

- **Note**: if any new disinfectant is added in the schedule, then its validation report or observation is recorded and attached with the disinfectant validation report as an Annexure.

7.0 EVALUATION:

All the data generated during validation study reviewed in detail to conclude,

7.1 The effective swabbing method with achievement of minimum 70% recovery of microbial population,

8.0 CONCLUSION:

On the basis of evaluation of collected data, the conclusion of following parameters shall be drawn.

8.1 The effective swabbing method with effective swabbing time to attain 70% recovery of Microbial population.

9.0 DEVIATION AND CHANGE CONTROL:

If, any deviation is observed during execution of activity or deviate the procedure from approved protocol or any change control is observed, then the complete information is recorded in the deviation form as per the SOP No. SOP/HA/017 (Title: SOP on handling of deviation) or as per SOP No. SOP/HA/010 (Title: SOP on change control procedure).