Protocol for Validation of Disinfection Efficacy of 70% Isopropyl Alcohol

Name of the Process/ Method/Study	Protocol for validation of disinfection efficacy of 70% Isopropyl Alcohol
Name of the Product	70% Isopropyl Alcohol
Validation Study Location	
Area / Department	Quality Control
Protocol Effective Date	

Validation Started On	**Validation Completed On**
Validation Completed On	
Validation No. *	**Initial**

***Note:** For first time study mention “Initial” and for Re-Validation mention the serial number starting from “01”.

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1. TABLE OF CONTENTS:

S.No.	Details	Page No.
1.	Table of Contents	2 of 10
2.	Abbreviations	3 of 10
3.	Objective	3 of 10
4.	Scope	3 of 10
5.	Intended Use of Process/ Method	3 of 10
6.	Responsibility	3 of 10
7.	Introduction	4 of 10
8.	Documentation	4 of 10
9.	Process/ Method Description	4 of 10
10.	Training record for Validation	5 of 10
11.	Material required for Validation	5 of 10
12.	Validation Activity Plan	5 of 10
13.	Sampling Plan	8 of 10
14.	Acceptance Criteria	9 of 10
15.	Safety/ Precautions	9 of 10
16.	Re-Validation Criteria	9 of 10
17.	References	9 of 10
18.	Enclosures	9 of 10
19.	Protocol Approval	10 of 10

2. ABBREVIATIONS:

- IPA - Isopropyl Alcohol
- ID No. - Identification Number
- MVP - Master Validation Plan
- QA - Quality Assurance
- QC - Quality Control
- SOP - Standard Operating Procedure
- S. No. - Serial Number
- VG - Validation Group

3. OBJECTIVE:

Objective of this study is to validate the disinfection efficacy of the 70% Isopropyl Alcohol which is used as hand disinfectant as well as surface disinfectant in the facility.

4. SCOPE:

Scope of this protocol and study is limited up to validation of disinfection efficacy of 70% Isopropyl Alcohol.

5. INTENDED USE OF METHOD:

70% Isopropyl Alcohol is used as the hand disinfectant for disinfection of the hands while working in the manufacturing or microbiology area in order to avoid the carry over of the external contamination in the manufacturing facility. 70% IPA is also used as the surface disinfectant in clean area.

6. RESPONSIBILITY:

Department	Responsibility
Quality control (Microbiology)	To Execute the activity as per the protocol, compile the results and prepare the report.
Engineering	To provide the required utility/ maintenance support.
QA	To review the validation protocol, activity and report.

7. INTRODUCTION:

70% Isopropyl Alcohol is used as the hand disinfectant for disinfection of the hands while working in the manufacturing or microbiology area in order to avoid the carry over of the external contamination in the manufacturing facility. 70% IPA is also used as the surface disinfectant in clean area.

- Composition of 70% Isopropyl Alcohol
  Each 100 mL contains:

  2-propanol : 70 ml

  Purified water : 30 ml

8. DOCUMENTATION:

- All documentation work shall be completed concurrently during execution of the validation activity.
- Use indelible blue ink for the recording.
- Fill complete information in the format provided.
- Do not leave any blank space.
- Enter "NA" in space that is not applicable by making cross line from the left top corner of page to right bottom corner of the page.
- Correct the wrong entry by writing "RE" with drawing single line through incorrect data, record the correct data with sign and date.
- All the reports / supporting data generated during the validation activity shall be enclosed to record of results with sign and date of concerned personnel on each page of the enclosure. Mention protocol reference number and enclosure number on first page of each enclosure.
- Record the deviations observed during the execution in the deviation column of the record of results.
- After protocol execution, report shall be prepared with following details.
  - Discussion of all study in observations.
  - Discussion of any deviations observed and their impact on validation status with corrective and preventive actions.
  - Conclusion with recommendation, if any.

9. PROCESS/ METHOD DESCRIPTION:

- Hand disinfection shall be done at the time of entering the facility or after handling of microorganisms. It shall be also done periodically while working in the clean area.
- Approximately 1to 2 mL of the 70% Isopropyl Alcohol shall be taken on the hand with the help of spray or pump and it shall be rubbed on the hands. It shall be allowed the contact time of 30 seconds.
- In 30 seconds hands shall almost dried.
- In case of surface disinfection, surface shall be wiped with wet mop with 70% IPA and it shall be allowed to air dry.

10. TRAINING RECORD FOR VALIDATION:

Fill the training record in record of result for those who are performing the validation activity. Include concerned personnel from User, Engineering, QC and QA.

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11. EQUIPMENTS/MATERIAL REQUIRED FOR VALIDATION:

11.1 Equipments

- LAF bench
- Autoclave
- Incubators
- Garment cabinet
- Pass box

11.2 Materials

- Cultures
  - Escherichia coli ATCC 8739
  - Staphylococcus aureus ATCC 6538
  - Bacillus subtilis ATCC 19659
  - Candida albicans ATCC 10231
  - Aspergillus niger ATCC 16404
- Letheen broth
- Letheen agar
- 70% Isopropyl Alcohol
- Soyabean casein digest medium
- Soyabean casein digest agar
- Test tubes
- Pipette – 1 mL
- Graduated pipette – 10mL
- Sterile Petri plates 90 mm diameter

12. VALIDATION PLAN:

The proposed validation of disinfection of 70% Isopropyl Alcohol shall be done in following steps:

- Preparation of inoculum
- Dilution of disinfectants
- Procedure for disinfectant validation

12.1 Preparation of inoculum:

- A loop full culture of all the organisms for which the inoculum is to be prepared shall be inoculated in 10 ml of sterile Soyabean casein digest medium.
- Incubate the inoculated tubes of bacterial culture at 30°C to 35°C for 18 to 24 hours and fungal and yeast cultures 20°C to 25°C for 3 days.

- After incubation swirl the culture tube to make it uniform and serially dilute it as follow:

  1 mL above culture + 9 mL sterile saline ----------→ (solution A) (dilution factor 10)

  1 mL (solution A) + 9 mL sterile saline ----------→ (solution B) (dilution factor 100)

  1 mL (solution B) + 9 mL sterile saline ----------→ (solution C) (dilution factor 1000)

  1 mL (solution C) + 9 mL sterile saline ----------→ (solution D) (dilution factor 10000)

  1 mL (solution D) + 9 mL sterile saline ----------→ (solution E) (dilution factor 100000)

  1 mL (solution E) + 9 mL sterile saline ----------→ (solution F) (dilution factor 1000000)

  1 mL (solution F) + 9 mL sterile saline ----------→ (solution G) (dilution factor 10000000)

- Transfer 1 mL of the solution E, F and G to the sterile Petri plate in duplicate.
- Pour 20 mL of sterile Soyabean casein digest agar at temperature of around 40°C to each Petri plate and than rotate the plate to mix the inoculum with media.
- Incubate the bacterial plate at 30°C to 35°C for 48 to 72 hours and fungal plates for 20°C to 25°C for 3 to 5 days.
- As soon as the incubation period is over than observe the number of colonies in the plates and calculate number of organisms per ml by multiplying it with dilution factor.
- The dilution containing 1 x 10^7 organisms per mL shall be selected for challenge test.
- The dilutions of the organism shall be stored in the refrigerator till the evaluation is not over.

12.2 Dilution of disinfection:

- Generally the dilution which is used in the facility for disinfection, one lower and one upper dilution shall be prepared to evaluate the correct dilution of the disinfectant.
- Since 70% Isopropyl Alcohol is a hand wash & surface disinfectant and it is used in the facility at 70% level only so it shall be evaluated at concentration of 70%v/v.

12.3 Procedure for disinfectant validation:

- Take five test tubes and add 5 ml of undiluted 70% Isopropyl Alcohol solution to it.
- Add 0.5 ml inoculum of challenge test organism to one tube.
- As soon as the addition is completed shake the tube and pipette one of the test solutions in two sterile Petri plates.
- Wait for 30 seconds and then add again pipette the 1 ml test solution in two Petri plates.
- Allow the one minute (60 seconds) of contact time to over and pipette the 1 mL test solution in two Petri plates.
- Immediately after Pipetting the test solution 20 ml of sterile Letheen agar medium shall be added to each plate.

- Repeat the same procedure with all the organisms.
- Dilute the inoculum as follow:

  1 mL challenge inoculum + 9 mL sterile saline ----------→ (solution A) (dilution factor 10)

  1 mL (solution A) + 9 mL sterile saline ----------→ (solution B) (dilution factor 100)

  1 mL (solution B) + 9 mL sterile saline ----------→ (solution C) (dilution factor 1000)

  1 mL (solution C) + 9 mL sterile saline ----------→ (solution D) (dilution factor 10000)

  1 mL (solution D) + 9 mL sterile saline ----------→ (solution E) (dilution factor 100000)

  1 mL (solution E) + 9 mL sterile saline ----------→ (solution F) (dilution factor 1000000)
- E and F solution from the above dilution shall be pipetted in the Petri plates.
- 20 mL Letheen broth shall be poured in plates and it shall be marked as positive control.
- Incubate all the Petri plates of bacterial culture at 30°C to 35°C for 48 to 72 hours and fungal plates for 20°C to 25°C for 3 to 5 days.
- Observe all the plates after incubation and note down the reading in the record of result.

13. SAMPLING PLAN:

- 500 mL of 70% IPA shall be taken as sample for the validation of disinfectant.

14. ACCEPTANCE CRITERIA:

- The number of microorganisms shall be reduced up to 5 log of the initial population in 30 second of contact time.

15. SAFETY PRECAUTIONS:

All the safety precautions specified in the MSDS of 70% Isopropyl Alcohol shall be followed and cultures shall be handled with care.

16. RE VALIDATION CRITERIA:

Revalidation of disinfectant shall be carried in following conditions:-

- Change in composition of disinfectant
- Once in three year to reassess the efficacy.

17. REFERENCES:

- Disinfectants and antiseptics USP (1072)

18. ENCLOSURES:

- NA

19. PROTOCOL APPROVAL

Prepared by:

Name	Department	Sign and Date

Reviewed by:

Name	Department	Sign and Date

Approved by QA:

Name	Department	Sign and Date