

Original Research Article

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Evaluation of Disinfectants by Surface Challenge test as per USP 1072 standard at Pharma Company Laboratory, India

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ABSTRACT

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Cleaning and disinfection of surfaces are essential steps for maintaining the cleanliness of pharmaceutical manufacturing operations. To validate the efficacy of disinfectants used in disinfection procedure, in order to reduce surface contaminations, we tested invitro the action of commercial disinfectants. Surface Challenge testing has been carried in a Pharmaceutical manufacturing laboratory in accordance with the United States Pharmacopeia <1072> Disinfectants and Antiseptics protocol. Surface Challenge tests assess the effectiveness of the selected disinfectant against surface-adhered microorganisms. The test organisms used include standard strains mentioned in USP <1072>. Our results showed a bactericidal, fungicidal, and sporicidal efficacy coherent to the acceptance criteria suggested by United States Pharmacopeia <1072>. The correct implementation of our cleaning and disinfection procedure, respecting stipulated concentrations and contact times, led to a reduction of more than 4 Log₁₀ for all microorganisms used. The qualification of procedure was carried out on a screening test in order to measure effectiveness of the disinfectants, chosen according to their principle of action.

Introduction

In pharmaceutical cleanroom cleaning the surface is necessary prior to the disinfection so that disinfectant to work efficiently. Microbial contamination of surfaces and environment is a fundamental aspect which requires constant monitoring and control, in order to minimize the contamination of microorganisms. The USP, requires stringent procedures to be followed during the

manufacturing of pharmaceutical preparations (as stated in USP Chapter <797>) [1]. One step towards achieving microbial control within a clean room is the use of defined cleaning techniques for walls, ceilings, floors and surfaces at working height together with the application of suitable detergents and disinfectants.

Disinfection process validation is defined as "establishing documented evidence that a disinfection process will consistently remove or inactivate known or

possible pathogens from inanimate objects" [4]. Disinfectants vary in their spectrum of activity, mode of action, and efficacy. To ensure the disinfectant demonstrates its suitability and effectiveness within the facility, the disinfection procedure utilized in cleanrooms should be validated in the specific manner in which they are used, on the type of surface material to which it is applied, by testing against standard test microorganisms as well as those that are typical environmental isolates, and to support the in-use expiry periods of the disinfectants. One of the more difficult tasks facing pharmaceutical organizations is with the selection of disinfectants, particularly in ensuring that the disinfectants selected are appropriate and that the effectiveness of the disinfectants are periodically assessed [2]. The need for a scientific rationale for the selection of disinfectants is outlined in the USP Chapter <1072> 'Selection of a Disinfectant for Use in a Pharmaceutical Manufacturing Environment' [10].

There is significant regulatory interest in this area as it forms a fundamental part of any production facility maintenance schedule [5]. Although we often use the terms disinfectant efficacy testing and disinfectant validation in the same context, it is very important to make a distinction between these two terms. Disinfectant efficacy testing is concerned with demonstrating that a product possesses antimicrobial activity under defined laboratory test conditions. It is the process that is used to compare the antimicrobial activity of a product against other products or known standards. The efficacy of disinfectants can be affected by a number of factors including pH, temperature, water hardness, organic soiling and dilution [4] [6]. Onsite Surface Challenge testing of disinfectants in a Pharmaceutical manufacturing laboratory was successfully carried out on the basis of their in house SOP (Standard Operating Procedure) and the results showed that the proposed test method was efficient for confirming the tested disinfectants' efficacy on test surfaces commonly found in Pharmaceutical manufacturing. Moreover, we observed that all chosen disinfectants used for the efficacy validation disinfection procedure were suitable for surface disinfection, since they reduced and controlled the spread of contamination in surfaces of sensitive areas.

Materials and Methods

Microorganisms

Standard strains of the test organisms of *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 11229), *Pseudomonas aeruginosa* (ATCC 9027) *Candida albicans* (ATCC 10231) and *Aspergillus brasiliensis* (ATCC 16404) [8] were obtained from National Collection of Industrial Microorganisms (NCIM), Pune, India.

Test Organism Suspension

Suspension of each of the test organisms was made by collecting a loopful of colony from each plate and inoculating in a sterile peptone water. The tubes of the subcultured organisms were incubated for bacteria at 30 - 35°C for 24 to 48 hours and for fungal at 20 - 25°C for 3-5 days. Adjust the cell density to approximately 10^6 - 10^7 CFU/ml using the diluent. For counting of fungal test suspension prepare 10^6 CFU/ml.

Disinfectant

The disinfectants products used in this testing were - Inficide II 256 (combination of DDAC and ADBAC), Inficide PQ+ (combination of DDAC and PHMB), Inficide 150 (Silver Hydrogen peroxide).

Culture Media and Reagents

Soyabean Casien Digest Agar (SCDA), Sabouraud Dextrose Agar (SDA), Dey/Engley Nutrient broth (DENB), 0.1% peptone water, Normal Saline (0.85% of sodium chloride solution), Phosphate Buffer Solution pH -7.0.

Accessories

Calibrated Micropipettes and Sterile Micropipette Tips, Sterile spreader, Membrane filtration assembly, Sterile filter cups, Sterile membrane filter papers - 0.45µm, Sterile Forceps, Measuring cylinder, Sterile Swab Sticks, Vortex mixer

Disinfectant Validation Study: Surface Challenge Test

Disinfectant Efficacy Validation testing is one of the key steps in the disinfectant validation process. These tests are very important because they determine the limitations of the disinfectant. Most importantly they help to

establish the nominal microbial kill times that will be required during routine use [2]. In this test surface coupons of 2 inch x 2 inch (or 25 cm²) for testing was selected from the various areas representing the surfaces in the facility.

Three sets of each test surface coupons for each disinfectant dilutions and each test organisms were used and labeled as - 1st used for test sample recovery, 2nd used for Positive control and 3rd used for Negative control. The test coupons were wrapped in aluminum foil and sterilized in autoclave. Epoxy coupons were sanitized. After sterilization, all the coupons were carefully unwrap in LAF area before testing. Add 0.1 mL of test culture suspension containing approximately 10⁶ - 10⁷ CFU/ml on the template of test surface coupons (1st and 2nd) and spreaded equally with spreader. After spreading the surfaces were kept in the LAF area for 30 seconds to 10 minutes for drying. Then hold the coupons in vertical position and spray the diluted disinfectant solution to cover all area of surface coupons without oversaturating it. Allow the disinfectant solution as per specified contact time on the template surface and recover by test challenge inoculums by swab method on the three surfaces with individual swab sticks. Wet the pre-sterile non-absorbent cotton swabs with 0.9% sterile saline and rotate & swab throughout the surface gently covering all the area of the surface in zigzag fashion. Immediately transfer the swab into a tube containing 10 ml DENB and mix well by vortexing for 30 sec. - 1 min. This procedure was repeated for all test organisms and with each disinfectant and their dilution concentration. After this proceed with filtration method by arranging the filtration assembly, attach the vacuum pump and filter the above DENB broth tube through 0.45µm membrane. Wash the membrane with 100 ml x 1 time with 0.1% Tween (Polysorbate) 80 in 0.1% peptone solution. Aseptically transfer the membrane on pre-incubated Soyabean Casien Digest Agar (SCDA) for bacterial growth and Sabourauds Dextrose Agar (SDA) for fungal growth. For bacteria culture plates were incubated at 30°C - 35°C for

24 to 48 hours and for fungal culture plates were incubated at 20 -25°C for 5 days. After incubation period colonies on membrane from all the plates are counted and recorded. The same was ascertained by negative control.. The difference in bacteria numbers between the treated sample and the positive control indicates the effectiveness of the disinfection, allowing the reduction log factor to be calculated as follows

Log Reduction = Log initial – Log after exposure

Also along with these two controls was carried out simultaneously as 1) A control to confirm that the neutralizing solution does not affect the test organisms and 2) A control for recovery validation [1] [10].

Acceptance Criteria

Since microorganisms vary in their susceptibility to disinfection procedures, USP <1072> “Disinfectants and Antiseptics” recommends an expectation of 3 log₁₀ of reduction for enveloped viruses, vegetative bacteria and fungi and ≥2 log₁₀ of reduction for non-enveloped viruses and bacterial spores [8].

Results and Discussion

The results obtained in this evaluation are presented in below tables.

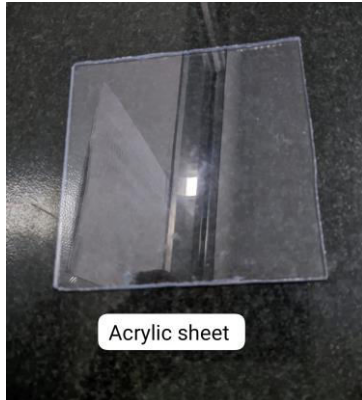
From the results obtained it is observed that all the test disinfectant products (i.e. Inficide II 256, Inficide PQ+ and Inficide 150) at respective dilution concentration showed good efficacy activity against standard test organisms in 5, 10 & 15 minutes. Therefore, this indicates that all test disinfectant product has excellent antimicrobial efficacy at test concentration. The use of these disinfectants may be means to reduce the contamination caused by the test microorganisms.

Table.1a Preparation of Disinfectant dilution concentration

Disinfectants	Dilution concentration	Preparation
Inficide II 256	0.2%, 0.4% & 0.6%	0.2% - 2ml in a litre of water 0.4% - 4ml in a litre of water 0.6% - 6ml in a litre of water
Inficide PQ+	0.5%, 1.0% & 1.5%	0.5% - 5ml in a litre of water 1.0% - 10ml in a litre of water 1.5% - 15ml in a litre of water

Inficide 150	5%, 10% & 20%	5% - 50ml in 950 ml of water 10% - 100ml in 900 ml of water 20% - 200ml in 800 ml of water
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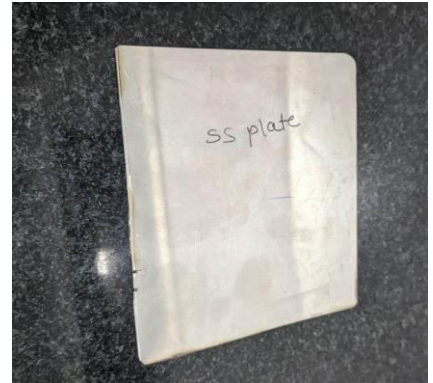
Figure.1 Test Surface Coupons



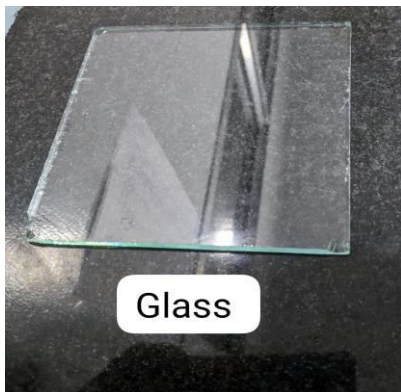
Acrylic



Epoxy



SS Plate



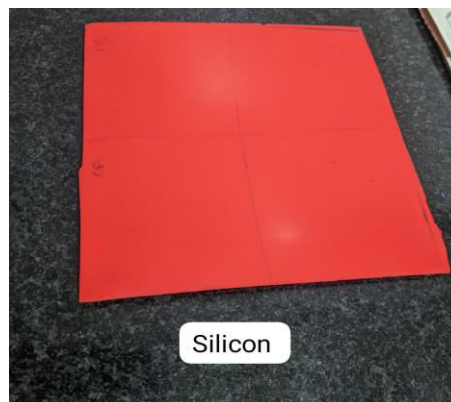
Glass



Teflon Plate



PVC Curtain



Silicon

Table.1b The validation results of Inficide II 256 disinfectant

Test Organisms	Disinf. Conc.	Contact Time	Surfaces													
			Acrylic sheet				Epoxy				SS Plate				Glass	
			Initial count		After Exposure		Log Reduction	After Exposure		Log Reduction	After Exposure		Log Reduction	After Exposure		Log Reduction
			CFU/ml	Log	CFU/ml	Log		CFU/ml	Log		CFU/ml	Log		CFU/ml	Log	
<i>S.aureus</i>	0.20%	5 mins.	94 x 10 ⁶	7.97	61	1.78	6.19	52	1.71	6.26	58	1.76	6.21	42	1.62	6.35
		10 mins.		7.97	25	1.39	6.58	24	1.38	6.59	29	1.46	6.51	23	1.36	6.61
		15 mins.		7.97	15	1.17	6.80	10	1.00	6.97	16	1.20	7.77	11	1.04	6.93
	0.40%	5 mins.	94 x 10 ⁶	7.97	7	0.84	7.13	Nil	-	7.97	2	0.30	7.67	Nil	-	7.97
		10 mins.		7.97	Nil	-	7.97	Nil	-	7.97	Nil	-	7.97	Nil	-	7.97
		15 mins.		7.97	Nil	-	7.97	Nil	-	7.97	Nil	-	7.97	Nil	-	7.97
	0.60%	5 mins.	94 x 10 ⁶	7.97	Nil	-	7.97	Nil	-	7.97	Nil	-	7.97	Nil	-	7.97
		10 mins.		7.97	Nil	-	7.97	Nil	-	7.97	Nil	-	7.97	Nil	-	7.97
		15 mins.		7.97	Nil	-	7.97	Nil	-	7.97	Nil	-	7.97	Nil	-	7.97
<i>E.coli</i>	0.20%	5 mins.	98 x 10 ⁶	7.99	58	1.76	6.23	30	1.47	6.52	47	1.67	6.32	38	1.60	6.39
		10 mins.		7.99	41	1.61	6.38	12	1.07	6.92	30	1.47	6.52	21	1.32	6.67
		15 mins.		7.99	22	1.34	6.65	8	0.9	7.09	21	1.32	6.67	10	1.00	6.99
	0.40%	5 mins.	98 x 10 ⁶	7.99	12	1.07	6.92	Nil	-	7.99	4	0.60	7.39	Nil	-	7.99
		10 mins.		7.99	2	0.30	7.69	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
		15 mins.		7.99	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
	0.60%	5 mins.	98 x 10 ⁶	7.99	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
		10 mins.		7.99	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
		15 mins.		7.99	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
<i>B.subtilis</i>	0.20%	5 mins.	90 x 10 ⁶	7.95	45	1.65	6.30	56	1.74	6.21	50	1.69	6.26	40	1.60	6.35
		10 mins.		7.95	23	1.36	6.59	34	1.53	6.42	31	1.49	6.46	25	1.40	6.55
		15 mins.		7.95	12	1.07	6.88	21	1.32	6.63	14	1.15	6.80	12	1.08	6.87
	0.40%	5 mins.	90 x 10 ⁶	7.95	6	0.77	7.18	11	1.04	6.91	5	0.70	7.25	7	0.85	7.10
		10 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
		15 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
	0.60%	5 mins.	90 x 10 ⁶	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
		10 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
		15 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
<i>P.aeruginosa</i>	0.20%	5 mins.	89 x 10 ⁶	7.95	54	1.73	6.22	48	1.68	6.27	62	1.79	6.16	55	1.74	6.21
		10 mins.		7.95	30	1.47	6.48	28	1.44	6.51	48	1.68	6.27	39	1.59	6.36
		15 mins.		7.95	11	1.04	6.91	10	1.00	6.95	29	1.46	6.49	26	1.41	6.54
	;lk0.40 %	5 mins.	89 x 10 ⁶	7.95	8	0.90	7.05	3	0.47	7.48	15	1.17	6.78	15	1.17	6.78
		10 mins.		7.95	1	0	7.95	Nil	-	7.95	3	0.47	7.48	4	0.60	7.35
		15 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
	0.60%	5 mins.	89 x 10 ⁶	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95

<i>C.albicans</i>	0.20%	10 mins.	86 x 10 ⁶	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
		15 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
		5 mins.		7.93	55	1.74	6.19	35	1.54	6.39	46	1.66	6.27	34	1.53	6.40
		10 mins.		7.93	21	1.32	6.61	20	1.30	6.63	20	1.30	6.63	19	1.28	6.65
		15 mins.		7.93	10	1.00	6.93	10	1.00	6.93	8	0.90	7.03	8	0.90	7.03
	0.40%	5 mins.	86 x 10 ⁶	7.93	4	0.60	7.33	6	0.77	7.16	Nil	-	7.93	Nil	-	7.93
		10 mins.		7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
		15 mins.		7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
	0.60%	5 mins.	86 x 10 ⁶	7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
		10 mins.		7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
		15 mins.		7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
<i>A.brasiliensis</i>	0.20%	5 mins.	89 x 10 ⁶	7.95	34	1.53	6.42	44	1.64	6.31	40	1.60	6.35	26	1.41	6.54
		10 mins.		7.95	20	1.30	6.65	26	1.41	6.54	21	1.32	6.63	10	1.00	6.95
		15 mins.		7.95	15	1.17	6.78	17	1.23	6.72	10	1.00	6.95	2	0.30	7.65
	0.40%	5 mins.	89 x 10 ⁶	7.95	9	0.95	7.00	8	0.90	7.05	1	0	7.95	Nil	-	7.95
		10 mins.		7.95	1	0	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
		15 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
	0.60%	5 mins.	89 x 10 ⁶	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
		10 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
		15 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
In house isolate (<i>Salmonella</i> sp.)	0.20%	5 mins.	91 x 10 ⁶	7.96	44	1.64	6.32	36	1.55	6.41	47	1.67	6.29	31	1.49	6.45
		10 mins.		7.96	31	1.49	6.47	22	1.34	6.62	26	1.41	6.55	19	1.27	6.69
		15 mins.		7.96	20	1.30	6.66	14	1.14	6.82	11	1.04	6.92	10	1.00	6.69
	0.40%	5 mins.	91 x 10 ⁶	7.96	15	1.17	6.79	7	0.84	7.12	3	0.47	7.49	Nil	-	7.96
		10 mins.		7.96	1	0	7.96	Nil	-	7.96	Nil	-	7.96	Nil	-	7.96
		15 mins.		7.96	Nil	-	7.96	Nil	-	7.96	Nil	-	7.96	Nil	-	7.96
	0.60%	5 mins.	91 x 10 ⁶	7.96	Nil	-	7.96	Nil	-	7.96	Nil	-	7.96	Nil	-	7.96
		10 mins.		7.96	Nil	-	7.96	Nil	-	7.96	Nil	-	7.96	Nil	-	7.96
		15 mins.		7.96	Nil	-	7.96	Nil	-	7.96	Nil	-	7.96	Nil	-	7.96

Surfaces								
Teflon			PVC Curtain			Silicon		
After Exposure		Log Reduction	After Exposure		Log Reduction	After Exposure		Log Reduction
CFU/ml	Log		CFU/ml	Log		CFU/ml	Log	
58	1.76	6.21	60	1.77	6.20	42	1.62	6.35
32	1.50	6.47	45	1.65	6.32	24	1.38	6.59
21	1.32	6.65	28	1.40	6.57	8	0.90	7.07
10	1.00	6.97	11	1.04	6.93	Nil	-	7.97
4	0.60	7.37	Nil	-	7.97	Nil	-	7.97
Nil	-	7.97	Nil	-	7.97	Nil	-	7.97

Nil	-	7.97	Nil	-	7.97	Nil	-	7.97
Nil	-	7.97	Nil	-	7.97	Nil	-	7.97
Nil	-	7.97	Nil	-	7.97	Nil	-	7.97
50	1.70	6.29	43	1.63	6.36	51	1.71	6.28
35	1.54	6.45	26	1.41	6.58	32	1.50	6.49
19	1.28	6.71	10	1.00	6.99	17	1.23	6.76
9	0.95	7.04	Nil	-	7.99	8	0.90	7.39
Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
48	1.68	6.27	51	1.71	6.24	57	1.75	6.20
20	1.30	6.65	28	1.44	6.51	30	1.48	6.47
11	1.04	6.91	16	1.20	6.75	10	1.00	6.95
6	0.77	7.18	5	0.70	7.25	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
42	1.62	6.33	55	1.74	6.21	59	1.77	6.18
22	1.34	6.61	29	1.46	6.49	36	1.55	6.40
14	1.14	6.81	8	0.90	7.05	24	1.38	6.57
7	0.84	7.11	Nil	-	7.95	18	1.25	6.70
1	0	7.95	Nil	-	7.95	9	0.95	7.00
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
45	1.65	6.28	40	1.60	6.33	54	1.73	6.20
20	1.30	6.63	26	1.41	6.52	29	1.46	6.47
6	0.77	7.16	13	1.11	6.82	17	1.23	6.70
Nil	-	7.93	8	0.90	7.03	6	0.77	7.16
Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
40	1.60	6.35	51	1.71	6.24	43	1.63	6.32
24	1.38	6.57	22	1.34	6.61	28	1.45	6.50
12	1.07	6.88	12	1.07	6.88	16	1.20	6.75
1	0	7.95	4	0.60	7.35	10	1.00	6.95

Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
47	1.67	6.29	44	1.64	6.33	52	1.71	6.25
29	1.46	6.50	21	1.32	6.64	30	1.47	6.49
16	1.20	6.76	14	1.15	6.81	21	1.32	6.64
10	1.00	6.96	3	0.48	7.48	9	0.95	7.01
1	0	7.96	Nil	-	7.96	Nil	-	7.96
Nil	-	7.96	Nil	-	7.96	Nil	-	7.96
Nil	-	7.96	Nil	-	7.96	Nil	-	7.96
Nil	-	7.96	Nil	-	7.96	Nil	-	7.96
Nil	-	7.96	Nil	-	7.96	Nil	-	7.96

Table.2 The validation results of Inficide PQ+ disinfectant

Test Organisms	Disinf. Conc.	Contact Time	Surfaces													
			Acrylic sheet				Epoxy				SS Plate				Glass	
			Initial count		After Exposure		Log Reduction	After Exposure		Log Reduction	After Exposure		Log Reduction	After Exposure		Log Reduction
			CFU/ml	Log	CFU/ml	Log		CFU/ml	Log		CFU/ml	Log		CFU/ml	Log	
<i>S.aureus</i>	0.50%	5 mins.	74 x 10 ⁶	7.86	51	1.70	6.16	48	1.68	6.18	41	1.41	6.45	36	1.55	6.31
		10 mins.		7.86	20	1.30	6.56	21	1.32	6.54	23	1.36	6.50	19	1.28	6.58
		15 mins.		7.86	10	1.00	6.86	6	0.77	7.09	12	1.07	6.79	2	0.30	7.56
	1.00%	5 mins.	74 x 10 ⁶	7.86	Nil	-	7.86	Nil	-	7.86	Nil	-	7.86	Nil	-	7.86
		10 mins.		7.86	Nil	-	7.86	Nil	-	7.86	Nil	-	7.86	Nil	-	7.86
		15 mins.		7.86	Nil	-	7.86	Nil	-	7.86	Nil	-	7.86	Nil	-	7.86
	1.50%	5 mins.	74 x 10 ⁶	7.86	Nil	-	7.86	Nil	-	7.86	Nil	-	7.86	Nil	-	7.86
		10 mins.		7.86	Nil	-	7.86	Nil	-	7.86	Nil	-	7.86	Nil	-	7.86
		15 mins.		7.86	Nil	-	7.86	Nil	-	7.86	Nil	-	7.86	Nil	-	7.86
<i>E.coli</i>	0.50%	5 mins.	50 x 10 ⁶	7.70	43	1.63	6.07	41	1.61	6.09	55	1.74	5.96	45	1.65	6.05
		10 mins.		7.70	34	1.53	6.17	25	1.40	6.30	29	1.46	6.24	23	1.36	6.34
		15 mins.		7.70	21	1.32	6.38	10	1.00	6.70	20	1.30	6.40	14	1.14	6.56
	1.00%	5 mins.	50 x 10 ⁶	7.70	11	1.04	6.66	2	0.30	7.40	8	0.90	6.80	Nil	-	7.70
		10 mins.		7.70	Nil	-	7.70	Nil	-	7.70	Nil	-	7.70	Nil	-	7.70
		15 mins.		7.70	Nil	-	7.70	Nil	-	7.70	Nil	-	7.99	Nil	-	7.99
	1.50%	5 mins.	50 x 10 ⁶	7.70	Nil	-	7.70	Nil	-	7.70	Nil	-	7.99	Nil	-	7.99
		10 mins.		7.70	Nil	-	7.70	Nil	-	7.70	Nil	-	7.99	Nil	-	7.99
		15 mins.		7.70	Nil	-	7.70	Nil	-	7.70	Nil	-	7.99	Nil	-	7.99
<i>B.subtilis</i>	0.50%	5 mins.	72 x 10 ⁶	7.85	38	1.58	6.27	41	1.61	6.24	30	1.48	6.37	37	1.57	6.28
		10 mins.		7.85	18	1.25	6.60	25	1.40	6.45	21	1.32	6.53	20	1.30	6.55

	1.00%	15 mins.	72 x 10 ⁶	7.85	10	1.00	6.85	12	1.08	6.77	11	1.04	6.81	9	0.95	6.90
		5 mins.		7.85	Nil	-	7.85	Nil	-	7.85	1	-	7.25	Nil	-	7.85
		10 mins.		7.85	Nil	-	7.85	Nil	-	7.85	Nil	-	7.85	Nil	-	7.85
		15 mins.		7.85	Nil	-	7.85	Nil	-	7.85	Nil	-	7.85	Nil	-	7.85
	1.50%	5 mins.	72 x 10 ⁶	7.85	Nil	-	7.85	Nil	-	7.85	Nil	-	7.85	Nil	-	7.85
		10 mins.		7.85	Nil	-	7.85	Nil	-	7.85	Nil	-	7.85	Nil	-	7.85
		15 mins.		7.85	Nil	-	7.85	Nil	-	7.85	Nil	-	7.85	Nil	-	7.85
<i>P.aeruginosa</i>	0.50%	5 mins.	70 x 10 ⁶	7.84	46	1.66	6.18	42	1.62	6.22	43	1.63	6.21	45	1.65	6.19
		10 mins.		7.84	27	1.43	6.41	21	1.32	6.52	28	1.44	6.40	19	1.28	6.56
		15 mins.		7.84	15	1.17	6.67	11	1.04	6.80	12	1.08	6.76	6	0.77	7.07
	1.00%	5 mins.	70 x 10 ⁶	7.84	5	0.70	7.14	Nil	-	7.84	1	0	7.84	Nil	-	7.84
		10 mins.		7.84	Nil	-	7.84	Nil	-	7.84	Nil	-	7.84	Nil	-	7.84
		15 mins.		7.84	Nil	-	7.84	Nil	-	7.84	Nil	-	7.84	Nil	-	7.84
	1.50%	5 mins.	70 x 10 ⁶	7.84	Nil	-	7.84	Nil	-	7.84	Nil	-	7.84	Nil	-	7.84
		10 mins.		7.84	Nil	-	7.84	Nil	-	7.84	Nil	-	7.84	Nil	-	7.84
		15 mins.		7.84	Nil	-	7.84	Nil	-	7.84	Nil	-	7.84	Nil	-	7.84
<i>C.albicans</i>	0.50%	5 mins.	65 x 10 ⁶	7.81	48	1.68	6.13	41	1.61	6.20	38	1.60	6.21	42	1.62	6.19
		10 mins.		7.81	20	1.30	6.51	24	1.38	6.43	14	1.15	6.66	18	1.30	6.51
		15 mins.		7.81	9	0.95	6.86	11	1.04	6.77	5	0.70	7.11	4	0.60	7.21
	1.00%	5 mins.	65 x 10 ⁶	7.81	2	0.30	7.51	1	0	7.81	Nil	-	7.81	Nil	-	7.81
		10 mins.		7.81	Nil	-	7.81	Nil	-	7.81	Nil	-	7.81	Nil	-	7.81
		15 mins.		7.81	Nil	-	7.81	Nil	-	7.81	Nil	-	7.81	Nil	-	7.81
	1.50%	5 mins.	65 x 10 ⁶	7.81	Nil	-	7.81	Nil	-	7.81	Nil	-	7.81	Nil	-	7.81
		10 mins.		7.81	Nil	-	7.81	Nil	-	7.81	Nil	-	7.81	Nil	-	7.81
		15 mins.		7.81	Nil	-	7.81	Nil	-	7.81	Nil	-	7.81	Nil	-	7.81
<i>A.brasiliensis</i>	0.50%	5 mins.	52 x 10 ⁶	7.72	38	1.60	6.12	36	1.55	6.17	31	1.49	6.23	30	1.48	6.24
		10 mins.		7.72	21	1.32	6.40	18	1.26	6.46	11	1.04	6.68	19	1.28	6.44
		15 mins.		7.72	10	1.00	6.72	7	0.84	6.88	3	0.48	7.24	6	0.77	6.95
	1.00%	5 mins.	52 x 10 ⁶	7.72	Nil	-	7.72	Nil	-	7.72	Nil	-	7.72	Nil	-	7.72
		10 mins.		7.72	Nil	-	7.72	Nil	-	7.72	Nil	-	7.72	Nil	-	7.72
		15 mins.		7.72	Nil	-	7.72	Nil	-	7.72	Nil	-	7.72	Nil	-	7.72
	1.50%	5 mins.	52 x 10 ⁶	7.72	Nil	-	7.72	Nil	-	7.72	Nil	-	7.72	Nil	-	7.72
		10 mins.		7.72	Nil	-	7.72	Nil	-	7.72	Nil	-	7.72	Nil	-	7.72
		15 mins.		7.72	Nil	-	7.72	Nil	-	7.72	Nil	-	7.72	Nil	-	7.72
In house isolate (<i>Salmonella</i> sp.)	0.50%	5 mins.	66 x 10 ⁶	7.81	46	1.66	6.15	41	1.61	6.20	48	1.68	6.13	34	1.53	6.28
		10 mins.		7.81	37	1.57	6.24	23	1.36	6.45	25	1.40	6.41	14	1.15	6.66
		15 mins.		7.81	22	1.34	6.47	12	1.08	6.73	16	1.20	6.61	5	0.70	7.11
	1.00%	5 mins.	66 x 10 ⁶	7.81	10	1.00	6.81	Nil	-	7.81	2	0.30	7.51	Nil	-	7.81
		10 mins.		7.81	Nil	-	7.81	Nil	-	7.81	Nil	-	7.81	Nil	-	7.81
		15 mins.		7.81	Nil	-	7.81	Nil	-	7.81	Nil	-	7.81	Nil	-	7.81
	1.50%	5 mins.	66 x 10 ⁶	7.81	Nil	-	7.81	Nil	-	7.81	Nil	-	7.81	Nil	-	7.81
		10 mins.		7.81	Nil	-	7.81	Nil	-	7.81	Nil	-	7.81	Nil	-	7.81
		15 mins.		7.81	Nil	-	7.81	Nil	-	7.81	Nil	-	7.81	Nil	-	7.81

Surfaces								
Teflon			PVC Curtain			Silicon		
After Exposure		Log Reduction	After Exposure		Log Reduction	After Exposure		Log Reduction
CFU/ml	Log		CFU/ml	Log		CFU/ml	Log	
49	1.69	6.17	54	1.73	6.13	40	1.60	6.26
34	1.53	6.33	41	1.61	6.25	21	1.32	6.54
22	1.34	6.52	26	1.41	6.45	11	1.04	6.82
10	1.00	6.86	17	1.23	6.63	Nil	-	7.86
Nil	-	7.86	Nil	-	7.86	Nil	-	7.86
Nil	-	7.86	Nil	-	7.86	Nil	-	7.86
Nil	-	7.86	Nil	-	7.86	Nil	-	7.86
Nil	-	7.86	Nil	-	7.86	Nil	-	7.86
Nil	-	7.86	Nil	-	7.86	Nil	-	7.86
52	1.72	5.98	46	1.66	6.04	38	1.58	6.12
31	1.49	6.21	20	1.30	6.40	22	1.34	6.36
15	1.18	6.52	10	1.00	6.70	9	0.95	6.75
4	0.60	7.10	Nil	-	7.70	Nil	-	7.70
Nil	-	7.70	Nil	-	7.70	Nil	-	7.70
Nil	-	7.70	Nil	-	7.70	Nil	-	7.70
Nil	-	7.70	Nil	-	7.70	Nil	-	7.70
Nil	-	7.70	Nil	-	7.70	Nil	-	7.70
Nil	-	7.70	Nil	-	7.70	Nil	-	7.70
42	1.62	6.23	45	1.65	6.25	33	1.52	6.33
25	1.40	6.45	27	1.43	6.42	16	1.20	6.65
16	1.20	6.65	11	1.04	6.81	3	0.48	7.37
7	0.85	7.00	Nil	-	7.85	Nil	-	7.85
Nil	-	7.85	Nil	-	7.85	Nil	-	7.85
Nil	-	7.85	Nil	-	7.85	Nil	-	7.85
Nil	-	7.85	Nil	-	7.85	Nil	-	7.85
Nil	-	7.85	Nil	-	7.85	Nil	-	7.85
Nil	-	7.85	Nil	-	7.85	Nil	-	7.85
50	1.70	6.14	35	1.54	6.30	41	1.61	6.23
32	1.51	6.33	21	1.32	6.52	28	1.48	6.36
20	1.30	6.54	6	0.77	7.07	14	1.15	6.69
12	1.07	7.77	Nil	-	7.84	Nil	-	7.84
Nil	-	7.84	Nil	-	7.84	Nil	-	7.84
Nil	-	7.84	Nil	-	7.84	Nil	-	7.84
Nil	-	7.84	Nil	-	7.84	Nil	-	7.84
Nil	-	7.84	Nil	-	7.84	Nil	-	7.84

Nil	-	7.84	Nil	-	7.84	Nil	-	7.84
40	1.60	6.21	34	1.53	6.28	44	1.64	6.17
23	1.36	6.45	19	1.28	6.53	27	1.43	6.38
11	1.04	7.77	10	1.00	6.81	10	1.00	6.81
Nil	-	7.81	2	0.30	7.51	1	0	7.81
Nil	-	7.81	Nil	-	7.81	Nil	-	7.81
Nil	-	7.81	Nil	-	7.81	Nil	-	7.81
Nil	-	7.81	Nil	-	7.81	Nil	-	7.81
Nil	-	7.81	Nil	-	7.81	Nil	-	7.81
Nil	-	7.81	Nil	-	7.81	Nil	-	7.81
45	1.65	6.07	48	1.68	6.04	35	1.54	6.18
21	1.32	6.40	26	1.41	6.31	20	1.30	6.42
10	1.00	6.72	15	1.18	6.54	9	0.95	6.77
Nil	-	7.72	8	0.90	6.82	Nil	-	7.72
Nil	-	7.72	Nil	-	7.72	Nil	-	7.72
Nil	-	7.72	Nil	-	7.72	Nil	-	7.72
Nil	-	7.72	Nil	-	7.72	Nil	-	7.72
Nil	-	7.72	Nil	-	7.72	Nil	-	7.72
Nil	-	7.72	Nil	-	7.72	Nil	-	7.72
51	1.70	6.11	40	1.60	6.21	32	1.51	6.30
28	1.44	6.37	20	1.30	6.51	15	1.18	6.63
19	1.28	6.53	11	1.04	6.77	2	0.30	7.51
8	0.90	6.91	Nil	-	7.81	Nil	-	7.81
Nil	-	7.81	Nil	-	7.81	Nil	-	7.81
Nil	-	7.81	Nil	-	7.81	Nil	-	7.81
Nil	-	7.81	Nil	-	7.81	Nil	-	7.81
Nil	-	7.81	Nil	-	7.81	Nil	-	7.81
Nil	-	7.81	Nil	-	7.81	Nil	-	7.81

Table.3 The validation results of Inficide 150 disinfectant

Test Organisms	Disinf. Conc.	Contact Time	Surfaces													
			Acrylic sheet				Epoxy				SS Plate				Glass	
			Initial count		After Exposure		Log Reduction	After Exposure		Log Reduction	After Exposure		Log Reduction	After Exposure		Log Reduction
			CFU/ml	Log	CFU/ml	Log		CFU/ml	Log		CFU/ml	Log		CFU/ml	Log	
<i>S.aureus</i>	5%	5 mins.	94 x 10 ⁶	7.97	40	1.60	6.37	39	1.59	6.38	50	1.69	6.28	46	1.66	6.31
		10 mins.		7.97	15	1.17	6.80	18	1.25	6.72	22	1.34	6.63	30	1.47	6.50
		15 mins.		7.97	6	0.77	6.20	8	0.90	7.07	11	1.04	6.93	14	1.14	6.83
	10%	5 mins.	94 x 10 ⁶	7.97	Nil	-	7.97	Nil	-	7.97	1	0	7.97	2	0.30	7.67
		10 mins.		7.97	Nil	-	7.97	Nil	-	7.97	Nil	-	7.97	Nil	-	7.97

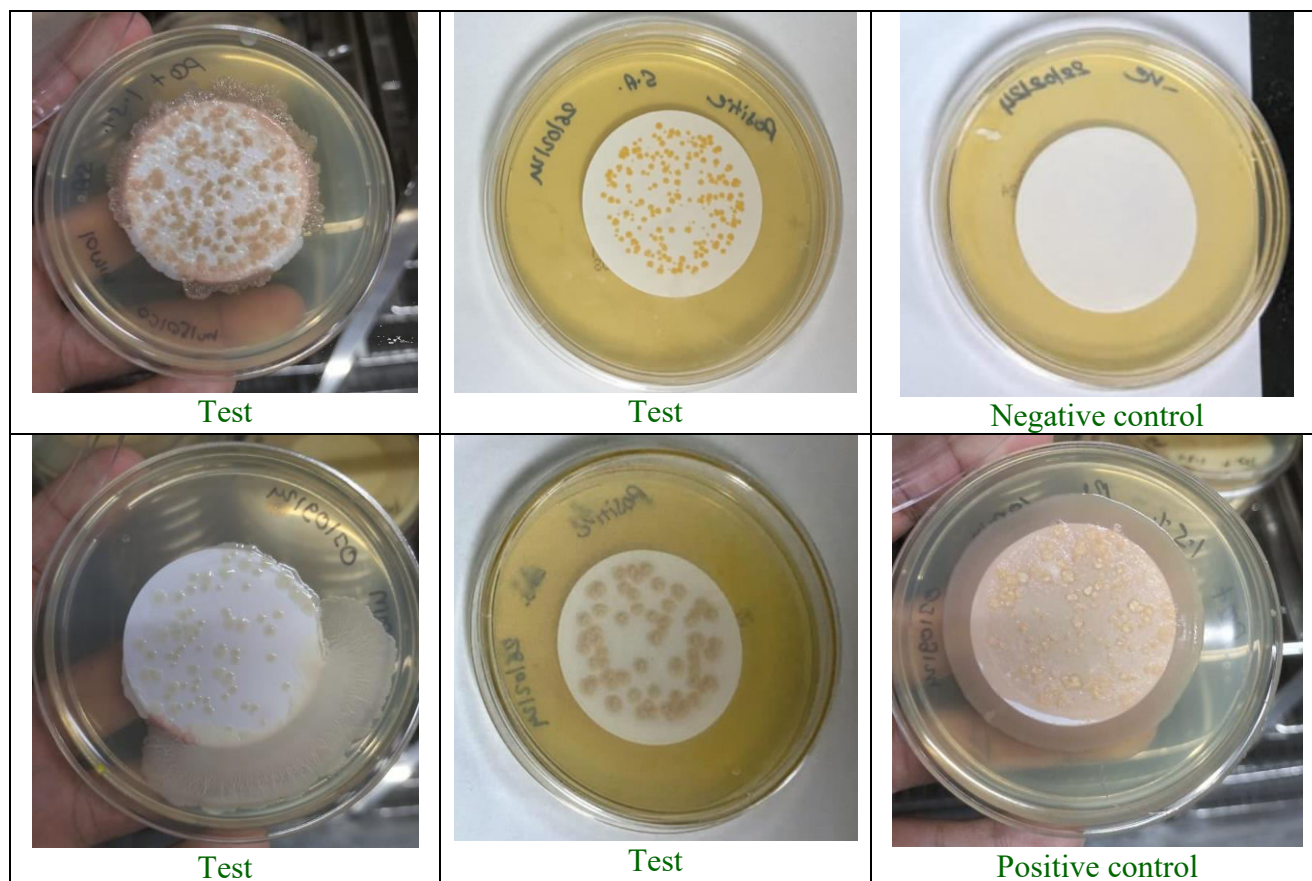
	20%	15 mins.	94 x 10 ⁶	7.97	Nil	-	7.97	Nil	-	7.97	Nil	-	7.97	Nil	-	7.97
		5 mins.		7.97	Nil	-	7.97	Nil	-	7.97	Nil	-	7.97	Nil	-	7.97
		10 mins.		7.97	Nil	-	7.97	Nil	-	7.97	Nil	-	7.97	Nil	-	7.97
		15 mins.		7.97	Nil	-	7.97	Nil	-	7.97	Nil	-	7.97	Nil	-	7.97
<i>E.coli</i>	5%	5 mins.	98 x 10 ⁶	7.99	52	1.72	6.27	42	1.62	6.37	51	1.71	6.28	36	1.55	6.44
		10 mins.		7.99	38	1.58	6.41	19	1.28	6.71	20	1.30	6.69	20	1.30	6.69
		15 mins.		7.99	21	1.32	6.67	3	0.48	7.51	9	0.95	7.04	5	0.70	7.29
	10%	5 mins.	98 x 10 ⁶	7.99	10	1.00	6.99	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
		10 mins.		7.99	4	0.60	7.39	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
		15 mins.		7.99	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
	20%	5 mins.	98 x 10 ⁶	7.99	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
		10 mins.		7.99	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
		15 mins.		7.99	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
		15 mins.		7.99	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
<i>B.subtilis</i>	5%	5 mins.	90 x 10 ⁶	7.95	46	1.66	6.29	52	1.71	6.24	48	1.68	6.27	40	1.60	6.35
		10 mins.		7.95	21	1.32	6.63	29	1.46	6.49	28	1.45	6.50	21	1.32	6.63
		15 mins.		7.95	11	1.04	6.91	14	1.15	6.80	12	1.08	6.87	13	1.11	6.84
	10%	5 mins.	90 x 10 ⁶	7.95	4	0.60	7.35	1	1.00	6.95	2	0.30	7.65	2	0.30	7.65
		10 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
		15 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
	20%	5 mins.	90 x 10 ⁶	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
		10 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
		15 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
		15 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
<i>P.aeruginosa</i>	5%	5 mins.	89 x 10 ⁶	7.95	58	1.76	6.19	52	1.71	6.24	51	1.70	6.25	48	1.68	6.27
		10 mins.		7.95	31	1.49	6.46	24	1.38	6.57	30	1.47	6.48	29	1.46	6.49
		15 mins.		7.95	22	1.31	6.64	13	1.11	6.84	21	1.32	6.63	15	1.17	6.78
	10%	5 mins.	89 x 10 ⁶	7.95	10	1.00	6.95	1	0	7.95	5	0.70	7.25	3	0.48	7.47
		10 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
		15 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
	20%	5 mins.	89 x 10 ⁶	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
		10 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
		15 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
		15 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
<i>C.albicans</i>	5%	5 mins.	86 x 10 ⁶	7.93	34	1.53	6.40	38	1.58	6.35	42	1.62	6.31	44	1.64	6.29
		10 mins.		7.93	18	1.26	6.67	21	1.32	6.61	22	1.34	6.59	19	1.28	6.65
		15 mins.		7.93	10	1.00	6.93	12	1.08	6.85	9	0.95	6.98	10	1.00	6.93
	10%	5 mins.	86 x 10 ⁶	7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
		10 mins.		7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
		15 mins.		7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
	20%	5 mins.	86 x 10 ⁶	7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
		10 mins.		7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
		15 mins.		7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
		15 mins.		7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
<i>A.brasiliensis</i>	5%	5 mins.	89 x 10 ⁶	7.95	31	1.49	6.46	36	1.55	6.40	40	1.60	6.35	35	1.54	6.41
		10 mins.		7.95	14	1.15	6.80	25	1.40	6.55	23	1.36	6.59	21	1.32	6.63
		15 mins.		7.95	5	0.70	7.25	12	1.07	6.88	10	1.00	6.95	12	1.08	6.87

In house isolate (<i>Salmonella</i> sp.)	10%	5 mins.	89 x 10 ⁶	7.95	Nil	-	7.95	4	0.60	7.35	Nil	-	7.95	1	0	7.95
		10 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
		15 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
	20%	5 mins.	89 x 10 ⁶	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
		10 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
		15 mins.		7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
	5%	5 mins.	91 x 10 ⁶	7.96	45	1.65	6.31	34	1.53	6.43	41	1.61	6.29	35	1.54	6.45
		10 mins.		7.96	30	1.48	6.48	20	1.30	6.66	20	1.30	6.55	14	1.14	6.69
		15 mins.		7.96	16	1.20	6.76	11	1.04	6.92	6	0.77	6.92	9	0.95	6.69
	10%	5 mins.	91 x 10 ⁶	7.96	9	0.95	7.01	Nil	-	7.96	Nil	-	7.96	Nil	-	7.96
		10 mins.		7.96	Nil	-	7.96	Nil	-	7.96	Nil	-	7.96	Nil	-	7.96
		15 mins.		7.96	Nil	-	7.96	Nil	-	7.96	Nil	-	7.96	Nil	-	7.96
	20%	5 mins.	91 x 10 ⁶	7.96	Nil	-	7.96	Nil	-	7.96	Nil	-	7.96	Nil	-	7.96
		10 mins.		7.96	Nil	-	7.96	Nil	-	7.96	Nil	-	7.96	Nil	-	7.96
		15 mins.		7.96	Nil	-	7.96	Nil	-	7.96	Nil	-	7.96	Nil	-	7.96

Surfaces								
Teflon			PVC Curtain			Silicon		
After Exposure		Log Reduction	After Exposure		Log Reduction	After Exposure		Log Reduction
CFU/ml	Log		CFU/ml	Log		CFU/ml	Log	
52	1.71	6.26	48	1.68	6.29	40	1.60	6.37
30	1.47	6.50	24	1.38	6.59	21	1.32	6.65
22	1.34	6.63	11	1.04	6.93	.3	0.47	7.50
10	1.00	6.97	2	0.30	7.67	Nil	-	7.97
Nil	-	7.97	Nil	-	7.97	Nil	-	7.97
Nil	-	7.97	Nil	-	7.97	Nil	-	7.97
Nil	-	7.97	Nil	-	7.97	Nil	-	7.97
Nil	-	7.97	Nil	-	7.97	Nil	-	7.97
Nil	-	7.97	Nil	-	7.97	Nil	-	7.97
45	1.65	6.34	43	1.63	6.36	50	1.69	6.30
31	1.49	6.50	22	1.34	6.64	26	1.41	6.58
14	1.14	6.85	10	1.00	6.99	15	1.17	6.82
1	0	7.99	Nil	-	7.99	2	0.30	7.69
Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
Nil	-	7.99	Nil	-	7.99	Nil	-	7.99
48	1.68	6.27	42	1.62	6.33	50	1.70	6.25
25	1.39	6.56	21	1.32	6.63	26	1.41	6.54
11	1.04	6.91	11	1.04	6.91	10	1.00	6.95

6	0.77	7.18	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
42	1.62	6.33	55	1.74	6.21	59	1.77	6.18
22	1.34	6.61	29	1.46	6.49	36	1.55	6.40
14	1.14	6.81	8	0.90	7.05	24	1.38	6.57
7	0.84	7.11	Nil	-	7.95	18	1.25	6.70
1	0	7.95	Nil	-	7.95	9	0.95	7.00
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
45	1.65	6.28	40	1.60	6.33	54	1.73	6.20
20	1.30	6.63	26	1.41	6.52	29	1.46	6.47
6	0.77	7.16	13	1.11	6.82	17	1.23	6.70
Nil	-	7.93	8	0.90	7.03	6	0.77	7.16
Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
Nil	-	7.93	Nil	-	7.93	Nil	-	7.93
40	1.60	6.35	51	1.71	6.24	43	1.63	6.32
24	1.38	6.57	22	1.34	6.61	28	1.45	6.50
12	1.07	6.88	12	1.07	6.88	16	1.20	6.75
1	0	7.95	4	0.60	7.35	10	1.00	6.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
Nil	-	7.95	Nil	-	7.95	Nil	-	7.95
47	1.67	6.29	44	1.64	6.33	52	1.71	6.25
29	1.46	6.50	21	1.32	6.64	30	1.47	6.49
16	1.20	6.76	12	1.15	6.81	21	1.32	6.64
10	1.00	6.96	Nil	-	7.96	10	1.00	6.96
Nil	-	7.96	Nil	-	7.96	Nil	-	7.96
Nil	-	7.96	Nil	-	7.96	Nil	-	7.96
Nil	-	7.96	Nil	-	7.96	Nil	-	7.96
Nil	-	7.96	Nil	-	7.96	Nil	-	7.96
Nil	-	7.96	Nil	-	7.96	Nil	-	7.96

Figure.2 Growth observed on some plates of results



In conclusion, the use of disinfectants will always be part of a pharmaceutical and healthcare facility cleaning programme [12]. Verifying that the routine disinfectant procedures are able to achieve control over the range of possible pathogens must always form a key part of the facility process qualification. Regulatory agencies are showing increased interest in data supporting the efficacy of manufacturing facilities' disinfection procedures [6]. Disinfection efficacy studies must be customized to each manufacturer's facility and procedures, and these studies can quickly become large and overwhelming [11]. The tested Disinfectant product showed excellent bactericidal, fungicidal and yeasticidal activity against the test organisms.

The responsibilities placed on the manufacturers to provide supporting data and the importance of ensuring that the overall validation reflects the way the products are used has also been highlighted. Validation does not have to be done in isolation and support and advice is widely available to ensure that it is performed to a satisfactory standard. The data generated in this

assessment have been reviewed and found acceptable by regulatory bodies [6]. We help to streamline and optimize a study to generate definitive data to support your disinfection regime. These data will provide a further layer of product safety specifically providing confidence in your ability to handle an unexpected contamination event in your facility. The control of contamination, the review of environmental monitoring data, the conducting and review of assessment data, the appropriate application, and continued supervision and training all combine to ensure success.

Surface tests can reflect in-use conditions like contact times, temperatures, use-dilutions, and surface properties.

Author Contributions

Imran Memon: Investigation, formal analysis, writing—original draft. Naushin I. Memon: Validation, methodology, writing—reviewing. Surjeet Samanta:—

Formal analysis, writing—review and editing. Rahul Mali: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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