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Study on Disinfectant Products Validation Assessment by Use Dilution as per USP 1072 Standard Method

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A B S T R A C T

Cleaning and disinfection assessment is an important part of cGMP in any pharmaceutical industry. To validate the efficacy of disinfectants used in disinfection procedure, in order to reduce surface contaminations, we tested invitro the action of commercial disinfectants. The qualification of procedure was carried out on a screening test in order to measure effectiveness of the test disinfectants. A clean surface becomes easier to disinfect and so the cleaning and disinfection programs complement each other. Disinfection efficacy and validation studies has been carried in accordance with the United States Pharmacopeia <1072> Disinfectants and Antiseptics protocol. The test organisms used include standard strains mentioned in USP <1072>. Standard Use Dilution test protocol was followed, before and after disinfection and the microbial load was assessed to calculate the Log_{10} reduction index. Subsequently, we developed and validated a disinfection procedure on using approximately 10^6–10^7 total colony forming units per test. 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 Log_{10} 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

Microbial contamination of surfaces and environment is a fundamental aspect which requires constant monitoring and control, in order to minimize the contamination of microorganisms. A disinfectant can be defined as a chemical which reduces the number of microorganisms present on a surface. Disinfectants vary in their spectrum of activity, mode of action, and efficacy. The first step of an efficient disinfection program is the choice of disinfectants that guarantee bactericidal, fungicidal, and sporicidal actions, as previously demonstrated by the manufacturer in compliance with United States Pharmacopoeia (USP) 35 Chapter <1072>.

A disinfection efficacy study is part of a manufacturing facility's overall contamination control program and should include these elements - Facility controls to

minimize the potential for contamination through testing raw materials for potential contaminants, flow of personnel and materials, including controlled zones identified by garments or other visual methods, Air handling flow, facility and equipment cleaning and disinfection, Monitoring the manufacturing environment to establish baseline flora, Trending environmental isolates and defining appropriate limits, Validating that the established disinfection procedures provide the expected level of disinfection, Verification that cleaning and disinfection procedures are documented in SOPs and that the procedures that are understood and replicated by all operators (Russell *et al.,* 2004; Sandle, 2014).

The design, validation and implementation of a documented and approved disinfectant programme must form a key part of any pharmaceutical production area qualification. There is significant regulatory interest in this area as it forms a fundamental part of any production facility maintenance schedule (Russell *et al.,* 2004). 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 (USP, 2012; Sandle, 2014).

"Disinfectant validation is the documented verification and implementation of procedures that have been shown to consistently control the range and levels of micro-organisms that may be encountered on the surfaces in a facility" (Sandle, 2017). Our results showed that the proposed test method was efficient for confirming the tested disinfectants' efficacy. Moreover, we observed that all chosen disinfectants used for the validation of our cleaning & 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)* (Sandle, 2017) 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 1.0 x 10^7 CFU/ml using the diluent. For counting of fungal test suspension prepare 1.0 - 1.5 x 10^7 CFU/ml.

Disinfectant

The disinfectants used in this study were In house products - Inficide II 256 (combination of DDAC and ADBAC), Inficide PQ+ (combination of DDAC and PHMB), Inficide LF 125 (combination of ADEBAC and ADBAC), Inficide BSP (Formaldehyde donor, Glutaraldehyde and BKC based), Inficide 150 (Silver Hydrogen peroxide), Inficide PAA (combination of Hydrogen Peroxide and Peracetic acid), Inficide CG+ (combination of Chlorhexidine Gluconate and Cetrimide) and Inficide QAC (BKC based).

Culture Media and Reagents

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

Disinfectant Validation Study

Efficacy 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 (Abraham, 2010).

The method employed for validation is a Use Dilution test method. 9ml of sterile Dey/Engley broth medium is taken in test tubes (number of test tubes may be decided on the basis of disinfectant product/s to be tested). Then 1 mL of diluted disinfectant/s / sanitizing solution is added to the 9 mL of sterile Dey/Engley broth medium.

Allow the tubes to stand for 20 minutes. After 20 minutes, inoculate 0.1 mL of test culture suspension containing NMT 100 CFU of Challenge Organism in respective test tube. After the specified exposure time of 5 and 10 minutes, surviving microorganisms were recovered by drawing an aliquot and neutralizing it.

After neutralization, 1.0ml of neutralized mixture was taken in duplicates and transfer each sample into separate Petri plate containing 12.0 to 15.0ml of 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. Count and determine the number of CFU (Colony Forming Unit) for each plate.

Average count was taken as CFU/ml. The same was ascertained by dilution blank. Test was carried out in duplicate and average count was taken as CFU/ml (Sandle, 2017).

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 three controls was carried out simultaneously as 1) A positive control with no disinfectant. 2) A control to confirm that the neutralizing solution does not affect the bacteria and 3) A control for recovery validation (Rottjakob, 2013; Russell *et al.,* 2004).

Acceptance Criteria

Since microorganisms vary in their susceptibility to disinfection procedures, USP <1072> "Disinfectants and Antiseptics" recommends an expectation of 3 $\log_{10}$ of reduction for enveloped viruses, vegetative bacteria and fungi and ≥2 $\log_{10}$ of reduction for non-enveloped viruses and bacterial spores (Sandle, 2017).

Results and Discussion

The results obtained in this assessment is presented in below tables.

From the results obtained it is observed that all the test disinfectant products at respective dilution concentration showed good efficacy activity against standard test organisms in 5 & 10 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.

The use of disinfectants will always be part of a pharmaceutical and healthcare facility cleaning programme (USP, 2012). 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 (USP, 2017). Disinfection efficacy studies must be customized to each manufacturer's facility and procedures, and these studies can quickly become large and overwhelming (Sandle, 2014). 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 (Sandle, 2017).

In this study 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.

Table.1 Preparation of Disinfectant dilution concentration

Disinfectants	Dilution concentration	Preparation
Inficide II 256	0.4%	4ml in a litre of water
Inficide PQ+	1.0%	10ml in a litre of water
Inficide LF 125	1.5%	15ml in a litre of water
Inficide BSP	2.0%	20ml in a litre of water
Inficide 150	10.0%	100ml in a litre of water
Inficide PAA	1.0%	10ml in a litre of water
Inficide CG+	1.0%	10ml in a litre of water
Inficide QAC	2.5%	25ml in a litre of water

Table.2 The validation results of Inficide II 256 disinfectant at 0.4% concentration

Product	Test Organisms	Exposure Time	Initial count		After Exposure		Log Reduction
			CFU/ml	Log	CFU/ml	Log	
Inficide II 256	*S.aureus*	5 mins.	2.84×10^5	5.45	48	1.68	**3.77**
		10 mins.	2.84×10^5	5.45	< 10	< 1	**>4.45**
	E.coli	5 mins.	1.90×10^5	5.27	16	1.20	**4.07**
		10 mins.	1.90×10^5	5.27	< 10	< 1	**>4.27**
	B.subtilis	5 mins.	2.16×10^5	5.33	25	1.39	**3.87**
		10 mins.	2.16×10^5	5.33	< 10	< 1	**>4.33**
	P.aeruginosa	5 mins.	2.12×10^5	5.32	18	1.25	**4.06**
		10 mins.	2.12×10^5	5.32	< 10	< 1	**>4.33**
	C.albicans	5 mins.	1.84×10^5	5.26	22	1.34	**3.92**
		10 mins.	1.84×10^5	5.26	< 10	< 1	**>4.26**
	A.brasiliensis	5 mins.	1.72×10^5	5.23	32	1.50	**3.73**
		10 mins.	1.72×10^5	5.23	< 10	< 1	**>4.23**

Table.3 The validation results of Inficide PQ+ disinfectant at 1.0% concentration

Product	Test Organisms	Exposure Time	Initial count		After Exposure		Log Reduction
			CFU/ml	Log	CFU/ml	Log	
Inficide PQ+	*S.aureus*	5 mins.	2.84×10^5	5.45	30	1.47	**3.98**
		10 mins.	2.84×10^5	5.45	< 10	< 1	**>4.45**
	E.coli	5 mins.	1.90×10^5	5.27	25	1.39	**3.85**
		10 mins.	1.90×10^5	5.27	< 10	< 1	**>4.27**
	B.subtilis	5 mins.	2.16×10^5	5.33	39	1.59	**3.74**
		10 mins.	2.16×10^5	5.33	< 10	< 1	**>4.33**
	P.aeruginosa	5 mins.	2.12×10^5	5.32	30	1.47	**3.85**
		10 mins.	2.12×10^5	5.32	< 10	< 1	**>4.32**
	C.albicans	5 mins.	1.84×10^5	5.26	12	1.07	**4.18**
		10 mins.	1.84×10^5	5.26	< 10	< 1	**>4.26**
	A.brasiliensis	5 mins.	1.72×10^5	5.23	24	1.38	**3.84**
		10 mins.	1.72×10^5	5.23	< 10	< 1	**>4.23**

Table.4 The validation results of Inficide LF 125 disinfectant at 1.5% concentration

Product	Test Organisms	Exposure Time	Initial count		After Exposure		Log Reduction
			CFU/ml	Log	CFU/ml	Log	
Inficide LF125	*S.aureus*	5 mins.	2.84×10^5	5.45	21	1.32	**4.13**
		10 mins.	2.84×10^5	5.45	< 10	< 1	**>4.45**
	E.coli	5 mins.	1.90×10^5	5.27	27	1.43	**3.84**
		10 mins.	1.90×10^5	5.27	< 10	< 1	**>4.27**
	B.subtilis	5 mins.	2.16×10^5	5.33	26	1.41	**3.91**
		10 mins.	2.16×10^5	5.33	< 10	< 1	**>4.33**
	P.aeruginosa	5 mins.	2.12×10^5	5.32	19	1.27	**4.05**
		10 mins.	2.12×10^5	5.32	< 10	< 1	**>4.32**
	C.albicans	5 mins.	1.84×10^5	5.26	14	1.14	**4.12**
		10 mins.	1.84×10^5	5.26	< 10	< 1	**>4.26**
	A.brasiliensis	5 mins.	1.72×10^5	5.23	11	1.04	**4.19**
		10 mins.	1.72×10^5	5.23	< 10	< 1	**>4.23**

Table.5 The validation results of Inficide BSP disinfectant at 2.0% concentration

Product	Test Organisms	Exposure Time	Initial count		After Exposure		Log Reduction
			CFU/ml	Log	CFU/ml	Log	
Inficide BSP	*S.aureus*	5 mins.	2.84×10^5	5.45	26	1.41	**4.04**
		10 mins.	2.84×10^5	5.45	< 10	< 1	**>4.45**
	E.coli	5 mins.	1.90×10^5	5.27	18	1.25	**4.02**
		10 mins.	1.90×10^5	5.27	< 10	< 1	**>4.27**
	B.subtilis	5 mins.	2.16×10^5	5.33	20	1.30	**4.03**
		10 mins.	2.16×10^5	5.33	< 10	< 1	**>4.33**
	P.aeruginosa	5 mins.	2.12×10^5	5.32	24	1.38	**3.93**
		10 mins.	2.12×10^5	5.32	< 10	< 1	**>4.32**
	C.albicans	5 mins.	1.84×10^5	5.26	11	1.04	**4.22**
		10 mins.	1.84×10^5	5.26	< 10	< 1	**>4.26**
	A.brasiliensis	5 mins.	1.72×10^5	5.23	15	1.17	**4.06**
		10 mins.	1.72×10^5	5.23	< 10	< 1	**>4.23**

Table.6 The validation results of Inficide 150 disinfectant at 10% concentration

Product	Test Organisms	Exposure Time	Initial count		After Exposure		Log Reduction
			CFU/ml	Log	CFU/ml	Log	
Inficide 150	*S.aureus*	5 mins.	2.84×10^5	5.45	12	1.07	**4.38**
		10 mins.	2.84×10^5	5.45	< 10	< 1	**>4.45**
	E.coli	5 mins.	1.90×10^5	5.27	15	1.17	**4.10**
		10 mins.	1.90×10^5	5.27	< 10	< 1	**>4.27**
	B.subtilis	5 mins.	2.16×10^5	5.33	13	1.11	**4.22**
		10 mins.	2.16×10^5	5.33	< 10	< 1	**>4.33**
	P.aeruginosa	5 mins.	2.12×10^5	5.32	12	1.07	**4.25**
		10 mins.	2.12×10^5	5.32	< 10	< 1	**>4.32**
	C.albicans	5 mins.	1.84×10^5	5.26	14	1.14	**4.12**
		10 mins.	1.84×10^5	5.26	< 10	< 1	**>4.26**
	A.brasiliensis	5 mins.	1.72×10^5	5.23	11	1.04	**4.19**
		10 mins.	1.72×10^5	5.23	< 10	< 1	**>4.23**

Table.7 The validation results of Inficide PAA disinfectant at 1% concentration

Product	Test Organisms	Exposure Time	Initial count		After Exposure		Log Reduction
			CFU/ml	Log	CFU/ml	Log	
Inficide PAA	*S.aureus*	5 mins.	2.84 x 10^5	5.45	< 10	< 1	**>4.45**
		10 mins.	2.84 x 10^5	5.45	< 10	< 1	**>4.45**
	E.coli	5 mins.	1.90 x 10^5	5.27	14	1.14	**4.13**
		10 mins.	1.90 x 10^5	5.27	< 10	< 1	**>4.27**
	B.subtilis	5 mins.	2.16 x 10^5	5.33	12	1.07	**4.26**
		10 mins.	2.16 x 10^5	5.33	< 10	< 1	**>4.33**
	P.aeruginosa	5 mins.	2.12 x 10^5	5.32	23	1.36	**3.95**
		10 mins.	2.12 x 10^5	5.32	< 10	< 1	**>4.32**
	C.albicans	5 mins.	1.84 x 10^5	5.26	16	1.20	**4.06**
		10 mins.	1.84 x 10^5	5.26	< 10	< 1	**>4.26**
	A.brasiliensis	5 mins.	1.72 x 10^5	5.23	19	1.27	**3.95**
		10 mins.	1.72 x 10^5	5.23	< 10	< 1	**>4.23**

Table.8 The validation results of Inficide CG+ disinfectant at 1% concentration

Product	Test Organisms	Exposure Time	Initial count		After Exposure		Log Reduction
			CFU/ml	Log	CFU/ml	Log	
Inficide CG+	*S.aureus*	5 mins.	2.84 x 10^5	5.45	31	1.49	**3.95**
		10 mins.	2.84 x 10^5	5.45	< 10	< 1	**>4.45**
	E.coli	5 mins.	1.90 x 10^5	5.27	21	1.32	**3.95**
		10 mins.	1.90 x 10^5	5.27	< 10	< 1	**>4.27**
	B.subtilis	5 mins.	2.16 x 10^5	5.33	25	1.39	**3.94**
		10 mins.	2.16 x 10^5	5.33	< 10	< 1	**>4.33**
	P.aeruginosa	5 mins.	2.12 x 10^5	5.32	33	1.51	**3.81**
		10 mins.	2.12 x 10^5	5.32	< 10	< 1	**>4.32**
	C.albicans	5 mins.	1.84 x 10^5	5.26	19	1.27	**3.99**
		10 mins.	1.84 x 10^5	5.26	< 10	< 1	**>4.26**
	A.brasiliensis	5 mins.	1.72 x 10^5	5.23	14	1.14	**4.09**
		10 mins.	1.72 x 10^5	5.23	< 10	< 1	**>4.23**

Table.9 The validation results of Inficide QAC disinfectant at 2.5% concentration

Product	Test Organisms	Exposure Time	Initial count		After Exposure		Log Reduction
			CFU/ml	Log	CFU/ml	Log	
Inficide QAC	*S.aureus*	5 mins.	2.84 x 10^5	5.45	15	1.17	**4.28**
		10 mins.	2.84 x 10^5	5.45	< 10	< 1	**>4.45**
	E.coli	5 mins.	1.90 x 10^5	5.27	34	1.53	**3.74**
		10 mins.	1.90 x 10^5	5.27	< 10	< 1	**>4.27**
	B.subtilis	5 mins.	2.16 x 10^5	5.33	30	1.47	**3.86**
		10 mins.	2.16 x 10^5	5.33	< 10	< 1	**>4.33**
	P.aeruginosa	5 mins.	2.12 x 10^5	5.32	24	1.38	**3.94**
		10 mins.	2.12 x 10^5	5.32	< 10	< 1	**>4.32**
	C.albicans	5 mins.	1.84 x 10^5	5.26	18	1.25	**4.01**
		10 mins.	1.84 x 10^5	5.26	< 10	< 1	**>4.26**
	A.brasiliensis	5 mins.	1.72 x 10^5	5.23	11	1.04	**4.19**
		10 mins.	1.72 x 10^5	5.23	< 10	< 1	**>4.23**

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.

References

Abraham, Z. (2010). Disinfectant Qualification – A Multifaceted Study, Microbiologics.

Marc, R. (2013), Disinfectant Efficacy Testing for Critical Environments – Presentation, Steris Corporation.

Rottjakob, D. (2013). Disinfection qualification testing—considerations for the aseptic and cleanroom manufacturing environment.

Russell, Hugo & Ayliffe's, (2004), Principles and Practice of Disinfection, Preservation & Sterilization, 4th ed, Blackwell Publishing, chapter 7: 220 – 237.

Sandle, T. (2014) Fungal contamination of pharmaceutical products: the growing menace, *European Pharmaceutical Review,* 19 (1): 68-71.

Sandle, T. (2016), The CDC Handbook: A Guide to Cleaning and Disinfecting Cleanrooms, 2nd Edition, Grosvenor House Publishing: Surrey, UK

Sandle, T. (2017), The European approach to disinfectant qualification, La Vague: 45 – 48.

United States Pharmacopeia Forum USP <1116> Microbiological evaluation of clean rooms and other controlled environmets. 2012

USP General Chapter <1072> "Disinfectants and Antiseptics",(2017):3792-3795.

Wilfried, P. (2004), Directory of Microbicides for the Protection of Materials – A Handbook, Kluwer Academic Publishers.

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