

Disinfectant Efficacy Testing of Imago & Getter Disinfectant on non-porous surfaces using Surface Challenge Test

Sachin D. Joshi, Dr. Imran Memon, Dr. Tahur Shaikh, Idris Khan, Surjeet Samanta
Komal Kumari, Romil Dagha

Imago & Getter, Technical Team, Mumbai, India

Abstract - Disinfectants are used to maintain the bioburden of any facility under limits and making them free from any external or internal pathogenic intervention. The cleanroom area of any pharmaceutical facility comprises of different surface, these surfaces are easier to disinfect and so the cleaning and disinfection programs complement each other. The Disinfection efficacy and validation studies are carried in consistent with the United State Pharmacopeia <1072> Disinfectants and Antiseptics protocol. This study was aimed to generate data to provide a high degree of assurance that the disinfection program will consistently yield results that meet pre-determined specification by using different types of Imagard brand disinfectant. The recommended concentration of all the disinfectant at precise time i.e., 10 minutes showed excellent log reduction against the standard test organisms. The results proved that Disinfectant Imagard HD, Imagard IG PRO 401, Imagard IL 15, Imagard AS 10, Imagard SF 25 and Imagard Plus are effective against the standard test organisms. These data add a layer of product safety and generate confidence in the customer's ability to deal with an unexpected contamination event.

Keywords- Disinfection, Imagard HD, Imagard IG PRO 401, Imagard AS 10, Standard test organisms, United State Pharmacopeia <1072>, bioburden.

I. INTRODUCTION

Disinfectants are chemical agents applied to non-living objects in order to destroy or remove vegetative forms of harmful organisms like bacteria, viruses, fungi, mold or mildews living on the objects. The "active ingredient" in any qualified disinfectant formula is what kills pathogens, usually by disrupting or damaging their cells and by other shock methods. Active ingredients are usually aided by other ingredients with various purposes. Contamination prevention in an aseptic manufacturing facility begins by choosing the most suitable chemical agents for removing environmental (in-house) microorganisms [1].

Obtaining the highest confidence that aseptic, cleanroom, and other critical manufacturing environments are properly cleaned, sanitized and disinfected is paramount in assuring the production of safe and effective pharmaceutical products and medical devices. It is for this reason that the U.S. Food and Drug Administration (USFDA) requires complying manufacturers to qualify and validate the disinfection procedures used in their respective manufacturing environment [2]. A disinfection efficacy study is part of any pharmaceutical manufacturing facility's overall contamination control

program which should include elements such as primarily determination of raw material's quality, the integrity of the manufacturing process, verification of proper cleaning and disinfection procedures and should be documented in SOPs, the procedures should be understood and replicated by all operators and personnel [3]. Disinfectant efficacy testing is concerned with demonstrating that a product possesses antimicrobial activity under defined laboratory test conditions. Designing validation, implementation of documents and approved disinfectant programme must form basis of any pharmaceutical production area qualification [4]. The efficacy of disinfectants can be affected by a number of factors including pH, temperature, organic soiling, water hardness and several dilutions [5].

As per principal by the European Committee for Standardization (CEN/TC 216), the disinfectants should be tested in several stages like preliminary suspension tests to verify whether a product deserves the qualification 'disinfectant', and tests on surfaces that mimic practical conditions [6]. Hence surface challenge test are widely considered for disinfection efficacy tests. This paper is intended to provide an overview of disinfection efficacy testing, standards test, guidelines and highlight their significance within the pharmaceutical industry,

healthcare etc for considerations that must be addressed when designing and executing these studies.

II. MATERIALS AND METHODS

1. Preparation of Disinfectant concentration:

The disinfectants were obtained from Imago & Getter, Mumbai. Imagard HD was diluted as 100ml in 900ml of Deionised water to obtain 10%, Imagard IG PRO 401 was diluted as 4ml in 1 litre of Deionised water to obtain 0.4%, Imagard AS 10 was diluted as 10ml in 1 litre of Deionised water to obtain 1.0%, Imagard IL 15 was diluted as 15ml in 1 litre of Deionised water to obtain 1.5%, Imagard SF 25 was diluted as 15ml in 1 litre of Deionised water to obtain 1.5%, Imagard plus was diluted as 20ml in 1 litre of Deionised water to obtain 2.0%.

2. Test Organism and its Suspension:

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) [7] were obtained from National Collection of Industrial Microorganisms (NCIM), Pune, India. Suspension of each of the test organisms was made by collecting a loopful of colony from each plate and inoculating in 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 1.0×10^7 CFU/ml using the diluent. For counting of fungal test suspension prepare $1.0 - 1.5 \times 10^7$ CFU/ml.

3. Surface coupons used

Coupons of size 5cm × 5cm (2" × 2") are used in disinfection efficacy studies which are mimic representative of facility actual surfaces. According to guidelines it is important that the coupons are representative of the surfaces in respective facility. The type of surfaces as well as the condition of surfaces should be representative. The most typical surfaces include Stainless Steel 316, Epoxy coated, Glass, PU, Vinyl flooring or curtain, Fibreglass, plastics and Terrazzo tiles [7].

4. Disinfection Efficacy Study:

This testing was done according to USP <1072> Guideline for the Surface Challenge Test [8]. The disinfectants were diluted as per concentration recommendation for cleanroom and qualified areas which was kept at room temperature. Add 0.1 ml of the challenge inoculum (containing around 10^7 CFU/ml) of *Bacillus subtilis* suspension to all coupons; one coupon will serve as positive control or initial count. Allow the culture suspension to dry on the surface of the coupons.

Use the method of application to be used on the surfaces of coupons (such as mopping by a wet mop or spraying) for the selected disinfectant agent as per the manufacturer recommended concentration. Allow to stand for 10 minutes. After contact time, take the sample of the culture surfaces of positive control coupon of each type of surfaces with sterile swab. Transfer the swab to 10 mL of Dey/Engley broth. Vortex the tubes containing swab for about 30 seconds. Perform serial dilution up to 10^{-3} in 9 mL of Dey/Engley broth. Arrange the sterile filter holders having 0.45 µ membrane filters on the manifold and assemble the manifold to the vacuum source.

Filter 1 ml of each culture dilution and rinse the membrane with 1 x 100 ml of the sterile 0.1% peptone water. After rinsing, place each membrane filter on the surfaces of individual pre-incubated sterile Tryptone Soya Agar plates with neutralizers. Incubate the plate at 30-35°C for 3 days for bacteria and at 20-25° C for 5 days for yeast and fungi. Similarly follow the above step for all challenged organisms. Keep one contact plate of Tryptone Soya Agar plate as negative control. For positive control take the sample of the culture surfaces of positive control coupon of each type of surfaces with sterile swab.

Transfer the swab to 10 mL of Dey/Engley broth. Vortex the tubes containing swab for about 30 seconds. Perform serial dilution up to 10^{-3} in 9 mL of Dey/Engley broth. After incubation, examine the plates and count the no. of colonies (CFU) on each plate [8] [9]. Calculate the Log reduction

$$\text{Final Log Reduction} = \text{Log (Initial Count)} - \text{Log (Final count)}.$$

5. 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 [7].

III. RESULTS AND DISCUSSION

The results obtained in this study for the disinfectant Imagard HD, Imagard IG PRO 401, Imagard IL 15, Imagard AS 10, Imagard SF 25 and Imagard Plus on the various test microorganisms are shown in respective tables:

Table I: The validation results of Imagard HD disinfectant (10 %).

Table 1: The validation results of Imagard HD disinfectant (10 %)

Sample Identification	Test Organism	Exposure time	Antimicrobial activity (Log Reduction)						
			SS 316	Epoxy	PU	Glass	Vinyl	Terrazzo tiles	Plastic
Imagard HD	<i>E. coli</i>	10 min	>4.34	>4.35	>4.23	>4.11	>4.12	>4.14	>4.21
	<i>S. aureus</i>		>4.27	>4.21	>4.32	>4.24	>4.27	>4.23	>4.21
	<i>B. subtilis</i>		>4.44	>4.40	>4.39	>4.42	>4.41	>4.42	>4.46
	<i>A. baumannii</i>		>4.08	>4.11	>4.02	>4.13	>4.06	>4.04	>4.30
	<i>C. albicans</i>		>4.34	>4.17	>4.09	>4.15	>4.10	>4.11	>4.12
	<i>P. aeruginosa</i>		>4.29	>4.31	>4.30	>4.27	>4.23	>4.32	>4.27

Table II: The validation results of Imagard IG PRO 401 disinfectant (0.4 %).

Table 2: The validation results of Imagard IG PRO 401 disinfectant (0.4 %)

Sample Identification	Test Organism	Exposure time	Antimicrobial activity (Log Reduction)						
			SS 316	Epoxy	PU	Glass	Vinyl	Terrazzo tiles	Plastic
Imagard IG PRO 401	<i>E. coli</i>	10 min	>4.44	>4.20	>4.20	>4.11	>4.14	>4.14	>4.16
	<i>S. aureus</i>		>4.71	>4.21	>4.38	>4.64	>4.24	>4.21	>4.40
	<i>B. subtilis</i>		>4.54	>4.35	>4.35	>4.42	>4.42	>4.32	>4.63
	<i>A. baumannii</i>		>4.33	>4.22	>4.50	>4.53	>4.64	>4.54	>4.17
	<i>C. albicans</i>		>4.30	>4.19	>4.09	>4.15	>4.21	>4.20	>4.19
	<i>P. aeruginosa</i>		>4.24	>4.31	>4.50	>4.27	>4.12	>4.42	>4.30

Table III: The validation results of Imagard IL 15 disinfectant (1.5%)

Table 3: The validation results of Imagard IL 15 disinfectant (1.5%)

Sample Identification	Test Organism	Exposure time	Antimicrobial activity (Log Reduction)						
			SS 316	Epoxy	PU	Glass	Vinyl	Terrazzo tiles	Plastic
Imagard IL 15	<i>E. coli</i>	10 min	>4.25	>4.28	>4.27	>4.21	>4.22	>4.28	>4.32
	<i>S. aureus</i>		>4.29	>4.25	>4.38	>4.34	>4.29	>4.26	>4.31
	<i>B. subtilis</i>		>4.32	>4.39	>4.35	>4.22	>4.31	>4.32	>4.36
	<i>A. baumannii</i>		>4.02	>4.01	>4.06	>4.03	>4.07	>4.03	>4.06
	<i>C. albicans</i>		>4.11	>4.04	>4.10	>4.13	>4.08	>4.12	>4.13
	<i>P. aeruginosa</i>		>4.21	>4.21	>4.20	>4.23	>4.20	>4.28	>4.20

Table IV: The validation results of Imagard AS 10 disinfectant (1.0%).

Table 4: The validation results of Imagard AS 10 disinfectant (1.0%)

Sample Identification	Test Organism	Exposure time	Antimicrobial activity (Log Reduction)						
			SS 316	Epoxy	PU	Glass	Vinyl	Terrazzo tiles	Plastic
Imagard AS 10	<i>E. coli</i>	10 min	>4.33	>4.15	>4.14	>4.16	>4.11	>4.17	>4.12
	<i>S. aureus</i>		>4.28	>4.26	>4.24	>4.20	>4.21	>4.25	>4.27
	<i>B. subtilis</i>		>4.31	>4.32	>4.30	>4.33	>4.31	>4.34	>4.32
	<i>A. baumannii</i>		>4.04	>4.06	>4.01	>4.07	>4.02	>4.07	>4.09
	<i>C. albicans</i>		>4.13	>4.12	>4.16	>4.11	>4.12	>4.09	>4.13
	<i>P. aeruginosa</i>		>4.32	>4.35	>4.37	>4.30	>4.34	>4.33	>4.35

Table V: The validation results of Imagard SF 25 disinfectant (2.5%).

Table 5: The validation results of Imagard SF 25 disinfectant (2.5%)

Sample Identification	Test Organism	Exposure time	Antimicrobial activity (Log Reduction)						
			SS 316	Epoxy	PU	Glass	Vinyl	Terrazzo tiles	Plastic
Imagard SF 25	<i>E. coli</i>	10 min	>4.23	>4.18	>4.24	>4.21	>4.22	>4.20	>4.24
	<i>S. aureus</i>		>4.35	>4.27	>4.23	>4.15	>4.21	>4.24	>4.26
	<i>B. subtilis</i>		>4.32	>4.35	>4.30	>4.34	>4.36	>4.32	>4.34
	<i>A. baumannii</i>		>4.12	>4.17	>4.13	>4.17	>4.16	>4.11	>4.15
	<i>C. albicans</i>		>4.19	>4.14	>4.18	>4.16	>4.21	>4.20	>4.17
	<i>P. aeruginosa</i>		>4.30	>4.37	>4.30	>4.38	>4.33	>4.39	>4.34

Table VI: The validation results of Imagard Plus disinfectant (2%).

Table 6: The validation results of Imagard Plus disinfectant (2%)

Sample Identification	Test Organism	Exposure time	Antimicrobial activity (Log Reduction)						
			SS 316	Epoxy	PU	Glass	Vinyl	Terrazzo tiles	Plastic
Imagard Plus	<i>E. coli</i>	10 min	>4.24	>4.30	>4.28	>4.26	>4.27	>4.25	>4.25
	<i>S. aureus</i>		>4.35	>4.32	>4.34	>4.37	>4.30	>4.38	>4.29
	<i>B. subtilis</i>		>4.28	>4.20	>4.29	>4.22	>4.21	>4.24	>4.26
	<i>A. baumannii</i>		>4.01	>4.03	>4.04	>4.03	>4.05	>4.02	>4.06
	<i>C. albicans</i>		>4.12	>4.15	>4.17	>4.11	>4.13	>4.14	>4.10
	<i>P. aeruginosa</i>		>4.22	>4.26	>4.20	>4.24	>4.26	>4.21	>4.23

From the results obtained it is observed that the Imagard products from Imago & Getter gave more than log 4 reduction at contact time of 10 minutes. Therefore, this indicates that all the test disinfectants have excellent antimicrobial efficacy at recommended concentration and time on test surfaces. The use of all the mentioned disinfectants may be means to reduce the contamination caused by the test microorganisms.

IV. CONCLUSION

The use of disinfectants will always be part of a pharmaceutical and healthcare facility cleaning programme [10]. 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 [5]. Disinfection efficacy studies must be customized to each manufacturer's facility and procedures, and these studies can quickly become large and overwhelming [11]. 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 & support and advice is widely available to ensure that it is performed to a satisfactory standard. The data generated in this study 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.

REFERENCES

- [1]. Rottjakob, D. (2013). Disinfection qualification testing—considerations for the aseptic and cleanroom manufacturing environment.
- [2]. Abraham, Z. (2010). Disinfectant Qualification – A Multifaceted Study, Microbiologics.
- [3]. Sandle, T. (2016), The CDC Handbook: A Guide to Cleaning and Disinfecting Cleanrooms, 2nd Edition, Grosvenor House Publishing: Surrey, UK
- [4]. Determination of the effectiveness of disinfectants used in the aseptic areas of, Technological University of Havana.
- [5]. Sandle, T. (2017), The European approaches to disinfectant qualification, La Vague: 45 – 48.
- [6]. Van Klinger, B., Koller, W., Bloomfield, S. F., Böhm, R., Cremieux, A., Holah, J. & Rödder, H. J. (1998). Assessment of the efficacy of disinfectants on surfaces. *International biodeterioration & biodegradation*, 41(3-4), 289-296.
- [7]. USP General Chapter <1072> “Disinfectants and Antiseptics”, (2017): 3792-3795.
- [8]. Peleazar, J.M., Chan, E.C.S. and Krieg, R.N. (2006), *Microbiology*, New Delhi: 5th ed, Tata McGraw-Hill publishing Company Ltd., 469-490.
- [9]. Severs, Y. D. and Lamontagne, M. C. (2002), A literature review of disinfectants, Technical Report, Defence R&D Canada – Toronto.
- [10]. Sandle, T. (2014) Fungal contamination of pharmaceutical products: the growing menace, *European Pharmaceutical Review*, 19 (1): 68-71.
- [11]. Vina, P., Rubio, S. and Sandle, T. (2011), „Selection and Validation of Disinfectants“, in Saghee, M. R., Sandle, T and Tidswell, E. C. (Eds.) (2011), *Microbiology and Sterility Assurance in Pharmaceuticals and Medical devices*, New Delhi: Business Horizon: 219 – 236.