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Study to Evaluate Efficacy and Validation Test of Imagard ID 401 and Imagard BIQUAT Disinfectant by USP 1072

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Abstract: ***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 Imagard ID 401 or Imagard BIQUAT or both disinfectants. A clean surface is easier to disinfect and so the cleaning and disinfection programs complement each other. Disinfection efficacy and validation studies are carried in consistent with the United State Pharmacopeia <1072> Disinfectants and Antiseptics protocol. The test organisms used include standard strains mentioned in USP <1072> [8]. Two different concentrations of both disinfectants showed excellent log reduction against the test organisms at different time interval. The results proved that Disinfectant Imagard ID 401 and Imagard BIQUAT are effective against the standard test organisms and stable for 7 days after dilution. 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 ID 401, Imagard BIQUAT, United State Pharmacopeia <1072>, effective, log reduction, contamination

1. Introduction

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 [5] [6]. 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 [3]. 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] [5].

"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" [8].
In this paper we have discussed the standards test, guidelines and highlight their significance within the pharmaceutical industry, healthcare etc. The tasks that should be considered in order to validate the disinfectant products and cleaning programme are outlined.

2. Materials and Methods

Disinfectant
Imagard ID 401 (combination of DDAC and ADBAC) and Imagard BIQUAT (combination of DDAC and PHMB) product was obtained from IMAGO & GETTER, Mumbai.

Preparation of Disinfectant concentration
Imagard ID-401 was diluted as 4ml in 1 litre and 8ml in 1 litre of Deionised water to obtain 0.4% and 0.8% respectively.
Imagard BIQUAT was diluted as 10ml in 1 litre and 15ml in 1 litre of Deionised water to obtain 1.0% and 1.5% respectively.

Reagents
Dey/Engley (D/E) broth, 0.1% peptone water, Normal Saline (0.85% of sodium chloride solution), Phosphate Buffer Solution pH -7.0.

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 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.

Surfaces

5cm × 5cm (2" × 2") coupons of representative facility surfaces are used in disinfection efficacy studies. It is important that the coupons are representative of the surfaces in the facility. The types of surfaces as well as the condition of the surfaces should be representative.

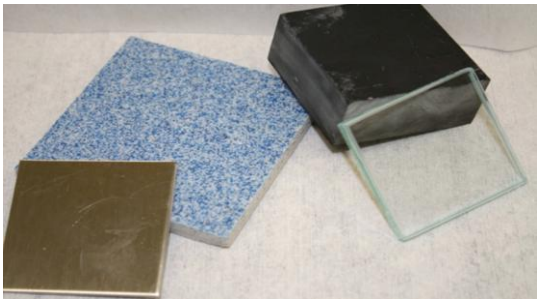

Figure 1: Different Surface material for testing

Typical surfaces include SS 316, Glass, Epoxy coated, PU, Fibreglass, Vinyl flooring or curtain, Terrazzo tiles, plastics etc.[8]

Disinfection Efficacy Study

Efficacy testing is one of the key steps in the disinfectant validation process. Typical disinfection efficacy studies involve replication of the surface disinfection procedure at small scale to verify the clearance of spiked culture suspensions. The culture suspension is dried onto a small coupon of a surface material (in duplicate) that is representative of surfaces in the manufacturing facility. The disinfectant is applied to one of the coupon mimicking the procedure used in the facility, and any remaining culture is neutralised and recovered and quantitated as CFU/ml using spread plate or pour plate assay [8] [10]. A general purpose or broad spectrum efficacy is claimed if the disinfectant exhibit activity against a range of Gram positive & Gram negative bacteria, yeast and mold. Whereas a high level efficacy is claimed if the disinfectant exhibit activity of bactericidal, fungicidal, tuberculocidal and sporicidal [11].

Disinfectant Validation Study

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 [1].

The method employed for validation is a suspension test method. Take 10ml of diluted disinfectant product of concentration 0.4% and 0.8% of Imagard ID 401 & 1.0% and 1.5% of Imagard BIQUAT respectively in a container kept at room temperature. Aliquot was drawn from all the dilution on test start day (Day 1) and was inoculated with 0.1ml of test organism (approximately 10^7 CFU/ml). After the specified exposure time of 2, 5 and 10 minutes, surviving microorganisms were recovered by drawing an aliquot and neutralizing it. After neutralization, take 1.0ml of neutralized mixture in duplicates and transfer each sample into separate Petri plate containing 12.0 to 15.0ml of Soyabean Casien Digest Agar (SCDA) cooled at 42°C±2°C 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 also the same test procedure is carried for all dilutions after 168 hours (i.e. 7 days) and average count was taken as CFU/ml [8]. 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 [2] [7].

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 [8].

3. Results and Discussion

The results obtained in this study for the disinfectant Imagard ID 401 at 0.4% on the various test microorganisms for Day 1 and after 168 hours (i.e. 7 days) is presented in Table 1 and Table 2.

Table 1: The validation results of Imagard ID 401 disinfectant on Day 1 (at 0.4% dilution)

Product Identification	Test Organisms	Exposure Time	Initial count		After Exposure		Log Reduction
			CFU/ml	Log	CFU/ml	Log	
Imagard ID 401	S.aureus	2 mins.	1.55 x 10^5	5.19	1.16 x 10^2	2.06	3.13
		5 mins.	1.55 x 10^5	5.19	32	1.50	3.69
		10 mins.	1.55 x 10^5	5.19	< 10	< 1	>4.19
	E.coli	2 mins.	2.75 x 10^5	5.43	2.64 x 10^2	2.42	3.01
		5 mins.	2.75 x 10^5	5.43	30	1.47	3.96
		10 mins.	2.75 x 10^5	5.43	< 10	< 1	>4.43
	B.subtilis	2 mins.	1.86 x 10^5	5.26	1.72 x 10^2	2.23	3.03
		5 mins.	1.86 x 10^5	5.26	33	1.51	3.75
		10 mins.	1.86 x 10^5	5.26	< 10	< 1	>4.26
	P.aeruginosa	2 mins.	2.06 x 10^5	5.31	1.84 x 10^2	2.26	3.05

		5 mins.	2.06×10^5	5.31	19	1.27	4.04
		10 mins.	2.06×10^5	5.31	< 10	< 1	>4.31
	C.albicans	2 mins.	2.10×10^5	5.32	2.60×10^2	2.41	2.91
		5 mins.	2.10×10^5	5.32	41	1.61	3.71
		10 mins.	2.10×10^5	5.32	< 10	< 1	>4.32
	A.brasiliensis	2 mins.	1.92×10^5	5.28	1.44×10^2	2.15	3.13
		5 mins.	1.92×10^5	5.28	22	1.34	3.94
		10 mins.	1.92×10^5	5.28	< 10	< 1	>4.28

Table 2: The validation results of Imagard ID 401 disinfectant after 168 hours (at 0.4% dilution)

Product Identification	Test Organisms	Exposure Time	Initial count		After Exposure		Log Reduction
			CFU/ml	Log	CFU/ml	Log	
Imagard ID 401	S.aureus	2 mins.	1.48×10^5	5.17	1.20×10^2	2.07	3.10
		5 mins.	1.48×10^5	5.17	48	1.68	3.49
		10 mins.	1.48×10^5	5.17	< 10	< 1	>4.17
	E.coli	2 mins.	2.84×10^5	5.45	2.80×10^2	2.44	3.01
		5 mins.	2.84×10^5	5.45	36	1.55	3.90
		10 mins.	2.84×10^5	5.45	< 10	< 1	>4.45
	B.subtilis	2 mins.	2.04×10^5	5.30	1.84×10^2	2.26	3.04
		5 mins.	2.04×10^5	5.30	40	1.60	3.70
		10 mins.	2.04×10^5	5.30	< 10	< 1	>4.30
	P.aeruginosa	2 mins.	1.96×10^5	5.29	1.76×10^2	2.24	3.05
		5 mins.	1.96×10^5	5.29	30	1.47	3.82
		10 mins.	1.96×10^5	5.29	< 10	< 1	4.29
	C.albicans	2 mins.	2.00×10^5	5.30	2.46×10^2	2.39	2.91
		5 mins.	2.00×10^5	5.30	44	1.64	3.66
		10 mins.	2.00×10^5	5.30	< 10	< 1	>4.30
	A.brasiliensis	2 mins.	1.72×10^5	5.23	1.66×10^2	2.22	3.01
		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

The results obtained for the disinfectant Imagard ID 401 at 0.8% on the various test micro-organisms for Day 1 and after 168 hours (i.e.7 days) is presented in Table 3 and Table 4.

T**able 3:** The validation results of Imagard ID 401 disinfectant on Day 1 (at 0.8% dilution)

Product Identification	Test Organisms	Exposure Time	Initial count		After Exposure		Log Reduction
			CFU/ml	Log	CFU/ml	Log	
Imagard ID 401	S.aureus	2 mins.	1.55×10^5	5.19	1.04×10^2	2.01	3.18
		5 mins.	1.55×10^5	5.19	20	1.30	3.89
		10 mins.	1.55×10^5	5.19	< 10	< 1	>4.19
	E.coli	2 mins.	2.75×10^5	5.43	2.44×10^2	2.38	3.05
		5 mins.	2.75×10^5	5.43	25	1.39	4.04
		10 mins.	2.75×10^5	5.43	< 10	< 1	>4.43
	B.subtilis	2 mins.	1.86×10^5	5.26	1.22×10^2	2.08	3.18
		5 mins.	1.86×10^5	5.26	18	1.25	4.01
		10 mins.	1.86×10^5	5.26	< 10	< 1	>4.26
	P.aeruginosa	2 mins.	2.06×10^5	5.43	1.54×10^2	2.18	3.25
		5 mins.	2.06×10^5	5.43	16	1.20	4.23
		10 mins.	2.06×10^5	5.43	< 10	< 1	4.43
	C.albicans	2 mins.	2.10×10^5	5.31	2.02×10^2	2.30	3.01
		5 mins.	2.10×10^5	5.31	30	1.47	3.84
		10 mins.	2.10×10^5	5.31	< 10	< 1	>4.31
	A.brasiliensis	2 mins.	1.92×10^5	5.28	1.16×10^2	2.06	3.22
		5 mins.	1.92×10^5	5.28	14	1.14	4.14
		10 mins.	1.92×10^5	5.28	< 10	< 1	>4.28

Table 4: The validation results of Imagard ID 401 disinfectant after 168 hours (at 0.8% dilution)

Product Identification	Test Organisms	Exposure Time	Initial count		After Exposure		Log Reduction
			CFU/ml	Log	CFU/ml	Log	
Imagard ID 401	S.aureus	2 mins.	1.48×10^5	5.17	1.28×10^2	2.10	3.07
		5 mins.	1.48×10^5	5.17	39	1.59	3.58
		10 mins.	1.48×10^5	5.17	< 10	< 1	>4.17
	E.coli	2 mins.	2.84×10^5	5.45	2.78×10^2	2.44	3.01
		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
	B.subtilis	2 mins.	2.04×10^5	5.30	1.64×10^2	2.21	3.09

		5 mins.	2.04×10^5	5.30	32	1.50	3.80
		10 mins.	2.04×10^5	5.30	< 10	< 1	>4.30
	P.aeruginosa	2 mins.	1.96×10^5	5.29	1.44×10^2	2.15	3.14
		5 mins.	1.96×10^5	5.29	25	1.39	3.90
		10 mins.	1.96×10^5	5.29	< 10	< 1	4.29
	C.albicans	2 mins.	2.00×10^5	5.30	2.04×10^2	2.30	3.00
		5 mins.	2.00×10^5	5.30	35	1.54	3.76
		10 mins.	2.00×10^5	5.30	< 10	< 1	>4.30
	A.brasiliensis	2 mins.	1.72×10^5	5.23	1.12×10^2	2.04	3.19
		5 mins.	1.72×10^5	5.23	17	1.23	4.00
		10 mins.	1.72×10^5	5.23	< 10	< 1	>4.23

From the results obtained it is observed that test product as Imagard ID 401 has shown 4 log reduction on Day 1 and after 168 hours at 0.4% and 0.8% dilution against standard test organisms in 10 minutes. Therefore, this indicates that disinfectant Imagard ID 401 has excellent antimicrobial efficacy at both the concentration.

Also the results obtained in this study for the disinfectant Imagard BIQUAT at 1.0% on the various test microorganisms for Day 1 and after 168 hours (i.e. 7 days) is presented in Table 5 and Table 6.

Table 5: The validation results of Imagard BIQUAT disinfectant on Day 1 (at 1.0% dilution)

Product Identification	Test Organisms	Exposure Time	Initial count		After Exposure		Log Reduction
			CFU/ml	Log	CFU/ml	Log	
Imagard BIQUAT	S.aureus	2 mins.	1.64×10^5	5.21	1.54×10^2	2.18	3.03
		5 mins.	1.64×10^5	5.21	36	1.55	3.66
		10 mins.	1.64×10^5	5.21	12	1.07	4.14
	E.coli	2 mins.	2.26×10^5	5.35	2.01×10^2	2.30	3.05
		5 mins.	2.26×10^5	5.35	33	1.51	3.84
		10 mins.	2.26×10^5	5.35	< 10	< 1	>4.35
	B.subtilis	2 mins.	2.40×10^5	5.38	2.20×10^2	2.34	3.04
		5 mins.	2.40×10^5	5.38	39	1.59	3.79
		10 mins.	2.40×10^5	5.38	< 10	< 1	>4.38
	P.aeruginosa	2 mins.	1.56×10^5	5.19	1.66×10^2	2.22	2.97
		5 mins.	1.56×10^5	5.19	38	1.57	3.62
		10 mins.	1.56×10^5	5.19	15	1.17	4.02
	C.albicans	2 mins.	2.14×10^5	5.33	1.96×10^2	2.29	3.04
		5 mins.	2.14×10^5	5.33	44	1.64	3.69
		10 mins.	2.14×10^5	5.33	< 10	< 1	>4.33
	A.brasiliensis	2 mins.	1.48×10^5	5.17	$1.24x\ 10^2$	2.09	3.08
		5 mins.	1.48×10^5	5.17	26	1.41	3.76
		10 mins.	1.48×10^5	5.17	< 10	< 1	>4.17

Table 6: The validation results of Imagard BIQUAT disinfectant after 168 hours (at 1.0% dilution)

Product Identification	Test Organisms	Exposure Time	Initial count		After Exposure		Log Reduction
			CFU/ml	Log	CFU/ml	Log	
Imagard BIQUAT	S.aureus	2 mins.	1.32×10^5	5.12	1.26×10^2	2.10	3.02
		5 mins.	1.32×10^5	5.12	41	1.61	3.51
		10 mins.	1.32×10^5	5.12	< 10	< 1	>4.12
	E.coli	2 mins.	1.64×10^5	5.21	1.62×10^2	2.20	3.01
		5 mins.	1.64×10^5	5.21	38	1.57	3.64
		10 mins.	1.64×10^5	5.21	< 10	< 1	>4.21
	B.subtilis	2 mins.	1.40×10^5	5.14	1.24×10^2	2.09	3.05
		5 mins.	1.40×10^5	5.14	26	1.41	3.73
		10 mins.	1.40×10^5	5.14	< 10	< 1	>4.14
	P.aeruginosa	2 mins.	1.90×10^5	5.27	1.80×10^2	2.25	3.02
		5 mins.	1.90×10^5	5.27	37	1.56	3.71
		10 mins.	1.90×10^5	5.27	< 10	< 1	>4.27
	C.albicans	2 mins.	1.76×10^5	5.34	1.95×10^2	2.29	3.05
		5 mins.	1.76×10^5	5.34	47	1.67	3.67
		10 mins.	1.76×10^5	5.34	12	1.07	4.27
	A.brasiliensis	2 mins.	1.08×10^5	5.03	1.03×10^2	2.01	3.02
		5 mins.	1.08×10^5	5.03	31	1.49	3.54
		10 mins.	1.08×10^5	5.03	< 10	< 1	>4.03

The results obtained for the disinfectant Imagard BIQUAT at 1.5% on the various test micro-organisms for Day 1 and after 168 hours (i.e.7 days) is presented in Table 7 and Table 8.

Table 7: The validation results of Imagard BIQUAT disinfectant on Day 1 (at 1.5% dilution)

Product Identification	Test Organisms	Exposure Time	Initial count		After Exposure		Log Reduction
			CFU/ml	Log	CFU/ml	Log	
Imagard BIQUAT	S.aureus	2 mins.	1.64×10^5	5.21	1.20×10^2	2.07	3.15
		5 mins.	1.64×10^5	5.21	28	1.44	3.77
		10 mins.	1.64×10^5	5.21	< 10	< 1	>4.21
	E.coli	2 mins.	2.26×10^5	5.35	1.94×10^2	2.28	3.07
		5 mins.	2.26×10^5	5.35	29	1.46	3.89
		10 mins.	2.26×10^5	5.35	< 10	< 1	>4.35
	B.subtilis	2 mins.	2.40×10^5	5.38	2.10×10^2	2.31	3.07
		5 mins.	2.40×10^5	5.38	32	1.50	3.88
		10 mins.	2.40×10^5	5.38	< 10	< 1	>4.38
	P.aeruginosa	2 mins.	1.56×10^5	5.19	1.40×10^2	2.14	3.05
		5 mins.	1.56×10^5	5.19	18	1.25	3.94
		10 mins.	1.56×10^5	5.19	< 10	< 1	>4.19
	C.albicans	2 mins.	2.14×10^5	5.33	1.90×10^2	2.27	3.06
		5 mins.	2.14×10^5	5.33	36	1.55	3.78
		10 mins.	2.14×10^5	5.33	< 10	< 1	>4.33
	A.brasiliensis	2 mins.	1.48×10^5	5.17	1.04×10^2	2.01	3.16
		5 mins.	1.48×10^5	5.17	19	1.27	3.90
		10 mins.	1.48×10^5	5.17	< 10	< 1	>4.17

Table 8: The validation results of Imagard BIQUAT disinfectant after 168 hours (at 1.5% dilution)

Product Identification	Test Organisms	Exposure Time	Initial count		After Exposure		Log Reduction
			CFU/ml	Log	CFU/ml	Log	
Imagard BIQUAT	S.aureus	2 mins.	1.32×10^5	5.12	1.10×10^2	2.04	3.08
		5 mins.	1.32×10^5	5.12	38	1.57	3.55
		10 mins.	1.32×10^5	5.12	< 10	< 1	>4.12
	E.coli	2 mins.	1.64×10^5	5.21	1.80×10^2	2.25	2.96
		5 mins.	1.64×10^5	5.21	33	1.51	3.70
		10 mins.	1.64×10^5	5.21	< 10	< 1	>4.21
	B.subtilis	2 mins.	1.40×10^5	5.14	1.24×10^2	2.09	3.05
		5 mins.	1.40×10^5	5.14	26	1.41	3.73
		10 mins.	1.40×10^5	5.14	< 10	< 1	>4.14
	P.aeruginosa	2 mins.	1.90×10^5	5.27	1.56×10^2	2.19	3.08
		5 mins.	1.90×10^5	5.27	35	1.54	3.73
		10 mins.	1.90×10^5	5.27	< 10	< 1	>4.27
	C.albicans	2 mins.	1.76×10^5	5.34	1.46×10^2	2.16	3.18
		5 mins.	1.76×10^5	5.34	40	1.60	3.74
		10 mins.	1.76×10^5	5.34	< 10	< 1	>4.34
	A.brasiliensis	2 mins.	1.08×10^5	5.03	1.05×10^2	2.02	3.01
		5 mins.	1.08×10^5	5.03	24	1.38	3.65
		10 mins.	1.08×10^5	5.03	< 10	< 1	>4.03

From the results obtained it is observed that test product as Imagard BIQUAT has shown 4 log reduction on Day 1 and after 168 hours at 1.0% and 1.5% dilution against standard test organisms in 10 minutes. Therefore, this indicates that disinfectant Imagard BIQUAT has excellent antimicrobial efficacy at both the concentration. The use of Imagard ID 401 and Imagard BIQUAT disinfectants may be means to reduce the contamination caused by the test microorganisms.

4. Conclusion

The use of disinfectants will always be part of a pharmaceutical and healthcare facility cleaning programme [6]. 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 [4]. Disinfection efficacy studies must be customized to each manufacturer's facility and procedures, and these studies can quickly become large and overwhelming [10].

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 study have been reviewed and found acceptable by regulatory bodies [9]. 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.

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