Analysis Report

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924980

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Item:	Analysis of disinfection of surfaces using a UVC-box according DS/EN 13697:2015 + A1:2019, modified version.
Period:	Test performed: 7 – 18 May 2019
Storage:	The test material will be destroyed after 3 months, unless otherwise agreed in writing.
Test results:	The results of the analysis and the method(s) used concern only the sample(s) analysed or the sub-sample(s) selected for analysis.
Terms:	This analysis was carried out in accordance with Danish Technological Institute's General Terms and Conditions regarding Commissioned Work Accepted by Danish Technological Institute. The test results solely apply to the tested item. This analysis report may be quoted in extract only if the Laboratory for Chemistry and Microbiology has granted its written consent.
Date/place:	25 May 2020 Danish Technological Institute, Aarhus Laboratory for Chemistry and Microbiology
Signature:	Digitally signed by: Helle Stendahl Andersen Date: 2020.05.25 16:21:12 +02'00' Helle Stendahl Andersen Business Manager

Introduction

The efficacy of treatment of a surface contaminated with bacteria using UVC-light in an UVC-box was tested according to a modified version of DS/EN 13697:2015 + A1:2019.

When tested in accordance with the method and under the required test conditions, the products shall demonstrate ≥log 4 reductions in cfu counts of the tested bacteria.

Test procedure

For each test organism, the test suspension was mixed with an inhibitory substance (organic material) and transferred to a stainless-steel surface and dried at 37°C until visibly dry.
The surface with the dried bacteria was placed in the UVC-box for 2 min.

The stainless-steel plate was subsequently washed to recover the bacteria. The number of surviving microorganisms was quantitated and compared with a drying sample where discs with dried bacteria had been placed at room temperature.

The bacteria were chosen according to the obligatory bacteria for the medical area according to EN 16615:2017 and EN 17272:2020.

Experimental conditions

Test organisms: *For use in the medical area*	*Pseudomonas aeruginosa* ATCC 15442 *Staphylococcus aureus* ATCC 6538 *Enterococcus hirae* ATCC 10541 *Acinetobacter baumanii* ATCC 19606
Extra bacteria strain	*Salmonella typhimurum (enterica)* ATCC 13311
Contact time:	2 min. ± 10sec.
Test temperature:	Room temperature (20-25°C)
Incubation of test organisms:	(37 ± 1) °C for 48 hours
Interfering substances	simulated clean conditions 0.3 g/L bovine albumin for P. aeruginosa, which is sensitive to drying, 8.5 g/L skimmed milk was used
Solution for washing the discs:	saline-peptone solution (SPO)
Test surface:	Stainless steel surface (2 cm diameter, Grade 2 B 1.4301 (EN 10088-1), EN 10 088-2. The test surfaces had been cleaned and sterilized.
Determination per bacteria:	double determination
UVC-box: SE-No.	 2005011

Results

	Contact time: 2 min	**Accepted acc. DS/EN 13969**
Testorganisme	**Log reduction**	**Requirement: ≥log 4 reduction**
P. aeruginosa	5.8	Accepted
S. aureus	4.7	Accepted
E. hirae	4.6	Accepted
A baumanii	5.3	Accepted
S. typhimurum	5.3	Accepted

Table 1 . To pass the test, the product must show a ≥log 4 reduction for the tested bacteria.

See enclosure 1-10 for detailed results.

Conclusion:

Treatment of a surface contaminated with bacteria for 2 min. using the UVC-box fulfil the requirements for bactericidal activity under simulated clean conditions according to DS/EN 13697: 2015 + A1:2019.

Analysis method

The samples were analysed according to:
Modified version of DS/EN 13697:2015 + A1:2019: Quantitative non-porous surface test for the evaluation of bactericidal and fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional – Test methods and requirements without mechanical action (Phase 2, step 2).

Test bacteria were chosen, and samples were analysed according to:
Modified version of DS/EN 17272:2020: Quantitative Carrier test for Airborne Room Disinfection by Automated Processes - Determination of Bactericidal, Fungicidal,Yeasticidal, Sporicidal, Tuberculocidal, Mycobactericidal, Virucidal and Phagocidal Activities in the Medical Area, Veterinary Area and Food, Industrial, Domestic and Institutional Areas - Test Methods and Requirements Phase 2, Step2)

Test bacteria were chosen according to:
DS/EN 16615: :2015. Chemical disinfectants and antiseptics - Quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action employing wipes in the medical area (4- field test) - Test method and requirements (phase 2, step 2)

Enclosure 1

Product concentration / Exposure time	UVC-light / 2 min.

Test suspension N ***Pseudomonas aeruginosa*** **ATCC 15442**	**Dilutions**	**Microbial count of plates**		**N [cells/mL] Log(N)**	**$5 \cdot 10^7 \leq N \leq 2 \cdot 10^9$ $7.7 \leq \log(N) \leq 9.3$**	**N [cells/metal disc] Log(N)**
	10^{-6}	>330	>330	$4.95 \cdot 10^8$	$7.7 \leq$ **8.7** ≤ 9.3 Accepted	$2.48 \cdot 10^7$
	10^{-7}	43	56	8.69	**8.7**	7.39

Control plates ***Pseudomonas aeruginosa*** **ATCC 15442**	**Dilutions**	**Microbial count of plates T1**		**T1: [cells/metal disc]** $\geq 1 \cdot 10^6$ CFU/disc **Log(T1)**	**Microbial count of plates T2**		**T2: [cells/metal disc]** $\geq 1 \cdot 10^6$ CFU/disc **Log(T2)**
	10^{-1}	>330	>330	$2.11 \cdot 10^6$ Accepted	>330	>330	$6.75 \cdot 10^6$ Accepted
	10^{-2}	210	199		>330	>330	
	10^{-3}	30	26	6.33	69	66	6.83
	10^{-4}	4	3		6	8	

Test ***Pseudomonas aeruginosa*** **ATCC 15442**	**Dilutions/ filtration volume**	**Microbial count of plates, Test 1**		**Microbial count of plates, Test 2**		**Result**	**n'1+n'2**	**Log(n'1+n'2)**	**Log reduction T = 6.58**
	10^0	<1	<1	<1	2	Test 1	$2.30 \cdot 10^0$	0.36	6.22
	10^{-1}	<1	<1	<1	1	Test 2	$1.40 \cdot 10^1$	1.15	5.43
	10^{-2}	<1	<1	<1	<1				
	10^{-3}	<1	<1	<1	<1				
	10 mL	<1		1		**Average**	**$8.15 \cdot 10^0$**	**0.75**	**5.8 ±0.6**
	87 mL	2		14					
n'2: CFU/metal disc		<1		4					

Table 2: n'1: CFU/membrane filter. n'2: CFU/disc. The lower limit is 14 CFU/ml. Results <14 CFU are included in the final calculation as<14. If <14/ml in the dilutions, only results from the filtration are used in the final calculation.

Enclosure 2

For validation, a metal disc with interfering substance was placed in the test room and exposed to UV-light for 2 minutes. The disc was transferred to the dilution media and a validation suspension was added to validate that the process did not have any effect on the test organism.

Method validation Product concentration	UVC-light / 2 min.

Test suspension N	**Dilutions**	**Microbial count of plates**		**N [cells/mL] Log(N)**
Pseudomonas aeruginosa **ATCC 15442**	10^{-6}	>330	>330	$4.95 \cdot 10^{8}$
	10^{-7}	43	56	8.69

Method validation **Neutralization-Dilution method** ***Pseudomonas aeruginosa*** **ATCC 15442**	**Dilutions**	**Microbial count of plates VC**		**VC_1: cells/mL/Log(VC_1)**
	10^{-7}	28	33	$3.04 \cdot 10^{8}$
				8.48

Method validation **Membrane filtration** ***Pseudomonas aeruginosa*** **ATCC 15442**	**Dilutions**	**Microbial count of plates VC**	**VC_1: cells/mL/Log(VC_1)**
	10^{-7}	33	$3.30 \cdot 10^{8}$
			8.52

Method validation **Inhibitory effect of metal disc cast in agarose gel** ***Pseudomonas aeruginosa*** **ATCC 15442**	**Dilution of test organism added to metal disc**	**Microbial count of plates**	**Metal disc cells/mL/Log_{10}**
	10^{-7}	24	$2.40 \cdot 10^{8}$
			8.38

1mL test suspension was added used for the validation of the inhibitory effect of the metal disc cast in agarose gel.

Results

Log_{10} for test suspension	**Log_{10} for VC for neutralization-dilution method**	**Log_{10} for VC for membrane filtration method**	**Log_{10} for test organism added to metal disc**
8.69	8.48	8.52	8.38

Table 3: Resumé of the method validation for test with P. aeruginosa.

Comments

The neutralizer did not have any toxic effect against the test organism.
The membrane filtration and disc cast in the agarose gel did not have any significant inhibitory toxic effect against the test organism.

Enclosure 3

Product concentration / Exposure time	UVC-light / 2 min.

Test suspension N ***Staphylococcus aureus*** **ATCC 6538**	**Dilutions**	**Microbial count of plates**		**N [cells/mL]** **Log(N)**	**$5 \cdot 10^7 \leq N \leq 2 \cdot 10^9$** **$7.7 \leq \log(N) \leq 9.3$**	**N [cells/metal disc]** **Log(N)**
	10^{-6}	>330	>330	$3.57 \cdot 10^9$	$7.7 \leq$ **9.6** ≤ 9.3 Accepted*	$1.78 \cdot 10^8$
	10^{-7}	387	320	9.55	**9.6**	8.25

**The test suspension is slightly higher than expected but is accepted as it is still possible to show ≥log 4 reduction.*

Control plates ***Staphylococcus aureus*** **ATCC 6538**	**Dilutions**	**Microbial count of plates** **T1**		**T1: [cells/metal disc]** $\geq 1 \cdot 10^6$ CFU/disc **Log(T1)**	**Microbial count of plates** **T2**		**T2: [cells/metal disc]** $\geq 1 \cdot 10^6$ CFU/disc **Log(T2)**
	10^{-1}	>330	>330	$3.18 \cdot 10^7$ Accepted	>330	>330	$1.57 \cdot 10^7$ Accepted
	10^{-2}	>330	>330		>330	>330	
	10^{-3}	293	333	7.50	154	159	7.19
	10^{-4}	40	33		12	20	

Test ***Staphylococcus aureus*** **ATCC 6538**	**Dilutions/ filtration volume**	**Microbial count of plates, Test 1**		**Microbial count of plates, Test 2**		**Result**	**n'1+n'2**	**Log(n'1+n'2)**	**Log reduction T = 7.35**
	10^0	2	1	<1	1	Test 1	$5.83 \cdot 10^2$	2.77	4.58
	10^{-1}	2	2	<1	<1	Test 2	$2.91 \cdot 10^2$	2.46	4.88
	10^{-2}	1	1	<1	<1				
	10^{-3}	<1	1	<1	<1				
	10 mL	58		29		**Average**	**$4.37 \cdot 10^2$**	**2.61**	**4.7±0.2**
	87 mL	>165		>165					
n'2: CFU/metal disc		3		1					

Table 4: n'1: CFU/membrane filter. n'2: CFU/disc. The lower limit is 14 CFU/ml. Results <14 CFU are included in the final calculation as<14. If <14/ml in the dilutions, only results from the filtration are used in the final calculation.

Enclosure 4

For validation, a metal disc with interfering substance was placed in the test room and exposed to UV-light for 2 minutes. The disc was transferred to the dilution media and a validation suspension was added to validate that the process did not have any effect on the test organism.

Method validation Product concentration	UVC-light / 2 min.

Test suspension N	**Dilutions**	**Microbial count of plates**		**N [cells/mL] Log(N)**
Staphylococcus aureus **ATCC 6538**	10^{-6}	>330	>330	$3.57 \cdot 10^{9}$
	10^{-7}	387	320	9.55

Method validation **Neutralization-Dilution method**	**Dilutions**	**Microbial count of plates VC**		**VC_1: cells/mL/Log(VC_1)**
Staphylococcus aureus **ATCC 6538**	10^{-7}	369	363	$3.66 \cdot 10^{9}$
				9.56

Method validation **Membrane filtration**	**Dilutions**	**Microbial count of plates VC**	**VC_1: cells/mL/Log(VC_1)**
Staphylococcus aureus **ATCC 6538**	10^{-7}	>165	$\geq 1.65 \cdot 10^{9}$
			≥9.22

Method validation **Inhibitory effect of metal disc cast in agarose gel**	**Dilution of test organism added to metal disc**	**Microbial count of plates**	**Metal disc cells/mL/Log_{10}**
Staphylococcus aureus **ATCC 6538**	10^{-7}	298	$2.98 \cdot 10^{9}$
			9.47

Results

Log_{10} for test suspension	**Log_{10} for VC for neutralization-dilution method**	**Log_{10} for VC for membrane filtration method**	**Log_{10} for test organism added to metal disc**
9.55	9.56	≥9.22	9.47

Table 5: Resumé of the method validation for test with S. aureus.

Comments

The neutralizer did not have any toxic effect against the test organism.
The membrane filtration and disc cast in the agarose gel did not have any significant inhibitory toxic effect against the test organism.

Enclosure 5

Product concentration / Exposure time	UVC-light / 2 min.

Test suspension N ***Enterococcus hirae*** **ATCC 10541**	**Dilutions**	**Microbial count of plates**		**N [cells/mL]** **Log(N)**	**$5 \cdot 10^7 \leq N \leq 2 \cdot 10^9$** **$7.7 \leq \log(N) \leq 9.3$**	**N [cells/metal disc]** **Log(N)**
	10^{-6}	>330	>330	$3.37 \cdot 10^9$	$7.7 \leq$ **9.5** ≤ 9.3 Accepted*	$1.68 \cdot 10^8$
	10^{-7}	364	309	9.53	**9.5**	8.23

**The test suspension is slightly higher than expected but is accepted as it is still possible to show ≥log 4 reduction.*

Control plates ***Enterococcus hirae*** **ATCC 10541**	**Dilutions**	**Microbial count of plates** **T1**		**T1: [cells/metal disc]** $\geq 1 \cdot 10^6$ CFU/disc **Log(T1)**	**Microbial count of plates** **T2**		**T2: [cells/metal disc]** $\geq 1 \cdot 10^6$ CFU/disc **Log(T2)**
	10^{-1}	>330	>330	$6.55 \cdot 10^7$ Accepted	>330	>330	$4.40 \cdot 10^7$ Accepted
	10^{-2}	>330	>330		>330	>330	
	10^{-3}	>330	>330	7.82	>330	>330	7.64
	10^{-4}	69	62		40	48	

Test ***Enterococcus hirae*** **ATCC 10541**	**Dilutions/ filtration volume**	**Microbial count of plates, Test 1**		**Microbial count of plates, Test 2**		**Result**	**n'1+n'2**	**Log(n'1+n'2)**	**Log reduction T = 7.73**
	10^0	21	20	12	9	Test 1	$2.06 \cdot 10^3$	3.31	4.42
	10^{-1}	4	3	1	2	Test 2	$1.05 \cdot 10^5$	3.02	4.71
	10^{-2}	<1	<1	<1	<1				
	10^{-3}	<1	<1	<1	<1				
	10 mL	>165		>165		**Average**	**$1.55 \cdot 10^3$**	**3.17**	**4.6±0.2**
	87 mL	>165		>165					
n'2: CFU/metal disc		5		1					

Table 6: n'1: CFU/membrane filter. n'2: CFU/disc. The lower limit is 14 CFU/ml. Results <14 CFU are included in the final calculation as<14. If <14/ml in the dilutions, only results from the filtration are used in the final calculation.

Enclosure 6

For validation, a metal disc with interfering substance was placed in the test room and exposed to UV-light for 2 minutes. The disc was transferred to the dilution media and a validation suspension was added to validate that the process did not have any effect on the test organism.

Method validation Product concentration	UVC-light / 2 min.

Test suspension N	**Dilutions**	**Microbial count of plates**		**N [cells/mL] Log(N)**
Enterococcus hirae **ATCC 10541**	10^{-6}	>330	>330	$3.37 \cdot 10^9$
	10^{-7}	364	309	9.53

Method validation **Neutralization-Dilution method**	**Dilutions**	**Microbial count of plates VC**		**VC_1: cells/mL/Log(VC_1)**
Enterococcus hirae **ATCC 10541**	10^{-7}	343	375	$3.59 \cdot 10^9$
				9.55

Method validation **Membrane filtration**	**Dilutions**	**Microbial count of plates VC**	**VC_1: cells/mL/Log(VC_1)**
Enterococcus hirae **ATCC 10541**	10^{-7}	>165	$\geq 1.65 \cdot 10^9$
			≥9.22

Method validation **Inhibitory effect of metal disc cast in agarose gel**	**Dilution of test organism added to metal disc**	**Microbial count of plates**	**Metal disc cells/mL/Log_{10}**
Enterococcus hirae **ATCC 10541**	10^{-7}	275	$2.75 \cdot 10^9$
			9.44

1mL test suspension was added used for the validation of the inhibitory effect of the metal disc cast in agarose gel.

Results

Log_{10} for test suspension	**Log_{10} for VC for neutralization-dilution method**	**Log_{10} for VC for membrane filtration method**	**Log_{10} for test organism added to metal disc**
9.53	9.55	≥9.22	9.44

Table 7: Resumé of the method validation for test with E. hirae.

Comments

The neutralizer did not have any toxic effect against the test organism.
The membrane filtration and disc cast in the agarose gel did not have any significant inhibitory toxic effect against the test organism.

Enclosure 7

Product concentration / Exposure time	UVC-light / 2 min.

Test suspension N ***Acinetobacter baumanii*** **ATCC 19606**	**Dilutions**	**Microbial count of plates**		**N [cells/mL]** **Log(N)**	**$5 \cdot 10^7 \leq N \leq 2 \cdot 10^9$** **$7.7 \leq \log(N) \leq 9.3$**	**N [cells/metal disc]** **Log(N)**
	10^{-6}	218	211	$2.15 \cdot 10^8$	$7.7 \leq$ **8.3** ≤ 9.3 Accepted	$1.07 \cdot 10^7$
	10^{-7}	23	11	8.33	**8.3**	7.03

Control plates ***Acinetobacter baumanii*** **ATCC 19606**	**Dilutions**	**Microbial count of plates** **T1**		**T1: [cells/metal disc]** $\geq 1 \cdot 10^6$ CFU/disc **Log(T1)**	**Microbial count of plates** **T2**		**T2: [cells/metal disc]** $\geq 1 \cdot 10^6$ CFU/disc **Log(T2)**
	10^{-1}	>330	>330	$1.14 \cdot 10^6$ Accepted	>330	>330	$1.04 \cdot 10^6$ Accepted
	10^{-2}	118	109		104	103	
	10^{-3}	7	12	6.05	12	11	6.01
	10^{-4}	1	2		1	<1	

Test ***Acinetobacter baumanii*** **ATCC 19606**	**Dilutions/ filtration volume**	**Microbial count of plates, Test 1**		**Microbial count of plates, Test 2**		**Result**	**n'1+n'2**	**Log(n'1+n'2)**	**Log reduction T = 6.03**
	10^0	2	<1	<1	<1	Test 1	$1.80 \cdot 10^0$	0.07	5.96
	10^{-1}	<1	<1	<1	1	Test 2	$2.59 \cdot 10^1$	1.42	4.62
	10^{-2}	<1	<1	<1	1				
	10^{-3}	<1	<1	<1	<1				
	10 mL	<1		2		**Average**	**$1.35 \cdot 10^1$**	**0.74**	**5.3 ±0.9**
	87 mL	1		5					
n'2: CFU/metal disc		<1		<1					

Table 8: n'1: CFU/membrane filter. n'2: CFU/disc. The lower limit is 14 CFU/ml. Results <14 CFU are included in the final calculation as<14. If <14/ml in the dilutions, only results from the filtration are used in the final calculation.

Enclosure 8

For validation, a metal disc with interfering substance was placed in the test room and exposed to UV-light for 2 minutes. The disc was transferred to the dilution media and a validation suspension was added to validate that the process did not have any effect on the test organism.

Method validation Product concentration	UVC-light / 2 min.

Test suspension N	**Dilutions**	**Microbial count of plates**		**N [cells/mL] Log(N)**
Acinetobacter baumanii **ATCC 19606**	10^{-6}	218	211	$2.15 \cdot 10^{8}$
	10^{-7}	23	11	8.33

Method validation **Neutralization-Dilution method**	**Dilutions**	**Microbial count of plates VC**		**VC_1: cells/mL/Log(VC_1)**
Acinetobacter baumanii **ATCC 19606**	10^{-7}	26	22	$2.39 \cdot 10^{8}$
				8.38

Method validation **Membrane filtration**	**Dilutions**	**Microbial count of plates VC**	**VC_1: cells/mL/Log(VC_1)**
Pseudomonas aeruginosa **ATCC 15442**	10^{-7}	22	$2.20 \cdot 10^{8}$
			8.34

Method validation **Inhibitory effect of metal disc cast in agarose gel**	**Dilution of test organism added to metal disc**	**Microbial count of plates**	**Metal disc cells/mL/Log_{10}**
Acinetobacter baumanii **ATCC 19606**	10^{-7}	19	$1.90 \cdot 10^{8}$
			8.28

1mL test suspension was added used for the validation of the inhibitory effect of the metal disc cast in agarose gel.

Results

Log_{10} for test suspension	**Log_{10} for VC for neutralization-dilution method**	**Log_{10} for VC for membrane filtration method**	**Log_{10} for test organism added to metal disc**
8.33	8.38	8.34	8.28

Table 9: Resumé of the method validation for test with A. baumanii.

Comments

The neutralizer did not have any toxic effect against the test organism.
The membrane filtration and disc cast in the agarose gel did not have any significant inhibitory toxic effect against the test organism.

Enclosure 9

Product concentration / Exposure time	UVC-light / 2 min.

Test suspension N ***Salomonella typhimurum*** **ATCC 13311**	**Dilutions**	**Microbial count of plates**		**N [cells/mL] Log(N)**	**$5 \cdot 10^7 \leq N \leq 2 \cdot 10^9$ $7.7 \leq \log(N) \leq 9.3$**	**N [cells/metal disc] Log(N)**
	10^{-6}	>330	>330	$4.60 \cdot 10^8$	$7.7 \leq$ **8.7** ≤ 9.3 Accepted	$9.00 \cdot 10^6$
	10^{-7}	36	56	8.66	**8.7**	6.95

Control plates ***Salomonella typhimurum*** **ATCC 13311**	**Dilutions**	**Microbial count of plates T1**		**T1: [cells/metal disc]** $\geq 1 \cdot 10^6$ CFU/disc **Log(T1)**	**Microbial count of plates T2**		**T2: [cells/metal disc]** $\geq 1 \cdot 10^6$ CFU/disc **Log(T2)**
	10^{-1}	279	266	$2.75 \cdot 10^5$ Accepted*	192	232	$2.19 \cdot 10^5$ Accepted
	10^{-2}	40	21		30	27	
	10^{-3}	4	1	5.44	2	4	5.34
	10^{-4}	1	2		2	<1	

**The concentration test control discs after drying is slightly lower than expected but is accepted as it is still possible to show ≥log 4 reduction.*

Test ***Salomonella typhimurum*** **ATCC 13311**	**Dilutions/ filtration volume**	**Microbial count of plates, Test 1**		**Microbial count of plates, Test 2**		**Result**	**n'1+n'2**	**Log(n'1+n'2)**	**Log reduction T = 5.39**
	10^0	<1	<1	<1	<1	Test 1	$1.15 \cdot 10^0$	0.06	5.33
	10^{-1}	<1	<1	<1	<1	Test 2	$\leq 1.15 \cdot 10^0$	≤0.06	≥5.33
	10^{-2}	<1	<1	<1	<1				
	10^{-3}	<1	<1	<1	<1				
	10 mL	<1		<1		**Average**	**$\leq 1.40 \cdot 10^1$**	**≤1.15**	**5.3±0.0**
	87 mL	1		*					
n'2: CFU/metal disc		<1		<1					

*Table 10: n'1: CFU/membrane filter. n'2: CFU/disc. The lower limit is 14 CFU/ml. Results <14 CFU are included in the final calculation as<14. If <14/ml in the dilutions, only results from the filtration are used in the final calculation. *Contamination. Not used in the final calculation.*

Enclosure 10

For validation, a metal disc with interfering substance was placed in the test room and exposed to UV-light for 2 minutes. The disc was transferred to the dilution media and a validation suspension was added to validate that the process did not have any effect on the test organism.

Method validation Product concentration	UVC-light / 2 min.

Test suspension N	**Dilutions**	**Microbial count of plates**		**N [cells/mL] Log(N)**
Salomonella typhimurum **ATCC 13311**	10^{-6}	>330	>330	$4.60 \cdot 10^{8}$
	10^{-7}	36	56	8.66

Method validation **Neutralization-Dilution method**	**Dilutions**	**Microbial count of plates VC**		**VC_1: cells/mL/Log(VC_1)**
Salomonella typhimurum **ATCC 13311**	10^{-7}	34	32	$3.30 \cdot 10^{8}$
				8.52

Method validation **Membrane filtration**	**Dilutions**	**Microbial count of plates VC**	**VC_1: cells/mL/Log(VC_1)**
Salomonella typhimurum **ATCC 13311**	10^{-7}	43	$4.30 \cdot 10^{8}$
			8.63

Method validation **Inhibitory effect of metal disc cast in agarose gel**	**Dilution of test organism added to metal disc**	**Microbial count of plates**	**Metal disc cells/mL/Log_{10}**
Salomonella typhimurum **ATCC 13311**	10^{-7}	50	$5.00 \cdot 10^{8}$
			8.70

1mL test suspension was added used for the validation of the inhibitory effect of the metal disc cast in agarose gel.

Results

Log_{10} for test suspension	**Log_{10} for VC for neutralization-dilution method**	**Log_{10} for VC for membrane filtration method**	**Log_{10} for test organism added to metal disc**
8.66	8.52	8.63	8.70

Table 11: Resumé of the method validation for test with S. typhimurum.

Comments

The neutralizer did not have any toxic effect against the test organism.
The membrane filtration and disc cast in the agarose gel did not have any significant inhibitory toxic effect against the test organism.