Analysis Report

REPORT NUMBER:
764771.1

DANISH
TECHNOLOGICAL
INSTITUTE

Teknologiparken
Kongsvang Allé 29
DK-8000 Aarhus C
+45 72 20 20 00
Info@teknologisk.dk
www.teknologisk.dk

Encl.: 7
Init.: HSA/SHSM

Assignor: Helge Grosch
Infuser ApS
Universitetsparken 7
DK-4000 Roskilde

Item: Determination of bactericidal activity for aerial surface disinfection processes according to NF T72-281 (Phase 2, step 2)

Sampling: The assignor

Period: Samples received: August 2017
Test performed: 24 July – 13 September 2017

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: 26 September 2017
Danish Technological Institute, Aarhus
Laboratory for Chemistry and Microbiology

Signature: Helle Stendahl Andersen
Senior Specialist

Procedure
The efficacy of ozone on contaminated surfaces was tested according to NF T-72-281, 1st ed., 2014-11.

A bacterial suspension was mixed with a solution of skimmed milk powder to simulate the presence of organic material.

50µl of a test suspension was transferred to a stainless steel surface and dried at 37°C until visibly dry. The metal discs were then placed in an airtight room and exposed to ozone at a fixed concentration for 60 min.

After the device had been stopped, the metal discs were left in the test room until the O_3 concentration had dropped to <0.1ppm.

The stainless steel plates were subsequently transferred to a neutralizing agent to neutralize the effect of the product. The number of surviving microorganisms was quantified and compared with a control sample in which a similarly treated stainless steel surface was placed in a room without being exposed to ozone for the same time.

When tested in accordance with the test method under the required test conditions, the product shall demonstrate ≥ log 5 reductions in viable counts for bacteria.

For the bacteria *Proteus mirabellis* it is only possible to get an estimated log reduction as they did not grow as single colonies.

Product:	Ozone
Device:	STERISAFE Pro
Serial No.:	#0002
Manufacturer:	Infuser ApS

Experiment conditions

Test organisms:	*Pseudomonas aeruginosa* ATCC 15442 *Enterococcus faecium* ATCC 6057 *Proteus mirabilis* ATCC 14153
Product concentration:	80 ppm
Build-up time:	ca. 30-45 min.
Exposure time:	60 min.
Cleaning (until <0.1 ppm):	ca. 60 min.
Specifications for test room:	$74m^3$ No ventilation and the room must be airtight The airtightness was confirmed by an O_3 alarm (Gas Alert Extreme BW)
Distance from device to organisms:	3.9 m ±0.39 m
Test temperature:	(24 ± 2) °C
Temperature sensor:	EL-USB-1, temperature data logger
Humidity:	84-85% RH (enclosure 11)
Humidity sensor:	EL-USB 2, RH/temp data logger
Test surface:	1.4301 (EN 10088-1) stainless steel discs, 4 cm in diameter with Grade 2 B with finish on both sides (acc. EN 10088-2)
Interfering substances:	100g/L skimmed milk
Neutralizer:	Na-thiosulphat 5g/L Polysorbat 80 30g/L Lecithin 3g/L Saponin L-histidine Dissolved in 0.25mmol phosphate buffer
Incubation conditions:	
Bacteria:	(37 ± 1) °C for 48 hours at trypton soya agar (TSA)

Results

Test organism	Test 1 Log reduction 80 ppm for 60min	Temperature/ relative humidity during the exposure of ozone
E. faecium	6.73 ±0.17	(25 ±2) °C; 84-85 %RH
P. aeruginosa	6.99 ±0.21	(25 ±2) °C; 84-85 %RH
P. mirabilis	≥5.8*	(24 ±1) °C; 83-84 %RH

*Table 1: The product has to achieve ≥ 5 log reduction for bacteria. The results are given as the log reduction ± the standard deviation. *Results are estimated.*

For all results, see Enclosure 1 – 7.

Conclusion

It was possible to achieve ≥5 log reduction for both the gram-positive bacteria *E. faecium* and for the two gram-negative bacteria *P. aeruginosa* and *P. mirabilis*. The result for *P. mirabilis* is only an estimated result.

Comments

For *P. aeruginosa* there is a possible outlier that has not been included in the calculation (see enclosure 3).

Analysis method

The samples were analysed according to Danish Technological Institute's method: MA 700-03.

Reference method: NF T72-281:2014.

Enclosure 1

Product concentration / Exposure time	Test 1: 80 ppm for 60 min.

Test suspension N ***Enterococcus faecium* ATCC 6057**	**Dilutions**	**Microbial count of plates**		**N [cells/ml] Log(N)**	**$5 \cdot 10^7 \leq N \leq 2 \cdot 10^9$ $7.7 \leq N \leq 9.3$**	**N [cells/metal disc] Log(N)**
	10^{-6}	212	252	$2.37 \cdot 10^8$	$7.7 \leq$ **8.38** ≤ 9.3 Accepted	$1.19 \cdot 10^7$
	10^{-7}	25	33	8.38		7.07
	10^{-8}	0	3			

Control plates ***Enterococcus faecium* ATCC 6057**	**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)**	**T [cells/metal disc] Log(T)**
	10^{-3}	58	72	$6.50 \cdot 10^6$ Accepted	78	60	$6.90 \cdot 10^6$ Accepted	$6.70 \cdot 10^6$
	10^{-4}	3	17		8	7		
	10^{-5}	1	<1	6.81	1	2	6.84	6.83
	10^{-6}	<1	<1		<1	<1		

Test ***Enterococcus faecium* ATCC 6057**	**Dilutions/ Filtration volume**	**Microbial count of plates, Test 1**		**Microbial count of plates, Test 2**		**Microbial count of plates, Test 3**		**Result**	**n'1+n'2**	**Log(n'1+n'2)**	**Log reduction T = 6.83**
	10^0	<1	<1	3	12	<1	<1	Test 1	2	0.30	6.52
	10^{-1}	<1	<1	<1	4	<1	<1	Test 2	1	0	6.83
	10^{-2}	<1	<1	1	<1	<1	<1	Test 3	1	0	6.83
	10^{-3}	<1	<1	<1	<1	<1	<1				
	10ml	<1		<1		<1		**Average**	**1.33**	**0.10**	**6.73 ±0.17**
	87ml	<1		<1		<1					
n'2: CFU/metal disc		2		1		1					

Calculated according to NF T72-281:2014, 5.6.6.

Enclosure 2

Method validation Product concentration	80 ppm

Test suspension N	**Dilutions**	**Microbial count of plates**		**N [cells/ml] / Log(N)**
***Enterococcus faecium* ATCC 6057**	10^{-6}	212	252	$2.37 \cdot 10^8$
	10^{-7}	25	33	8.38
	10^{-8}	0	3	

Method validation Neutralization-Dilution method	**Dilutions**	**Microbial count of plates VC_1**		**VC_1: cells/ml/Log(VC_1)**
***Enterococcus faecium* ATCC 6057**	10^{-7}	21	22	$2.15 \cdot 10^8$
				8.33

VC = validation control

Method validation Membrane filtration	**Dilutions**	**Microbial count of plates VC**	**VC_1: cells/ml/Log(VC_1)**
***Enterococcus faecium* ATCC 6057**	10^{-7}	20	$2.00 \cdot 10^8$
			8.30

Results

Log_{10} for test suspension	**Log_{10} for VC for neutralization-dilution method**	**Log_{10} for VC for membrane filtration method**
8.38	8.33	8.30

Enclosure 3

Product ncentration / Exposure time	Test 1: 80 ppm for 60 min.

Test suspension N ***Pseudomonas aeruginosa* ATCC 15442**	**Dilutions**	**Microbial count of plates**		**N [cells/ml] Log(N)**	**$5 \cdot 10^7 \leq N \leq 2 \cdot 10^9$ $7.7 \leq N \leq 9.3$**	**N [cells/metal disc] Log(N)**
	10^{-6}	>330	>330	$1.87 \cdot 10^9$	7.7≤**9.27**≤9.3 Accepted	$9.36 \cdot 10^7$
	10^{-7}	166	184	9.27		7.97
	10^{-8}	32	30			

Control plates ***Pseudomonas aeruginosa* ATCC 15442**	**Dilutions**	**Microbial count of plates T1**		**T1: [cells/metal disc]** ≥$1 \cdot 10^6$ CFU/disc **Log(T1)**	**Microbial count of plates T2**		**T2:[cells/metal disc]** ≥$1 \cdot 10^6$ CFU/disc **Log(T2)**	**T [cells/metal disc] Log(T)**
	10^{-3}	162	137	$1.50 \cdot 10^7$ Accepted	123	129	$1.26 \cdot 10^7$ Accepted	$1.37 \cdot 10^7$
	10^{-4}	7	11		10	12		
	10^{-5}	2	1	7.17	<1	1	7.10	7.14
	10^{-6}	<1	1		<1	1		

Test ***Pseudomonas aeruginosa* ATCC 15442**	**Dilutions/ Filtration volume**	**Microbial count of plates, Test 1***		**Microbial count of plates, Test 2**		**Microbial count of plates, Test 3**		**Result**	**n'1+n'2**	**Log(n'1+n'2)**	**Log reduction T = 7.14**
	10^0	10	9	1	<1	<1	<1	Test 1	$3.38 \cdot 10^2$	4.24	2.53
	10^{-1}	5	5	<1	<1	<1	<1	Test 2	1	0	7.14
	10^{-2}	<1	<1	<1	<1	<1	<1	Test 3	2	0.30	6.84
	10^{-3}	1	<1	<1	<1	<1	<1				
	10ml	51		1		<1		**Average**	**2**	**0.15**	**6.99 ±0.21**
	87ml	>165		<1		<1					
n'2: CFU/metal disc		8		<1		2					

*Calculated according to NF T72-281:2014, 5.6.6.*The colonies for test 1 were difficult to count. Due to a suspected contamination, the results from test 1 are not used for calculating the log reduction.*

Enclosure 4

Method validation Product concentration	80 ppm

Test suspension N	**Dilutions**	**Microbial count of plates**		**N [cells/ml] / Log(N)**
***Pseudomonas aeruginosa* ATCC 15442**	10^{-6}	>330	>330	$1.87 \cdot 10^9$
	10^{-7}	166	184	9.27
	10^{-8}	32	30	

Method validation Neutralization-Dilution method	**Dilutions**	**Microbial count of plates VC_1**		**VC_1: cells/ml/Log(VC_1)**
***Pseudomonas aeruginosa* ATCC 15442**	10^{-7}	97	111	$2.08 \cdot 10^9$
				9.32

VC = validation control

Method validation Membrane filtration	**Dilutions**	**Microbial count of plates VC**	**VC_1: cells/ml/Log(VC_1)**
***Pseudomonas aeruginosa* ATCC 15442**	10^{-7}	70	$7.00 \cdot 10^8$
			8.85

Results

Log_{10} for test suspension	**Log_{10} for VC for neutralization-dilution method**	**Log_{10} for VC for membrane filtration method**
9.27	9.32	8.85

Enclosure 5

Product concentration / Exposure time	Test 1: 80 ppm for 60 min.

Test suspension N ***Proteus mirabilis* ATCC 14153**	**Dilutions**	**Microbial count of plates**		**N [cells/ml] Log(N)**	**$5 \cdot 10^7 \leq N \leq 2 \cdot 10^9$ $7.7 \leq N \leq 9.3$**	**N [cells/metal disc] Log(N)**
	10^{-6}	+	+	$1.00 \cdot 10^8$	7.7≤**8.00**≤9.3 Accepted	$1.19 \cdot 10^7$
	10^{-7}	+	+	8.00		7.0
	10^{-8}	<1	<1			

Control plates ***Proteus mirabilis* ATCC 14153**	**Dilutions**	**Microbial count of plates T1**		**T1: [cells/metal disc]** ≥$1 \cdot 10^6$ CFU/disc **Log(T1)**	**Microbial count of plates T2**		**T2:[cells/metal disc]** ≥$1 \cdot 10^6$ CFU/disc **Log(T2)**	**T [cells/metal disc] Log(T)**
	10^{-3}	+	+	$5 \cdot 10^6$ Accepted	+	+	$5 \cdot 10^6$ Accepted	$5 \cdot 10^6$
	10^{-4}	+	+		+	+		
	10^{-5}	<1	<1	6.0	<1	<1	6.0	6.0
	10^{-6}	<1	<1		<1	<1		

Test ***Proteus mirabilis* ATCC 14153**	**Dilutions/ Filtration volume**	**Microbial count of plates, Test 1**		**Microbial count of plates, Test 2**		**Microbial count of plates, Test 3**		**Result**	**n'1+n'2**	**Log(n'1+n'2)**	**Log reduction T = 6.0**
	10^0	+	<1	<1	+	<1	<1	Test 1	1	0	6.0
	10^{-1}	<1	<1	<1	<1	<1	<1	Test 2	1	0	6.0
	10^{-2}	<1	<1	<1	<1	<1	<1	Test 3	3	0.5	5.5
	10^{-3}	<1	<1	<1	<1	<1	<1				
	10ml	<1		1		<1		**Average**	**1.67**	**0.2**	**5.8**
	87ml	<1		<1		<1					
n'2: CFU/metal disc		1		<1		3					

Results for P. mirabilis is given as "+" when growth was observed or "<1" when no growth was observed. It was not possible to count single colonies. Results are estimated.

Enclosure 6

Method validation Product concentration	80 ppm

Test suspension N	**Dilutions**	**Microbial count of plates**		**N [cells/ml] / Log(N)**
***Proteus mirabilis* ATCC 14153**	10^{-6}	+	+	$1 \cdot 10^8$
	10^{-7}	+	+	8.0
	10^{-8}	<1	<1	

Method validation Neutralization-Dilution method	**Dilutions**	**Microbial count of plates VC_1**		**VC_1: cells/ml/Log(VC_1)**
***Proteus mirabilis* ATCC 14153**	10^{-6}	+	+	$1 \cdot 10^8$
				8.0
	10^{-7}	+	+	
	10^{-8}	+	+	

VC = validation control

Method validation Membrane filtration	**Dilutions**	**Microbial count of plates VC**	**VC_1: cells/ml/Log(VC_1)**
***Proteus mirabilis* ATCC 14153**	10^{-7}	+	$1 \cdot 10^8$
			8.0

Results

Log_{10} for test suspension	**Log_{10} for VC for neutralization-dilution method**	**Log_{10} for VC for membrane filtration method**
8.0	8.0	8.0

Results for P. mirabilis are given as "+" when growth was observed or "<1" when no growth was observed. It was not possible to count single colonies. Results are estimated.

Enclosure 7

Temperature and humidity were measured with EL-USB-1temp data logger.

***P. aeruginosa* & *E. faecium* 80 ppm for 60 min.**				
	Placing the test organisms in the test room Starting STERISAFE	**Starting the exposure at 80ppm O_3**	**End of exposure at 80ppm O_3 Starting cleaning**	**Removing the test organisms from the test room at <0.1 ppm O_3**
Temperature	24 °C	24 °C	26 °C	27 °C
Humidity	56.0% RH	85% RH	84% RH	64% RH
Measures O3-concentration	<0.01 ppm	80 ppm	80 ppm	<0.1 ppm

***P. mirabilis* 80 ppm for 60min.**				
	Placing the test organisms in the test room Starting STERISAFE	**Starting the exposure at 80ppm O_3**	**End of exposure at 80ppm O_3 Starting cleaning**	**Removing the test organisms from the test room at <0.1 ppm O_3**
Temperature	23 °C	23 °C	24 °C	25 °C
Humidity	58% RH	84% RH	84% RH	60% RH
Measures O3-concentration	<0.01 ppm	80 ppm	80 ppm	<0.1 ppm