Analysis Report

REPORT NUMBER:
789802.1

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Assignor:	Helge Grosch Infuser ApS Universitetsparken 7 DK-4000 Roskilde
Item:	Determination of bactericidal activity against *Staphylococcus aureus* for aerial surface disinfection processes according to NF T72-281 (Phase 2, step 2)
Sampling:	The assignor
Period:	Samples received: 26 January 2018 Test performed: 26 – 29 January 2018
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:	23 February 2018 Danish Technological Institute, Aarhus Laboratory for Chemistry and Microbiology
Signature:	Helle Stendahl Andersen Senior Consultant

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 80ppm for 2.5 hours.

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.

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

Experiment conditions

Test organisms:	*Staphylococcus aureus* ATCC 6538
Product concentrations:	80 ppm
Build-up time:	ca. 30 min.
Exposure time:	2.5 hours
Cleaning (until <0.1 ppm):	ca. 90 min.
Specifications for test room:	74m³ 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:	(17 ± 0.5) °C

Temperature sensor:	EL-USB-1, temperature data logger
Humidity:	77-82% RH (enclosure 5)
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-thiosulphate 3g/L Polysorbat 80 30g/L Lecithin 5g/L Saponin 30g/L L-histidine 1g/L Dissolved in 0.25mmol phosphate buffer
Rinsing liquid	Na-thiosulphate 5g/L Polysorbat 80 30g/L Lecithin 3g/L
Incubation conditions:	(37 ± 1) °C for 48 hours at tryptone soya agar (TSA)

Results

Test organism	Log reduction 80 ppm for 2.5 hours	Temperature/ relative humidity during the exposure of ozone
S. aureus	6.80 ±0.17	(17 ±0.5) °C; 77-82%RH

Table 1: The product has to achieve ≥ 5 log reduction for bacteria.

Conclusion

It was possible to achieve ≥5 log reduction for *S. aureus,* under the given test conditions with an exposure time of 2.5 hours.60 min.

With STERISAFE Pro it was possible to achieve a full bactericidal activity against *S. aureus* with 2.5 hours of exposure under the achieved test conditions.

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 2.5 hours

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	313	$3.05 \cdot 10^8$	$7.7 \leq$ **8.48** ≤ 9.3 Accepted	$1.53 \cdot 10^7$
	10^{-7}	24	37	8.48		7.18
	10^{-8}	-	-			

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)**	**T [cells/metal disc] Log(T)**
	10^{-3}	96	76	$8.60 \cdot 10^6$ Accepted	68	80	$7.40 \cdot 10^6$ Accepted	$7.98 \cdot 10^6$
	10^{-4}	18	8		9	11		
	10^{-5}	1	2	6.93	1	<1	6.87	6.90
	10^{-6}	<1	<1		<1	<1		

Test ***Staphylococcus aureus* ATCC 6538**	**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 = 6.90**
	10^0	<1	<1	<1	<1	1	1	Test 1	1	0	6.90
	10^{-1}	<1	<1	<1	<1	<1	<1	Test 2	2	0.30	6.60
	10^{-2}	<1	<1	<1	<1	<1	<1	Test 3	<1	0	≥6.90
	10^{-3}	<1	<1	<1	<1	<1	<1				
	10ml	1		2		<1		**Average**	**1.33**	**0.10**	**6.80 ±0.17**
	87ml	<1		<1		<1					
n'2: CFU/metal disc		<1		<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)**
***Staphylococcus aureus* ATCC 6538**	10^{-6}	>330	313	$3.05 \cdot 10^8$
	10^{-7}	24	37	8.48
	10^{-8}	-	-	

Method validation Neutralization-Dilution method	**Dilutions**	**Microbial count of plates VC_1**		**VC_1: cells/ml/Log(VC_1)**
***Staphylococcus aureus* ATCC 6538**	10^{-7}	21	24	$2.25 \cdot 10^8$
				8.35

VC = validation control

Method validation Membrane filtration	**Dilutions**	**Microbial count of plates VC**	**VC_1: cells/ml/Log(VC_1)**
***Staphylococcus aureus* ATCC 6538**	10^{-7}	Clear growth. Colonies not countable	

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}	30	$3.00 \cdot 10^8$
			8.48

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**
8.48	8.35	Clear growth	8.48

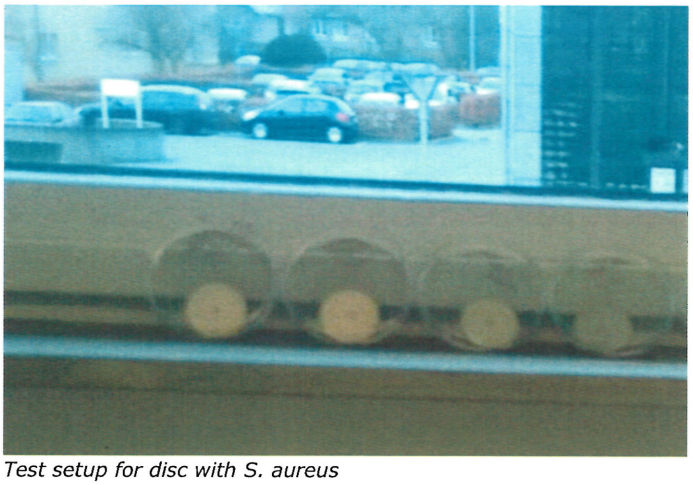
Enclosure 3

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

S. aureus **80 ppm for 2.5 hours**				
	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 <0.1ppm O_3**
Temperature	17.5 °C	16.5 °C	17.0 °C	16.5 °C
Humidity	52.0% RH	77.5% RH	81.5% RH	78.5% RH
Measures O3-concentration	<0.01 ppm	80 ppm	80 ppm	<0.1ppm

Enclosure 4

Test setup
The Sterisafe placed 3.9 meters away from the metal discs with the test organisms.
The metal discs were placed in the window.



Test setup for disc with S. aureus