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Infuser ApS
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Analysis Report No. 638325

Assignment:	Determination of bactericidal, fungicidal and sporicidal activity for aerial surface disinfection processes according to NF T72-281 (Phase 2, step 2)
Sampling by:	The client
Sample (s) received:	-
Test performed:	11 – 30 March 2015
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.

This analysis was carried out in accordance with Danish Technological Institute's General Terms and Conditions regarding Commissioned Work Accepted by Danish Technological Institute. This analysis report may be quoted in extract only if the Laboratory for Chemistry and Microbiology has approved the extract in writing.

The Laboratory for Chemistry and Microbiology

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

A bacterial, fungal or spore 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 a certain time.

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 2 hours.

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 and ≥ log 4 reductions in viable counts for fungi.

Product:	Ozone
Device:	STERISAFE
Serial No.:	Proto002
Manufacturer:	Infuser ApS

Experiment conditions

Test organisms:	<u>Bacteria</u> *Escherichia coli* ATCC 10541 *Listeria monocytogenes* ATCC 15313 <u>Fungus</u> *Candida albicans* ATCC 10231	
Product concentrations:	80 ppm	
Exposure time:	Test 1: Test 2:	1h 2h 30min
Time between device was stopped & transfer of metal discs to neutralizer:	Test 1: Test 2:	40-45 min 1h 5min
Total exposure time:	Test 1: 2h 37min Test 2: 4h 15min	

The total exposure time is incl. the time for reaching 80 ppm and the time after exposure before the concentration in the room had reached ≤ 0.1ppm and the metal discs were further treated.

Specifications for test room:	$48m^3$ No ventilation and airtight room The airtightness was confirmed by an O_3 alarm (Gas Alert Extreme BW)
Distance from device to organisms:	3.0 m ±0.3 m
Test temperature:	(20 ± 2) °C
Temperature sensor:	EL-USB-1, temperature data logger
Humidity:	80% RH – 92% RH
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	36 °C ± 1 °C for 48 hours at trypton soya agar
Fungus	30 °C ± 1 °C for 48 hours at malt extract agar

Test Results

For the tests, all controls and verification tests were within the basic limits. For all results, see Enclosure 1 – 13.

Test organism	Log reduction 80 ppm for 1h	Log reduction 80 ppm for 2h 30min
Escherichia coli ATCC 10536	≥6.22	≥6.18
Listeria monocytogenes ATCC 15313	≥7.70	≥6.97
Candida albicans ATCC 10231	3.65	4.17

Table 1: the product has to achieve ≥ 5 log reduction for bacteria and ≥ 4 log reduction for fungi.

Conclusion

The test was performed with an ozone concentration at 80ppm for 1hour and 2h 30min, respectively, with STERISAFE, proto002 from Infuser ApS.

For the two bacteria, the gram-negative *Escherichia coli* and the gram-positive *Listeria monocytogenes,* it was possible to achieve ≥ log 5 reduction with an exposure time of 1 hour.

For the fungus *Candida albicans* a ≥ log 4 reduction was achieved with an exposure time of 2h 30 min.

Ozone generated with STERISAFE, proto002 from Infuser ApS is suitable to reduce bacteria and fungi at 80ppm with an exposure time of 1h for bacteria and 2h 30min for fungus.