NATIONAL PUBLIC HEALTH CENTRE

TEST RECORD

BSI EN 17272:2020
Testing of the effectiveness of airborne surface disinfection.
Determination of bactericidal and fungicidal effects.

Registration number: 3593-2/2021/JIF

1. Equipment under test		
Name:	ExOzone® EX-SR-100	
Type:	ExOzone® EX-SR-100	
Client:	OzonExtrade Kft.	
Address: Tax number:	1141 Budapest, Cinkotai út 123. 24342704-2-42	
Contact information:	lajos.balasko@ozonextrade.hu / +36/20 950 93 71	
Manufacturer:	OzonExtrade Kft. 1141 Budapest, Cinkotai út 123.	
Composition: *biocidal agent*	ozone (CAS number: 10028-15-6); (EC number: 233-069-2) produced by a generator from the oxygen of the surrounding air	
Method of generation:	„in situ”	
2. Testing and testing conditions		
Method:	MSZ EN 17272:2020 (modified)	
Test micro-organisms: For the testing of bactericidal activities:	*Pseudomonas aeruginosa* ATCC 15442 *Staphylococcus aureus* ATCC 6538 *Enterococcus hirae* ATCC 10541 *Escherichia coli* ATCC 10536	= CIP 103-467 (DSM 937) = CIP 4.83 (DSM 799) = CIP 5855 (DSM 3320) = CIP 54127 (DSM 682)
For the testing of fungicidal activities:	*Candida albicans* ATCC 1023 *Aspergillus brasiliensis* ATCC 16404	= IP 4872 (DSM 1386) = IP 1431-83 (DSM 1988)
Culture medium used:	Brilliant Green Agar Plate; Eosin Methylene Blue Agar Plate; TSA Agar Plate; Sabouraud Glucose Agar Plate; 6% Salty Blood Agar Plate;	serial number: 004211463 serial number: 006211540 serial number: 808211472 serial number: 255211464 serial number: 157211460
Incubation temperature:	Bacteria: 37±1 °C; Fungi: 30±1 °C;	
Incubation time:	Bacteria: 24-48 h; Fungi: 48-96 h	

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3. Varying test parameters	
Test room:	Sealed and draught-free laboratory room with a cubic capacity of 81.6 m^3 and a floor area of 16 m^2
Test room temperature:	21 ± 2 °C
Test room humidity:	41 ± 2% relative humidity
Carrier used:	Glass plate 5x5 cm with a track width of 3 mm
Quantity applied to the carrier:	50 µl suspension per carrier
Plate count in the test suspension of bacteria	1.04 * 10^9 – 2.93 * 10^9 cfu/ml
Plate count in the test suspension of fungi	3.00 * 10^7—3.10 * 10^7 cfu/ml
Load (clean circumstances)	0.3 g/1 BSA
Duration of exposure:	The test carriers were exposed for 60, 120, 180 and 240 minutes. Date and time of removal of the carriers: 14 December 2020 11:21; 12:21; 13:21; 14:21
Period of testing:	14-18 December 2020

The testing according to the standard EN 17272:2020 was carried out in the laboratory of the National Public Health Centre, located at Mezzanine 1-8, Building B, Albert Flórián út 2-6, 1097 Budapest, between 10:21 am and 14:21 pm on 14 December 2020. The walls of the room with a cubic capacity of 81.6 m^3 (5 x 3.2 x 5.1 m) are covered with tiles up to the ceiling, and they are equipped with a plastic window and a door made of fibreboard. Before the tests, the room, including the exhaust system, and the openings, holes and discharges of various dimensions, has been sealed with materials (like silicone, aluminium etc.) into which the ozone is not able to diffuse. Fresh air had been let into the room before the testing in order to reach the temperature and humidity according to the standard. During the tests, the carriers were contaminated with 50 µl suspension of the selected dilution member (Bacteria 10^{-3} and Fungi 10^{-2}) of the four bacteria and the two fungi under test. The drying time was 1 hour 5 minutes. Next the glass plates were transported in sterile plastic Petri dishes within the building, then they were placed in the four corners of the test room in a distance of 50 cm from the boundary wall surfaces at a height of 1.2-1.5 m.

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4. Placement of the test preparations

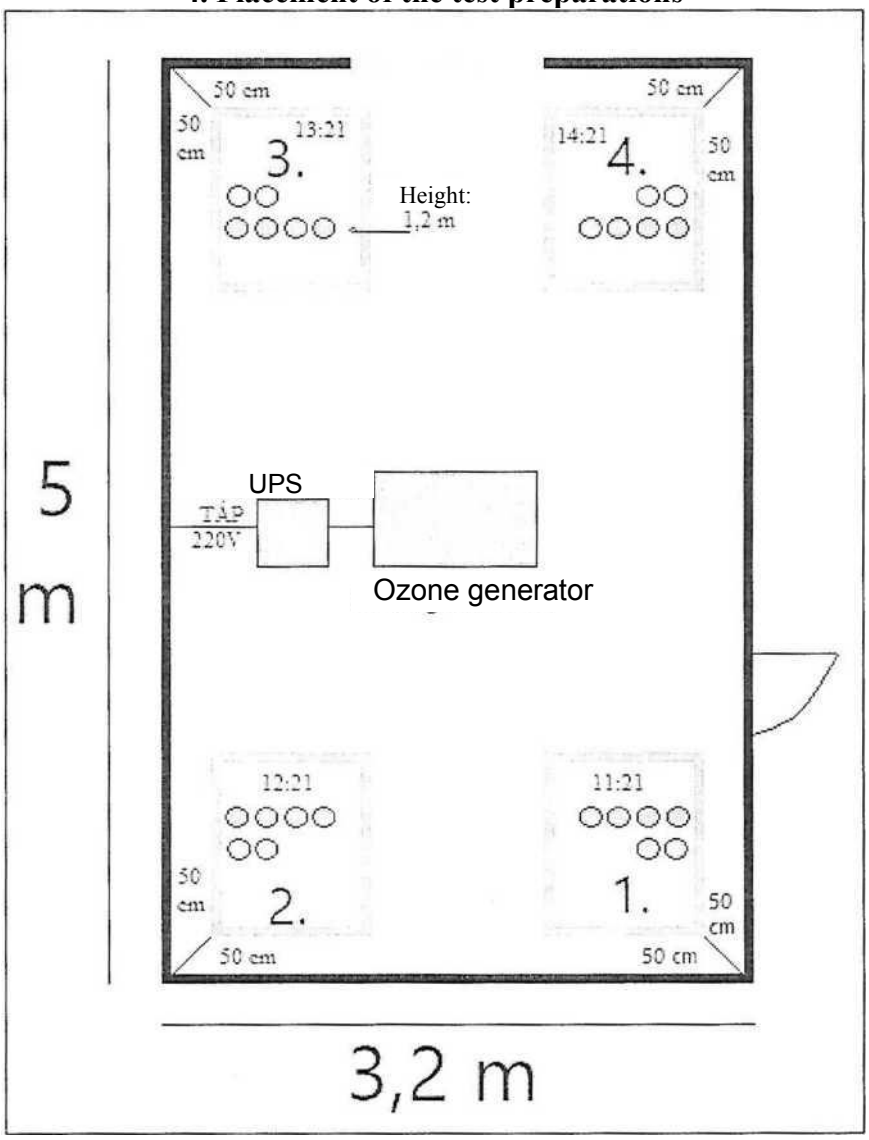

Figure 1 – Schematic layout of the test room – Drawing made by us.

NOTE 1 The control carriers were put at a place with similar temperature and humidity as those of the test room, but they had not been exposed to the automatized disinfection process.
NOTE 2 The removal method of the test organisms followed the sequence indicated on the drawing.
NOTE 3 The numbering of the corners is the same as the sequence of removing the samples.

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5. Process of ozone disinfection

Test procedure:	1. Ozone production (using the ozone generator ExOzone® EX-SR-100). The said equipment operated for 240 minutes and generated ozone from the atmospheric oxygen during this time. 2. Time of removal of the test samples 1. 11:21 2. 12:21 3. 13:21 4. 14:21 3. Ventilation Ventilation for 24 hours to change air several times in order to eliminate the characteristic irritating ozone smell absorbed.

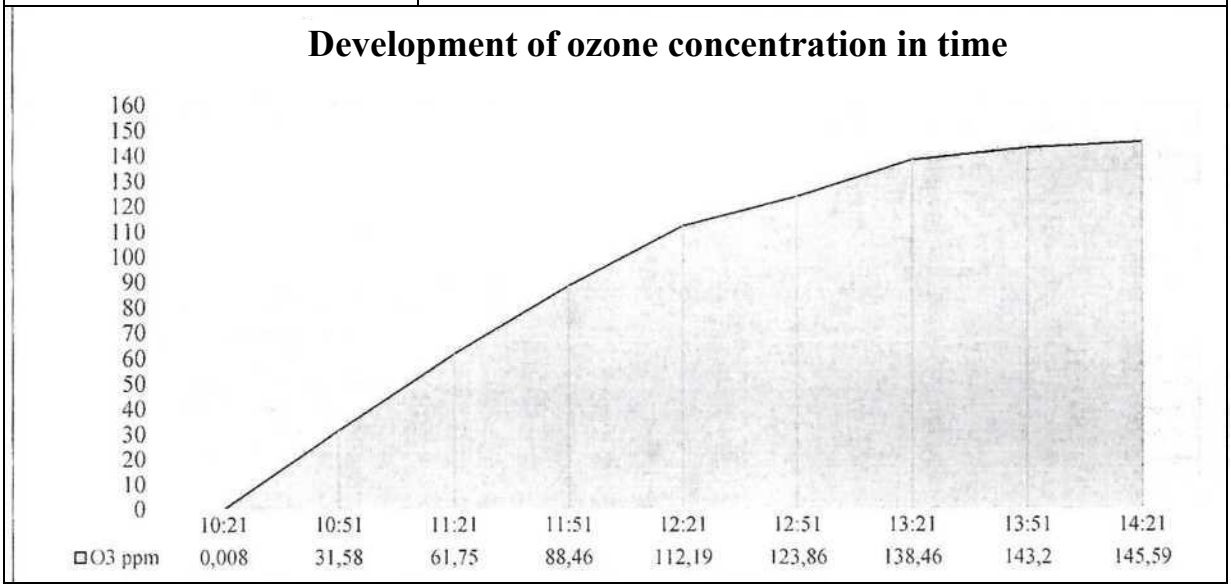

Figure 2 – Development of ozone concentration in the atmosphere of the test room

NOTE 1 The concentration of ozone (O_3) was measured by the manufacturing company. The NPHC cannot take responsibility for the reliability of the measurement data.

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6. Test results

Test organism	Initial cell count (cfu/ml)		Dilution member used Bacteria: 10^{-3} (cfu/ml) Fungi: 10^{-2} (cfu/ml)		Cell count at the end of the test (cfu/ml)		Logarithm of cell count decrease (R) at the end of the test (cfu/ml)
	1 ml	LOG 10	1 ml	LOG10	Control	Carrier	
P. aeruginosa ATCC 15442	$1.04* 10^9$	9.01	$1.04* 10^6$	6.01	>330	0	>6.01
S. aureus ATCC 6538	$1.20*10^9$	9.08	$1.20* 10^6$	6.08	>330	0	>6.08
E. hirae ATCC 10541	$1.60* 10^9$	9.20	$1.60*10^6$	6.20	>330	0	>6.20
E. coli ATCC 10536	$2.93* 10^9$	9.47	$2.93 * 10^6$	6.47	>330	0	>6.47
Candida albicans ATCC 1023	$3.10*10^{7*}$	7.49	$3.10* 10^5$	5.49	>330	0	>5.49
Aspergillus brasiliensis ATCC 16404	$3.00* 10^7$	7.48	$3.00* 10^5$	5.48	>330	0	>5.48
Condition of disinfectant effect: R≥5 for bacteria; R≥4 for fungi No colony propagated again after the incubation time.							

NOTE 1 Diagnosis of the carriers to determine the count of living cells: the carriers were placed on selective culture medium for 30 minutes immediately after the end of the test. Then the carriers were removed from the culture medium and the culture medium was exposed to an incubation temperature suitable for the culture of the given micro-organism. Bacteria: 37±1 °C; Fungi: 30±1 °C; Bacteria: 24-48 hours; Fungi: 48-72 hours. After the incubation time, the generated colonies were counted.
NOTE 2 The applicability of the impression sampling of the carriers was tested, and it was found that the impression sample fixed on the selective culture medium had no measurable inhibitive effect.
NOTE 3 Decrease of the required magnitude could only be achieved in the case of test carriers subjected to 240-minute exposure.

7. Assessment

We tested the bactericidal and fungicidal effects of using the **ozone generator ExOzone® EX-SR-100** at room temperature (21±2°C), with an organic matter load of 0.3 g/litre bovine albumin and exposure time of 240 minutes. In the range of 90-146 ppm (parts per million), the equipment decreased the number of the living cells of the micro-organisms *Staphylococcus aureus* ATCC 6538, *Enterococcus hirae* ATCC 10541, *Escherichia coli* ATCC 10536 and *Pseudomonas aeruginosa* ATCC 15442 by a magnitude of 5 (lg) at least, as well as the number of the living cells of *Candida albicans* ATCC 10231 and the number of spores of *Aspergillus brasiliensis* ATCC 16404 by a magnitude of 4 (lg) at least.

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According to the test results, the ozone generator of the type ExOzone® EX-SR-100 achieves the required bactericide and fungicide effects under the given test conditions.

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Róbert Heck
Public Health Inspector

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Dr. Márta Milassin
Biologist

Illegible signature
Dr. Ágnes Dánielisz
Head of Department

Budapest, 13 January 2021

The test results apply exclusively to the given type of the test device/equipment: ExOzone® EX-SR-100.
This Test Record may only be copied in its full version.