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Accredited laboratory according to STN EN ISO/IEC 17025:2017

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VALIDATION REPORT ON TESTING THE ANTIMICROBIAL EFFICACY OF OZONE GENERATOR IN ROOM DISINFECTION WITH APPARATUS G-ULTRA FROM THE COMPANY GRIZZLY

Revision number 1.0

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TEST INFORMATION

Name of the apparatus: G ultra

Manufacturer: Ozone generator with power 80 000 mg/h wit power ventilator 3500m^3/h

Height: 96cm

Weight: 58kg

Supply voltage: 230V/50Hz

Siren noise: 98dB

Suitable for room: to 200m^2

Ozone disinfection: - Power 80 000mg/h

- Number ozone ceramic plates: 72
- Ventilator: 3500m^3/h

Ozone output: 480 000 mg / h

Test Conditions

Ozone disinfection

Date: 3.11.2021-5.11.2021

Testing rooms: Microbiological laboratory – dressing room

Room Temperature: 21,9°C

Humidity: 39%

Area of the test rooms: 11,6m^2

Contact time of the apparatus: 5min+45min ventilation and 15min+45ventilation

Carrier type: plastic (LITEN MB 71 – HDPE – hight density polyethylene) round carries – nonporous and carrier diameter was 3cm

Position of the carriers:

- three carriers 2,6m horizontally from the apparatus and 1m from the ground from each test organisms
- three carriers 2,6m vertically from the apparatus and 1m from the ground from each test organisms

Microbial culture:

- *Escherichia coli* ATCC 25922
- *Staphylococcus aureus* ATCC 6538P
- Spore – *Bacillus subtilis* ATCC 6633

Dilution solution: Tryptone salt

Incubation medium: TSA - Tryptone soy agar (*Staphylococcus aureus, Bacillus subtilis*)

MCA- MacConkey Agar (*Escherichia coli)*

Incubation temperature: 36±2°C

Incubation Time: 45±3h

METHOD:

The protocol was done following the principle of standard EVE EN 17272:2020 – Chemical disinfectants and antiseptics – Methods of airborne room disinfection by automated process – Determination of bactericidal, mycobactericidal, sporicidal, fungicidal, yeasticidal, virucidal and phagocidal activities. And the second standard was used ASTM E3135-18 Standard Practice for determining Antimicrobial Efficacy of Ultraviolet Germicidal Irradiation Against Microorganisms on Carriers with Simulated Soil.

Ozone disinfection

1. **Preparation of bacterial culture**

Preparation of stock subculture a solid agar *Staphylococcus aureus* ATCC 6538P on TSA medium and *Escherichia coli* ATCC 25922 on MCA medium. It was prepared the third subculture – each subculture was incubated 24h at 36±1°C. From the subculture were prepared dilution of bacterial cells – concentration from $5x10^7$ to $2x10^9$ CFU/ml

Preparation of stock subculture of *Bacillus subtilis* ATCC6633 was according to SOP (4460). The work concentration of spore was $5x10^6$ CFU

2. **Preparation of inoculum and treatment with UVC and ozone generator carriers**

From the bacterial culture were prepared working dilution 10^{-6}, 10^{-7}, 10^{-8} in dilution solution (tryptone salt). 50µl of the working subculture was transferred on plastic nonporous carriers and dried in incubator at 36±1°C. The dried carriers with bacterial culture were incubate maximum 120 minutes at 37°C. Carrier were exposure to production UVC radiation in 1 distance and 2 times - **Table 1. and Table 2.**

There were prepared two positive controls:

- Positive control 1 – prepared from inoculum
- Positive control 2 – without exposure of UVC irradiation in experimental time 10 and 20 minutes

Carriers were collected to sterile Petri dish after exposure to UVC radiation. The test carriers were transferred to Erlenmeyer flask with volume of tryptone salt 100ml. The flasks were mixture a few seconds and scrub to carrier surface with sterile tip. 100µl were transferring from previous dilution into 900µl sterile tryptone salt. And 10µl of final concentration were inoculated to Petri dish with TSA medium for *Staphylococcus aureus* and *Bacillus subtilis* and MCA for *Escherichia coli.* The Petri dish were incubated at 30 – 37°C for 45±3h.

3. Experimental data and calculation

After cultivation were obtained colonies of microorganisms. Each experimental setup (distance and time dependent carriers) was done duplicates. Each experimental concentration was evaluated and calculate according to standard EVE EN 17272:2020 – Chemical disinfectants and antiseptics – Methods of airborne room disinfection by automated process – Determination of bactericidal, mycobactericidal, sporicidal, fungicidal, yeasticidal, virucidal and phagocidal activities.

Experimental groups were compared to Positive control 2. The log reduction (effectiveness of the Spectra Infinity) was calculated from the normalized values.

RESULTS SUMMARY

Ozone disinfection

Bacteria and spore	Test position	CFU/ml	Reduction
Escherichia coli	Positive control 2	5,00E+08	-
	2,6M1H	0	8,7
	2,6M1V	0	8,7
Staphylococcus aureus	Positive control 2	3,60E+07	-
	2,6M1H	0	7,6
	2,6M1V	0	7,6
Bacillus subtilis - spores	Positive control 2	1,30E+06	-
	2,6M1H	0	6,1
	2,6M1V	0	6,1

Table 1. Account of colony *E. coli, S. aureus* and *B. subtilis spores* after 5 minutes ozone exposure + 45 minutes ventilation. Effect of ozone generator Spectra Infinity in one distance.

Bacteria and spore	Test position	CFU/ml	Reduction
Escherichia coli	Positive control 2	5,00E+08	-
	2,6M1H	0	8,7
	2,6M1V	0	8,7
Staphylococcus aureus	Positive control 2	3,60E+07	-
	3M1H	0	7,6
	3M1V	0	7,6
Bacillus subtilis - spores	Positive control 2	1,30E+06	-
	3M1H	0	6,1
	3M1V	0	6,1

Table 2. Account of colony *E. coli, S. aureus* and *B. subtilis spores* after 15 minutes ozone exposure + 45 minutes ventilation. Effect of ozone generator Spectra Infinity in one distance.

DISCUSSION:

The reduction of *Staphylococcus aureus* ATCC 6538P was log7,6 (99,999%) in each distance and time. According to standard EVE EN 17272:2020 – Chemical disinfectants and antiseptics – Methods of airborne room disinfection by automated process – Determination of bactericidal, mycobactericidal, sporicidal, fungicidal, yeasticidal, virucidal and phagocidal activities, Spectra Infinity can be used in the medical area. In the case of *Escherichia coli* ATCC 25922 was reduction log8,7 (99,999%) in each distance and time. The device can be used in medical area, because reduction with compare to control was higher as 5. The apparatus Spores *Bacillus subtilis* had log6,1 reduction. Spectra Infinity as proven by experiment enables log7 reduction of *S. aureus*, log8 reduction of *E. coli* and log6 reduction of *Bacillus subtilis* spores. The apparatus Spectra Infinity can be used in medical area for room disinfection, because efficiency was higher like log5.