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VALIDATION REPORT ON TESTING THE ANTIMICROBIAL EFFICACY OF THE UVC – RADIATION IN ROOM DISINFECTION WITH APPARATUS SPECTRA 360 ANTIMAGNETIC FROM THE COMPANY GRIZZLY

Revision number 1.0

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TEST INFORMATION

Name of the apparatus: SPECTRA 360 ANTIMAGNETIC

Manufacturer: UVC radiation

Height: 1,65m + base diameter 50cm

Weight: 30kg

UVC power: 1160W

Supply voltage: 230V/50Hz

UVC lamps: 8 pieces – 145W, Philips

Siren noise: 98dB

Suitable for room: to 100m^2

Test Conditions

Date: 3.11.2021-5.11.2021

Testing room: Microbiological Laboratory – Laboratory for medium preparing

Room Temperature: 21,9°C

Humidity: 39%

Area of the test room: 20m^2

Contact time of the apparatus: 10min and 20min

Carrier type: plastic (LITEN MB71 – HDPE- hight density polyethylene) round carries – nonporous and carrier diameter was 3cm

Position of the carriers:

- three carriers 3m horizontally from the apparatus and 1m from the ground from each testing organisms
- three carriers 3m vertically from the apparatus and 1m from the ground from each testing organisms
- three carriers 5m horizontally from the apparatus and 1m from the ground from each testing organisms
- three carriers 5m vertically from the apparatus and 1m from the ground from each testing 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.

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 UVC treatment of 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 360 Antimagnetic) was calculated from the normalized values.

RESULTS SUMMARY:

Bacteria and spore	Test position	CFU/ml	Reduction
Escherichia coli	Positive control 2	5E+08	-
	3M1H	0	8,7
	3M1V	0	8,7
	5M1H	0	8,7
	5M1V	0	8,7
Staphylococcus aureus	Positive control 2	3,6E+07	-
	3M1H	0	7,5
	3M1V	0	7,5
	5M1H	0	7,5
	5M1V	0	7,5
Bacillus subtilis - spores	Positive control 2	1,3E+06	-
	3M1H	4,3E+04	4,63
	3M1V	0	6,1
	5M1H	0,2	-0,7
	5M1V	0	6,1

Table 1. Account of colony *E. coli, S. aureus* and *B. subtilis spores* after 10 minutes exposure of UVC radiation from the apparatus Spectra 360 Antimagnetic in two distance and different positions.

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

Table 2. Account of colony *E. coli, S. aureus* and *B. subtilis spores* after 20 minutes exposure of UVC radiation from the apparatus Spectra 360 Antimagnetic in two distance and different positions.

DISCUSSION:

The reduction of *Staphylococcus aureus* ATCC 6538P was log 7,5 (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 360 Antimagnetic can be used in the medical area. In the case of *Escherichia coli* ATCC 25922 was reduction log 8,7 (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. In the log reduction of *Bacillus subtilis* ATCC 6633 spores was evaluated different log reduction. Sensibility of *Bacillus subtilis* spores is independent on the time of exposure with the apparatus and distance the apparatus. In the distance 3 meter was log4,63 and distance 5meters was minus log reduction compare to control. The time 20minutes had log6,1 reduction compares to control. Reduction of *Bacillus subtilis* spores after exposure UVC radiation by 20minutes had efficacy in both distances. The apparatus Spectra 360 Antimagnetic as proven by experiment enables log7 reduction of *S. aureus*, log8 reduction of *E. coli* and different log reduction of *Bacillus subtilis* spores – depend on the time of exposure and distance.