



Microbiological laboratory

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

Accreditation certificate holder č. S-264

The testing of disinfection efficacy UVC radiation, Ozone generator and air filtration with apparatus Spectra Infinity from the company – Grizzly, s.r.o.

Revision number 2.0

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Date report: 01.03. 2022

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TEST INFORMATION

Name of the apparatus: SPECTRA INFINITY

Manufacturer: Spectra infinity is professional device that combine three technologies:

1. Open UVC emitter with UVC power 1 760W (360° radiation)
2. Close UVC emitter with UVC power 440W with power ventilator 1320m³/h
3. Ozone generator with power 80 000 mg/h wit power ventilator 1320m³/h

Height: 1,76m + base diameter 50cm

Weight: 76kg

Max. power: 2200W

Supply voltage: 230V/50Hz

Siren noise: 98dB

Suitable for room: to 200m²

Open UVC system: - UVC power: 1760W

- Type of tube: Philips PL-L 55 2G11 or OSRAM HNS 55 2G11
- Quantity of tubes 32
- Lamp life 20 000h

Close UVC system: - UVC power 440W

- Type of tube: Philips PL-L 55 2G11 or OSRAM HNS 55 2G11
- Quantity of tubes 8
- Lamp life 20 000h
- Ventilator 1320m³/h

Ozone disinfection: - Power 80 000mg/h

- Number ozone ceramic plates: 16
- Life: 8 000h
- Ventilator: 1320m³/h

Test Conditions

Open UVC system

Date: 5.11.2021-7.11.2021

Testing room: Microbiological Laboratory – Laboratory for incubation and identification of microorganisms

Room Temperature: 20,4°C

Humidity: 40,2%

Area of the test room: 29m²

Contact time of the apparatus: 10min and 20min

Carrier type: plastic (LITEN MB 71 – HDPE – high 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

Close UVC system

Date: 25.01.2022

Room Temperature: 20,7°C

Humidity: 39,2 %

The test points: The points were defined according to norm EN 17 141:2022

Contact time of the apparatus: 20,40 and 60 minutes

Carrier type: without carriers – evaluated with Sampler air – gas flow meter

Measuring points:

- According to standard EN 17 141:2020 was defined one measuring points in the test room (three independents measurements in the point)
- Measurement point – one point – three measurements, hermetic close room

Identification of microorganisms species – see Table 3.

Incubation medium: TSA - Tryptone soy agar – universal medium for identification of microorganisms

Incubation temperature: 36±2°C

Incubation Time: 45±3h

Ozone disinfection

Date: 5.11.2021-7.11.2021

Testing rooms: Office building

Room Temperature: 20,4°C

Humidity: 40,2%

Area of the test rooms: 18,5m²

Contact time of the apparatus: 20min+45min ventilation and 40min+45ventilation

Carrier type: plastic (LITEN MB 71 – HDPE – high 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. The third standard was used EN 17 141:2020 Cleanrooms and associated controlled environments – biocontamination control.

Open UVC system

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 $36\pm 1^\circ\text{C}$. From the subculture were prepared dilution of bacterial cells – concentration from 5×10^7 to 2×10^9 CFU/ml

Preparation of stock subculture of *Bacillus subtilis* ATCC6633 was according to SOP (4460). The work concentration of spore was 5×10^6 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).

50 μl of the working subculture was transferred on plastic nonporous carriers and dried in incubator at $36\pm 1^\circ\text{C}$. The dried carriers with bacterial culture were incubate maximum 120 minutes at 37°C . Carrier were exposure to production UVC radiation in 2 distance and 2 times - **Table 1.** and **Table 2.**

There were prepared two positive controls:

- Positive control 1 – prepared from inoculum
- Positive control 2 – carriers 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. 100 μl were transferring from previous dilution into 900 μl sterile tryptone salt. And 10 μl 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 $30 - 37^\circ\text{C}$ for $45\pm 3\text{h}$.

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.

Close UVC system

1. Preparation of medium and the apparatus

There were used TSA (Tryptone soy agar) medium **L7293 exp.23.5.2022 (certified medium)**. Temperature of medium was warmed to room temperature. The clean of air was defined with Sampler Air Lite in the room after using of the apparatuses Spectra Infinity.

The one measurement point was defined based on the room size. The air was measured in 0 minutes – as positive control – species control of the microorganisms in evaluated room.

The experimental measurements were after 20,40- and 60-minutes activity of the apparatuses from the company Grizzly, s.r.o. – closed UVC radiation.

The TSA plates were incubated by $36\pm 1^{\circ}\text{C}$ for $45\pm 3\text{h}$.

2. Experimental data and calculation

The account of CFU (colony forming unit) per m^3 were defined after incubation and there was identified species of microorganisms in the measurement area – Table 4.

According standard ASTM E3135-18 Standard Practice for determining Antimicrobial Efficacy of Ultraviolet Germicidal Irradiation Against Microorganisms on Carriers with Simulated Soil were evaluated reduction and efficacy of the apparatus Spectra Infinity.

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 $36\pm 1^{\circ}\text{C}$. From the subculture were prepared dilution of bacterial cells – concentration from 5×10^7 to 2×10^9 CFU/ml

Preparation of stock subculture of *Bacillus subtilis* ATCC6633 was according to SOP (4460). The work concentration of spore was 5×10^6 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). 50 μl of the working subculture was transferred on plastic nonporous carriers and dried in incubator at $36\pm 1^{\circ}\text{C}$. 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 5. and Table 6.**

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:

Open UVC system

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

Bacteria and spore	Test position	CFU/ml	Reduction
<i>Escherichia coli</i>	Positive control 2	5,00E+08	-
	3M1H	0	8,7
	3M1V	0	8,7
	5M1H	0	8,7
	5M1V	4,50E+08	0,05
<i>Staphylococcus aureus</i>	Positive control 2	3,60E+07	-
	3M1H	0	7,6
	3M1V	0	7,6
	5M1H	0	7,6
	5M1V	0	7,6
<i>Bacillus subtilis</i> - spores	Positive control 2	1,30E+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 Infinity in two distance and different positions.

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

Close UVC system

Table 3. The account of CFU per m³ and identification of microorganisms species in the samples of the air in 20, 40 and 60 minute.

Measurement point	0'	20'	40'	60'
	CFU/m ³	CFU/m ³	CFU/m ³	CFU/m ³
1 st measure Identification	290	0	0	0
	Coagulase negative <i>Staphylococci</i> , <i>Micrococcus luteus</i> , <i>Bacillus</i> spp., <i>Trichoderma</i> spp.	-	-	-
2 nd measure Identification	340	0	0	0
	Coagulase negative <i>Staphylococci</i> , <i>Micrococcus luteus</i> , <i>Bacillus</i> spp., <i>Trichoderma</i> spp.	-	-	-
3 th measure Identification	325	0	0	0
	Coagulase negative <i>Staphylococci</i> , <i>Micrococcus luteus</i> , <i>Bacillus</i> spp., <i>Bacillus</i> <i>subtilis</i> , <i>Aspergillus</i> <i>niger</i>	-	-	-

Table 4: Time dependent reduction CFU/m³ after using Spectra Infinity

Measurement point	0' (CFU/m ³)	20' (CFU/m ³)	40' (CFU/m ³)	60' (CFU/m ³)
1 st measure	290	0	0	0
2 nd measure	340	0	0	0
3 th measure	325	0	0	0
Average	318,33	0	0	0
Reduction (%)		100%	100%	100%

Ozone disinfection

Table 5. Account of colony *E. coli*, *S. aureus* and *B. subtilis* spores after 20 minutes ozone exposure + 45 minutes ventilation. Effect of ozone generator Spectra Infinity in one distance.

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

Table 6. Account of colony *E. coli*, *S. aureus* and *B. subtilis* spores after 40 minutes ozone exposure + 45 minutes ventilation. Effect of ozone generator Spectra Infinity in one distance.

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

DISCUSSION:

Open UVC system

The reduction of *Staphylococcus aureus* ATCC 6538P was log 7,6 (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 log 8,7 (99,999%) in one distance (3m) and both times. In the distance 5m was different results, in the time 10 minutes and vertical position was not reduction log 8,7; but only log0,05. The device can be used in medical area, because reduction with compare to control was higher as 5, but using of the function open UVC radiation is time and distance dependent. In the log reduction of *Escherichia coli* ATCC 25922 is independent on the time of exposure with the apparatus and distance the apparatus. *Bacillus subtilis* ATCC 6633 spores was evaluated as log 6,1 the reduction with using Spectra Infinity. The apparatus Spectra Infinity as proven by experiment enables log7 reduction of *S. aureus*, log8 reduction of *E. coli* - depend on the time and distance, log6 reduction of *Bacillus subtilis* spores.

Close UVC system

The apparatus SPECTRA Infinity had efficiency in air purification. The reduction of microorganism colonies was 100% after 20 minutes flirtation of air. Spectra Infinity – close UVC radiation reduced identified species of bacteria's and microscopic fibrous fungi. The apparatus had efficacy of the air disinfection. There were reduced spores (*Bacillus subtilis*) fungi (*Aspergillus niger*), pathogen and non-pathogen species of microorganisms in the normal air in the test room.

Ozone generator

The reduction of *Staphylococcus aureus* ATCC 6538P was log 7,6 (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 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. 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 than log5.