Testing of antimicrobial efficacy of three different disinfection procedures

Case study

Antimicrobial activity

Bacteriophage MS2

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VALIDATION REPORT ON PERFORMED ANALYSIS

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The presented test results refer exclusively to the herein defined test objects. Any extracts of this report are possible only with written approval of Microbium d.o.o. The subject report shall not be reproduced or modified without written consent of Microbium L.LC.

Summary of results

1) The tested air disinfection device AirLight ULTRA QUITE achieves total elimination of microorganisms in the efficiency test after contact time of:

10 s

6,26 log (>99,9999 % efficiency) reduction on the reference test strain *Escherichia phage* MS2 DSM 13767

2) The tested product DEZINSEPT DERM+ achieves in the efficiency test after contact time of:

60 min

0,19 log reduction on the reference test strain MS2 DSM 13767

3) The tested device Philips MASTER LEDtube 600mm HO 8W 840 T8 achieves total elimination of microorganisms in the efficiency test after contact time of:

60 min

7,04 log reduction on the reference test strain MS2 DSM 13767

Expert opinion

The tested air disinfection device with the respective trade name **AirLight ULTRA displays a sufficient level of virucidal activity** in accordance with standard EN 15714: 2019 at airflow of 300 m^3/h and at the contact time of 10 s in relation with the reference test strains *Escherichia phage* MS2.

The tested disinfectant **DEZINSEPT DERM+ displays an insufficient level of virucidal activity** in accordance with standard EN 14476:2013+A2:2019 in clean conditions and at room temperature 20 °C at the contact time of 60 min in relation with the reference test strains *Escherichia phage* MS2.

The tested air disinfection device with the respective trade name **Philips MASTER LEDtube 600mm HO 8W 840 T8 displays a sufficient level of virucidal activity** in accordance with standard EN 17272:2020 at the contact time of 60 min relation with the reference test strains *Escherichia phage* MS2.

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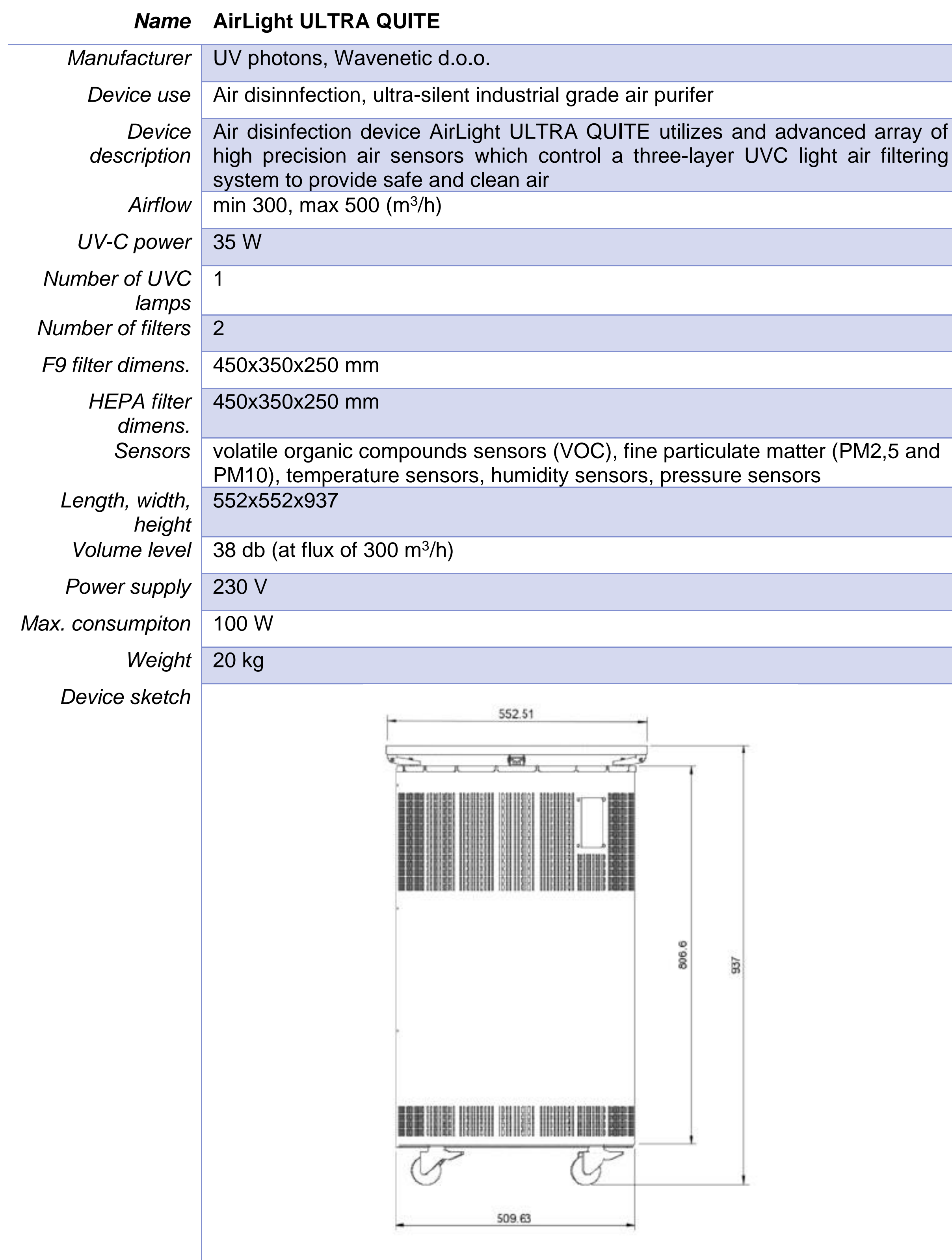
Study intention

After the COVID-19 pandemic outbreak, the global use for disinfectant products and devices is rapidly growing. Disinfectants can be split into two different principles of disinfection: physical methods (UV-C light irradiation, filtration, heating) or chemical methods (fumigation, chemicals).

In presented study, we compared three different principles of disinfection, namely:

1. air disinfection device AirLight ULTRA QUITE of the company Wavenetic d.o.o.,
2. UVC lamp integrated in microbiological chamber MC9-2 of the company Iskra Pio d.o.o.,
3. disinfectant DEZINSEPT DERM+ of the company Irbis d.o.o.

In the presented study, we used a reference strain of *Escherichia phage* MS2 (DSM 13767, German Collection of Microorganisms and Cell Cultures) and we followed the protocols of four standards most suitable for each type of disinfection tested.

Name	**AirLight ULTRA QUITE**
Manufacturer	UV photons, Wavenetic d.o.o.
Device use	Air disinnfection, ultra-silent industrial grade air purifer
Device description	Air disinfection device AirLight ULTRA QUITE utilizes and advanced array of high precision air sensors which control a three-layer UVC light air filtering system to provide safe and clean air
Airflow	min 300, max 500 (m³/h)
UV-C power	35 W
Number of UVC lamps	1
Number of filters	2
F9 filter dimens.	450x350x250 mm
HEPA filter dimens.	450x350x250 mm
Sensors	volatile organic compounds sensors (VOC), fine particulate matter (PM2,5 and PM10), temperature sensors, humidity sensors, pressure sensors
Length, width, height	552x552x937
Volume level	38 db (at flux of 300 m³/h)
Power supply	230 V
Max. consumpiton	100 W
Weight	20 kg
Device sketch	

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Name	**DEZINSEPT DERM+, medical products**	
Manufacturer	IRBIS d.o.o.	
Product type	PT1: Hand desinfection	
Aggregate state	Fluid biocidal product	
Color characteristics	Colourless fluid	
Chemical composition	Active substance	Concentration
	Ehtanol	70g / 100g

Name	**Philips MASTER LEDtube 600mm HO 8W 840 T8, integrated into the protective microbiological chamber MC 9-2**
Manufacturer	Iskra PIO d.o.o./Philips
Microbiological chamber use	In laboratories that manipulate chemical and biological substances and in areas that require maximum protection of the operator, the environment and the product of work.
Device description	We tested a UVC lamp that is built into the chamber and is designed to disinfect the surface.
UV-C lamp	Philips MASTER LEDtube 600mm HO 8W 840 T8
Number of UV-C lamps	1
Length, width, height	603x26x26 mm
Luminous Efficacy	131 lm/w
Luminous Flux	1,050 lm
Voltage	230 V
Power	8 W
Device picture	

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Summary of experimental conditions

Summary of experimental conditions	Laboratory	Analytic laboratory of Microbium L.LC.
	Date of analysis	12. - 16.3.2022, 18. - 23.3.2022
	Room temperature	20 °C
	Relative humidity	57 %
	Standards used*	EN 15714:2019 Method of evaluating the UV dose to airborne microorganisms transiting in-duct ultraviolet germicidal irradiation devices 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 EN 14476:2013+A2:2019 Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of virucidal activity in the medical area - Test method and requirements (Phase 2/Step 1) ASTM E3135-18 Standard Practice for Determining Antimicrobial Efficacy of Ultraviolet Germicidal Irradiation Against Microorganisms on Carriers with Simulated Soil
	Analytical method	Plaque assay
	Diluent	dH_2O z 0,9 % NaCl
Microbial test strain and culture data	Reference microbial test strain	*Escherichia phage* MS2 DSM 13767
	Host strain for phagocidal activities	*Escherichia coli* DSM 5695
	Incubation medium	TSA (Tryptic Soy Agar) LB soft agar (0,7 % agar)
	Incubation temperature	36±2 °C
	Incubation time	24 h±2h

*The protocol was done following the principle of four standards. The entire methodology is described in the Methods section.

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Reference test strain Escherichia phage MS2

Bacteriophages are a group of viruses that specifically infect bacteria. They are ubiquitous in the environment and are recognized as the most abundant biological agent on earth (Hatfull and Hendrix., 2011). It is estimated that there are about 10^{31} bacteriophages on Earth. Bacteriophages are bacterial viruses that infect a susceptible bacterium, replicate in it, and cause lysis. Bacteriophages can be used as surrogates for animal / human viruses.

Testing of antimicrobial efficacy of elimination devices on human viruses is difficult and associated with high cost (special media, maintenance on host cell lines) and requires second, third or even fourth laboratory biosafety level. Working in laboratories with higher biosafety levels requires more expensive materials and equipment, additional requirements regarding safety rules, preventive and corrective measures. Therefore, working with human pathogens is more demanding both financially and technically. In 2020 and 2021, the use of bacteriophages as surrogates for human viruses grow practically exponentially. The MS2 virus is used as a surrogate for noroviruses. Noroviruses (viruses of the Caliciviridae group) are known causes of human gastroenteritis and are the leading cause of diarrhea.

Escherichia phage MS2 belongs to the group Leviviridae. It is associated with noroviruses (Caliciviridae) by some structural similarities:

- The genome is a single-stranded RNA molecule.
- Both MS2 and norovirus are non-enveloped viruses. Viruses are composed of a single protein coat, which consists of symmetrically arranged viral proteins.
- The entire viral coat consists of several units of viral protein.

In the presented study, we used a reference strain of *Escherichia phage* MS2 (DSM 13767, German Collection of Microorganisms and Cell Cultures). The performance results of the tested air disinfection device on phage MS2 DSM 13767 can be extrapolated to the operation of the same device under the same conditions on Vertebrate viruses without viral envelope.

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Results

1. Air disinfection device AirLight ULTRA QUITE

Efficiency test - MS2

Repetition	Dilution	Vc1	Vc2	Average Vc1, Vc2	Average PFU/ml	log Na	Log N0	log reduction (N0-Na)	efficiency (%)	Result
1	10^{-0}	0	0	0	0,00E+00	0,00	6,26	**6,26**	**>99,9999**	**total elimination**
	10^{-1}	0	0	0						
2	10^{-0}	0	0	0	0,00E+00	0,00	6,26	**6,26**	**>99,9999**	**total elimination**
	10^{-1}	0	0	0						

Control - MS2

Test suspension N	Dilution	Vc1	Vc2	Average Vc1, Vc2	Average PFU/ml	log N
	10^{-6}	>330	>330	>330	6,50E+09	9,81
	10^{-7}	74	56	65		

Control 1	Dilution	Vc1	Vc2	Average Vc1, Vc2	Average PFU/ml	Average PFU (x40 ml)	log N0
	10^{-1}	>330	>330	>330	4,20E+04	1,68E+06	6,23
	10^{-2}	39	44	42			

Control 2	Dilution	Vc1	Vc2	Average Vc1, Vc2	Average PFU/ml	Average PFU (x40 ml)	log N0
	10^{-1}	>330	>330	>330	4,90E+04	1,96E+06	6,29
	10^{-2}	55	43	49			

Control average	Control 1	Control 2	Average PFU/ml	Average PFU (x40 ml)	log N0
	4,20E+04	4,90E+04	4,55E+04	1,82E+06	6,26

Sterility test	Dilution	Vc1	Vc2	Average Vc1, Vc2	Average PFU/ml	Result
	10^{-0}	0	0	0	0,00E+00	valid
	10^{-1}	0	0	0		

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2. Disinfection product DEZINSEPT DERM+, medical products

Test results

Disinfection product	*Product concentration (%)*	*Contact time*	*Dilution*	*Vc1*	*Vc2*	*Average Vc1, Vc2*	*Average PFU/ml*	*Na = average PFU/ml x10*	*log Na*	*Log N0*	*log reduction (N0-Na)*	*Criterion*	*Result*
DEZINSEPT DERM+	80 % dilution	1 min	10^{-4}	143	175	175	1,90E+07	1,90E+08	8,28	8,32	**0,04**	**≥4**	**invalid**
			10^{-5}	18	23	20,5							
		5 min	10^{-3}	>330	>330	>330	1,17E+07	1,17E+08	8,07	8,32	**0,25**	**≥4**	**invalid**
			10^{-4}	105	129	117							
		15 min	10^{-4}	155	128	142	2,06E+07	2,06E+08	8,31	8,32	**0,01**	**≥4**	**invalid**
			10^{-5}	21	33	27							
		30 min	10^{-4}	167	144	155,5	2,32E+07	2,32E+08	8,37	8,32	**-0,05**	**≥4**	**invalid**
			10^{-5}	45	18	31,5							
		60 min	10^{-4}	122	148	135	1,35E+07	1,35E+08	8,13	8,32	**0,19**	**≥4**	**invalid**
			10^{-5}	<14	<14	<14							

Controls and validation

	Dilution	*Vc1*	*Vc2*	*Average Vc1, Vc2*	*Average PFU/ml*	*log N*
Test suspension N	10^{-6}	193	223	208	2,39E+09	9,38
	10^{-7}	34	19	27		

	Dilution	*Vc1*	*Vc2*	*Average Vc1, Vc2*	*Average PFU/ml*	*N0 = average PFU/ml x10*	*log N0*
Test N0 (control)	10^{-4}	183	155	169	2,09E+07	2,09E+08	8,32
	10^{-5}	23	27	25			

	Dilution	*Vc1*	*Vc2*	*Average Vc1, Vc2*	*Average PFU/ml*	*0,5 x Nv average PFU/ml*
Validation suspension Nv	10^{-2}	145	159	152	2,11E+05	1,06E+05
	10^{-3}	23	30	27		

	Dilution	*Vc1*	*Vc2*	*Average Vc1, Vc2*	*Average PFU/ml*	*A = average PFU/ml x10*	*A is ≥ 0,5 X average Nv*
Control of experimental condition A	10^{-1}	89	143	116	1,16E+04	1,16E+05	valid
	10^{-2}	<14	<14	<14			

	Dilution	*Vc1*	*Vc2*	*Average Vc1, Vc2*	*Average PFU/ml*	*B = average PFU/ml x10*	*B is ≥ 0,5 X average Nv*
Control of neutralisation B	10^{-1}	167	145	156	2,23E+04	2,23E+05	valid
	10^{-2}	30	28	29			

	Dilution	*Vc1*	*Vc2*	*Average Vc1, Vc2*	*Average PFU/ml*	*C = average PFU/ml x10*	*C is ≥ 0,5 X average Nv*
Method validation	10^{-1}	133	151	142	2,16E+04	2,16E+05	valid
	10^{-2}	34	24	29			

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3. Philips MASTER LEDtube 600mm HO 8W 840 T8

Test results

Contact time	Dilution	Vc1	Vc2	Average Vc1, Vc2	Average PFU/ml	log Na	Log N0	log reduction (N0-Na)	Criterion	Result
5 min	10^{-3}	83	45	64						
	10^{-4}	<14	<14	<14	6,40E+05	5,81	7,04	**1,24**	**≥4**	**invalid**
15 min	10^{-2}	73	123	98						
	10^{-3}	<14	<14	<14	9,80E+04	4,99	7,04	**2,05**	**≥4**	**invalid**
30 min	10^{-1}	173	153	163						
	10^{-2}	19	28	23,5	1,99E+04	4,30	7,04	**2,74**	**≥4**	**invalid**
60 min	10^{-0}	0	0	0						
	10^{-1}	0	0	0	0,00E+00	0,00	7,04	**7,04**	**≥4**	**valid**

Controls and validation

Test suspension N	Dilution	Vc1	Vc2	Average Vc1, Vc2	Average PFU/ml	log N
	10^{-6}	193	223	208	2,39E+09	9,38
	10^{-7}	34	19	27		

Test N0 control	Dilution	Vc1	Vc2	Average Vc1, Vc2	Average PFU/ml	log N0
	10^{-4}	93	127	110	1,10E+07	7,04
	10^{-5}	<14	<14	<14		

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Discussion

Air disinfection device **AirLight ULTRA QUITE** consists of three barriers for microorganisms. At the air influx system is a F9 filter and prefilters ePM10 particles and ePM2,5 particles, at the air outlet system is a HEPA H13 filter and captures viruses, bacteria, and tiny microorganisms inside the filter´s internal surface and the UVC light breaks down the DNA and RNA of pathogens and thereby inactivates them. In the efficiency test, when inoculating the virus suspension into the device, we did not detect any virus particles that would pass through the device, which means total elimination of virus. The calculated log reduction was 6.26, which means more than 99.9999% efficiency. The particles transfer through the device occurs in a couple of seconds, which means that once the microorganisms are in contact with the device, it is immediately trapped in the HEPA filter and removed from the atmosphere. The device operated at a flow rate of 300 m^3/h, which means that entire air in a room of 60 m^3 is replaced within 12 minutes.

Ethanol-based disinfectants are the most widely used biocidal product on the market. 70% ethanol is a fluid biocidal product and is used as hand sanitizers or for hard-surface disinfection. The effectiveness of 70 % ethanol has been tested according to ISO standard EN 14476:2013+A2:2019 Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of virucidal activity in the medical area - Test method and requirements. In the efficacy test we calculated log reduction of virus MS2 of only 0.25. Results indicate that MS2 is highly resistant to 70 % ethanol. These results confirm previous study “Improved inactivation of nonenveloped enteric viruses and their surrogates by a novel alcohol-based hand sanitizer” by Macinga et al. (2008) demonstrating ethanol resistance of MS2 virus. This broad, multilaboratory study describes the antiviral performance of a different hand sanitizer. Presented results show that disinfectants based on ethanol and isopropanol did not cause the biocidal effect of MS2 virus. In contrast, they were able to inactivate the virus MS2 within 60 seconds using 100 ppm sodium hypochlorite.

UV-C irradiation has been used extensively in disinfecting drinking water, wastewater, air, pharmaceutical products, and surfaces. Viruses as a domain have a broad range of resistance to the germicidal UV light and the MS2 bacteriophage seems to be in the upper quadrant of the range of resistance. For a log reduction of bacteriophage count of 4 a dosage of about 120 mJ/cm^2 is required. The biocidal effect of the Philips MASTER LEDtube 600mm HO 8W 840 T8 UVC lamp was tested in accordance with standard EN 17272:2020 and the ASTM E3135-18 standard. In the efficacy test we calculated log reduction of virus MS2 of 2.04 at 15 min UVC exposure time. After 30 minutes of irradiation, we achieved 2.74 log reduction and after 60 minutes we achieved complete elimination of microorganisms with 7.04 log reduction.

In the presented study, we found that the fastest method tested for the elimination of MS2 virus is the method of air disinfection with the tested device AirLight ULTRA QUIET, where we achieved complete elimination of the virus just in 10 seconds. 70 % ethanol did not cause the biocidal effect of MS2 virus. Disinfection with UVC irradiation displays sufficient level of virucidal activity with log 7 reduction of MS2 virus, but with limitation of long exposure time - 60 minutes of irradiation was required for the total elimination of MS2 virus.

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Methods

1.) Preparation of the bacteriophage suspension

The bacteriophage MS2 was previously propagated on the host bacterium Escherichia coli DSM 5695. The bacteriophages were propagated in liquid culture in LB medium. One colony of overnight culture of E. coli was added to an Erlenmeyer flask with 100 ml of LB medium. The Erlenmeyer flask was incubated on an orbital shaker at 36 °C for 2 h and then 10 µl of bacteriophage stock culture was added. Bacteriophages were incubated for further 6 h. Bacteriophage suspension was centrifuged at 6000 g for 10 min and the supernatant was filtered through a 0,22 µm filter. This was regarded as bacteriophage test suspension for testing of antiviral efficacy of air disinfection device. The final titre of the produced bacteriophages was determined by the plaque assay method. The plaque assay method protocol was as follows: 100 µl of overnight host culture of E. coli DSM 5695 was mixed with 100 µl of the sample, and then the mixture was added to 5 ml of soft agar, vortexed and poured onto solid TSA agar medium. The plates were incubated for 21±3 h at 36±2 °C.

This was regarded as test suspension for testing of antimicrobial efficacy of air disinfection devices and antiseptics.

2.) Device testing – AirLight ULTRA QUITE

Device description

Air disinfection device **AirLight ULTRA QUITE** utilizes an advanced array of high precision air sesnors which control a three-layer UVC light air filtering system to provide safe and clean air. The F9 filter prefilters up to 95 % of ePM10 and up to 80 % of ePM1 particles and extends the HEPA filter lifespan. The H13 HEPA filter captures viruses, bacteria, and tiny microorganisms inside the filter´s internal surface. The UVC light breaks down the DNA and RNA of pathogens and thereby inactivates them.

Effiency test

The testing of antimicrobial efficacy of the AirLight ULTRA QUITE device was performed by placing the device horizontally between two support carriers. At the air influx system on the upper part of the device, the filter F9 module was removed to ensure a sufficiently high flux of microorganisms. At the air influx system on the bottom of the device, a sterile bag was installed for the purpose of testing the antimicrobial efficacy. An incision was made into the bag and from the bag, the air efflux was connected to the microbiological chamber MC 9-2. Further on, a petri dish of 150mm radius was placed at the air efflux from the bag in the microbiological chamber MC 9-2. The petri dish contained 40mL of saline solution. The position of the petri dish perpendicular to the air flux with the solid agar medium facing the air current.

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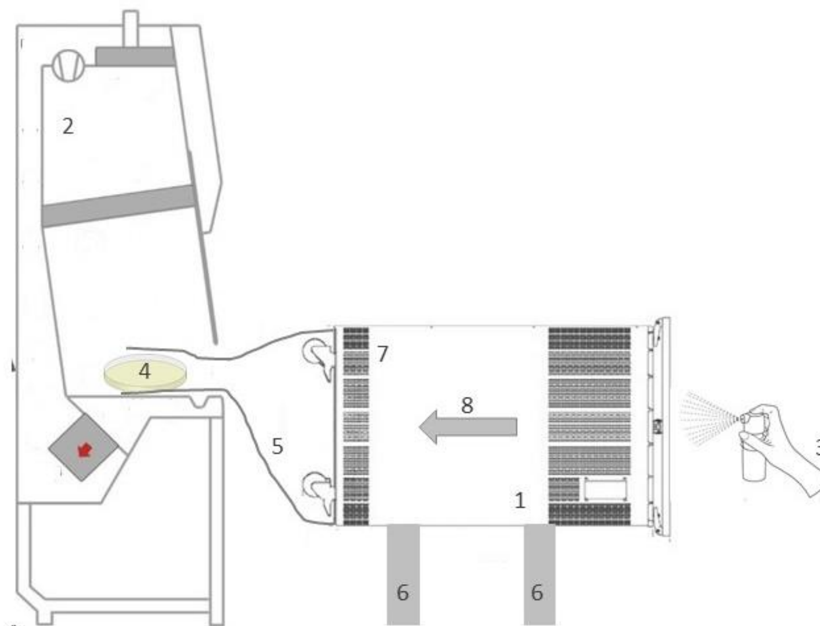

Scheme: schematic presentation of the device testing protocol.

At the upper part of the device, 10mL of test culture (*Escherichia phage* MS2 DSM 13767) was sprayed. Further on, the ventilator was set at the volume flux of 300 m^3/h. After the spraying of test cultures, the lid of the petri dishes was left off for an additional minute. Afterwards, the lid of the petri dish was positioned on the petri dish and the petri dishes with the air sample were stored until the analysis. All experimental setups were done in duplicates.

On the obtained bacteriophage suspensions, a 10-time dilution series was made and transferred to 5mL of soft agar containing the host bacterial strains. The mixed suspension in soft agar was then slightly mixed and transferred onto TSA solid agar. Afterwards, the petridishes containing bacteriophage samples were incubated 36±2 °C or 25±2 °C for 24±3h. After the incubation plaques (in the case of bacteriophages) were counted.

By comparing with the suspensions in the control petri dish, the reduction of the microorganisms was expressed as the logarithm with base 10 of the average number of microorganisms (obtained by duplicates or two technical repetitions) in the control petri dish (N_0) divided by the average number of microorganisms (obtained by duplicates or two technical repetitions) in each experimental setup (N_a). The reduction (Log R) can be calculated as:

$$LogR = Log\frac{N_0}{N_a} = logN_0 - N_a$$

Where N_0 is the average number of microorganisms (obtained by duplicates or two technical repetitions) in the control petri dish and N_a is the average number of microorganisms (obtained by duplicates or two technical repetitions) in each experimental setup

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Controls

Sterility test

The sterility test of the AirLight ULTRA QUITE device was performed by placing the device horizontally between two support carriers. At the air influx system on the upper part of the device, both of the filters, namely F9 filter and HEPA filter were removed. At the air influx system on the bottom of the device, a sterile bag was installed for the purpose of testing the antimicrobial efficacy. An incision was made into the bag and from the bag, the air efflux was connected to the microbiological chamber MC9-2. Further on, a petri dish of 140mm radius was placed at the air efflux from the bag in the microbiological chamber MC9-2. The petri dish contained 40mL of saline solution. The position of the petri dish was perpendicular to the air flux with the solid agar medium facing the air current. The device was turned on for 3 min and the ventilator was set at the volume flux of 300 m^3/h. The content of the petri dish was then analysed for the presence of bacteria and bacteriophages according to the methods described above. With sterility test we confirmed the methodology used and make sure that we do not introduce contamination into petri dish before the spraying of bacteria or bacteriophages.

Control petri dishes

The experimental set up for the control petri dishes is identical as efficiency test, except that we removed both filters - F9 filter and HEPA filter and turned off the UVC lamp. With control petri dishes or control experiments, we determined concentration of microorganisms which were transferred through the device and trapped in 40 ml of saline. By spraying each of the test suspensions of microorganisms, an aerosol is created, and part of the aerosol is lost at the inlet as we observed as droplets at the inlet into the fan. Part of aerosol is lost after transferring through the device, and part of the aerosol is likely to be lost in the sampling methodology as well. Control petri dishes were made in duplicates, where no significant differences in concentration were detected between individual duplicates.

3.) Chemical disinfectants testing - DEZINSEPT DERM+

Efficiency test

According to EN 14476: 2013 + A2: 2019 hand chemical disinfectants are test at 80 % concentration. Determination of bactericidal concentrations was done by mixing 1 ml of the interfering substance of concentration 3 g/ml with 1 ml of Test suspension and incubating for 2 min at room temperature. After the 2 min incubation, 8 ml of Dezinsept Derm+ was added to each of the test strain experimental setup mixtures. The mixtures interfering substance: test suspension: test substance were then incubated for the reference contact time, which was either 1min, 5min, 15min, 30 min and 60min. 1ml of each of the experimental setup mixtures were then added to a mixture of 8ml of the neutralizing solution (3 % Saponin and 0,5 % Tween in Leethen Broth) and 1ml of sterile distilled water (dH_2O). The mixtures were then incubated for 10s. On the obtained neutralized mixtures, a 10-time dilution series was made and transferred to 5mL of soft agar containing the host bacterial strains. The mixed suspension in soft agar was then slightly mixed and transferred onto TSA solid agar. Afterwards, the petri dishes containing bacteriophage samples were incubated 36±2 °C or 25±2 °C for 24±3h. After the incubation plaques were counted.

The log reduction factors Log (R) were determined as the difference between the log value of the average of the N_0 test (control) (obtained as the average over all dilution triplicates and over all technical

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repetitions) and the log value of the Na test - for the respective substance on the respective test strain - (obtained as the average over all dilutions (all dilution triplicates) and all technical repetitions)

$$Log\ R = logN_0 - logNa$$

N_0 is the average cell concentration of the N_0 control experiment over all dilutions (all dilution triplicates) and all technical repetitions of the experimental setup
Na is the average cell concentration of the Na test experiment over all dilutions and all technical repetitions of the experimental setup

Control

Control N0: N_0 test (control) was designed and executed in the same matter as the Test experiment »Na« with the sole divergence of substituting the test substance with 8ml of sterile distilled water (dH_2O).

Control A: In the first phase of the validation of the experimental conditions 1mL of the Interfering substance with concentration 3g/L was mixed with 1mL of the validation suspension and incubated for 2min. Afterwards, 8mL of sterile distilled water (dH_2O) was added to the mixture. The mixture was then incubated for 30s. After the 30s incubation, a 10-times dilution series was obtained from each of the mixtures and analysed with plaque assay method as described above.

Control B: The toxic effect of the neutralizer on the viability of the cell culture was by mixing 8ml of neutralization solution, 1ml of sterile distilled water (dH2O) and 1mL of validation suspension of each of the test strains. The mixture was then incubated for 10s. After the 10s incubation, a 10-times dilution series was obtained from each of the mixtures and analysed with plaque assay method as described above.

Control C: The control of the effectivity of the inhibitory effect of the testing substance was performed by mixing 1ml of the interfering substance, 1ml of sterile distilled water (dH_2O) and 8mL of the testing substance. The mixture was then incubated for 30s. After 30s incubation, 1mL of the mixtures were transferred to 8mL of the neutralizing solution. The mixtures in the neutralizing solution were incubated for 10s. Afterwards, 1mL of the validation suspensions was added. The mixture was then incubated for 10s. After the 10s incubation, a 10-times dilution series was obtained from each of the mixtures and analysed with plaque assay method as described above.

The obtained concentration of the viable cells from the control must be in accordance with the conditions mentioned in the Results section for each tested microbial strain.

3. Device testing - UVC lamp Philips MASTER LEDtube 600mm HO 8W 840 T8

Efficiency test

The testing of antimicrobial efficacy of the UVC lamp was performed by inoculating test carriers with MS2 bacteriophage test suspension. 50µL of each of test suspension was transferred on stainless steel carriers 9cm length and 2,5cm width (22,5cm^2 surface) and homogenously distributed on the carriers. The carriers were then air dried. Two test carriers were placed on the work surface in the microbiological protection chamber MC 9-2. The carriers were exposed to UVC lamp for a contact time of 5 min. After the 5 min exposure time, two carriers were transferred to 10mL of sterile distilled water (dH_2O) to obtain

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a suspension of viable cells. Then we followed the same protocol for contact time of 15 min, 30 min and 60 min. After disinfection, the samples were analysed with plaque assay method as described above.

The reduction (Log R) can be calculated as:

$$LogR = Log\frac{N_0}{N_a} = logN_0 - N_a$$

Where N_0 is the average number of microorganisms (obtained by duplicates or two technical repetitions) in the control carriers and N_a is the average number of microorganisms (obtained by duplicates or two technical repetitions) in each experimental setup.

Control

Control was designed and executed in the same matter as the efficiency test except that two test carriers with dried MS2 bacteriophages were not exposed to UVC lamps. The carriers were left on laboratory desk and after 30 min were transferred to 10mL of sterile distilled water (dH_2O) to obtain a suspension of viable cells. The samples were analysed with plaque assay method as described above.

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