Testing of antimicrobial efficacy (semi-field trial) of air disinfection device AirLight ULTRA QUIET of the company UV photons (Wavenetic d.o.o.)

Antimicrobial activity

Escherichia coli,

Staphylococcus aureus,

bacteriophage phi6,

bacteriophage MS2

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VALIDATION REPORT ON PERFORMED ANALYSIS

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Summary of results

The tested air disinfection device **AirLight ULTRA QUITE** achieves total elimination of microorganisms in the efficiency test.

5,94 log (>99,999 % efficiency) reduction on the reference test strain *Escherichia coli DSM18039*

5,87 log (>99,999 % efficiency) reduction on the reference test strain *Staphylococcus aureus* ATCC 6538

6,82 log (>99,9999 % efficiency) reduction on the reference test strain *Pseudomonas phage* phi6 DSM 21518

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

Expert opinion

The tested air disinfection device with the respective trade name **AirLight ULTRA displays a sufficient level of bactericidal and virucidal activity** in accordance with standard EN 15714: 2019 at airflow of 300 m^3/h in relation with the reference test strains: *Escherichia coli, Staphylococcus aureus, Pseudomonas phage* phi6 and *Escherichia phage* MS2.

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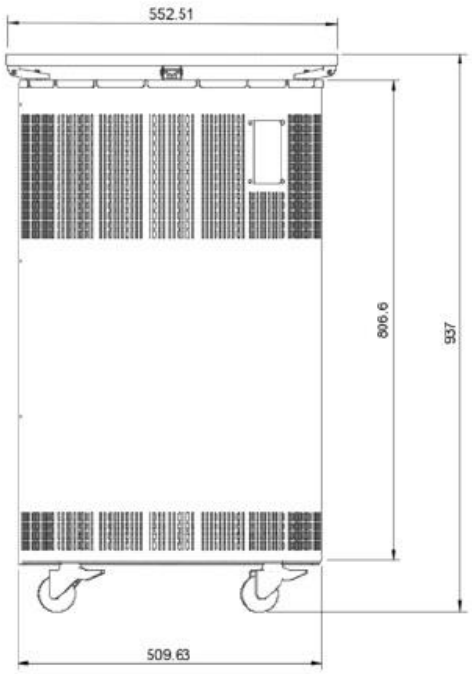
Description of the device

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 (m^3/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 m^3/h)
Power supply	230 V
Max. consumpiton	100 W
Weight	20 kg
Device sketch	

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Summary of experimental conditions

Summary of experimental conditions	Laboratory	Analytic laboratory of Microbium L.LC.
	Date of analysis	11.3.2022 - 18.3.2022
	Room temperature	20 °C
	Relative humidity	55 %
	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
	Spray volume (inoculum)	10 ml
	Sample volume (petri dish)	40 ml
	Airflow	300 m^3/h
	Analytical method	method for determining colony-forming units, plaque assay
	Diluent	dH_2O z 0,9 % NaCl
Microbial test strain and culture data	Reference microbial test strain	*Escherichia coli* DSM18039 *Staphylococcus aureus* ATCC 6538 *Pseudomonas phage* phi6 DSM 21518 *Escherichia phage* MS2 DSM 13767
	Host strain for phagocidal activities	*Pseudomonas sp.*DSM 21482 *Escherichia coli* DSM 5695
	Incubation medium	TSA (Tryptic Soy Agar) LB soft agar (0,7 % agar) CASO soft agar (0,7 % agar)
	Incubation temperature	36±2 °C, 25±1 °C
	Incubation time	24 h±2h, 48±3h

*The protocol was done following the principle of two standards. EN 15714:2019: we applied the test procedure for testing the air disinfection device with certain modifications. EN 17272: 2020: 2020 we applied the procedures for the preparation of microbial suspensions of bacteria and yeast and the procedures for the cultivation and quantification of microorganisms. The entire methodology is described in the Methods section.

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Description of the reference test strains

Reference test strain Escherichia coli

Enterobacteriaceae is a large, heterogeneous group of Gram-negative rods that includes bacteria that naturally inhabit the mammalian gut but are also one of the most common human pathogens. Enterobacterial infections, including *E. coli* infections, are among the most common cause of hospital-acquired infections (Radšel Medvešček in sod. 2002). Most *E. coli* strains in the human intestine are harmless, but some strains have acquired different virulence factors and cause a variety of infectious diseases, most commonly urinary tract infections and intestinal infections. Gram-negative bacteria are highly efficient at up-regulating or acquiring genes that code for mechanisms of antibiotic drug resistance. The emergence of enterobacterial resistance to most β-lactam antibiotics poses a serious public health challenge (Scheutz et al. 2005).

In the presented study, we used a reference strain of *Escherichia coli* (Migula 1895) Castellani and Chalmers 1919 (DSM 18039, German Collection of Microorganisms and Cell Cultures), which is used to test the effectiveness of elimination. The performance results of the tested air disinfection device on *E. coli* DSM 18039 can be extrapolated to the operation of the same device under the same conditions on pathogenic *E. coli* strains such as enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative (EAEC), enterohaemorrhagic *E. coli* (EHEC) and others.

Reference test strain Staphylococcus aureus

Staphylococcus aureus is a Gram-positive coccus and is found in normal human flora, located on the skin and mucous membranes (most often the nasal area). Up to 30 % of the human population are asymptomatically and permanently colonized with nasal *Staphylococcus aureus*. The nose is a key source of transmission of this microorganism (Wertheim et al., 2005). *S. aureus* is a clinically important pathogen and one of the most common causes of hospital-acquired infections, causing a series of serious pathological conditions such as skin infection, nosocomial pneumonia, endocarditis, sepsis, as well as toxic shock syndrome (Inokuchi et al., 2018). *S. aureus* bacteria are resistant to a wide range of antibiotics, thus limiting the number of therapeutic options against *S. aureus* infections.

In the presented study, we used a reference strain of *Staphylococcus aureus* subsp. aureus Rosenbach (ATCC 6538, American Type Culture Collection), used in ISO standards for the evaluation of microbial activity of chemical disinfectants. The performance results of the tested air disinfection device on *S. aureus* ATCC 6538 can be extrapolated to the operation of the same device under the same conditions on methicillin-resistant S. aureus (MRSA) and vancomycin-resistant *S. aureus* (VRSA).

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Reference test strain Pseudomonas phage phi6 in 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 bacteriophage Pseudomonas virus phi6 has been extensively used as a surrogate for Coronaviridae viruses and the MS2 virus is used as a surrogate for noroviruses (Bae in sod., 2008; Whitworth in sod., 2020; Machado in sod., 2021; Fedorenko in sod., 2020; Vatter in sod. 2021, Baros in sod. 2021, Ma in sod. 2021, Franke in sod. 2021; Aquino de Carvalho in sod. 2017, Prussin in sod. 2018, Ford, 2015; Dey at al,2021; Rockey in sod., 2020; Martins in sod., 2020). Noroviruses (viruses of the Caliciviridae group) are known causes of human gastroenteritis and are the leading cause of diarrhea. Viruses belonging to the family Coronaviridae are known to cause severe acute respiratory syndrome. Among them is SARS-CoV-2, the cause of the COVID-19 pandemic.

Pseudomonas phage phi6 belongs to the group Cystoviridae. It has several similar characteristics to **SARS-CoV-2**:

- Both viruses have an external viral envelope composed of lipids and proteins.
- Surface glycoproteins are present on the external viral envelope - part of the surface glycoprotein structure is outside the viral envelope and part of the structure is located within the viral envelope.
- The external viral envelopes are formed by coat proteins.
- On the inside of the viral coat is the capsid, which is a geometrically symmetrical viral structure constructed from a protein complex.
- The genome is an RNA molecule (RNA genome), in the case of SARS coronaviruses the RNA is single-stranded and in the case of Pseudomonas phage phi6 it is double-stranded.

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 *Pseudomonas phage* phi6 (DSM 21518, German Collection of Microorganisms and Cell Cultures). The performance results of the tested air disinfection device on *phage* phi6 DSM 21518 can be extrapolated to the operation of the same device under the same conditions on SARS-CoV-2 virus and other Vertebrate viruses with a viral envelope.

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. Escherichia coli DSM18039

Efficiency test - E. coli

Repetition	Dilution	Vc1	Vc2	Average Vc1, Vc2	Average CFU/ml	log Na	Log N0	log reduction (N0-Na)	efficiency (%)	Result
1	10^{-0}	0	0	0	0,00E+00	0,00	5,94	**5,94**	**>99,999**	**total elimination**
	10^{-1}	0	0	0						
2	10^{-0}	0	0	0	0,00E+00	0,00	5,94	**5,94**	**>99,999**	**total elimination**
	10^{-1}	0	0	0						

Control - E. coli

Test suspension N	Dilution	Vc1	Vc2	Average Vc1, Vc2	Average CFU/ml	log N
	10^{-6}	223	196	210	3,30E+09	9,52
	10^{-7}	44	45	45		

Control 1	Dilution	Vc1	Vc2	Average Vc1, Vc2	Average CFU/ml	Average CFU (x40 ml)	log N0
	10^{-1}	139	167	153	2,16E+04	8,64E+05	5,94
	10^{-2}	23	32	28			

Control 2	Dilution	Vc1	Vc2	Average Vc1, Vc2	Average CFU/ml	Average CFU (x40 ml)	log N0
	10^{-1}	148	162	155	2,18E+04	8,70E+05	5,94
	10^{-2}	25	30	28			

Control average	Control 1	Control 2	Average CFU/ml	Average CFU (x40 ml)	log N0
	2,16E+04	2,18E+04	2,17E+04	8,67E+05	5,94

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

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2. Staphylococcus aureus ATCC 6538

Efficiency test - S. aureus

Repetition	Dilution	Vc1	Vc2	Average Vc1, Vc2	Average CFU/ml	log Na	Log N0	log reduction (N0-Na)	efficiency (%)	Result
1	10^{-0}	0	0	0						
	10^{-1}	0	0	0	0,00E+00	0,00	5,87	**5,87**	**>99,999**	**total elimination**
2	10^{-0}	0	0	0						
	10^{-1}	0	0	0	0,00E+00	0,00	5,87	**5,87**	**>99,999**	**total elimination**

Kontrole - S. aureus

Test suspension N	Dilution	Vc1	Vc2	Average Vc1, Vc2	Average CFU/ml	log N
	10^{-6}	234	207	221	2,66E+09	9,42
	10^{-7}	33	28	31		

Control 1	Dilution	Vc1	Vc2	Average Vc1, Vc2	Average CFU/ml	Average CFU (x40 ml)	log N0
	10^{-1}	142	153	148	1,89E+04	7,56E+05	5,88
	10^{-2}	19	27	23			

Control 2	Dilution	Vc1	Vc2	Average Vc1, Vc2	Average CFU/ml	Average CFU (x40 ml)	log N0
	10^{-1}	158	147	153	1,82E+04	7,26E+05	5,86
	10^{-2}	19	23	21			

Control average	Control 1	Control 2	Average CFU/ml	Average CFU (x40 ml)	log N0
	1,89E+04	1,82E+04	1,85E+04	7,41E+05	5,87

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

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3. Pseudomonas phage phi6 DSM 21518

Efficiency test - phi6

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	6,82	**6,82**	**>99,9999**	**total elimination**
	10^{-1}	0	0	0						
2	10^{-0}	0	0	0	0,00E+00	0,00	6,82	**6,82**	**>99,9999**	**total elimination**
	10^{-1}	0	0	0						

Control - phi6

Test suspension N	Dilution	Vc1	Vc2	Average Vc1, Vc2	Average PFU/ml	log N
	10^{-6}	>330	>330	>330	1,75E+10	10,24
	10^{-7}	182	167	175		

Control 1	Dilution	Vc1	Vc2	Average Vc1, Vc2	Average PFU/ml	Average PFU (x40 ml)	log N0
	10^{-2}	143	12	78	2,46E+05	9,84E+06	6,99
	10^{-3}	<14	16	16			

Control 2	Dilution	Vc1	Vc2	Average Vc1, Vc2	Average PFU/ml	Average PFU (x40 ml)	log N0
	10^{-2}	78	93	86	8,40E+04	3,36E+06	6,53
	10^{-3}	<14	<14	<14			

Control average	Control 1	Control 2	Average PFU/ml	Average PFU (x40	log N0
	2,46E+05	8,40E+04	1,65E+05	6,60E+06	6,82

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

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4. Escherichia phage MS2 DSM 13767

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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Methods

1.) Preparation of the overnight culture/bacterial suspension

All commercial bacterial strains obtained either from the German Collection of Microorganisms and Cell Cultures were stored in the internally organized Microbium L.LC bank of bacterial strains. After rehydration and revitalization of the cell culture the stock cultures were prepared by cultivating *S. aureus* and *E. coli* on the TSA medium and further incubating for 21±3 h at 36±2 °C. After the overnight incubation, one colony of the tested microbial culture was transferred onto the LB medium and further incubated for 21±3 h at 36±2 °C. The overnight culture was then centrifuged at 6000 rcf for 10 min. The supernatant was decanted and the pellet was resuspended in a small volume. This was regarded as test suspension for testing of antimicrobial efficacy of air disinfection device.

2.) Preparation of the bacteriophage suspension

Phage phi6 was previously propagated on the host bacterium *Pseudomonas sp.* DSM 21482, with confluent plate method. Phage phi6 from the bacteriophage bank Microbium L.LC. was diluted to 10^6 PFU/ml and 100 µl of phage was transferred into microcentrifuge tubes. Bacterial culture of *Pseudomonas sp.* from the TSA plate was resuspended in saline solution to an optical density value of 1. 100 µl of host culture was added to microcentrifuge tubes with 100 µl of phage phi6 and mixed. The mixture of host culture and phages were added to 5 ml of soft CASO agar. The agar was poured onto TSA plates. The plates were incubated for 24 h at 25 ° C. Subsequently, 3 ml of saline solution was added and further incubated on an orbital shaker at 100 rpm for 4 hours at room temperature. Saline solution with phages was then recovered and the bacteriophage suspension was centrifuged at 6000 g for 10 min and 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 Pseudomonas sp. DSM 21482 was mixed with 100 µl of the phage 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 25±2 °C.

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.

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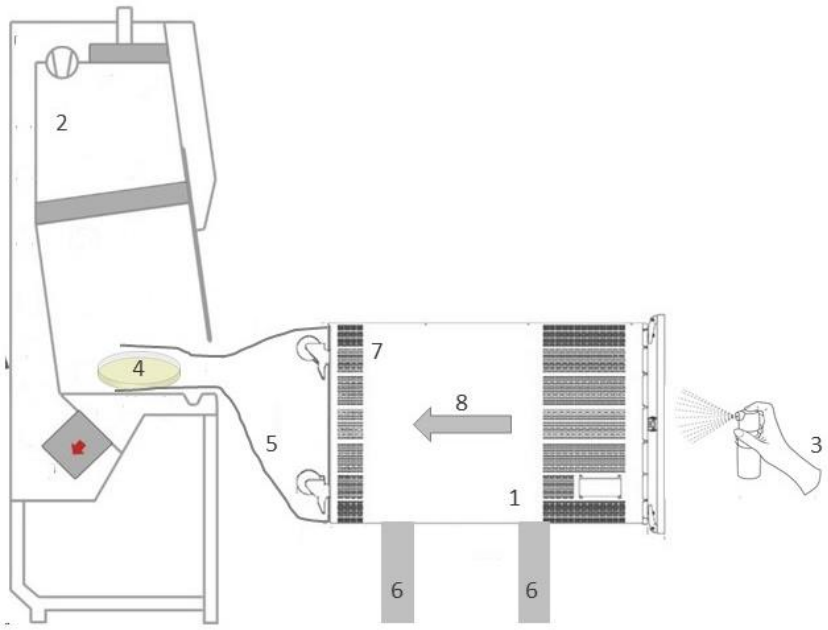
3.) Device testing

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.



Legend:

1 – tested device	5 – sterile bag
2 – safety cabinet	6 – supports
3 – spraying	7 – HEPA filter
4 – petri dish	8 – airflow

Scheme: schematic presentation of the device testing protocol.

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At the upper part of the device, 10mL of test cultures (*Escherichia coli* DSM18039, *Staphylococcus aureus* ATCC 6538, Pseudomonas phage phi6 DSM 21518 or *Escherichia phage* MS2 DSM 13767) were 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. Each test culture was tested on a separate day of analysis to avoid cross contamination.

On the obtained bacterial supensions in saline solution, a 10-time dilution series was generated. 100µL of each dilution along with the original suspension were transferred to TSA solid agar medium and incubated at 36±2 °C for 48±3h. On the obtained bacteriophage suspensions, a 10-time dilution series was made and transferred to 5mL of soft agar containing the hosp bacterial strains. The mixed suspension in soft agar was then slightly mixed and transferred onto TSA solud agar. Afterwards, the petridishes containing bacteriophage samples were incubated 36±2 °C or 25±2 °C for 24±3h. After the incubation colonies (In the case of bacterial test cultures) and 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 redustio (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

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 makd sure that we do not introduce contamination into petri dish before the spraying of bacteria or bacteriophages.

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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.

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