□ Elbrachtsweg 76 · 33332 Gütersloh
Telefon: (0 52 41) 3 07 20-0
Telefax: (0 52 41) 3 07 20-79
e-mail: info@biotec-gmbh.com

□ Leipziger Straße 27
09648 Mittweida · Telefon/Fax: (0 37 27) 97 63 11
e-mail: biotec-GmbH@web.de

biotec Umwelt-Analytik-Beratung-Service GmbH · Elbrachtsweg 76 · 33332 Gütersloh

biotec

Umwelt - Analytik - Beratung - Service GmbH

Gütersloh, 26.07.2021

Herr Suys
AED Distribution
Bedrijvenpark de Veert 13/004
2830 Willebroek
Belgium

Report on the research project B AIR V2 „inactivation analyse of airborne viruses"

Dear Mr. Suys,

enclosed you will find the report on the research project carried out by the „B AIR V2" you provided. Thank you for the exciting project. If you have any further questions, please do not hesitate to contact us.

Please note that results and statements naming the company biotec GmbH or individual employees in publications / brochures may not be published without the approval of biotec GmbH.

Kind regards

Dr. A. Bermpohl
Managing Director
biotec GmbH

M. Sc. Nathalie Brand
biotec GmbH

Final report

Research project AED Distribution

B AIR V2

Table of content

Project description

The „B AIR V2“ (Fig. 1) from AED Distribution company is a moveable air cleaner with a cylindrical shape with 1150 mm × 180 mm × 235 mm dimensions (manufacturer specification). The maximum air flow rate of the system is specified by the manufacturer as 117 m³/h. 2 UV-C tubes with 55 watts each are installed in the system.

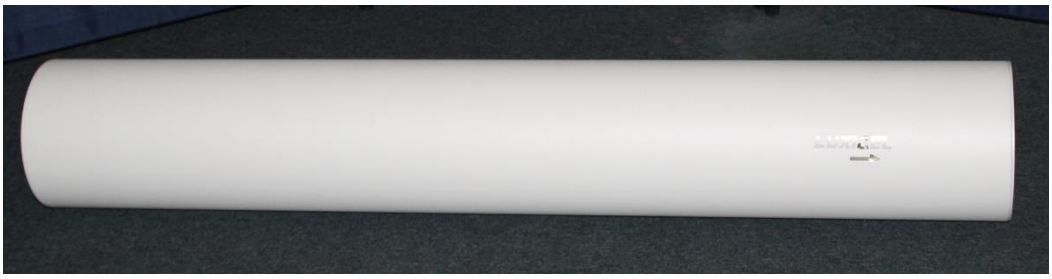


Figure 1: B AIR V2.

The inactivation of airborne viruses was investigated using the RNA viruses MS2 when they passed through the system once. The viruses are used as a coronavirus surrogate. MS2 (family: Leviviridae) is a bacteriophage which plats on *Escherichia coli*. It is an ssRNA virus with a 4 kB genome. The phages measure approx. 26 nm in diameter. SARS-CoV-2 is the causative agent of the current coronavirus pandemic, which causes COVID-19. The coronavirus is an enveloped RNA virus with an ssRNA genome.

With regard to the inactivation of viruses in the air by UV-C, the following D90 values (dose required for 90% inactivation) are available from the literature[1]:

Coronavirus	3 J/m²
MS2	3 – 61 J/m²

Data for the novel coronavirus SARS-CoV-2 are scarce and the inactivation rate is always dependent on other factors such as humidity and temperature, but it can be assumed that the tenacity to UV-C of the pathogen is comparable to the surrogate MS2 used.

In addition to the measurements described above, the depletion of the total number of germs was examined in a test room over 24 hours when the disinfection system was in operation. To determine possible outgassing from the system, ozone was determined as an individual parameter. Leaking radiation from UV systems represents a health hazard for end users. For this reason, the UV-C radiation at the air outlet of the system was measured with a radiometer.

[1] W. Kowalski, 2009. Springer-Verlag Berlin Heidelberg. Ultraviolet Germicidal Irradiation Handbook.

Material & Methods

Determination of the inactivation rate of viruses with MS2 as viral surrogate

Initially, a high-titer lysate of bacteriophage MS2 has been prepared. For this purpose, dilution series of the viral lysate were plated by agar assay with the target bacterium *Escherichia coli* (PC agar). The soft agar plates thus obtained have been incubated for up to 48 h at 25 °C. Based on the dilution step and the number of plaques present, the titer was determined. The dilution step with confluent lysis was used to produce a high titer lysate. For this purpose, 10 plates with confluent lysis were overlaid with SM (saline-magnesium) buffer (4 ml SM buffer, min. 4 h, 5 - 8 °C). The lysate prepared in this way was used for nebulization (Fig. 2).

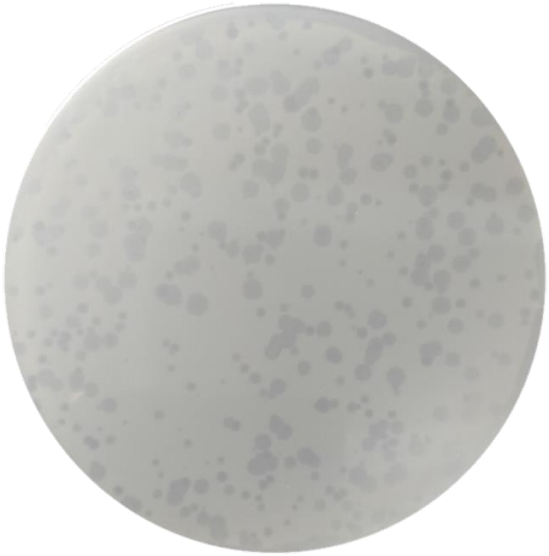


Figure 2: *Escherichia coli* lawn with plaques cause by infection with MS2.

For control the nebulization of the viruses, the B AIR V2 system was installed in an incubator tent (11,5 m^3). The quantified lysate has been introduced into the system via a nebulization head (Pari Boy TurboS, Pari GmbH, Starnberg, Germany). The absolute amount of nebulized viruses has been determined by differential weighing and subsequent calculation using the titer of the lysate.

The size of the generated particles per unit volume due to nebulization has been qualified via a laser particle monitor (Met One A2408-1-115-1 Laser Particle Counter, Met One Instruments, Inc., Oregon, USA) over a 1:1000 dilution distance and the number quantified (figure 3).

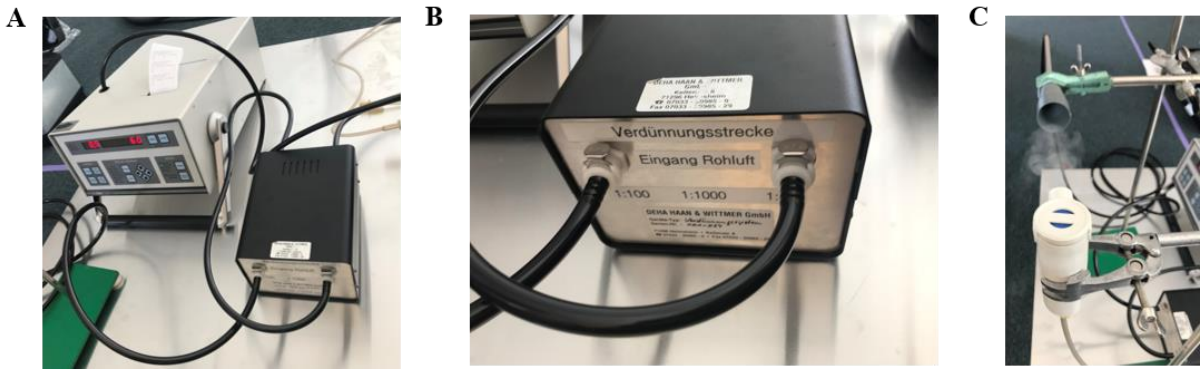


Figure 3: A Laser particle counter with connected dilution section. **B** Dilution section for the particles in the room air. **C** Nebulization by means of Pari Boy TurboS opposite the sampling head for particle counting (distance 20 cm).

The particle count yielded the particle sizes per cubic meter of air shown in figure 4. Averaged from 6 individual measurements, the differential values are as follows:

particle < 0,5 µm: $4{,}5 \times 10^7$ particle / m³

particle < 5,0 µm: $1{,}1 \times 10^5$ particle / m³

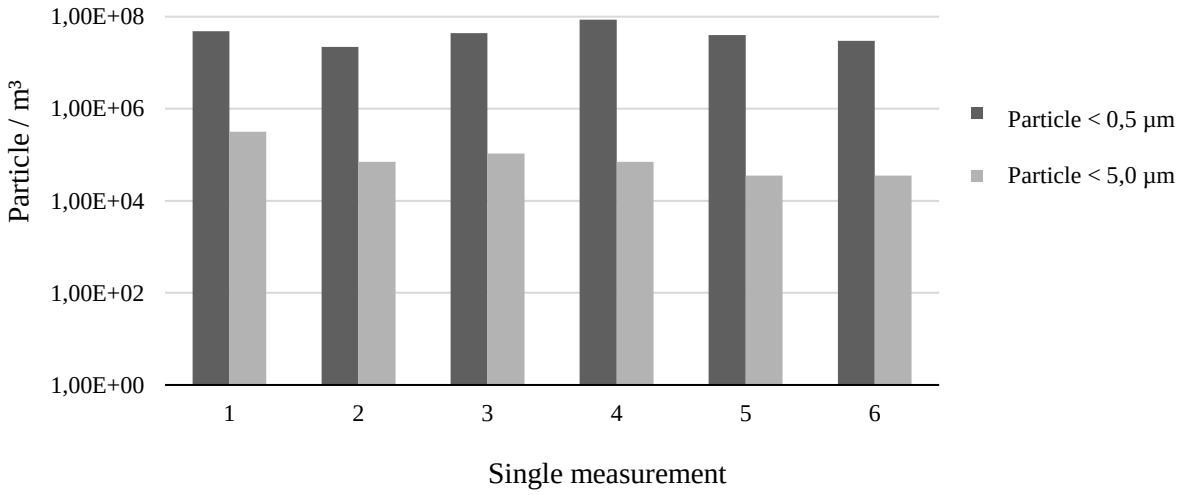


Figure 4: Individual measurements (with 3 measuring cycles each in the determination) of particles < 0.5 µm (dark gray) and < 5 µm (light gray) in particles/m3. The differential value is indicated.

Air sampling was performed via membrane filtration at the air outlet (gelatin filter pore size 3 µm, appendix A1) using the MD8 air sampler (figure 5, both Sartorius AG, Göttingen) at 6 m³/h for 10 min (1 m³ sampling volume, calibration protocol in appendix A1). The gelatin filter has been dissolved in SM buffer. The number of viruses collected was determined via a dilution series and agar assay.

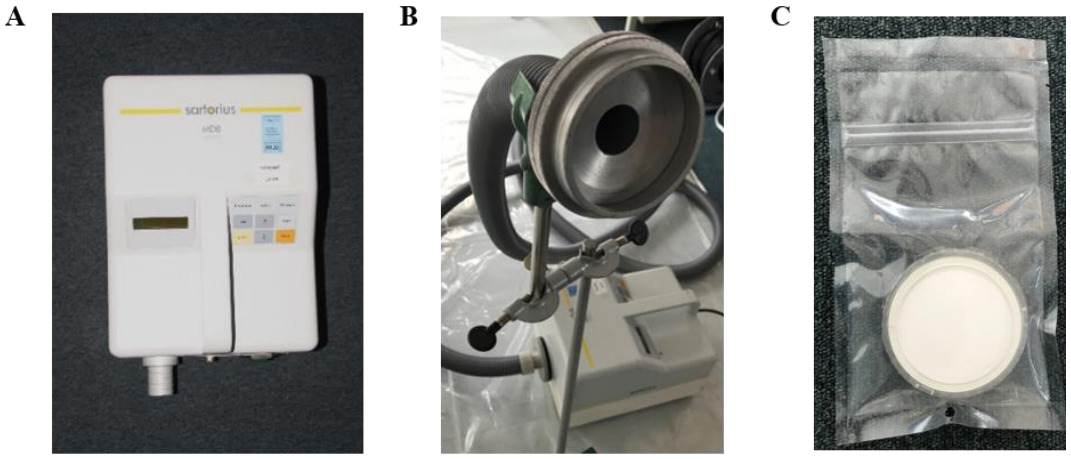


Figure 5: A MD8 air sampler, Sartorius GmbH. Test seal in blue documents the calibration of the system (valid until 03/22, see Appendix A1). **B** Sampling head for air aspiration through the gelatin membrane of the filter. **C** Gelatin filter, sterile packed for attachment to the sampling head.

In order to determine the effect of air disinfection by the B AIR V2 system, it was first determined how many bacteriophages could be detected in the outgoing air stream after passing through the system with UV-C radiation. After switching off the UV-C radiation (N_0), the experiment was repeated. By comparing the two values, the inactivation rate could be calculated. The virus suspension was nebulized directly at the air intake. At the air outlet, the viruses were collected via membrane filtration of the air. The entire experimental setup for the B AIR V2 system is outlined in figure 6.

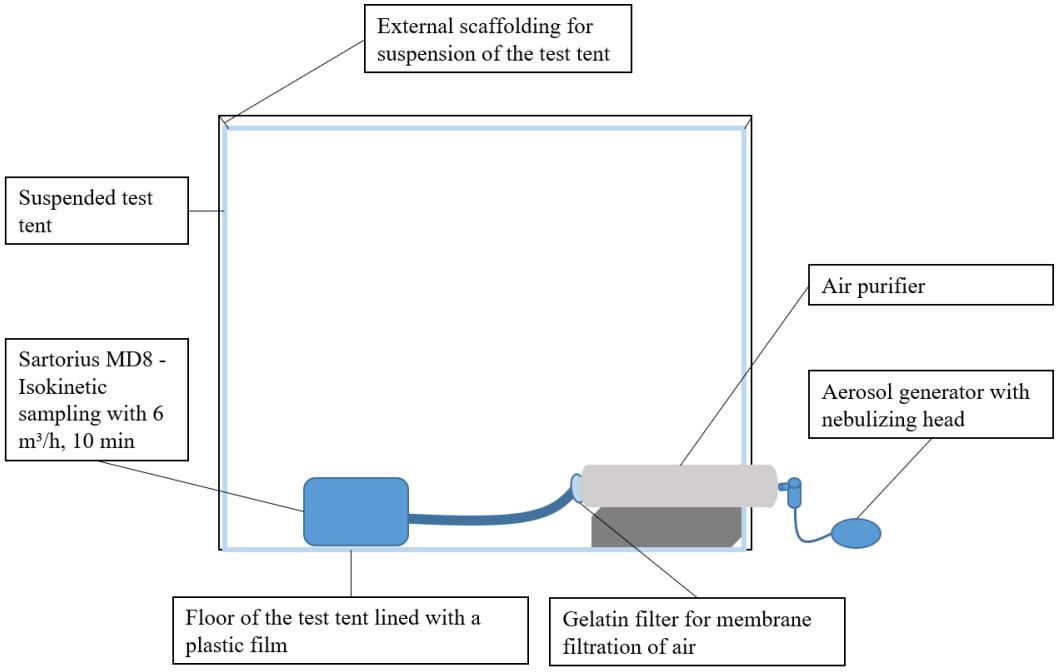


Figure 6: Schematic drawing of the experimental setup for the analysis of active viruses after passage through the B AIR V2 system.

To study viral reduction during single passage through system B AIR V2, viruses were introduced into the air stream as an aerosol from a nebulization head at the air inlet (figure 7 A). At the air outlet, the air was collected via an isokinetic membrane filtration (figure 7 B). This filter was then processed.

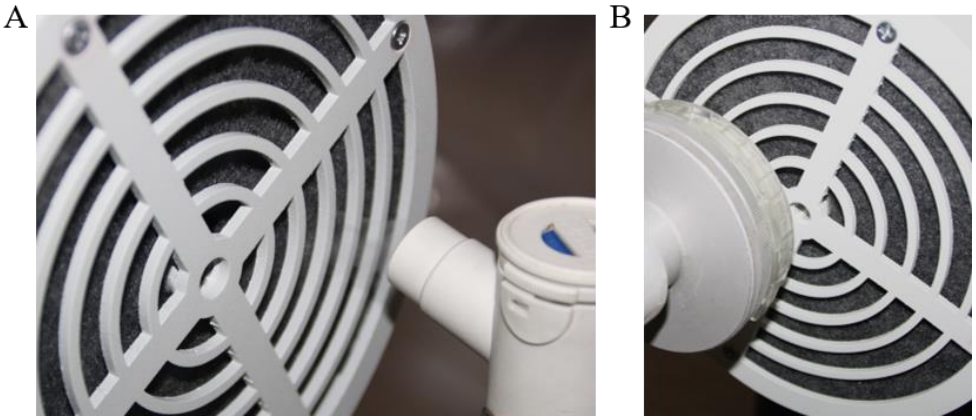


Figure 7: A Nebulization before air intake. **B** Sampling at the air outlet via membrane filtration (gelatin filter).

Determination of temperature and humidity

The measurement of temperature and humidity was read after 10 minutes of adaptation of a probe to the room climate in the measurement tent (testo 605 H1, Testo SE & Co. KGaA).

Ozone measurement

The ozone measurement was carried out with a Dräger pump (Drägerwerk AG & Co. KGaA, Lübeck, Germany) after 60 minutes of system start-up at the air outlet. Ozone concentrations in the range of 0,05 to 0,7 ppm can be detected with the test tubes used. The Dräger-Tubes are filled with a blue matrix. During the measurement, air is drawn through the tube. The ozone causes the matrix to change color (white). The pump with test tubes for ozone measurement is shown in Figure 8.

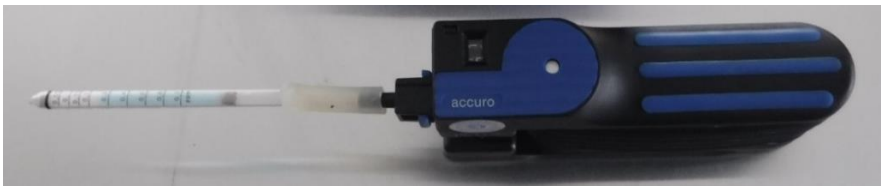


Figure 8: Dräger pump with test tube for ozone measurement.

Depletion of the total germ count in a defined space

The quantification of the total germ count of the air was performed by membrane filtration. In a defined sampling tent (11.37 m³), the air sample was collected using validated gelatin filters. After placing the gelatin filter on PC agar, the plates have been incubated for up to 48 h/30 °C. Subsequently, the visible colonies were counted. Sampling was performed in the test tent (figure 9 and figure 10) at 3 m³/h for 5 min (250 L sampling volume). A control sample (N_0) was taken before the B AIR V2 system was turned on. This N0 sample was compared with the samples taken at the time points 0.5 h, 1 h, 4 h and 24 h after switching on the air sterilizer.

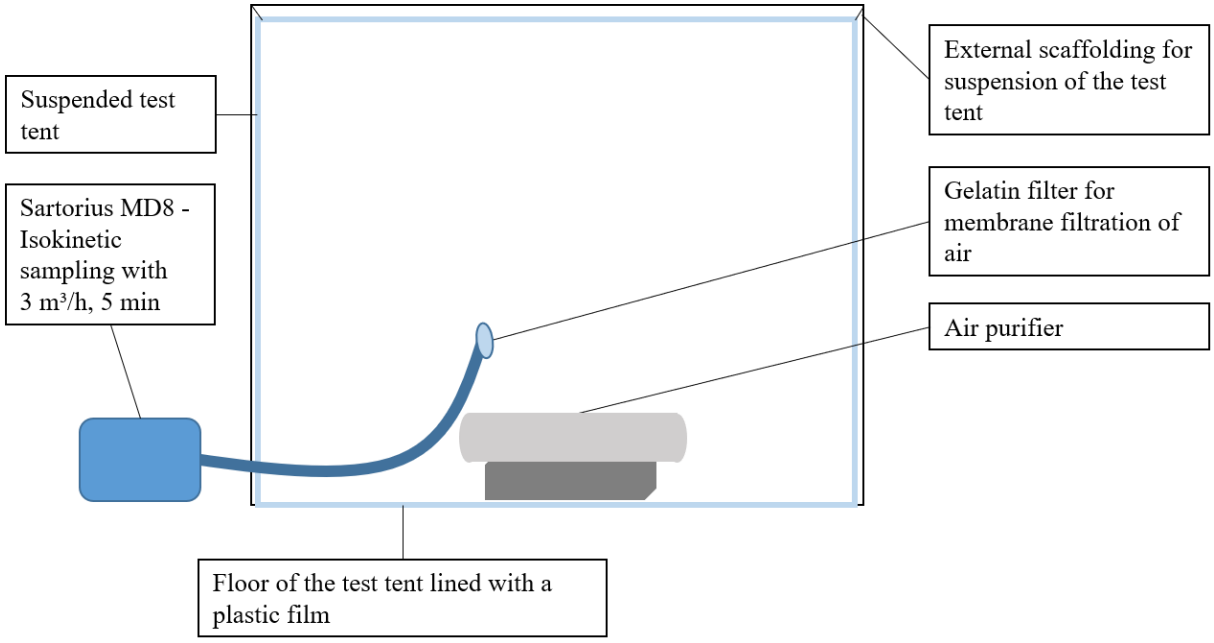


Figure 9: Sampling of the total bacterial count in the test tent with 11,37 m^3, B AIR V2 at a height of 30 cm.

Figure 10: Illustration of the experimental setup and representation of the positioning of the gelatin filter respectively of the B AIR V2 system.

Determination of radiation

The residual radiation at the air intake and the air outlet of the UV-C disinfection system was determined by means of a Radiometer RMD from Opsytec Dr. Gröbel (Ettlingen) (distance 0,5 cm and 30 cm). The Radiometer RMD (measuring range 0 - 10 mW/cm², resolution of 0,001 µW/cm²) is shown in figure 11.

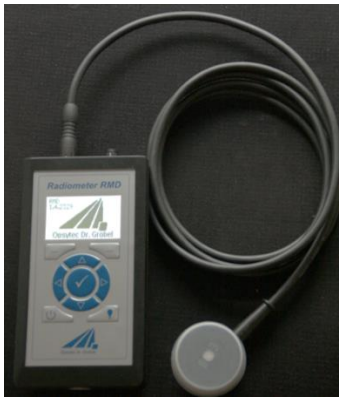


Figure 11: Radiometer RMD from the company Opsytec Dr. Gröbel.

Results

Reduction of MS2 in air during single passage through the B AIR V2 system

An MS2 lysate with > 10^{11} PFU/ml was used for the nebulization experiments. Table 1 summarizes the data obtained.

Table 1: Measured virus concentration at single passage through the air disinfection system in the supply air (PFU/m^3) with indication of log levels reduction of active viruses and associated inactivation rate, as well as temperature and relative humidity. UVC- radiation off: - (N0); UVC radiation on: +.

UVC	Temperature [°C]	Relative humidity [%]	Virus concentration [PFU/m^3]	Log stages reduction	Inactivation rate
-	24,0	62,9	$2{,}75 \times 10^8$	4,22	99,994 %
+	25,2	55,6	$1{,}60 \times 10^4$		

Determination of the ozone concentration

Ozone was measured using Dräger tubes, which measure an ozone content in the range between 0.05 - 0.7 ppm. Measurements were taken at the air outlet of the system. No ozone could be detected in the air outlet of the system with the measuring tube shown (no visually recognizable color change from blue to white of the test tube; see figure 12).

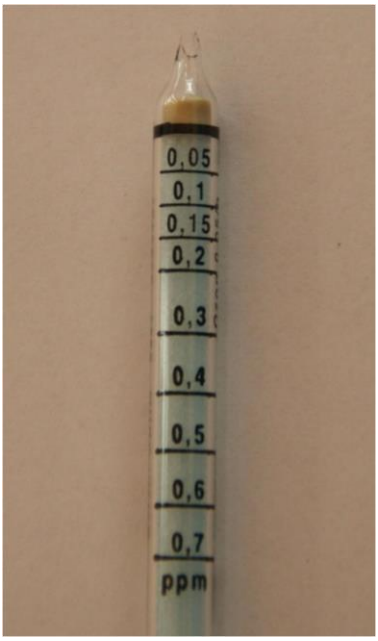


Figure 12: Dräger tubes after measuring the ozone content of the B AIR V2 system.

Depletion of the total germ count through operation of the B AIR V2 system

The reduction of the total germ count (GKZ) was investigated by operating the air purifier for 24 h in a defined sampling tent of 11,37 m³. At the time points 0,5 h, 1,0 h, 4,0 h and 24,0 h, an air sample has been taken from the room (250 L sampling volume). The total germ count was quantified at each sampling time point. The samples were compared with the N0 value. The results are shown in Figure 13.

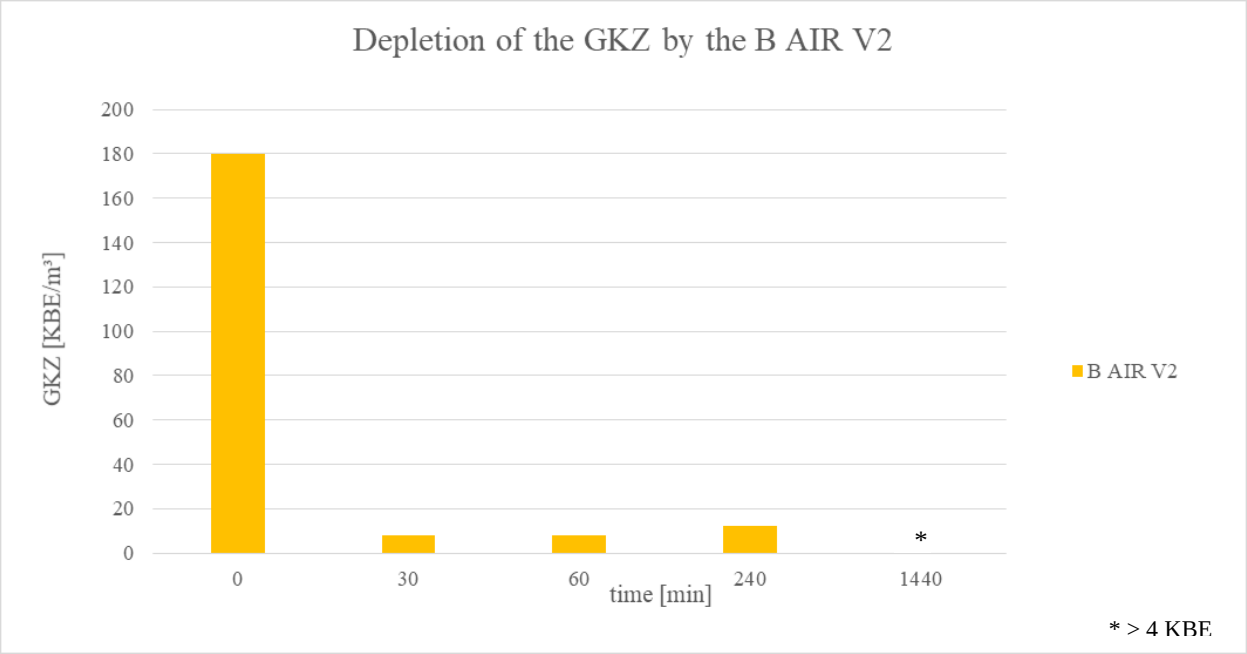


Figure 13: Plot of total germ counts in an 11,5 m³ tent at time points t0 (N0), t0,5 (30 min), t1 (60 min), t4 (240 min), and t24 (1440 min).

Table 2: Measured values of the total germ count (GKZ) in CFU/m^3 over 24 hours.

time [h]	Total germ count [KBE/m^3]
0	180
0,5	8
1	8
4	12
24	0

Testing of photobiological safety at the air inlet and air outlet

The residual radiation was determined at the air outlet and at the air inlet via a radiometer (Figure 14). Measurements were taken at a distance of 0,5 cm and 30 cm between the measuring probe and the B AIR V2 system.

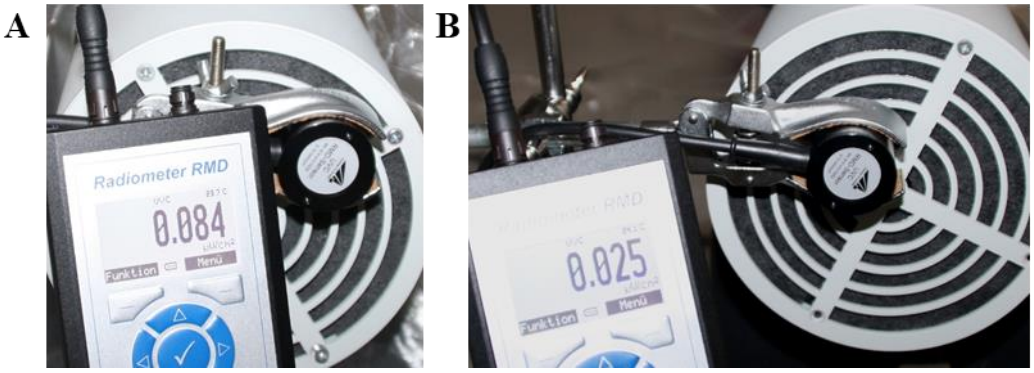


Figure 14: B AIR V2 System: A Measurement probe at the air inlet (0,5 cm). **B** Measurement at the air outlet (0,5 cm).

The measured values are listed in table 2.

Table 3: The measured values for the B AIR V2 from AED Distribution are shown. Measurements were taken with a distance of 0.5 cm or 30 cm between the measuring probe and the air inlet or air outlet.

Measurement position	**Distance[cm]**	**B AIR V2 UV-C radiation [µW/cm²]**
Air inlet	0,5	0,084
	30	< 0,0
Air outlet	0,5	0,025
	30	< 0,0

For the B AIR V2 system, the highest value was measured at a distance of 0,5 cm at the inlet.

Evaluation

The system "B AIR V2" of the company AED Distribution is an air disinfection system, which disinfects the air via UVC emitters. In this project, the reduction of MS2 viruses in single passage and the depletion of the total germ count by the system were investigated. Furthermore, a measurement of the photobiological emissions as well as the ozone concentration took place.

The reduction of viral material in the air stream has been studied using the surrogate virus MS2. The B AIR V2 system was able to reduce the viral load by 4,22 log levels under the given conditions with a single passage.

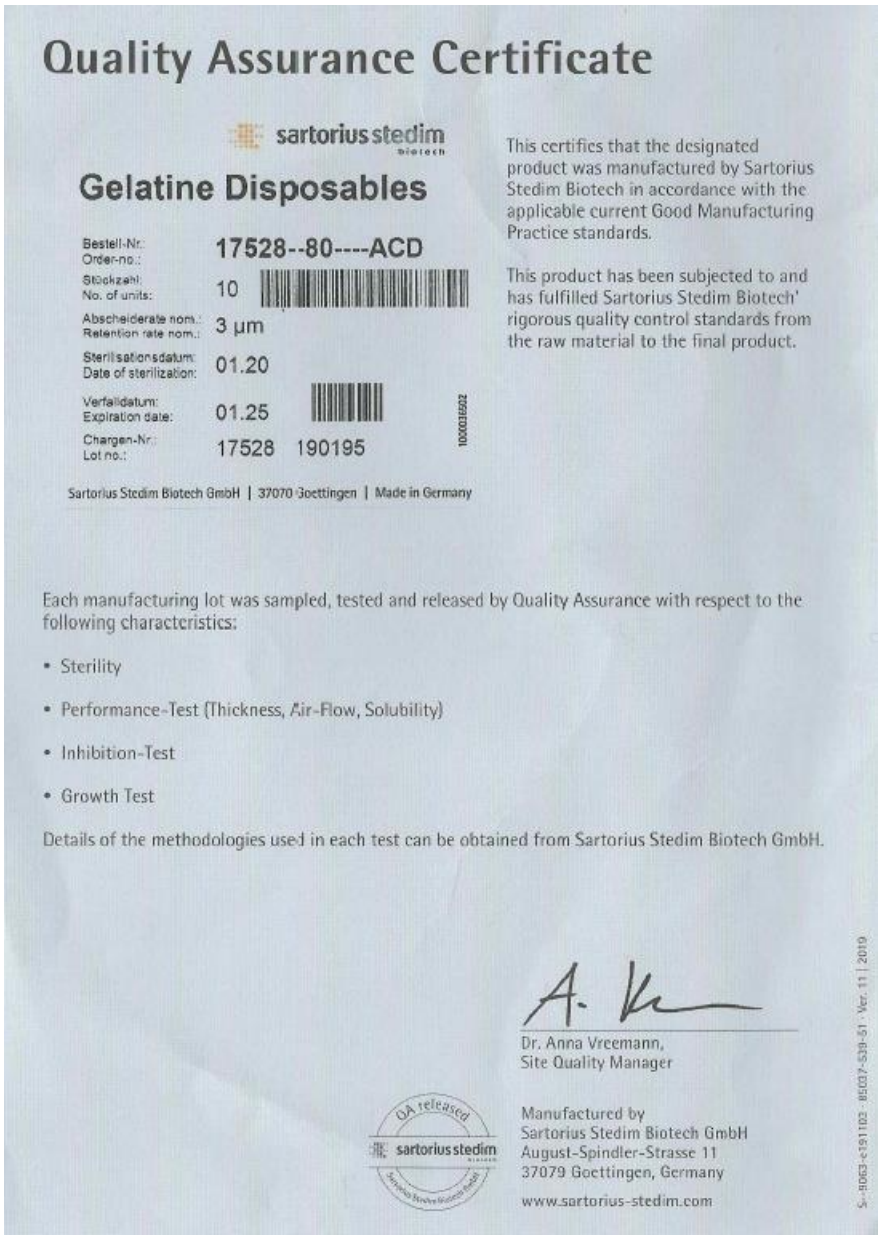
After one hour of operation, no ozone could be detected in the outlet area of the B AIR V2 in the tent with the test tube used (Dräger).

Furthermore, a reduction of total germs in a defined test tent (11,37 m^3) could be demonstrated. Already within one hour after commissioning, the GKZ was reduced by > 90%.

During the photobiological measurement, it was found that the guideline value for photobiological emissions of 0.3 µW/cm² (E DIN EN 60335-2-65/A2:2014), was not exceeded at either the air inlet or the air outlet.

Appendix

A1: Certificate gelatin filter and calibration record MD8



Quality Assurance Certificate

sartorius stedim biotech

Gelatine Disposables

Bestell-Nr.: Order-no.: 17528--80----ACD
Stückzahl: No. of units: 10
Abscheiderate nom.: Retention rate nom.: 3 µm
Sterilisationsdatum: Date of sterilization: 01.20
Verfalldatum: Expiration date: 01.25
Chargen-Nr.: Lot no.: 17528 190195

10000315922

Sartorius Stedim Biotech GmbH | 37070 Goettingen | Made in Germany

This certifies that the designated product was manufactured by Sartorius Stedim Biotech in accordance with the applicable current Good Manufacturing Practice standards.

This product has been subjected to and has fulfilled Sartorius Stedim Biotech' rigorous quality control standards from the raw material to the final product.

Each manufacturing lot was sampled, tested and released by Quality Assurance with respect to the following characteristics:

- Sterility
- Performance-Test (Thickness, Air-Flow, Solubility)
- Inhibition-Test
- Growth Test

Details of the methodologies used in each test can be obtained from Sartorius Stedim Biotech GmbH.

Dr. Anna Vreemann,
Site Quality Manager

QA released
sartorius stedim

Manufactured by
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen, Germany

www.sartorius-stedim.com

Sa-9063-e191102 · BEQ7-539-51 · Ver. 11 | 2019

A2: Calibration record MD8

SARTORIUS

Prüfprotokoll MD8® | Test Report MD8®

Modell Model:	MD8® airscan (16746)	Fabr. Nr. Serial no.:	12201007	Software version	2,7	Inventar / PM Nr. Inventar / PM no.:	-/-

Es wurden folgende Gerätefunktionen überprüft
The following functions have been checked

☒ Flow-Rate
Flow-rate

☒ Messzeit
Time measurement

Überprüfung der Flow-Rate: Beschreibung des Testablaufes	Zwischen dem Gelatinefilter und Lufteinlass des MD8 wird die Kalibriereinheit Durchfluss geschaltet. Je nach der am MD8 vorgewählten Luftdurchsatzmenge wird über einen festen Zeitraum die durchströmende Luftmenge gemessen. Das Messergebnis wird in m³/h ausgegeben. Während des Tests wird die Umgebungstemperatur konstant gehalten. Der Test wird bei Atmosphärendruck durchgeführt. Die Testzeit der einzelnen Tests beträgt 3 Minuten
Flow rate check: Description of the test run	A calibration unit, for airflow, is installed between the gelatin membrane filter and the air inlet of the MD8. The air volume sampled at an air flow rate preselected on the MD8 is measured over a specific period of time. The result is given in m³/h. A constant temperature is maintained during the test, which is carried out under atmospheric pressure. The time for each test is 3 minutes.
Messmittel: Measuring Equipment	Kalibriereinheit / Calibration unit: Type: RVG-ST G16,DN25 Serial no.: 75087961 Prüfmittel Nr. | Testing equipment no.: 19566/10.2020 gültig bis | valid until: 10.2021 Filterhalter zur Aufnahme des Gelatinefilter | Filter holder for again the gelatin filter Gelatinefilter vom Typ | Gelatin membrane filter type: 12602-080 ALK Digital-Stoppuhr / Digital Stop watch: Type: C-563 No.: CHR / 0229 SI Prüfmittel Nr. | Testing equipment no.: P-5572838 gültig bis | valid until: 02.2022

Messprotokoll | Test record

MD8 m³/h	Kalibriereinheit Calibration unit m³/h	Abweichung Deviation m³/h
8,00	7,95	-0,05
6,00	5,97	-0,03
5,00	5,04	0,04
4,00	4,01	0,01
2,50	2,51	0,01

Es wurden folgende Messzeiten überprüft (Strömungsbereich 2,5 m³/h)
The following air-sampling times were checked (flow 2,5 m³/h)

MD8 Zeit (Min:Sek) Time (min:sec)	Zeit (Stoppuhr) (Min:Sek) Time (Stop watch) (min:sec)	Abweichung (Sek.) Deviation (sec.)
2:00	2:00	0 ✓
3:00	3:01	1 ✓
5:00	5:02	2 ✓

Testergebnisse /|Test results

Die maximal gemessenen Luftdurchsatzmengen haben eine Abweichung von 0,6 % vom Maximalwert (8 m³/h).
The maximally measured air volume quantities have a deviation from 0,6 % from the maximum value (8 m³/h).
Die maximal zulässige Abweichung beträgt ≤± 5 %. | The maximum allowable deviation is ≤± 5 %.

Die maximal gemessene Messzeit hat eine Abweichung von 2 Sekunde(n).
The maximally measured gate time has a deviation from 2 second(s).
Die maximal zulässige Abweichung ist ≤± 3 Sekunden, bezogen auf jede einzelne Zeitmessung.
The maximum allowable deviation is ≤± 3 seconds, related to each individual time measurement.

Die Messergebnisse liegen innerhalb der Toleranzen
The results are within the specified tolerances

☒ Ja | Yes ☐ Nein | No

Bitte beachten Sie, dass wir eine Kalibrierung einmal im Jahr empfehlen. | Please note that we recommend a recalibration once a year.
Dieses Prüfprotokoll darf nur vollständig und unverändert weiterverbreitet werden. Prüfprotokolle ohne Unterschrift haben keine Gültigkeit.
This test report may only be distributed only completely and unchanged. Test reports without signature are not valid.

Nächste Kalibrierung / next calibration: März 2022 / March 2022 Datum / Date: 04. März 2021 / 04 March 2021 Prüfer | Tested by: F. Enders Unterschrift | Signature: Enders	Firma | Company: Kd-Nr. | Co-No.: 10055863 biotec Umwelt-Analytik-Beratung-Service GmbH Elbrachtsweg 76 33332 Gütersloh	Frank Enders Repair Center Central Europe Groner Siekanger 1 37081 Göttingen Tel.: 0551.308.3174 Fax:-3737 E-Mail: repaircenter-ce@sartorius.com

Sartorius Lab Instruments GmbH & CO KG, Otto-Brenner-Str. 20, 37079 Göttingen
Sartorius Lab Instruments GmbH & CO KG, Otto-Brenner-Str. 20, 37079 Goettingen

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