Evaluation of a prototype Medair air purifier for its ability to sterilise SARS-CoV-2 aerosols

Research report prepared
by QIMR Berghofer Medical Research Institute
for Medair Australia Pty Ltd

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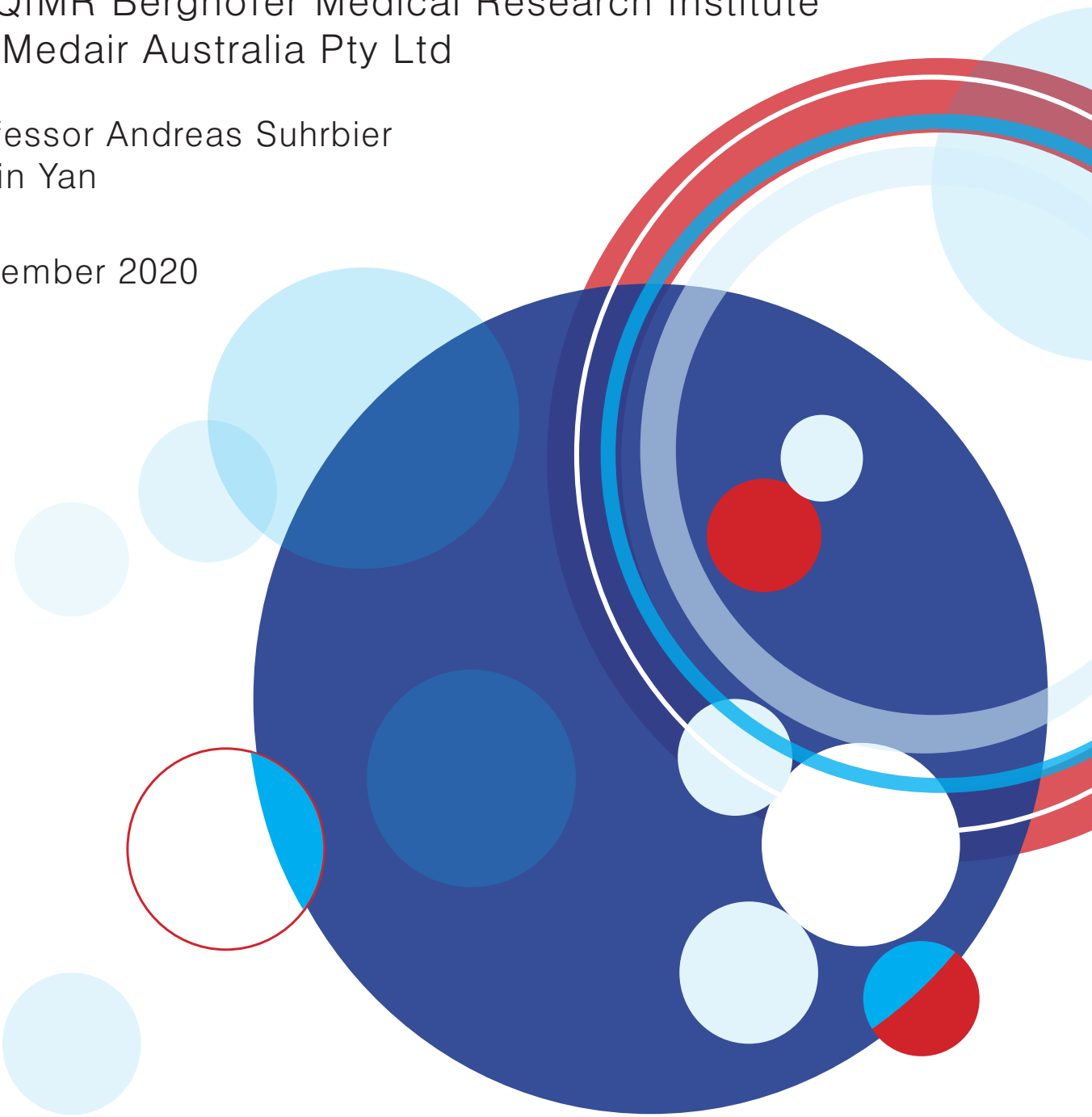

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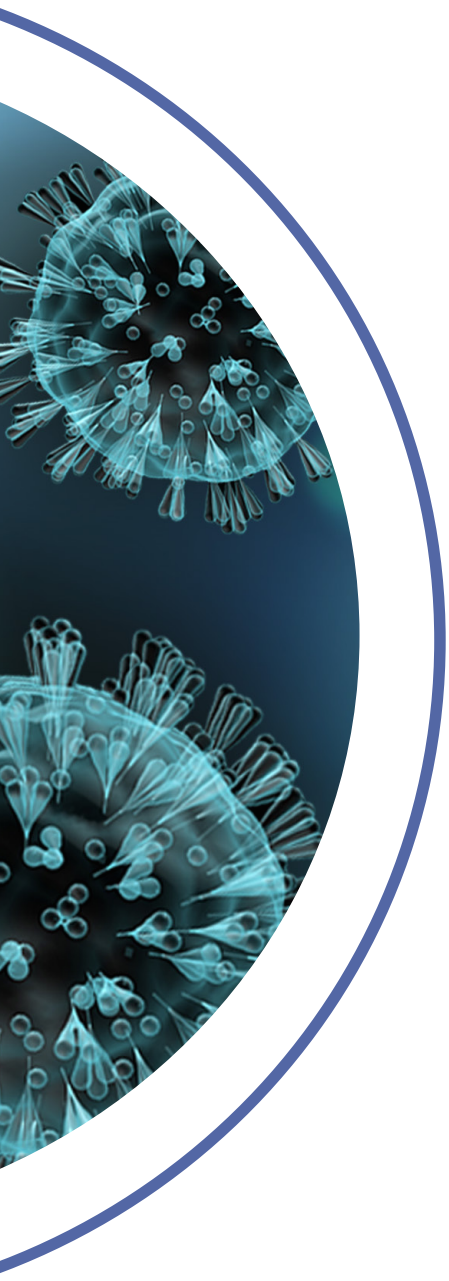

About QIMR Berghofer

QIMR Berghofer is one of Australias leading medical research institutes. Our mission is to translate research discoveries into clinical practice to improve the health of patients and the broader community. We achieve this through developing novel therapeutics, diagnostics, and prevention strategies for diseases with high unmet clinical needs. Moreover, we support the Australian lifescience industry by providing commercial partners access to our state-of-art research facilities and the expertise of our world leading scientists, through collaborations and contract research.

Located within the Herston Health Precinct and adjacent to the Royal Brisbane and Women's Hospital, QIMR Berghofer is home to over 1000 scientists, students, and support staff. QIMR Berghofer is uniquely positioned to deliver innovation in medical research with Q-Gen Cell Therapeutics, a GMP contract manufacturing facility, and Nucleus Network, a clinical research organisation on premise. For more information, please visit our website at www.qimrberghofer.edu.au.

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Disclaimer and disclosure

About the COVID-19 team at QIMR Berghofer

Professor Andreas Suhrbier

Professor Suhrbier leads the Inflammation Biology Group which has developed a number of mouse models used to gain new insights into the factors that regulate viral infections. These models are often utilised by commercial collaborators to test the efficacy of potential new viral interventions. In response to the global COVID−19 pandemic, the group established a specialised Biosafety Level 3 facility to undertake COVID−19 research using in vitro testing and vivo disease models. To date, a number of Australian and global lifescience companies have used this facility to evaluate the efficacy of their products.

During his career, Professor Suhrbier has been an inventor on 18 granted international patents: 13 have been commercialised and 11 led to human clinical trials. In addition, he has been a consultant for Sementis, Valneva, Abivax, Leo Pharma, GSK, Paradigm, WHO, Aventis Pasteur, CSL, Bavarian Nordic, Peplin, C−Bio, and Medair Australia.

Kexin Yan

Kexin Yan is a senior research assistant within the Inflammation Biology Group and is the author of 9 academic research publications.

Thuy T Le

Thuy Le is a highly experienced senior research assistant within the Inflammation Biology Group and is the author of 38 academic research publications.

Daniel J Rawle

Dr Rawle is a research officer who has recently joined the Inflammation Biology Group and is the author of 15 academic research publications.

Executive summary

This report was commissioned by Medair Australia Pty Ltd, a start-up company developing an air purifying device. The ability of Medair's prototype device to remove and/or sterilise SARS-CoV-2 present in aerosols was evaluated using an aerosol chamber and a $CCID_{50}$-based assay system with Vero E6 cells. The Medair device was found to sterilise and/or remove aerosols containing infectious SARS-CoV-2.

Introduction

Increasing evidence suggests that SARS-CoV-2 is able to be transmitted by aerosols. The wearing of masks has been employed to prevent aerosol transmission, however their effectiveness has been shown to be limited[1]. Transmission by aerosols is deemed to represent a risk in certain settings including prolonged exposure to infectious individuals, enclosed spaces with poor ventilation, and during high aerosol generating activities or procedures[2,3]. Settings that have these characteristics are common in society, for example healthcare centres and hospitality venues. Therefore, aerosol transmission of SARS-CoV-2 could play a significant role in the global pandemic.

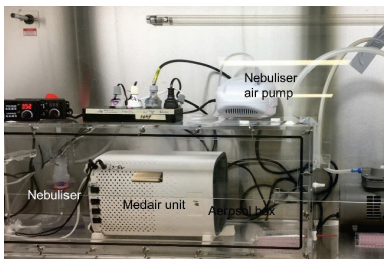
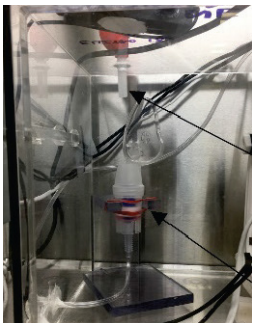
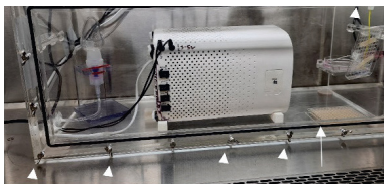
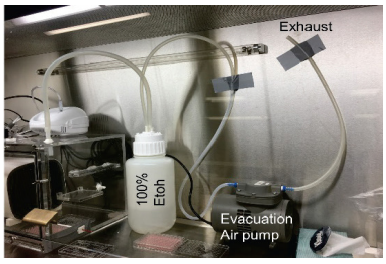
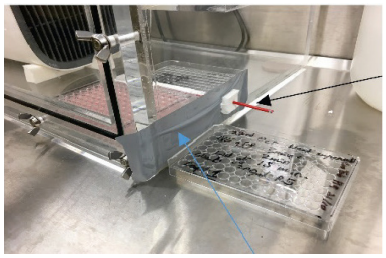
Medair Australia is a start-up company aiming to address this issue through developing an air purifying device to neutralise SARS-CoV-2 infectious aerosols. Medair's device is proposed to sterilise aerosols via its Negative Electrode and charge neutralisation unit. Initial testing conducted by QIMR Berghofer demonstrated that a prototype version of the device could reduce the presence of Getah virus (a non-pathogenic virus in humans[4,5]) containing aerosols or substantially render the aerosols non-infectious. In this report, the ability of Medair's prototype device to sterilise or remove SARS-CoV-2 virus containing aerosols was evaluated.

Methods

Materials

Testing was carried out in QIMR Berghofer's specialised Biosafety Level 3 (PC3) SARS-CoV-2 facility. A CCID50-based assay system, recently developed in-house[6], measuring cytopathic effects (CPE) in Vero E6 cells was used to quantify the presence of SARS-CoV-2 infectious aerosols.

To contain aerosols, a sealed Perspex box was designed in-house and constructed by Foremost Plastics. The box was placed inside a biosafety cabinet (BSC) and contained the Medair device, a nebulizer to create infectious aerosols, and a slot to introduce and remove 96 well plates containing medium and Vero E6 cells. The electronic controls on the exterior of the box allowed for control of the nebulizer, and the Medair device's fan and Negative Electrode (NE) to be switched on and off (Fig. 1).

	Mediar unit aerosol box inside Bio–Safety cabinet.
	Nebuliser (aerosol generator) inside aerosol box. Vent (air in when evacuation pump is on). Virus solution.
	Aerosol box sealed iwth front plate secured by 18 wing nuts (arrow heads) against a rubber seal (arrow).
	Venting aerosols from box through 100% ethanol and evacuation to the top of the BSC, with vent (above nebuliser) open and evacuation pump on.
	Lid removed from 96 well plate inside the box by rotation of red lever.

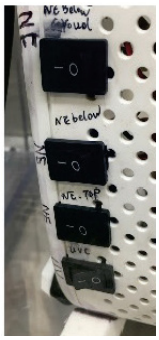
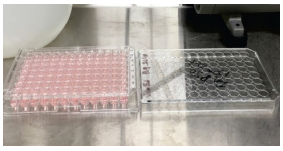
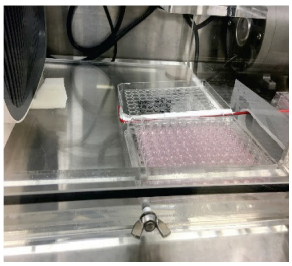
	Mediar unit Negative Electrode and fan voltage controls.
	Switch settings.
	In BSC (outside the box) plate lid off throughout experiment to detect infectious aerosols that may have escaped the box.
	Lid off collection 96 well plate.

Figure 1: Aerosol box and its components

Procedure

RUN 1 and 2

NE off. The nebulizer was loaded with 8 ml of SARS−CoV−2 (containing about 10^6 $log_{10}CCID_{50}$/ml of virus) in RPMI 1640 supplemented with 2% FCS and buffered with 10 mM HEPES. The box was sealed and the nebulizer was run for 3 mins while the Medair device fan was switched on (3.5 volts). The nebulizer was then switched off and the fan run for a further 2 mins. The lid on the 96 well plate inside the box containing Vero E6 with 200 µl of medium with 5% FCS (RUN 1) or 20 µl of medium with 5% FCS (RUN 2) was then lifted off (Fig. 1, red lever) and the fan run for a further 15 mins. The fan was switched off, aerosols were vented by switching on evacuation pump and opening vent above the nebulizer for 2 mins. The 96 well plate in the box was exchanged via the slot in the box while the pump maintained a negative pressure with the vent above the nebuliz−er closed. The 96 well plate was recovered, a new sterile lid was placed on the plate, the plate sprayed with 80% ethanol, and then transferred to an incubator in a sealed box and cultured for 6 days to evaluate viral cytopathic effects (CPE).

NE on. To evaluate the ability of the Medair device to sterilise SARS−CoV−2 virus containing aerosols, the NE was turned on (NE on, 18 volts) during the 2 min nebulization, 2 mins lid on, 15 min lid off, and 2 min venting periods. For the NE off experimental group (NE off) the NE was not turned on during these time periods. See Fig 2 for a summary of the procedure.

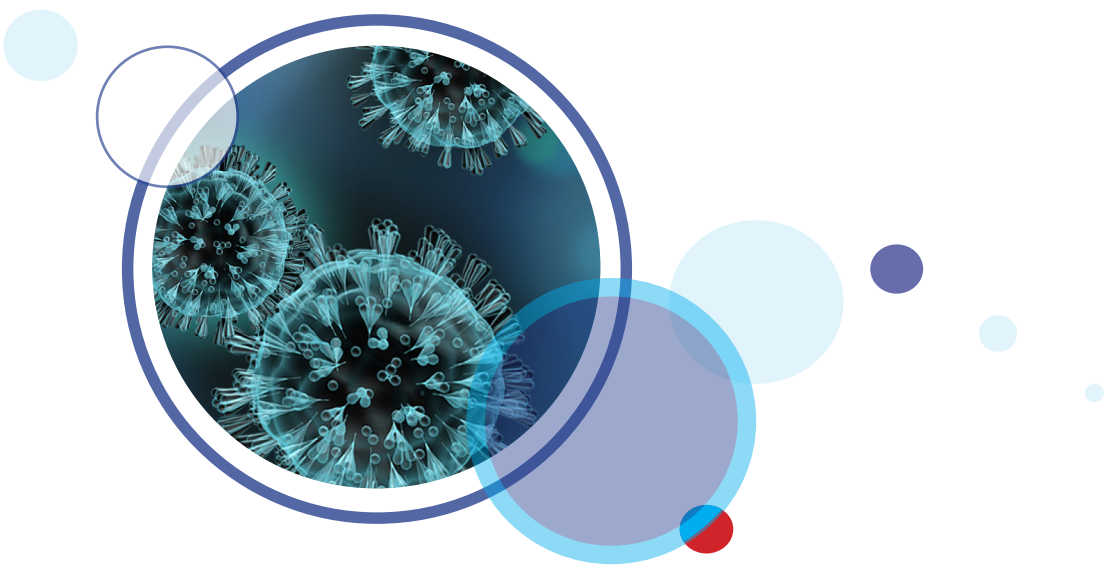
RUN 3

The procedure for this experiment was conducted as per RUN1, however the lid of the 96 well plate was removed prior to the nebuilzer, device fan, and NE being switched on. In this setting, some aerosols likely landed in the 96 well plate without passing through the Medair device.

In-BSC Control

A 96 well plate in the BSC was present, outside of the aerosol box, throughout the experiment to check for virus escape from the box. At the end of the experiment the lid was replaced and the plate incubated for six days to assess CPE.

All testing was approved by the QIMR Berghofer Biosafety Committee and relevant external regulatory bodies.



Results

RUN 1

To determine whether the Medair device removed or sterilised infectious aerosols, we generated aerosols containing SARS−CoV−2 using a nebulizer and quantified the presence of SARS−CoV−2 using a CCID50−based assay system measuring cytopathic effects (CPE) in Vero E6 cells. In this assay, reduced intensity of crystal violet staining in wells of a 96 well plate indicated the presence of SARS−CoV−2. When the Negative Electrode (NE) of the Medair device was switched off, 85/96 wells captured infectious aerosols (Fig. 2b). When the Negative Electrode (NE) was switched on, 0/96 wells captured infectious aerosols. In this setting the device removed 100% of infectious aerosols.

a

Time mins	Nebuliser	Fan	NE	Collector 96 well plate lid	Vent	Evacuation pump
NE OFF						
3	ON	ON	OFF	ON (closed)	CLOSED	OFF
2	OFF	ON	OFF	ON	CLOSED	OFF
15	OFF	ON	OFF	OFF (aerosol collection on)	CLOSED	OFF
2	OFF	OFF	OFF	OFF	OPEN	ON
Change plate	OFF	OFF	OFF	ON	CLOSED	ON
NE ON						
3	ON	ON	ON	ON	CLOSED	OFF
2	OFF	ON	ON	ON	CLOSED	OFF
15	OFF	ON	ON	OFF	CLOSED	OFF
2	OFF	OFF	ON	OFF	OPEN	ON
Change plate	OFF	OFF	OFF	ON	CLOSED	ON

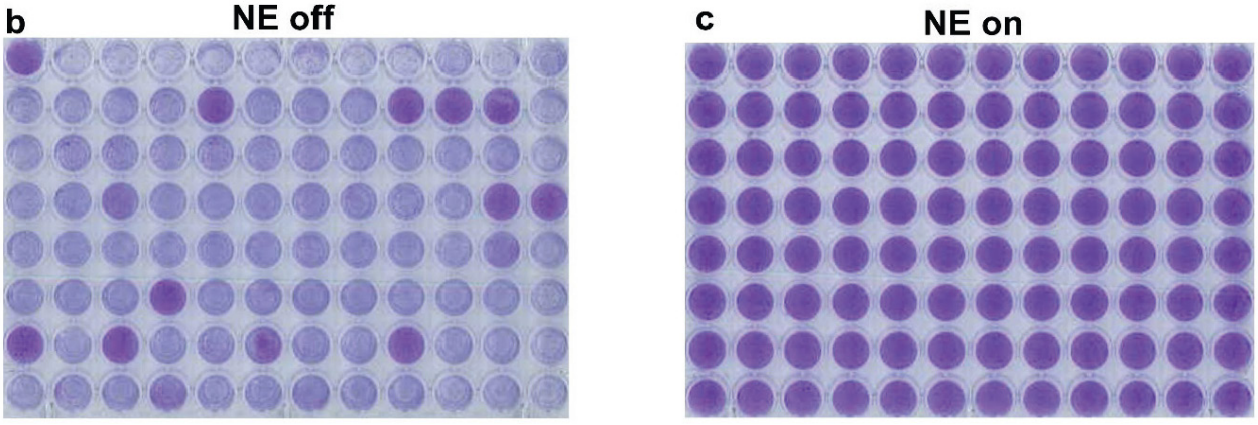


Figure 2. SARS-CoV-2 aerosol testing RUN 1: a) Switching sequence. b) Negative electrodes (NE) off. 85/96 wells captured infectious aerosols. Viral cytopathic effects (CPE) indicated by reduced crystal violet staining. c) NE on; 0/96 wells with CPE; no detection of infectious aerosols.

RUN 2

The experiment was repeated as for RUN 1 except that the volume in the 96 well plate was reduced to 20 µl. This nominally increased the detection limit by 10 fold (i.e. 20 ul vs 200 µl). With the NE off, 96/96 wells detected infectious virus. With NE on, 3/96 wells detected infectious virus (Fig. 3). Under these conditions the device thus reduced infectious aerosols by 96.9%.

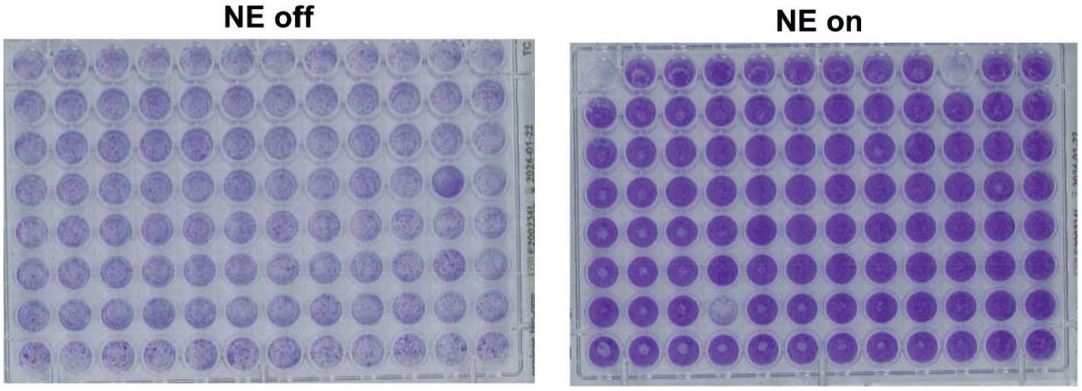


Figure 3. SARS-CoV-2 aerosol testing RUN 2. As for RUN 1 except the volume in the 96 well plate was reduced to 20 µl.

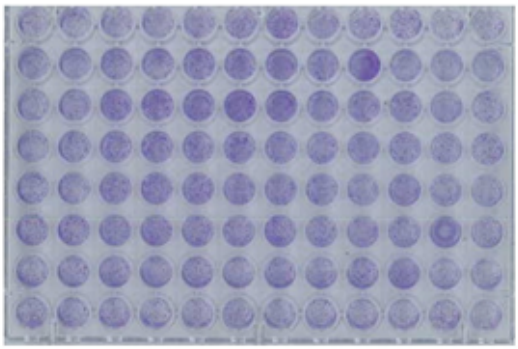
RUN 3

In RUN 1 and 2, it was possible that sterilization of aerosols occurred outside of the device. To determine whether aerosol sterilization was dependent on aerosols passing through the device while the NE was on, the lid of the 96 well plate was removed prior to the nebuilizer, fan and NE being turned on. In this setting, some aerosols could land on the 96 well plate without having passed through the device. With the NE off, all wells 96/96 contained infectious virus. In comparison, with the NE on, 59/96 captured infectious virus (Fig. 3c, d). Thus in this setting the device provided a 38.5% reduction in infectious aerosols. This experiment suggests that the aerosols need to pass into/through the device in order for them to be removed or to be sterilized.

a

Time mins	Nebuliser	Fan	NE	Collector 96 well plate lid	Vent	Evacuation pump
NE OFF						
5	ON	ON	OFF	OFF	CLOSED	OFF
2	OFF	OFF	OFF	OFF	OPEN	ON
Change plate	OFF	OFF	OFF	ON	CLOSED	ON
NE ON						
5	ON	ON	ON	OFF	CLOSED	OFF
2	OFF	OFF	ON	OFF	OPEN	ON
Change plate	OFF	OFF	OFF	ON	CLOSED	ON

b NE off



c NE on

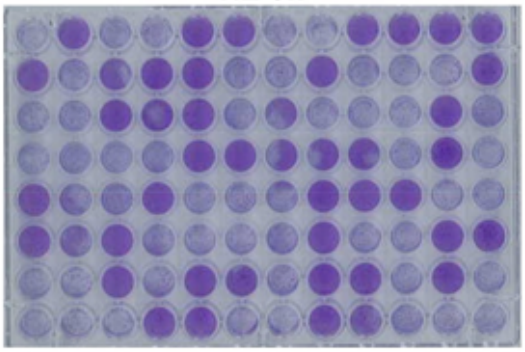


Figure 3. SARS-CoV-2 aerosol testing RUN 3: a) Switching sequence. b) Negative electrodes (NE) off. c) NE on.

In-BSC control

No infectious virus was detected by the in-BSC control plate (Fig. 4) present and open (lid off) throughout all the aforementioned runs, suggesting that infectious aerosols did not escape from the box during all the aforementioned runs.

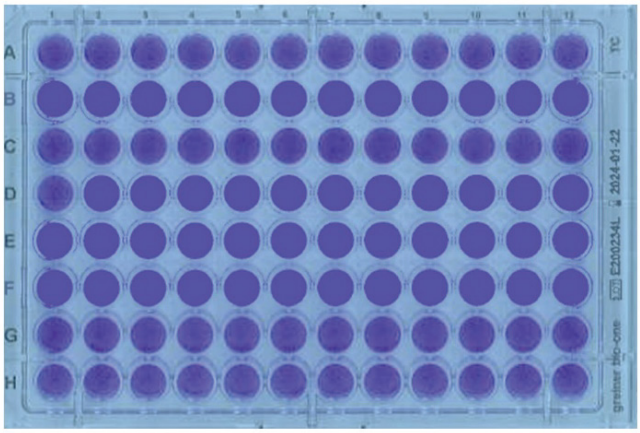


Figure 4. SARS-CoV-2 aerosol testing in-BSC control

Conclusion

The prototype Medair air device was able to substantially sterilise or remove infectious SARS−CoV−2 aerosols from the volume of air contained within the box.

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Lead author's declaration of authorship

Professor Andreas Suhrbier, 15th December 2020

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