

This is an Accepted Manuscript for *Infection Control & Hospital Epidemiology* as part of the Cambridge Coronavirus Collection.

DOI: 10.1017/ice.2021.103

**Article Type: Research Brief**

## **Removal of SARS-CoV-2 Bioaerosols using Ultraviolet Air Filtration**

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Short Title: SARS-CoV-2 and Air Purification

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Word count: 899

Engineering controls play an important role in reducing the spread of SARS-CoV-2 (1). Established technologies such as air filtration and novel approaches such as UV-C light or plasma air ionization carry the potential to support the fight against the pandemic (2). We tested the efficacy of an air purification system (APS) combining UV-C light and High Efficiency Particulate Air (HEPA) filtration in a controlled environment using SARS-CoV-2 as test organism. The APS successfully removed the virus from the air using UV-C light by itself and in combination with HEPA air filtration.

#### **METHODS:**

Testing was performed at the Battelle Biomedical Research Center (Columbus, Ohio) under three test groups: 1) Inactive test unit (control); 2) APS with UV-C light activated; 3) APS with UV-C light activated and HEPA filter in place. The ten-minute test runs in each group were repeated five times.

The APS consisted of a UV-C photolytic chamber, which incorporated four low-pressure UV-C germicidal lamps operating at 254nm wavelength, at a total irradiance of  $177.8 \mu\text{w}/\text{cm}^2$  and containing approximately 2400 transparent quartz tubular elements oriented randomly in the chamber (Aerobiotix, Dayton, OH). Air filtration was provided by a commercial-grade 99.97% efficacy HEPA filter.

The APS was placed inside a single pass air-testing unit (ATU; 12"x12" cross section) within a Biological Safety Level 3 cabinet (figure 1). The ATU consisted of a pre-APS, APS, and post-APS chamber. Airflow was controlled by fans in the ATU and recirculated at 588-623 L/min. A six-jet Collision nebulizer (BGI, Waltham, MA) generated an SARS-CoV-2 aerosol from liquid suspension

using HEPA and carbon capsule pre-filtered air (Pall Corp., Port Washington, NY). A total of  $1.57 \times 10^7$  TCID<sub>50</sub> of SARS-CoV-2 was nebulized into the pre-APS chamber at 0.4 mL/minute for 10 minutes, and mixed by baffles placed inside the chamber. Mixing air volume was 567 L/min. This represents a pre-APS chamber air concentration of 2,760 units/L, assuming full dissemination efficiency. An air particle sizer (APS Model 3321 with aerosol diluter [Model 3302A], TSI Inc., Shoreview, MN) determined the aerodynamic particles at 0.25 L/min for 10 seconds five minutes into the control run. Impinger samples (Model 7541, Ace Glass Inc.) were taken in the pre- and post-APS chambers during the control runs at  $6.0 \pm 0.5$  L/min for 10 minutes each and from the post-APS chamber for all subsequent runs. Temperature and humidity during all tests were 68-69°F and 48-49% RH, respectively.

SARS-CoV-2 virus was propagated by ATCC from BEi Resources NR-52281 lot 70034262 (ATCC, Manassas, VA) and concentrated to  $1.24 \times 10^7$  TCID<sub>50</sub>/mL in Minimum Essential Medium (Sigma Cat. No. 51416C, St. Louis, MO, USA;) + penicillin-streptomycin (Sigma Cat. No. P4333;) + 5% fetal bovine serum (Sigma Cat. No. F4135).

Samples were quantitated using VERO E6 cell culture (ATCC C1008 E6 [Cat. No. NR596]) to determine cytopathological effects (CPE). CPE was defined as cell rounding, fusion, or lysis and expressed as present in the cell culture. Analysis of results was performed using the Fisher-Boschloo test.

## **RESULTS:**

In the control group particle counts increased from 20 (pre-APS chamber) to 54 particles (post-APS chamber) reflecting the introduction of the SARS-CoV-2 aerosol. A similar, yet less

pronounced increase was observed in the second run - APS with UV-C light activated (79 to 101 particles). Placing a HEPA filter into the air-flow reduced the particle count significantly (79 to 5 particles). The mass mean aerodynamic diameter over all runs ranged from 0.80 to 1.17  $\mu\text{m}$  consistent with the Collison nebulizer particle dispersion characteristics (3).

For the viral culture tests three out of five runs collected from the pre-APS chamber samples and two out of five runs collected in the post-APS chamber showed CPE in the control group. No CPE were observed in the post-APS chamber samples for both the APS with UV-C light activated and APS with UV-C light and HEPA filtration. The one-sided Fisher–Boschloo test revealed a p-value of 0.0386.

## **CONCLUSIONS:**

The emergence of SARS-CoV-2 has accelerated the development and adoption of air purification technologies (4-7). In this study viable virus was detected in the control runs without UV-C light activation or HEPA filtration. Using an UV-C light system with and without HEPA filtration eliminated SARS-CoV-2.

This study holds several key advantages. Virus was detected by direct culturing, which unlike genomic studies depicts the presence of viable virus. The closed single-pass design assured that bioaerosols were contained in the sealed test unit reducing environmental effects such as decreased viability of the aerosolized virus through temperature and humidity, and adherence to chamber walls (8). Airflow was maximized to challenge the APS. This resulted in a SARS-CoV-2 pre-APS chamber air concentration of 2,760 TCID<sub>50</sub> units/L, assuming full dissemination efficiency. Even accounting for inefficiencies, the pre-APS chamber air concentration compared

favorably to published SARS-CoV-2 air concentrations samples in active patient rooms (6-74 TCID<sub>50</sub> units/L) (9).

There are limitations. Maximizing airflow resulted in rapid dilution of the aerosolized virus.

Therefore, although the presence of viable virus through CPE could be detected, quantification could not be performed. However, these findings were significant and are consistent with prior testing of the same device using MS2 virus (10).

To date, we are unaware of any APS having been directly challenged with SARS-CoV-2 bioaerosols (4-7). The APS successfully eliminated the virus within closely defined parameters reaching significance. Future studies should address the impact of APS on SARS-CoV-2 transmission in a clinical environment taking into account challenges from virus emissions by patients to airflow patterns.

**ACKNOWLEDGEMENTS:** Support for this study was provided by Aerobiotix, Dayton, OH.

**DISCLOSURE STATEMENTS:** Dr. Barnewall reports grants from Aerobiotix, Dayton, OH, during the conduct of the study. Dr. Bischoff reports grants from Aerobiotix, Dayton, OH, outside the submitted work.

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**LEGENDS:** Figure 1: Schematic of Testing Set-up

