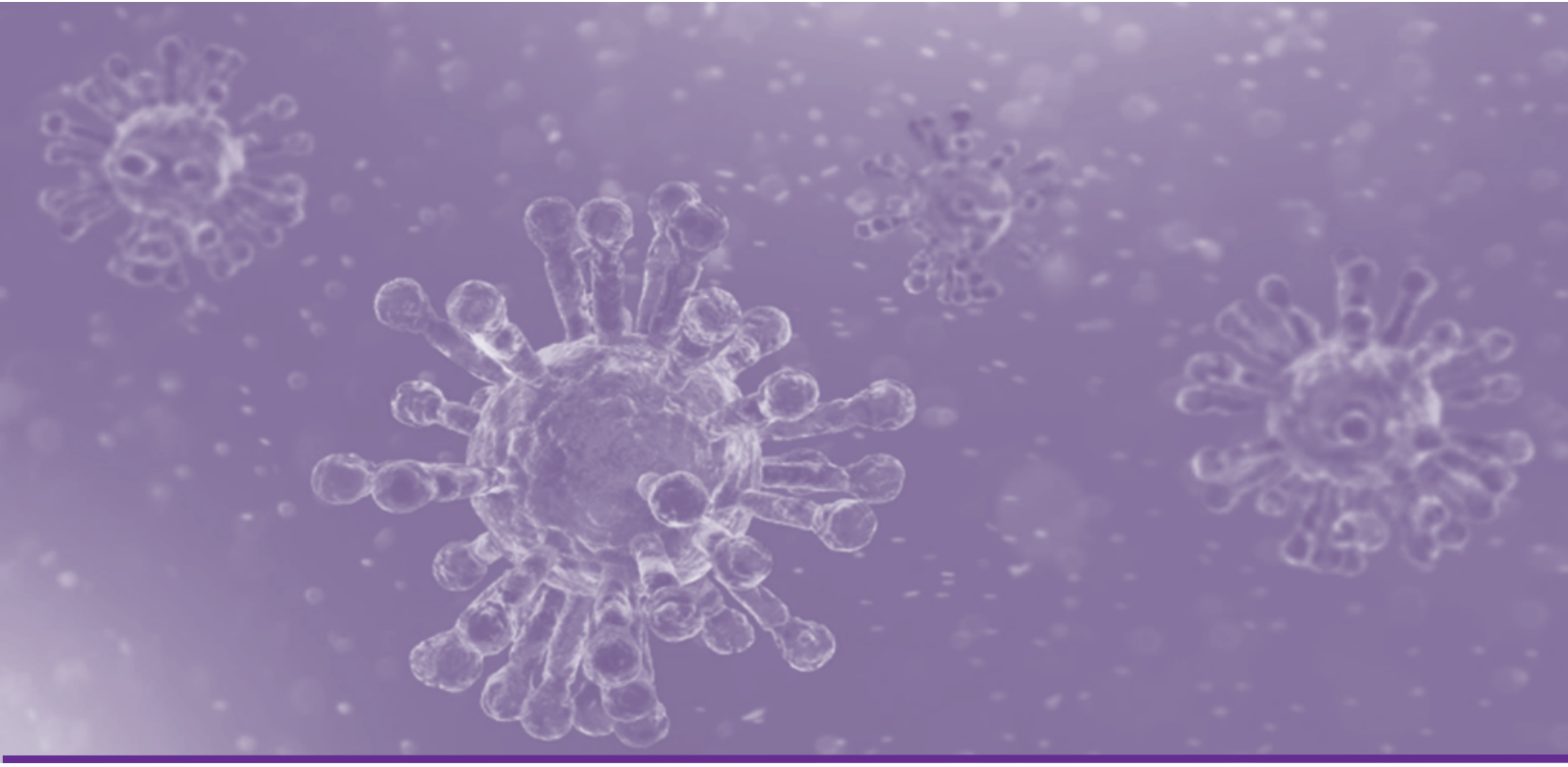

SARS-CoV-2 Bioaerosol Removal by the Aerobiotix Ultraviolet Filtration System

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ABSTRACT

Introduction: Multiple studies have confirmed the presence of airborne SARS-COV-2 as small-droplet bioaerosols. The emergence of SARS-CoV-2 has accelerated adoption of air disinfection technologies. The purpose of this study is to determine effectiveness of the Aerobiotix C-band ultraviolet (UVC) air filtration unit to remove SARS-CoV-2 bioaerosols under challenge conditions with viable virus.

Methods: Aerosolized SARS-CoV-2 was nebulized into a test setup consisting of prechamber, Aerobiotix photolytic chamber, and postchamber. Aerodynamic particle sizing and impinger samples were analyzed to document SARS-CoV-2 bioaerosols. For each test run, a total of 1.57 x 10^7 $TCID_{50}$ of SARS-CoV-2 was nebulized into the prechamber at 0.4 mL/minute for 10 minutes and confirmed with prechamber and postchamber sampling. Testing was performed under three test groups: (a) Inactive test unit (control); (b) Aerobiotix unit with UVC plus adjuvant HEPA; and (c) Aerobiotix test unit with UVC only.

Results: For aerodynamic particle sizing, median particle diameters of 1 µm ±0.2 were consistent with small bioaerosols described for airborne SARS-CoV-2. For viral cultures, there were 0/10 positive cultures in the Aerobiotix group, versus 2/5 positive cultures in the control group. Due to the nonparametric nature of the data, a one-tailed Fisher's exact test was utilized with a value of p=.0952. The presence of the HEPA filter under test conditions did not result in additional removal performance over UVC alone.

Conclusions: Within the limitations of this study, the Aerobiotix unit was successful in removing viable SARS- CoV-2 bioaerosols compared to controls.

INTRODUCTION

A growing body of evidence supports the presence of airborne SARS-CoV-2 as small droplet bioaerosols in contaminated environments, particularly acute healthcare settings.[11,17] Multiple studies have confirmed the presence of airborne SARS-COV-2 bioaerosols by both genetic and direct culturing techniques.[3,5-7, 13,18,19] The World Health Organization has specifically cautioned against airborne transmission from aerosol-generating procedures in hospital settings.[22,23]

Recently, viable SARS-CoV-2 virions were cultured from air samples collected 2 to 4.8 m away from hospitalized patients. The genomic sequence of airborne strains was identical to those isolated from the patient with an active infection. Estimates of viable viral concentrations ranged from 6 to 74 $TCID_{50}$ units/L of air.[10] The SARS-CoV-2 virus has been shown to be stable in airborne particles with a half-life exceeding one hour.[20]

Genomic studies have found positive air samples in 2 of 3 patient infection isolation rooms, with a viral load range of 1.8–3.4 RNA copies per L of air.[3] Additional studies have found 63% of air samples positive with a mean viral load of 2.9 copies/L, including in patient rooms and the hallway air. The highest viral loads were found in the presence of a patient receiving oxygen via nasal cannula (mean: 19 and 48 copies/L), indicating that this treatment may promote the spread of airborne virus.[19]

In order to reduce risk of airborne SARS-CoV-2 transmission, improvements in room ventilation have been advocated, including increased exchange rates, fresh air ventilation, and supplemental air filtration/ disinfection. Air disinfection utilizing ultraviolet germicidal irradiation in the C-band wavelengths (UVC) is a novel approach to reducing airborne viral loads. SARS-Cov-2 has been shown to be highly susceptible to UVC light.[8] Multiple UVC studies have demonstrated effectiveness against airborne virus including coronaviruses[21], vaccinia[15], Mycobacteria[24], influenza[15,16], and MERS-CoV.[1] However, no studies to date have directly demonstrated removal of viable SARS-CoV-2 bioaerosols using CUVC.

Recently, advanced techniques for air disinfection utilizing UVC have been developed. The Illuvia® system (Aerobiotix, USA) has been found to remove viable bioaerosols, MS2 virus, bacteria and spore-forming organisms using a novel combination of simultaneous mechanical and UVC photolytic air treatment (Fig. 1). This technique increases air elimination efficiency over UVC alone.[9,4,2] An additional benefit of this system over prior upper-room UVC lamps is that UVC radiation is contained to the interior of the device, preventing exposure to room occupants.

This study will examine the ability of the Aerobiotix technology to remove airborne SARS-CoV-2 in closed, single pass testing with bioaerosol challenge. Prior to this study, this device has not been tested against SARS-CoV-2 directly, nor are we aware of any other air decontamination device which has been directly challenged with viable SARS-CoV-2 aerosol to date.

Figure 1. The Illuvia air disinfection system.

MATERIALS AND METHODS

Testing was performed at Battelle Biomedical Research Center (BBRC, Ohio, USA). All procedures utilizing live virus occurred in a biosafety level III laboratory. Aerosolization of the virus was performed in a biosafety cabinet within the laboratory.

The test unit consisted of a UVC photolytic chamber, identical to one used in the Aerobiotix Illuvia® system which incorporated four low-pressure UVC germicidal lamps operating at 254nm wavelength, at a total irradiance of 17.8 mw/cm^2 and containing approximately 2400 transparent quartz tubular elements oriented randomly in the chamber. For test modalities with adjuvant filtration, a commercial-grade 99.97% efficacy HEPA filter was utilized downstream of the photolytic chamber (Fig. 2).

A Collison nebulizer loaded with SARS-CoV-2 suspension was used to generate bioaerosol into a 12” x 12” cross-section wind tunnel. The virus was propagated by American Type Culture Collection (ATCC) at ≥ 99% sequence identity to SARS-CoV-2, isolate USA-WA1/2020. The test atmosphere was mixed by baffles before being sampled by an impinger and Aerodynamic Particle Sizer (APS, TSI, Minnesota, USA). APS results are expressed as a raw count at sampling time of 10 seconds at a rate of 0.25 L/min. Impinger samples were analyzed to document SARS-CoV-2

bioaerosol concentration/and or presence while APS data documented aerosol count. Similar impinger and APS samples were collected at the test unit outlet to determine the presence of SARS-CoV-2 remaining.

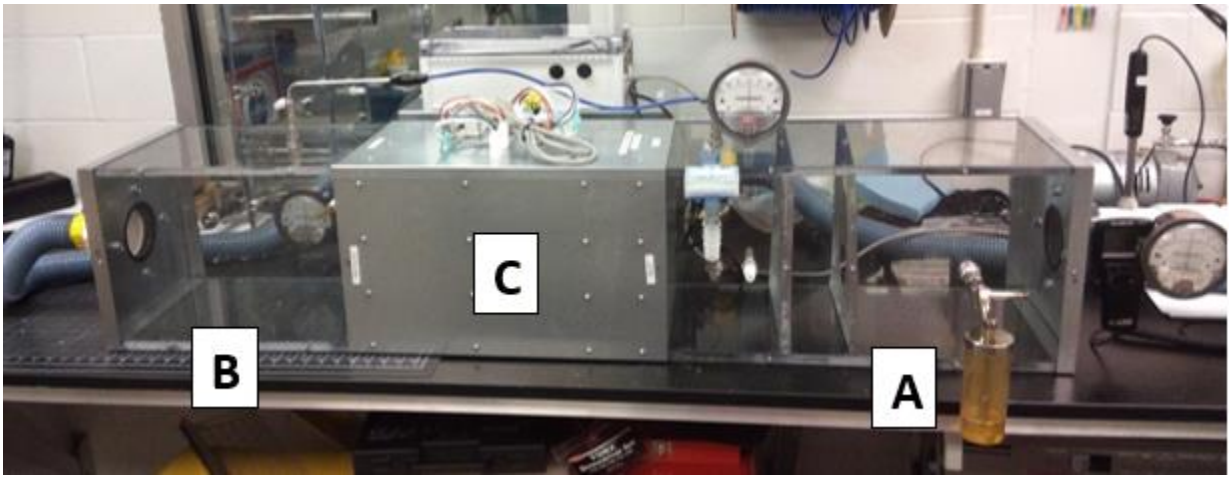

Figure 2. Test Setup demonstrating (a) Prechamber, (b) Postchamber (c) Test unit containing ultraviolet photolytic unit.

For the source virus, a $TCID_{50}$ assay was utilized for quantification of SARS-CoV-2. This quantitative assay relies on the presence and detection of cytocidal virus particles that replicate and release progeny virions into the media, which infect healthy cells. A cell monolayer is inoculated with serial dilutions of the virus of interest and observed for the presence of cytopathological effects (CPE). CPE manifests itself by the appearance of cell rounding, cell fusion (syncytia formation), or cell lysis. For the downstream virus, viral concentrations were too low for accurate quantification to occur, so the presence or absence of CPE in each sample was utilized as the endpoint.

For each test run, a total of 1.57×10^7 $TCID_{50}$ of SARS-CoV-2 was nebulized into the test unit prechamber at a rate 0.4 mL/minute for 10 minutes. Mixing air volume was 567 L per minute. This represents a prechamber air concentration of 2760 units/L, assuming full dissemination efficiency. The presence of viable virus in the prechamber was confirmed by impinger sampling.

Test system airflow was controlled by test unit fans and an external recirculating fan/HEPA unit. Recirculated air was HEPA filtered before being directed to the bioaerosol generation chamber. Airflow rate was recorded through the test system.

Testing was performed under three test runs at different test modes, with sampling into 5 viral culture wells per run. The test modes were:

a) Inactive test unit (control)

b) Aerobiotix (AEX) Mode A: Active test unit with UV plus adjuvant HEPA

c) Aerobiotix (AEX) Mode B: Active test unit with UV only

RESULTS

A. Aerodynamic Particle Sizer (APS) counts

GROUP	FUNCTION		APS COUNTS		PARTICLE MMAD (µm)	
	UVC	HEPA	US	DS	US	DS
CONTROL	-	-	17	45	1.17	0.88
AEX MODE A	+	-	66	84	1.02	1.16
AEX MODE B	+	+	66	4	1.08	0.8

Table 1: Particle Count Results. US = Upstream, DS = Downstream MMAD = mass median aerodynamic diameter, AEX = Aerobiotix. A (+) or (–) in the function column denotes presence or absence of the function, respectively. APS counts are a raw count with sampling time 10s at a rate of 0.25 L/min.

For particle counts, there was an increase from 17 to 45 in the control group, which confirms increased downstream aerosol load as nebulized particles are diffused through the system. This effect was also seen in AEX mode A, but to a lesser extent than control increasing from 66 to 84. For the AEX group, the presence of the mechanical filtration reduced the transmission of particles from upstream to downstream, 66 to 4. Median particle sizes of approximately 1 µm were consistent with small bioaerosols described for airborne SARS CoV-2.

B. Viral Culture

CULTURE	TEST RUN	FUNCTION		SARS-CoV-2 CPE
		UVC	HEPA	
1	CONTROL	-	-	+
2		-	-	+
3		-	-	0
4		-	-	0
5		-	-	0
6	AEX MODE A	+	-	0
7		+	-	0
8		+	-	0
9		+	-	0
10		+	-	0
11	AEX MODE B	+	+	0
12		+	+	0
13		+	+	0
14		+	+	0
15		+	+	0

Table 2: Viral culture results. A (+) culture denotes the presence of cytopathological effects manifested by the appearance of cell rounding, cell fusion (syncytia formation), or cell lysis. A (+) or (–) in the function column denotes presence or absence of the function, respectively. AEX = Aerobiotix. CPE= cytopathological effects (CPE).

For viral cultures, there were 0/10 positive cultures in the Aerobiotix groups, versus 2/5 positive cultures in the control group. Due to the nonparametric nature of the data, a one-tailed Fisher's exact test was utilized with a value of p=.0952. The presence of the HEPA filter under the test conditions did not result in additional elimination performance over UVC alone.

CONCLUSION

Within the limitations of this study, the Aerobiotix unit was successful in removing viable SARS-CoV-2 bioaerosols compared to controls.

DISCUSSION

The current emergence of SARS-CoV-2 has accelerated the interest and adoption of air disinfection technologies. To date, we are unaware of any air disinfection system being directly tested for reduction of airborne SARS-CoV-2 bioaerosols. This study holds several key advantages over prior investigations. Firstly, testing was analyzed via direct culturing, unlike genomic studies which depict the presence of RNA which may not be indicative of viable virus. Additionally, the closed single-pass design assured that bioaerosols were contained in the sealed test unit, negating any environmental effects arising from ambient measurements in open rooms.

This study has certain limitations. In this study, the SARS-CoV-2 prechamber air concentration was 2760 $TCID_{50}$ units/L, assuming full dissemination efficiency. However, aerosol systems may have significant inefficiencies due to decreased viability from to the nebulization process, adherence to chamber walls, and other factors. Even accounting for such inefficiencies, the prechamber air concentration compares favorably to published SARS-CoV-2 air concentrations samples in active patient rooms of 6-74 $TCID_{50}$ units/L.[10]

One of the goals of this study was to maximize air flow to provide the greatest challenge to the system possible. However, we noted that the aerosolized virus rapidly underwent dilution below quantifiable levels even at modest air volumes. Therefore, although the presence of viable virus through CPE could be detected, accurate quantification could not be performed. However, these findings are consistent with prior testing of the same device using MS2 virus. Furthermore, since this study represents a validation test of a specified environmental remediation device, the initial endpoint of elimination is arguably more important than quantification. Future studies using different flow rates or viral concentrations may yield additional data on performance against SARS-CoV-2 and other airborne pathogens.

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