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Bioaerosol Removal Efficiency of the Aerobiotix™ UV/Filtration Unit against SARS-CoV-2 Virus

Final Report

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SIGNATURES

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ABBREVIATIONS

APS	Aerodynamic Particle Sizer
ATU	Aerobiotix Test Unit
BBRC	Battelle Biomedical Research Center
CFM	Cubic Foot per Meter
GSD	Geometric Standard Deviation
HEPA	High Efficiency Particulate Air
L	Liter
LOQ	Limit of Quantitation
MMAD	Mass Median Aerodynamic Diameter
mL	Milliliter
min	Minute
MEM	Minimum Essential Medium
NA	Not Applicable
SOP	Standard Operating Procedures
µm	micrometer
UV	Ultraviolet

1 INTRODUCTION

Coronaviruses (CoVs) cause mild to severe upper-respiratory illness in humans. The common cold and Severe Acute Respiratory Syndrome (SARS) are both caused by coronaviruses. Coronaviruses are zoonotic, meaning they can spread from animals to humans. Severe coronavirus infections can cause pneumonia, kidney failure, and death (WHO.int, January 2020).

In 2019, an outbreak of the CoV disease (COVID-19) caused by a novel coronavirus (SARS CoV-2) was detected in Wuhan City, Hubei Province, China. The United States reported its first confirmed instance of the virus being spread person-to-person on January 30, 2020 (CDC.gov, February 2020). Based on the alarming levels of global spread and severity, the World Health Organization (WHO) declared COVID-19 a pandemic on March 11, 2020 (WHO.int, March 11, 2020). As of August 3, 2020, the Centers for Disease Control and Prevention (CDC) reported 4,601,526 confirmed and probable cases of COVID-19 in all 50 states and 56 jurisdictions, with 154,002 attributed and probable deaths (CDC.gov, July 9, 2020). The WHO have reported that the highest risk of disease is in older adults and populations with underlying health conditions, such as cardiovascular disease, chronic repository disease, and cancer (WHO int, March 11, 2020).

Recently it has been reported that, in addition to large respiratory droplets that can be a source of infection, small respiratory droplets or aerosols can also be a source of transmission for the SARS-CoV-2 virus including in health care settings (Morawska et al., 2020). This study examined the ability of a proprietary device provided by Aerobiotix (Aerobiotix Test Unit; ATU) to remove/inactivate SARS-CoV-2 virus from the atmosphere by using a combination of Ultraviolet (UV) light and High-Efficiency Particulate Absorbing (HEPA) filtration.

2 METHODS

Briefly, The Aerobiotix Test Unit (ATU) was tested for it's ability to remove virus from the atmosphere (Figure 1 and Figure 2). Air was supplied to the Collison nebulizer by an in-house air system filtered through a High Efficiency Particulate Air (HEPA) capsule filter and a carbon capsule filter (Pall Corp., Port Washington, NY). A six-jet Collison nebulizer with a precious fluid jar was used to generate a controlled delivery of SARS-CoV-2 from a liquid suspension. The virus laden aerosol entered the ATU upstream of the ATU ultraviolet photolytic unit (UV). 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 1.57×10^7 TCID50 of SARS-CoV-2 was nebulized into the prechamber at 0.4 mL/minute for 10 minutes and confirmed with prechamber and postchamber sampling. The virus laden test atmosphere was mixed by baffles in the prechamber prior to impinger sampling and entering the ATU. Total air flow was maintained and controlled by fans in the ATU and an external fan/HEPA unit to recirculate the air. The air flow was maintained between 20.8-22 CFM (588-623 L/min). Testing was performed under three test groups: 1) Inactive test unit (control;

UV off and HEPA filter removed); 2) Aerobiotix unit with UV on plus adjuvant HEPA; and 3) Aerobiotix test unit with UV only. The ATU was set up and tested within a Biological Safety Level 3 cabinet.

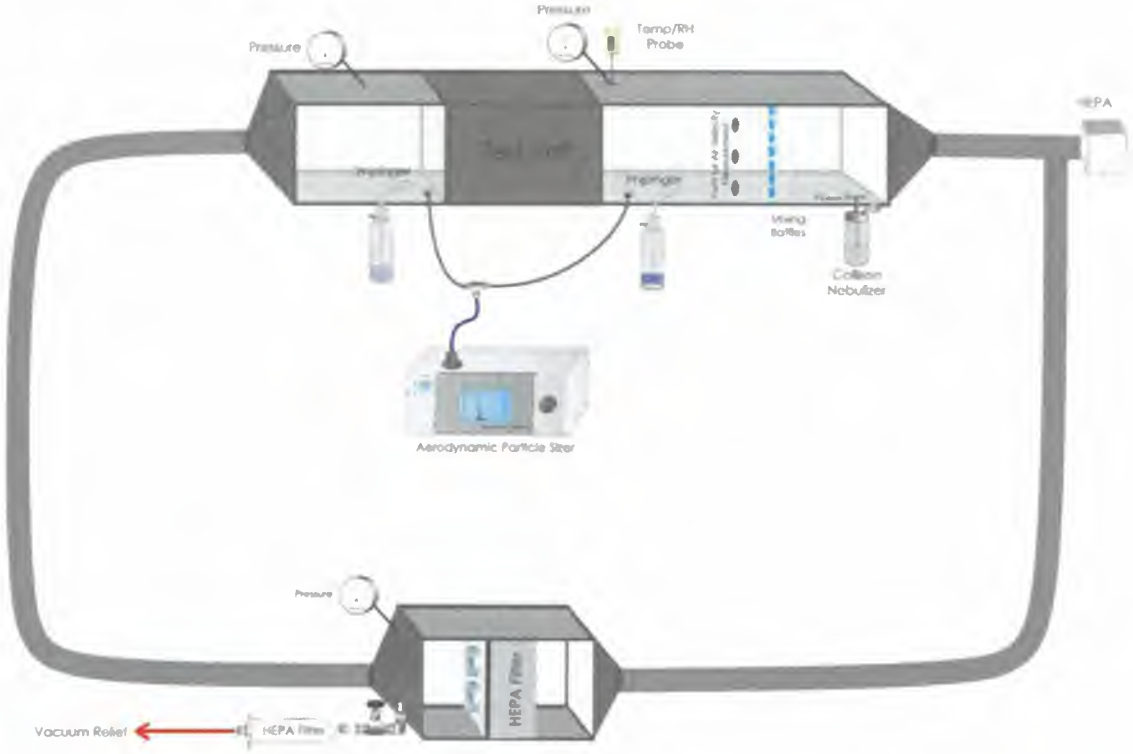
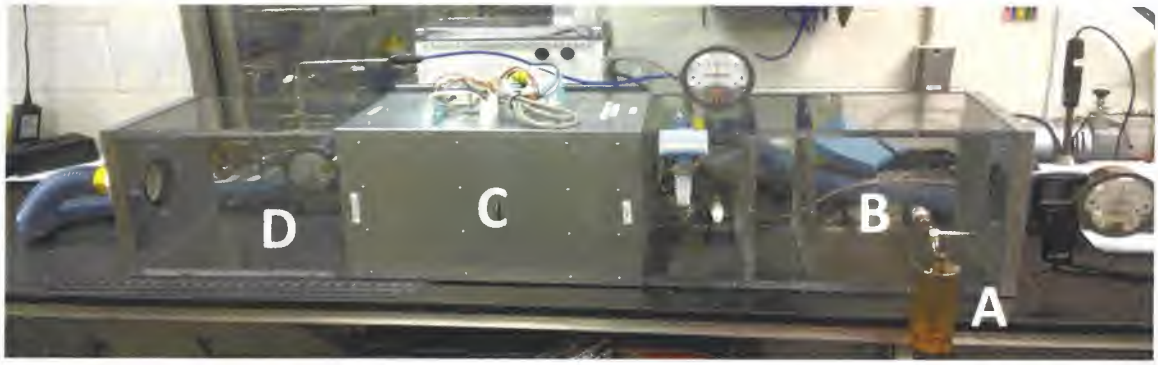


Figure 1. Aerobiotix Test Unit Diagram



Set up demonstrating (A) Collison nebulizer aerosol generator, (B) Prechamber, (C) Test Unit containing ultraviolet photolytic unit and HEPA filter, and (D), Postchamber.

Figure 2. Aerobiotix Test Unit

2.1 AEROSOL GENERATION PROCEDURES

The SARS-CoV-2 aerosol was produced by a modified Microbiological Research Establishment type six-jet Collison nebulizer (BGI, Waltham, MA) with a precious fluid jar (CH Technologies, Westwood, NJ). The Collison nebulizer is designed to generate aerosols that have an approximate mean diameter of 1-2 micrometers (μm). The nebulizer was characterized/qualified prior to each use to determine the head pressure (normally 18-24 pounds per square inch) that results in approximately 12 L/min of flow with an approximate use rate of 0.4 mL/minute.

2.2 SARS-COV-2 VIRUS AND TCID50 ASSAY

The SARS-CoV-2 virus was propagated by ATCC from BEI Resources NR-52281 lot 70034262. From ATTC we received at a titer of TCID50 ≥ 2 × 105 per mL and ≥ 99% sequence identity to SARS-CoV-2, isolate USA-WA1/2020 (GenBank: MN985325.1). The virus was concentrated to 1.24 x 10^7 $TCID_{50}$/mL for testing. The virus was prepared in media consisting of Minimum Essential Medium (MEM; 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 according to SOP BBRC.X-171. The SARS-CoV-2 TCID50 assay is designed to quantify virus 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. Cell monolayers were inoculated with serial dilutions of the virus of interest and observed for the presence of cytopathological effects (CPE) defined as the appearance of cell rounding, cell fusion (syncytia formation), or cell lysis.

2.3 AEROSOL SAMPLING PROCEDURES

The prechamber and postchamber atmosphere was sample for during selected tests and were collected into a glass impinger (Model 7541, Ace Glass Inc.) containing approximately 20 milliliters (mL) of MEM. A vacuum pump maintained a sampling rate of 6.0±0.5 L/min (≥18 inches of mercury) for each 10-minute test. After each test, the liquid was decanted and provided to the virology team for analysis by the $TCID_{50}$ assay.

2.4 AEROSOL SIZING PROCEDURES

Aerosol particle size was analyzed during each test from both the prechamber and postchamber using an Aerodynamic Particle Sizer (APS Model 3321, TSI Inc., Shoreview, MN) spectrometer with an aerosol diluter (Model 3302A, TSI Inc., Shoreview, MN). The aerosol diluter was used to reduce the incidence of particle coincidence and particle measurements above the accurate range of the instrument (20 μm). The APS dilution flow was adjusted to achieve APS sampling from the exposure chamber atmosphere at an approximate rate of 0.25 L/min (requiring a dilution flow of approximately 4.75 L/min for a total flow of

approximately 5.00 L/min). The APS sample was taken approximately five minutes into each test and sampled for 10 seconds.

The aerodynamic size of aerosol particles primarily dictates aerosol transport characteristics, and in the case of inhalation studies, the sites of lung deposition. The aerodynamic equivalent diameter is the diameter of a sphere with density of 1 gram per cubic centimeter, that has the same terminal settling velocity as the aerosol being evaluated. For inhalation exposures, the mass median aerodynamic diameter (MMAD) of the aerosol is typically reported along with the geometric standard deviation (GSD). Aerosol size distribution plays a critical role in inhalation studies. The biological effects of inhaled aerosols can be dependent upon the sites and degree of deposition within the respiratory tract. Further, the size and shape of inhaled aerosols is a critical factor in determining deposition mechanisms and the extent of penetration into the lung and alveolar regions. As a general rule, aerosols with aerodynamic particle sizes less than 5 μm are desired for inhalation studies requiring deposition in the alveoli (Schlesinger, 1985). Above this size, a larger portion of the aerosol is deposited in the upper respiratory tract (Hinds, 1999).

It is important to know the aerosol particle size since large particles containing viral organisms deposited in the upper respiratory tract may not cause disease, may require a higher quantity (dosage) to cause disease, or may cause only an upper respiratory disease. Therefore, if the objective is to maximize deep lung deposition, then an aerosol with a size on the order of 1 to 5 μm or lower, as opposed to larger aerosols, is desired. Figure 3 shows a representative APS particle size distribution graph for the virus aerosol.

3 RESULTS

The aerosol particle counts on the control group test increased from 17 in the prechamber (upstream) to 45 in the postchamber (downstream), which confirmed that the increase in the downstream aerosol load is likely do to the nebulized particles being diffused and more homogeneously dispersed through the system. This effect was also seen in the UV only mode, but to a lesser extent than control increasing from 66 to 84. For the UV and HEPA mode, the presence of the mechanical filtration reduced the transmission of particles from upstream to downstream from 66 to 4 which is expected from the mechanical capture of the aerosol onto the HEPA filter Table 1. The mass mean aerodynamic diameter of the aerosol particles ranges from 0.80 to 1.17 μm which is consistent with small bioaerosols described for airborne SARS-CoV-2 that would be deposited to the lung alveoli if inhaled. The $TCID_{50}$ quantitation for the Control was used as the representative value of what would be obtained in the prechamber (upstream) for the UV only and UV plus HEPA tests.

Table 1. $TCID_{50}$ and Aerosol Particle Size Summary Data

Sample ID	Counts (TCID50/mL) Location		APS Counts (particles) Location		Particles Size MMAD (um) Location	
	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream
HEPA and UV	NA	0	66	4	1.08	0.80
UV only*	NA	0	66	84	1.02	1.16
(Control) None**	1.31E+01	<LOQ[1]	17	45	1.17	0.88

Downstream denotes impinger sampled after the aerobiotix unit with the HEPA and UV.
Upstream denotes impinger sampled before the aerobiotix unit with the HEPA and UV
* HEPA was removed from the test
** HEPA removed and UV was off
<LOQ (=cytopathic effect observed in 2 of the 5 wells but below the LOQ)
Assigned Stock concentration nebulized = 1.24E+7 TCID50/mL, titer quantified on the test day 3.92E+6 TCID50/mL

As the $TCID_{50}$ results from tests with the Control were just above or below the LOQ the data can be evaluated in a different fashion. In the assay, 5 wells are inoculated with test sample to observe for cytopathic effect. In the Control tests, 3/5 wells from the prechamber (upstream) sample were observed with cytopathic effect and 2/5 in the postchamber (downstream) compared to no (0) cytopathic effected observed in the postchamber (downstream) sample for both the HEPA plus UV and UV alone (Table 2). These results suggest that the virus was either physically removed and/or inactivated. A representative graph of the particle size distribution is presented in Figure 3.

Table 2. SARS-CoV-2 Viral Culture Data

Culture Well	Test Run	Function		Cytopathic Effect (Downstream)
		UV	HEPA	
1	Control	(-)	(-)	(+)
2		(-)	(-)	(+)
3		(-)	(-)	0
4		(-)	(-)	0
5		(-)	(-)	0
6	UV only	(+)	(-)	0
7		(+)	(-)	0
8		(+)	(-)	0
9		(+)	(-)	0
10		(+)	(-)	0
11	UV plus HEPA	(+)	(+)	0
12		(+)	(+)	0
13		(+)	(+)	0
14		(+)	(+)	0
15		(+)	(+)	0

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.

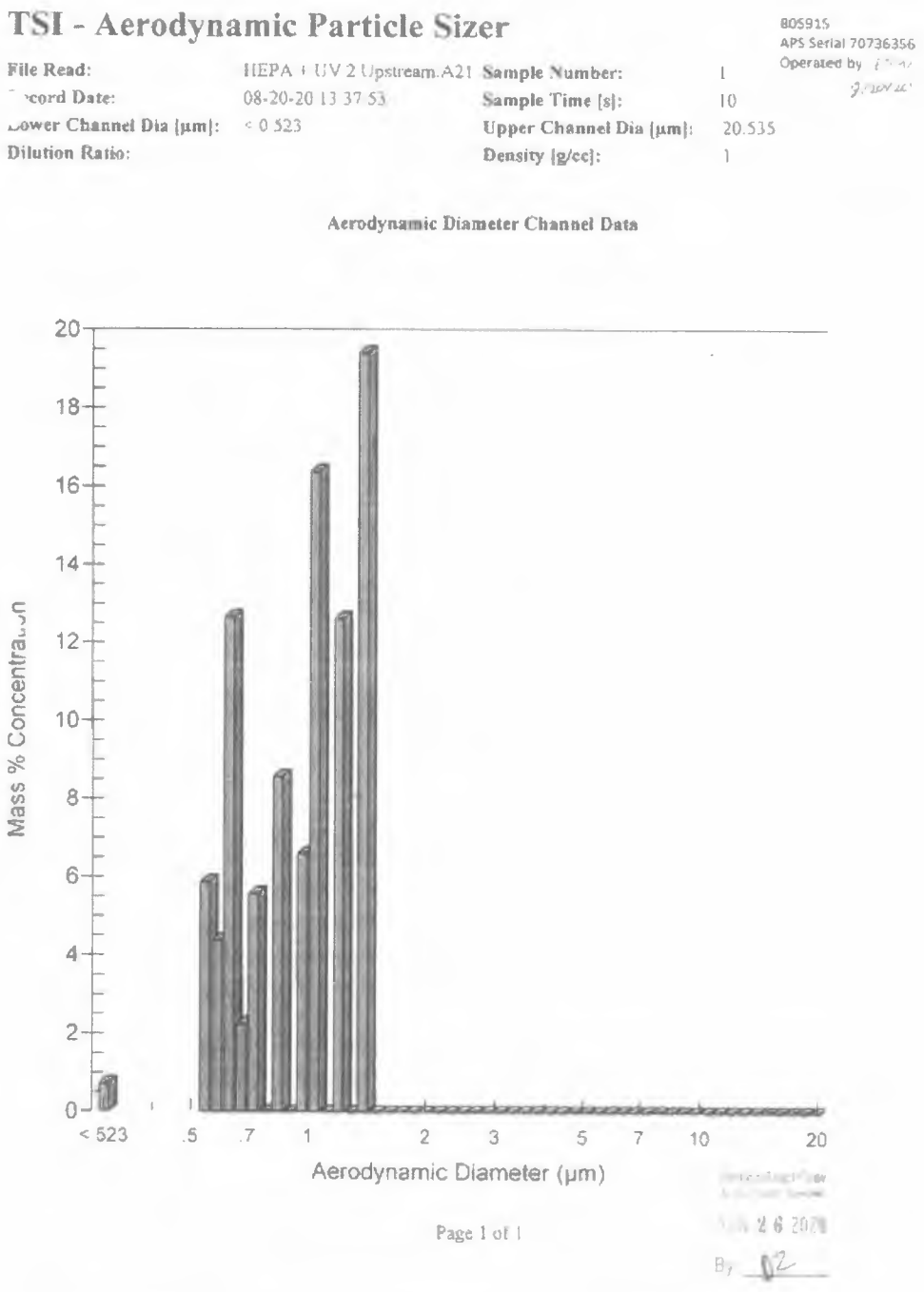


Figure 3. Representative Aerosol Mass Distribution Plot: Animal Exposures on 11/04/2019

4 CONCLUSION AND DISCUSSION

The Aerobiotix unit (ATU) was successful in removing viable SARS-CoV-2 bioaerosols when compared to controls.

This study had 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 TCID50 units/L.10

One of the goals of this study was to maximize recovery of viable virus aerosol to provide the greatest challenge to the system as possible. However, the results obtained of low viable virus recovery noted may have been caused by the aerosolized virus being rapidly diluted to near or below quantifiable levels in the $TCID_{50}$ assay even at modest air flow rates of 20.8-22 CFM (588-623 L/min). Therefore, although the presence of viable virus through CPE could be detected, accurate quantification $TCID_{50}$ assay could not be performed in some samples. This study represents a test of the ATU with the actual virus, the initial endpoint of elimination is equally or more important than the 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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