Airborne Inactivation of the Novel Coronavirus SARS-CoV-2 by Aerus Technology

Aerus Medical, LLC contract

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To

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Objective

SARS-CoV-2, the virus responsible for the COVID-19 pandemic, has had a profound, detrimental effect across the globe. The disease is associated with flu-like symptoms, persistent chest pain, respiratory complications/distress, confusion, and death. To date, there has been over 68 million cases of COVID-19 and over 1.5 million COVID19-related deaths worldwide, while in the United States alone, there has been approximately 15 million COVID19 cases and 284,000 COVID19-related deaths. In attempts to prevent the spread of the virus, several countries have implemented mandatory shutdowns of businesses and schools. While these measures have proven effective in controlling the spread of infection, they unfortunately have crippled several economies and are therefore unsustainable.

SARS-CoV-2 is believed to be transmitted by various means which includes direct, indirect, or close contact with infected people; aerosol transmission; and fomite transmission. In the case of aerosol transmission, SARS-CoV-2 virions become suspended in respiratory secretions or droplets expelled into the air by infected individuals. These droplets, due to their extremely small sizes, remain aerosolized for extended periods of time and are potentially inhaled by other individuals in the vicinity. Due to this route of aerosol transmission, the need for air purification devices capable of inactivating the virus within indoor environments is paramount.

Aerus Medical, LLC, has developed technology that can eliminate microbial contaminants by creating powerful oxidizers that can inactivate microbes. Aerus technology has demonstrated in lab tests the ability to eliminate surface SARS-CoV-2 (coated in a bio-film) by 99.98% in just 7 hours. In tests resulting in FDA Class II Medical Device Clearance, Aerus technology demonstrated 99.999% reductions in airborne MS2 Bacteriophage in just 30 minutes. These findings suggest Aerus technology would also be effective at eliminating airborne SARS-CoV-2.

The objective of this study was to test the ability of a device manufactured by Aerus Medical, LLC, to inactivate airborne SARS-CoV-2.

Experimental Procedure

Virus
SARS-CoV-2, strain USA_WA1/2020, was provided by the World Reference Center for Emerging Viruses and Arboviruses.

Plaque assay
Vero E6 cells were seeded in 6-well or 12-well plates to a confluency of 90-100%. Samples were serially 10-fold diluted in PBS. Diluted samples were plated onto the cells and left to incubate at 37ºC with 5% CO2 for one hour. After incubation, samples were overlaid with a mixture of 2X MEM (supplemented the 8% FBS and 2% pen/strep) and1.6% LE agarose. The plates were incubated overnight at 37ºC at 5% CO2. Plaques

were visualized with neutral red stain and a light box. The lower limit of detection (LLD) for the assay was 40 pfu/ml.

Aerosol generation and sampling

The aerosolization was performed using a Biaera aerosol control platform (Aero3G, Biaera Technologies, LLC) fitted with a custom-made 150-liter chamber manufactured by Biaera Technologies, LLC (**Figure 1**). The viral aerosol was generated using a 6-jet Collison nebulizer (flowrate set to 14.0 LPM), and it was combined with a standard volume of air to deliver a given concentration of virus to the chamber. For each nebulization, 10 ml of the viral inoculum at concentrations of 1-5 x 10^7 pfu/ml was used. The duration of aerosolization was 15 minutes. Aerosol samples were collected at 12.5 LPM for 5 minutes immediately following aerosolization and at various testing time-points using BioSamplers (SKC, Inc.) containing 20 ml of collection medium to determine the chamber viral concentrations. The total airflow to the aerosol setup was 50.0 LPM. Temperature and humidity were monitored during nebulization and sampling.

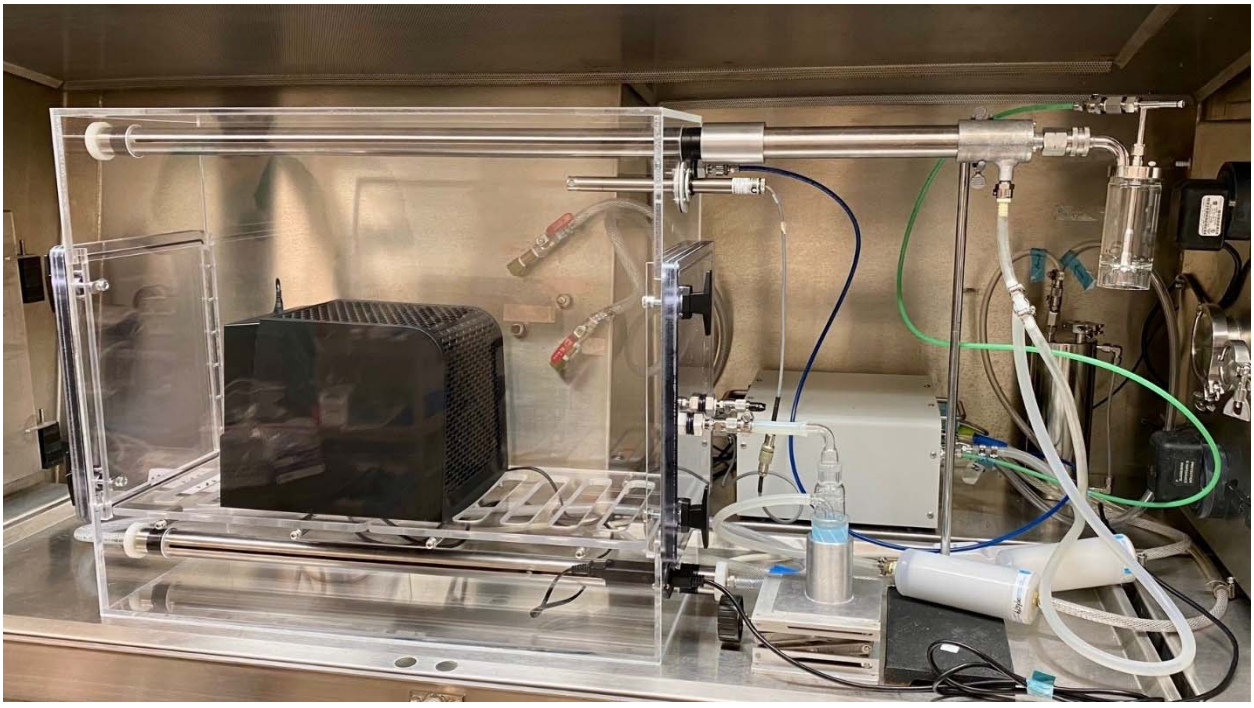

Figure 1. Aerosol testing setup

Aerus devices

Two devices manufactured by Aerus Medical were used in the study, an experimental device and a control device. These were the Aerus Pure & Clean and the Vollara Air & Surface Pro. The experimental device was equipped with the Aerus ActivePure technology capable of inactivating airborne viruses, as well as a fan used for air intake and output (all other technologies within the devices were either removed or deactivated). The control device was essentially identical to the experimental device, but the viral

inactivation ActivePure technology was removed from the control device. For operation, both devices were plugged into a 110V outlet within the chamber.

Aerosol testing timeline

The time and duration of aerosolization, device on-time, and sampling were performed according to **Figures 2A-C**. The device on-times tested were 3 and 10 minutes (**Figure 2A**), 15 and 30 minutes (**Figure 2B**), and 60 minutes (**Figure 2C**). These timelines were used for both the experimental (using the experimental device) and control (using the control device) runs. A test run with a timeline similar to **Figure 2A** was also performed wherein the control device was not turned on at t=0. This was done to ascertain the degree at which the aerosol droplets settle without air circulation being generated by the fan within the devices.

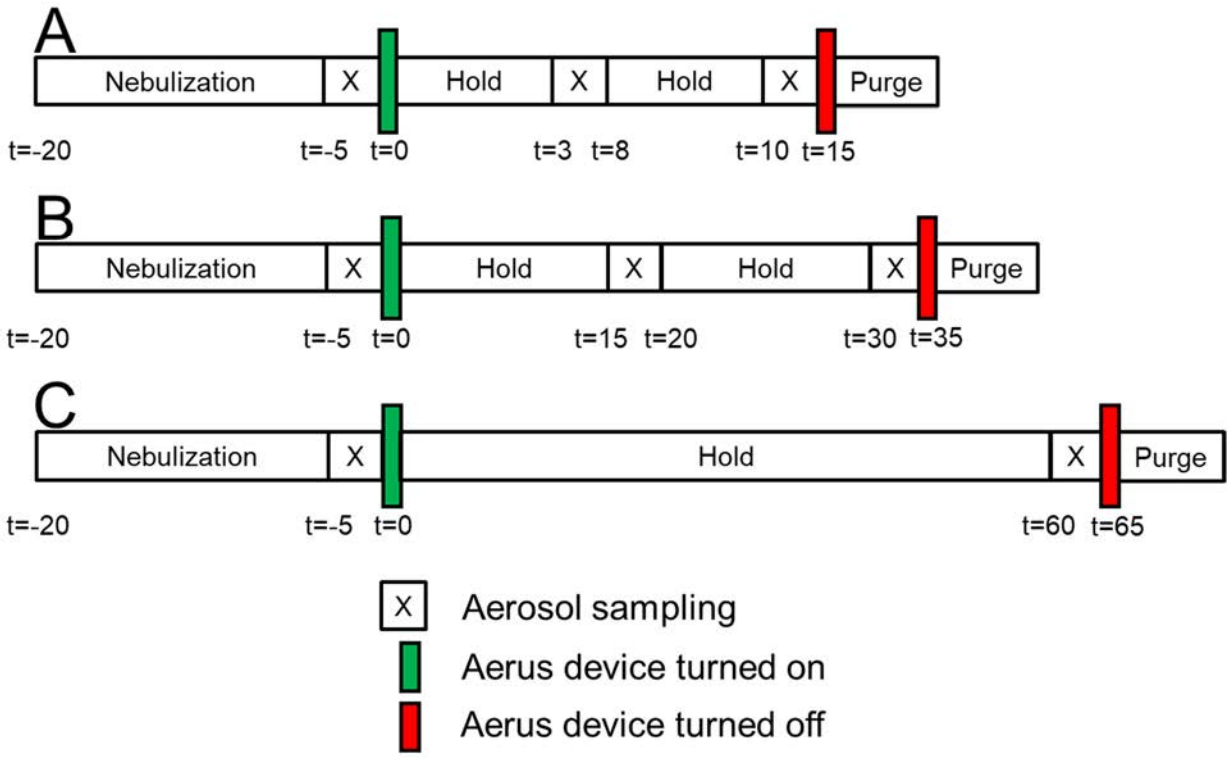

Figure 2. Testing timelines

Results

During the aerosol and sampling runs, the average humidity level was approximately 70%, and the temperature was approximately 22ºC. **Table 1** provides the log reductions (relative to the viral concentration at t=0) in viral concentration for the control and experimental devices with the various on-times. All device on-times were tested in triplicate except for the 3-minute (unit off) control, 10-minute (unit off) control, 30-minute control, and 60-minute control. In the case of the experimental device, the measured viral concentrations were below the plaque assay LLD (40 pfu/ml or 1.6 logs) for all but one

sample collection. Therefore, the range in log reduction was ≥2.87 to ≥3.38 which equates to ≥99.87 to ≥99.96%. However, since no plaque were detected in nearly every case, the percent inactivation could be ≥99.99% (≥4 logs). The log reductions in viral concentration for the control runs ranged from 0.61 to 2.91; however, the viral concentration of one sample collection (60-minute control) did fall below the assay LLD therein giving a log reduction of ≥3.18. The true net reduction in viral concentration could not be determined since the experimental device inactivated the virus to undetectable levels. Moreover, the true net log reduction for the runs over 3 minutes would be misleading since a greater portion of the virus settled in the test chamber as time passed. Because of this, it is likely that more of the virus was inactivated than could be measured. The study raw data is provided in the Appendix.

Table 1. Log reduction in viral concentration

Test unit	Device on-time	Log reduction from t=0	Test unit	Device on-time	Log reduction from t=0
Control device	3 minutes	0.84	Experimental device	3 minutes	3.38
		0.68			≥2.98
		0.61			≥2.87
	10 minutes	1.49		10 minutes	≥3.38
		1.27			≥2.98
		1.15			≥2.87
	15 minutes	1.49		15 minutes	≥3.38
		1.15			≥2.91
		1.18			≥2.92
	30 minutes	2.91		30 minutes	≥3.38
		1.91			≥2.91
		----			≥2.92
	60 minutes	2.42		60 minutes	≥3.22
		≥3.18			≥2.97
		----			≥2.87
	3 minutes (unit off)	0.61			
	10 minutes (unit off)	1.18			

Summary

The Aerus technology inactivated airborne SARS-CoV-2 to undetectable levels. The results show that, when accounting for the LLD, the percent reduction in virus was ≥99.87 to ≥99.96%; however, since no virus was detected after using the experimental device, the true percent reduction was likely greater than 99.99% in every case. The true net reduction could not be determined due to the LLD of the quantitation assay, but this too was likely greater than 99.99%.

Appendix

Raw data

Plate #	Sample	Sample Description	Run Date	Plaque Assay Date	Stain & Read Date	Dilution Read (10^{-x})	PFU Count	µl/Well	PFU/ml	Log_{10} PFU/ml
1	post-nebulization	Nebulizer #1	11/16/2020	11/16/2020	11/18/2020	4	442	250	1.77E+07	7.25
2	post-nebulization	Nebulizer #2	11/16/2020	11/16/2020	11/18/2020	4	389	250	1.56E+07	7.19
3	post-nebulization	Nebulizer #3	11/16/2020	11/16/2020	11/18/2020	4	402	250	1.61E+07	7.21
4	post-nebulization	Nebulizer #4	11/16/2020	11/16/2020	11/18/2020	4	370	250	1.48E+07	7.17
5	post-nebulization	Nebulizer #5	11/16/2020	11/16/2020	11/18/2020	4	270	250	1.08E+07	7.03
6	post-nebulization	Nebulizer #6	11/16/2020	11/16/2020	11/18/2020	4	297	250	1.19E+07	7.07
7	0-minute experimental	BioAnalyzer #1-1	11/16/2020	11/16/2020	11/18/2020	3	24	250	9.60E+04	4.98
8	3-minute experimental	BioAnalyzer #1-2	11/16/2020	11/16/2020	11/18/2020	1	1	250	4.00E+01	1.60
9	10-minute experimental	BioAnalyzer #1-3	11/16/2020	11/16/2020	11/18/2020	1	0	250	<4.00E+01	<1.60
10	0-minute experimental	BioAnalyzer #2-1	11/16/2020	11/16/2020	11/18/2020	3	24	250	9.60E+04	4.98
11	15-minute experimental	BioAnalyzer #2-2	11/16/2020	11/16/2020	11/18/2020	1	0	250	<4.00E+01	<1.60
12	30-minute experimental	BioAnalyzer #2-3	11/16/2020	11/16/2020	11/18/2020	1	0	250	<4.00E+01	<1.60
13	0-minute experimental	BioAnalyzer #3-1	11/16/2020	11/16/2020	11/18/2020	2	166	250	6.64E+04	4.82
14	60-minute experimental	BioAnalyzer #3-2	11/16/2020	11/16/2020	11/18/2020	1	0	250	<4.00E+01	<1.60
15	0-minute control	BioAnalyzer #4-1	11/16/2020	11/16/2020	11/18/2020	2	104	250	4.16E+04	4.62
16	3-minute control	BioAnalyzer #4-2	11/16/2020	11/16/2020	11/18/2020	1	151	250	6.04E+03	3.78
17	10-minute control	BioAnalyzer #4-3	11/16/2020	11/16/2020	11/18/2020	1	34	250	1.36E+03	3.13
18	0-minute control	BioAnalyzer #5-1	11/16/2020	11/16/2020	11/18/2020	2	80	250	3.20E+04	4.51
19	15-minute control	BioAnalyzer #5-2	11/16/2020	11/16/2020	11/18/2020	1	26	250	1.04E+03	3.02
20	30-minute control	BioAnalyzer #5-3	11/16/2020	11/16/2020	11/18/2020	1	1	250	4.00E+01	1.60
21	0-minute control	BioAnalyzer #6-1	11/16/2020	11/16/2020	11/18/2020	2	79	250	3.16E+04	4.50
22	60-minute control	BioAnalyzer #6-2	11/16/2020	11/16/2020	11/18/2020	1	3	250	1.20E+02	2.08
23	post-nebulization	Nebulizer #1	11/17/2020	11/17/2020	11/19/2020	5	70	250	2.80E+07	7.45
24	post-nebulization	Nebulizer #2	11/17/2020	11/17/2020	11/19/2020	5	62	250	2.48E+07	7.39
25	post-nebulization	Nebulizer #3	11/17/2020	11/17/2020	11/19/2020	5	68	250	2.72E+07	7.43
26	post-nebulization	Nebulizer #4	11/17/2020	11/17/2020	11/19/2020	5	37	250	1.48E+07	7.17

27	post-nebulization	Nebulizer #5	11/17/2020	11/17/2020	11/19/2020	5	91	250	3.64E+07	7.56
28	post-nebulization	Nebulizer #6	11/17/2020	11/17/2020	11/19/2020	5	79	250	3.16E+07	7.50
29	0-minute experimental	BioAnalyzer #1-1	11/17/2020	11/17/2020	11/19/2020	2	96	250	3.84E+04	4.58
30	3-minute experimental	BioAnalyzer #1-2	11/17/2020	11/17/2020	11/19/2020	1	0	250	<4.00E+01	<1.60
31	10-minute experimental	BioAnalyzer #1-3	11/17/2020	11/17/2020	11/19/2020	1	0	250	<4.00E+01	<1.60
32	0-minute experimental	BioAnalyzer #2-1	11/17/2020	11/17/2020	11/19/2020	2	81	250	3.24E+04	4.51
33	15-minute experimental	BioAnalyzer #2-2	11/17/2020	11/17/2020	11/19/2020	1	0	250	<4.00E+01	<1.60
34	30-minute experimental	BioAnalyzer #2-3	11/17/2020	11/17/2020	11/19/2020	1	0	250	<4.00E+01	<1.60
35	0-minute experimental	BioAnalyzer #3-1	11/17/2020	11/17/2020	11/19/2020	2	93	250	3.72E+04	4.57
36	60-minute experimental	BioAnalyzer #3-2	11/17/2020	11/17/2020	11/19/2020	1	0	250	<4.00E+01	<1.60
37	0-minute control	BioAnalyzer #4-1	11/17/2020	11/17/2020	11/19/2020	2	115	250	4.60E+04	4.66
38	3-minute control unit off	BioAnalyzer #4-2	11/17/2020	11/17/2020	11/19/2020	2	28	250	1.12E+04	4.05
39	10-minute control unit off	BioAnalyzer #4-3	11/17/2020	11/17/2020	11/19/2020	1	75	250	3.00E+03	3.48
40	0-minute control	BioAnalyzer #5-1	11/17/2020	11/17/2020	11/19/2020	2	155	250	6.20E+04	4.79
41	3-minute control	BioAnalyzer #5-2	11/17/2020	11/17/2020	11/19/2020	2	32	250	1.28E+04	4.11
42	10-minute control	BioAnalyzer #5-3	11/17/2020	11/17/2020	11/19/2020	1	83	250	3.32E+03	3.52
43	0-minute control	BioAnalyzer #6-1	11/17/2020	11/17/2020	11/19/2020	2	152	250	6.08E+04	4.78
44	60-minute control	BioAnalyzer #6-2	11/17/2020	11/17/2020	11/19/2020	1	0	250	<4.00E+01	<1.60
45	post-nebulization	Nebulizer #1	11/19/2020	11/19/2020	11/21/2020	4	232	250	9.28E+06	6.97
46	post-nebulization	Nebulizer #2	11/19/2020	11/19/2020	11/21/2020	4	192	250	7.68E+06	6.89
47	post-nebulization	Nebulizer #3	11/19/2020	11/19/2020	11/21/2020	4	202	250	8.08E+06	6.91
48	post-nebulization	Nebulizer #4	11/19/2020	11/19/2020	11/21/2020	4	236	250	9.44E+06	6.97
49	post-nebulization	Nebulizer #5	11/19/2020	11/19/2020	11/21/2020	5	18	250	7.20E+06	6.86
50	post-nebulization	Nebulizer #6	11/19/2020	11/19/2020	11/21/2020	4	141	250	5.64E+06	6.75
51	0-minute experimental	BioAnalyzer #1-1	11/19/2020	11/19/2020	11/21/2020	2	74	250	2.96E+04	4.47
52	3-minute experimental	BioAnalyzer #1-2	11/19/2020	11/19/2020	11/21/2020	0	0	250	<4.00E+01	<1.60
53	10-minute experimental	BioAnalyzer #1-3	11/19/2020	11/19/2020	11/21/2020	0	0	250	<4.00E+01	<1.60
54	0-minute experimental	BioAnalyzer #2-1	11/19/2020	11/19/2020	11/21/2020	2	82	250	3.28E+04	4.52
55	15-minute experimental	BioAnalyzer #2-2	11/19/2020	11/19/2020	11/21/2020	0	0	250	<4.00E+01	<1.60
56	30-minute experimental	BioAnalyzer #2-3	11/19/2020	11/19/2020	11/21/2020	0	0	250	<4.00E+01	<1.60
57	0-minute experimental	BioAnalyzer #3-1	11/19/2020	11/19/2020	11/21/2020	2	74	250	2.96E+04	4.47

58	60-minute experimental	BioAnalyzer #3-2	11/19/2020	11/19/2020	11/21/2020	0	0	250	<4.00E+01	<1.60
59	0-minute control	BioAnalyzer #4-1	11/19/2020	11/19/2020	11/21/2020	2	52	250	2.08E+04	4.32
60	3-minute control	BioAnalyzer #4-2	11/19/2020	11/19/2020	11/21/2020	1	128	250	5.12E+03	3.71
61	10-minute control	BioAnalyzer #4-3	11/19/2020	11/19/2020	11/21/2020	1	37	250	1.48E+03	3.17
62	0-minute control	BioAnalyzer #5-1	11/19/2020	11/19/2020	11/21/2020	2	71	250	2.84E+04	4.45
63	15-minute control	BioAnalyzer #5-2	11/19/2020	11/19/2020	11/21/2020	1	22	250	8.80E+02	2.94
64	0-minute control	BioAnalyzer #6-1	11/19/2020	11/19/2020	11/21/2020	2	49	250	1.96E+04	4.29
65	15-minute control	BioAnalyzer #6-2	11/19/2020	11/19/2020	11/21/2020	1	32	250	1.28E+03	3.11
66	30-minute control	BioAnalyzer #6-3	11/19/2020	11/19/2020	11/21/2020	1	6	250	2.40E+02	2.38