Evaluation of a Bio-Aerosol Sampler in Collection Efficiency of Aerosolized SARS-CoV-2

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Introduction

The threat of COVID-19 infection and high rate of transmission, associated with severe illness, and fatalities, has created a severe threat to health care personnel, first responders, and general populations worldwide. This pandemic has brought about a need for rapid development of technology to detect and quantify airborne microbes. Here we describe the characterization of a bio-aerosol sampler and its efficiency in collecting aerosolized SARS-CoV-2. The device uses electro-kinetic flow to sample air at high rates and capture bio-aerosols on the grounded electrodes. For testing, three devices were operated in a chamber filled with SARS-CoV-2 bio-aerosols. The bio-aerosols were recovered from the electrodes and assayed via RT-qPCR and $TCID_{50}$ to quantify the viral collection efficiency.

Aerosol Testing Methods and Materials

Aerosol testing was performed using an aerosol test system fabricated out of Plexiglas. The test system was housed in the Class III Biosafety Cabinet for all conducted tests. The bio-aerosol test system is fabricated for nebulizer adaptation, aerosol and sample dilution air displacement filtration, air supply regulation and control, exhaust flow regulation, aerosol sampling, particle size measurement, and temperature and humidity monitoring. SARS-CoV-2 aerosol nebulizer generation was provided with flow and pressure regulated tank supplied breathing grade air.

The three bio-samplers were placed in the center of the cabinet floor and at equal distances from the cabinet walls. A Collison 6 jet nebulizer was used for aerosol generation and was filled with 10 ml of viral stock for each test. The nebulizer was supplied with tanked breathing grade air at a pressure of 26 psi at a flow rate of approximately 15 L/min. Following a ten minute aerosol generation period with only an air recirculation fan operational, the nebulizer was turned off, and aerosol viral reference sampling from the chamber was initiated along with simultaneous operation of the three bio-samplers for 30 minutes. Aerosol collection tests were conducted in triplicate.

An aerosol reference sample was collected from the chamber using low flow midget impinger (Ace Glass, Inc.) filled with 10 ml of sterile DMEM/F12 (Gibco). The single impinger sample was taken over the entirety of each 30 minute test period following termination of bio-aerosol generation for post-test analysis of the viable and non-viable virus.

Additionally, an APS 3321 (Aerosol Particle Sizer) was used to pull a 30 second aerosol sample from the system at t = 0, t = 10, and t = 20 minutes post aerosol generation. The APS is an aerodynamic time of flight particle measurement instrument that provides accurate particle size analysis, and has a dynamic particle size measurement range of 0.3 to 20 µm. The APS provides mass median aerodynamic diameter (“MMAD”), Geometric Standard Deviation (“GSD”), total sample aerosol mass (mg/cc), and aerosol particle counts (#/cc) in real time.

Each of the bio-samplers had two aerosol collection plates. For each test, a collection plate cassette containing two test strips was loaded into each device. Following each test, recovered impinger and plate strip samples were collected and divided for PCR and $TCID_{50}$ analysis.

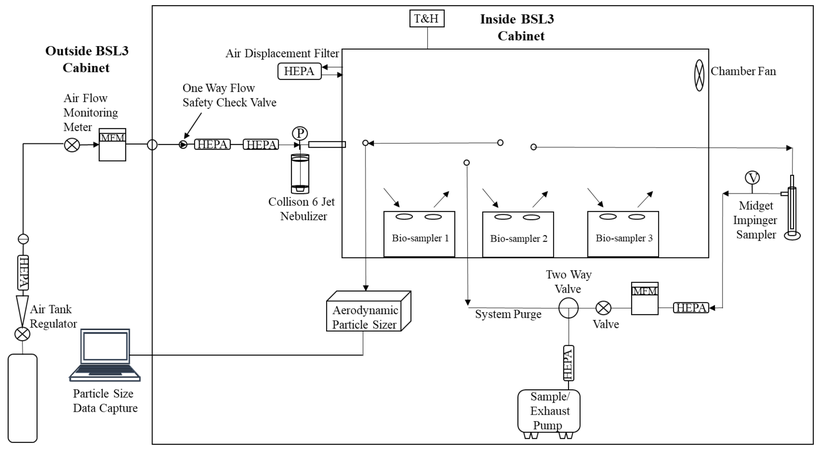
PCR Methods and Materials

Verification of viral aerosol collection was performed via RT-qPCR on extracts prepared from test samples using a Qiagen QIAamp Viral RNA Mini kit (Cat # 52904), with a 140 µL of DMEM wash as input and 60 µL elution from the silica-based spin column. Dilutions of the SARS-CoV-2 virus stock used for aerosol generation challenge, neat Test Device sample extracts, and impinger reference samples were subsequently analyzed by RT-qPCR utilizing an RUO version of the N1 assay from the CDC’s 2019-nCoV Real-Time PCR Diagnostic Panel. The RT-qPCR reaction was performed using SuperScript III One-Step RT-qPCR master mix (Life Technologies, Cat # 11732020) and the N1 assay primers and probes, resulting in a 20 µL total reaction volume (15 µL of master mix and a 5 µL sample add) and run on a Bio-Rad CFX96 system.

$TCID_{50}$ Methods and Materials

Test strips were placed in conical tubes and vortex extracted in 10 ml of DMEM/F12 for analysis. Extracts and impinger reference samples were diluted 1:10 down a 24-well plate in DMEM/F12 to assess the $TCID_{50}$ of the samples. These dilutions were incubated approximately 45 minutes, after which DMEM/F12 supplemented with 5% FBS was added to cells to feed them for the next four to five days. This incubation period allowed the virus to adsorb to cells without interference from FBS.

Aerosol Test System



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Results

	PCR					$TCID_{50}$					
Sample ID	Triplicate Sample Std. Dev.	Quant (copies/mL)	Log_{10} (copies/mL)	Average Quant (copies/mL)	Average Log_{10} Copies/mL	$TCID_{50}$/ml	Log_{10} $TCID_{50}$/mL	Average $TCID_{50}$/mL	Average Log_{10} $TCID_{50}$/mL	%Viable vs. Non-viable	Normalized Collection Efficiency*
1-1	0.03	4.4E+06	6.64			3.51E+01	1.55				
1-2	0.04	5.4E+06	6.73	4.3E+06	6.62	4.14E+00	0.62	2.03E+01	1.17	0.000476	16.2%
1-3	0.04	3.0E+06	6.48			2.16E+01	1.33				
2-1	0.09	4.5E+06	6.65			4.32E+01	1.64				
2-2	0.15	2.6E+06	6.41	4.3E+06	6.61	3.51E+01	1.55	3.99E+01	1.60	0.000929	16.3%
2-3	0.07	5.8E+06	6.76			4.14E+01	1.62				
3-1	0.08	2.0E+06	6.30			4.14E+00	0.62				
3-2	0.05	3.1E+06	6.49	2.5E+06	6.40	2.98E+00	0.47	4.00E+00	0.59	0.000158	9.60%
3-3	0.13	2.5E+06	6.40			4.88E+00	0.69				
Impinger 1	0.08	8.4E+05	5.92			≤3.51E-01	≤-0.45	Below Detection Limit			
Impinger 2	0.08	4.9E+05	5.69	6.8E+05	5.82	≤3.51E-01	≤-0.45				
Impinger 3	0.04	7.0E+05	5.85			≤5.16E-01	≤-0.29				
Viral Stock	N/A	7.8E+08	8.89	7.8E+08	N/A	3.16E+06	6.50	N/A	N/A	0.405128	N/A

*Normalized % PCR collection efficiency of device sampling (~109 L/min) vs. reference impinger sample (1.4 L/min)(copies/mL x 2 test strips per bio-sampler)

APS Sample	Test 1			Test 2			Test 3		
Time (min)	Particle Counts	Mass (mg/m^3)	Median Diameter (µm)	Particle Counts	Mass (mg/m^3)	Median Diameter (µm)	Particle Counts	Mass (mg/m^3)	Median Diameter (µm)
0	1708970	9.20	3.23	1754445	9.38	3.16	1786277	9.53	3.18
10	3810	5.71E-03	1.46	10355	1.53E-02	1.45	2541	3.39E-03	1.39
20	35	6.43E-05	1.52	44	4.28E-05	1.17	4	1.46E-06	0.647

Conclusions

- The bio-samplers were fairly consistent and reproducible in aerosol collection.
- A high rate of aerosol particle collection was observed with a minimum reduction in particle counts of 99.41% within 10 minutes of operation, and to near non - detectible background levels within 20 minutes of operation.
- No viable virus was detected in the impinger samples. This is likely due to the low flow rate of the impingers when compared to the devices.
- The average viable vs. non-viable virus from the collection plates was 0.000521%, compared to 0.405% from the viral stock. This indicates a loss of viability of approximately 3 logs as a result of the nebulization and collection processes.
- A collection efficiency of approximately 16% was determined for the first two devices, and approximately 10% for the third.
- The efficiency and consistency observed from the bio-samplers indicate that they could be a powerful tool for monitoring indoor air for the presence of harmful microbes such as SARS-CoV-2.