Collection of SARS-CoV-2 Virus from the Air of a Clinic Within a University Student Health Care Center and Analyses of the Viral Genomic Sequence

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Revision

Submitted to

Aerosol & Air Quality Research – Technical Note

May 21, 2020

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SUPPLEMENTARY MATERIAL

Sampling Site - The site chosen for the pilot study, was the respiratory infection evaluation area of the University of Florida's Student Health Care Center (SHCC). This particular area of the SHCC was chosen at the onset of the COVID-19 outbreak in Alachua County, Florida to evaluate all potential COVID-19 patients of the SHCC as 1) it could be accessed without entering other parts of the SHCC building, 2) had a separate air handling system, 3) had an adequate number of patient rooms (five) to perform evaluations located away from staff office space, and 4) had a covered outside area which could be used as a waiting room with patient chairs spaced 6 feet apart. Only patients suspicious for COVID-19 with symptoms, travel history or known exposure to COVID-19 positive patients were allowed in the area for evaluation. When a patient came to the evaluation area, they were required to wear a surgical mask and asked to wait in one of the outside waiting room chairs. Once an inside evaluation room became available, the masked patient was allowed to enter through the main entrance, walked down a hallway and placed in an available room and the door was closed behind them. The air sampling device was placed near this hallway, approximately 3 meters from nearest patient traffic (see Figure S1). Patients were evaluated in the rooms with doors closed. Providers wore goggles or face shields, N-95 masks, gowns and gloves. Patients remained in their evaluation room for SARS-CoV-2 testing when it was deemed clinically necessary and RNA nasopharyngeal, and occasionally oral pharyngeal, swabs depending on availability were used to collect their sample. After evaluation, patients exited while wearing their surgical mask, by walking down the same hallway (again 3 meters from the sampling unit) to the same main entrance door before leaving the area.

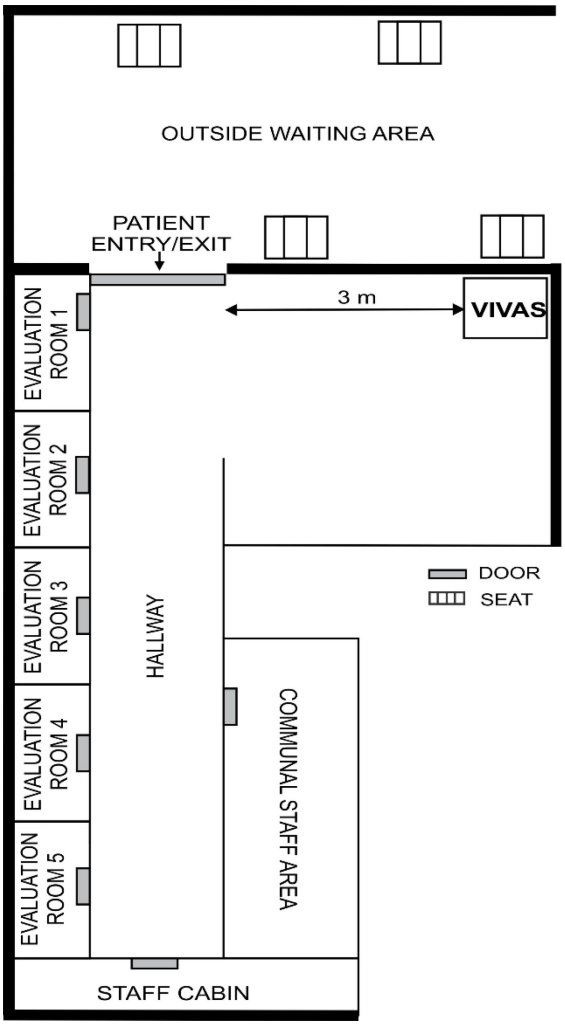

Figure S1 Schematic layout of the sampling site

Air Sampling - The VIVAS, which collects airborne particles using a laminar-flow water vapor condensation method (Pan et al., 2016), was used. Details of its design and performance evaluation were reported elsewhere (Lednicky et al., 2016b; Pan et al., 2016). In brief, the process encapsulates small particles into larger droplets, thus enabling efficient collection of these enlarged particles through gentle impaction (Hering et al., 2005). It contains 8 parallel growth tubes and has four main sections - the conditioner, initiator, moderator and collector. Aerosol particles enter each tube are first cooled to 4 °C, and then subsequently enter the initiator maintained at 40 °C. Under those operating temperatures, particles as small as 5 nm form

droplets greater than 6.6 µm in diameter (Tilly et al., 2019). The moderator, held at 32 ºC, reduces excess water vapor entering the collector. These particles encapsulated in water then travel through 24 nozzles (3 per growth tube) held at 37 ºC to impinge onto 1.5 mL of collection fluid maintained at 25 ºC.

The aerosol inlet was at ~ 1.5 m above ground, and the sampler was operated at 6.5 L min^{-1} for 1 hr, resulting in a sampling volume of 390 L. Between two air samplings, a negative control run was performed by collecting air through a HEPA-filtered airflow (for a total of four air-samplings, two without, and two with HEPA-filters at the intake tube). A 35 mm Petri dish was used as the collection vessel. The liquid collection media consisted of 1.5 mL of 1× phosphate buffered saline (PBS) with 0.5% (w/v) bovine albumin fraction V and a final concentration of 0.2 M sucrose. After the 1-hr collection period, the Petri dishes were sealed with parafilm, placed in a Styrofoam cooler with an ice-pack, then transported to a BSL2-enhanced laboratory at the University of Florida Emerging Pathogens Institute (EPI) where they were aseptically transferred to sterile plastic cryotubes with O-ring seals, and the tubes thereafter stored in a locked -80 ºC freezer for analyses at a later time.

Detection of SARS-CoV-2 genomic RNA in collection media – After the samples were thawed on ice, virus genomic RNA (vRNA) was extracted from virions in collection media in a Class II biosafety cabinet in a BSL2-plus room at the EPI by analysts wearing appropriate personal protective equipment (chemically impervious Tyvek lab coats and gloves) and using powered-air purifying respirators. The purification of vRNA from 140 µL aliquots of the collection media was accomplished using a QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA, USA), with purified RNA eluted from the RNA-binding silicone column in a volume of 80 µL. Twenty-five µL (final volume) real-time reverse-transcription polymerase chain reaction (rtRT-PCR) tests

were performed in a BioRad CFX96 Touch Real-Time PCR Detection System using 5 µL of purified vRNA and rtRT-PCR primers and the probe listed in Table S1. The assay that was used was designed by J. Lednicky for a previous study aimed at the discovery of coronaviruses in bats and does not detect common human alpha- or betacoronaviruses. Using this rtRT-PCR detection system, the limit of detection is about 1.5 SARS CoV-2 genome equivalent per 25 µL rtRT-PCR assay. The rtRT-PCR tests were performed using the following parameters: 400 nM final concentration of forward and reverse primers and 100 nM final concentration of probe using SuperScriptTM III One-Step RT-PCR system with PlatinumTM Taq DNA Polymerase (Thermo Fisher Scientific). Cycling conditions were 20 minute at 50 °C for reverse transcription step, followed by 2 minutes at 95 °C for Taq polymerase activation step, then 44 cycles of 15 seconds at 95 °C of denaturing, 30 seconds at 57 °C for annealing, and 20 seconds at 68 °C for extension.

Table S1. SARS-CoV-2 N-gene rtRT-PCR detection system.

Primer/probe name	Description	Oligonucleotide sequence (5' to 3')	Label
Led-N-F	SARS CoV-2 N Forward Primer	5' – GGGAGCAGAGGCGGCAGTCAAG - 3'	None
Led-N-R	SARS CoV-2 N Reverse Primer	5' – CATCACCGCCATTGCCAGCCATTC – 3'	None
Led-N-Probe	SARS CoV-2 N Probe	5' FAM - CCTCATCACGTAGTCGCAACAGTTC-BHQ1-3'	FAM, BHQ1

The TaqMan® probe is 5'-end labeled with the reporter molecule 6-carboxyfluorescein (FAM) and with quencher Black Hole Quencher 1 (BHQ-1) at the 3'- end.

Cell line for virus isolation - Vero E6 cells, obtained from the American Type Culture Collection (catalog no. ATCC CRL-1586), was used for virus isolation. They were maintained in cell culture medium comprised of aDMEM (advanced Dulbecco's modified essential medium, Invitrogen, Carlsbad, CA) supplemented with 10% low antibody, heat-inactivated, gamma-irradiated fetal bovine serum (FBS, Hyclone, GE Healthcare Life Sciences, Pittsburgh, PA), L-

alanine, L-glutamine dipeptide supplement (GlutaMAX,), and 50 μg mL^{-1} penicillin, 50 μg mL^{-1} streptomycin, 100 μg mL^{-1} neomycin (PSN antibiotics, Invitrogen) with incubation at 37 ˚C in 5% CO_2.

Quantification of SARS CoV-2 genomes in sampled air – To estimate the quantity of virus genome equivalents collected from the air, a 6-log standard curve was run using 10-fold dilutions of a calibrated single-stranded oligonucleotide DNA template of the SARS-CoV-2 N-gene that had been obtained from IDT Technologies, Inc. The rtRT-PCR Ct value for the aliquot of purified RNA collected from virus in the air was 39.15 which corresponds to about 2 genome equivalents in a 25 μL rtRT-PCR assy. To get the no. of SARS-CoV-2 genome equivalents in 1,489 μL (the final volume of the collection media), the ff. calculation was made:

(a) The number of virus genome equivalents in 140 μL (the volume of collection media from which RNA was purified):

No. of virus genome equivalents (based on Ct for a 5 μL reaction) x 16 = total no. of virus genomes in 140 μL. The factor 16 arises from 80 μL (volume of total RNA purified from 140 μL of collection medium)/5 μL (volume tested) = 16. Thus, 2 x 16 = 32 viral genome equivalents/140 μL of purified RNA.

(b) The total amount of virus genome equivalents in the collection media:

% of total volume tested = 1,489/140 = 9.4%

Therefore, the number of viral genome equivalents detected was (32 x 100)/9.4 = 340 genomes

(c) Number of genomes per liter of air = 340 genome equivalents/390 L of sampled air = 0.87 virus genome equivalents L^{-1} of air.

Virus culture – Attempts to isolate SARS-C0V-2 were performed in a BSL3 laboratory. Analysts wore powered air-purifying respirators and used BSL3 work practices for virus isolation. Prior to inoculation, the Vero E6 cell culture medium of cells at 80% confluency in a

T-25 flask (growing surface 25 cm^2) was removed and inoculum containing 100 µL of VIVAS collection medium that had been filtered through a sterile 0.45 µm filter and mixed with 400 µL aDMEM with 3% FBS, GlutaMAX, and PSN antibiotics was added to the monolayer. The inoculated cell cultures were incubated at 37 ˚C in 5% CO_2, and rocked every 15 minutes for 1 hour, after which 5 mL of complete cell growth medium with 3% FBS was added. A mock-infected cell culture was maintained in parallel with the other cultures. The cell cultures were refed every three days by the replacement of 2 mL of spent media with aDMEM with 3% FBS and observed daily for an observation period of one month before being judged negative for virus isolation. When virus-induced cytopathic effects (CPE) were evident in 50% of the cells, the remaining cells were scraped, and both scraped cells and spent media collected and stored at -80 °C for further analyses by rtRT-PCR.

Sanger sequencing – The complete genome sequence of SARS-CoV-2 in the environmental sample was determined using a genome walking strategy similar to the one described previously (Lednicky et al., 2016a). Briefly, cDNA was produced using AccuScript high-fidelity reverse transcriptase (Agilent Technologies, Santa Clara, CA) and sequence-specific primers based on SARS-CoV-2 that had been posted in GISAID (https://www.gisaid.org/) early during the outbreak. The resulting cDNA was amplified by PCR with Q5 polymerase (New England BioLabs) and gene-specific primers. The 5′ and 3′ ends of the SARS-CoV-2 genome were determined using a Rapid Amplification of cDNA Ends (RACE) kit (Life Technologies, Inc., Carlsbad, CA, USA). The resulting sequences were assembled with Sequencher DNA sequence analysis software version 2.1 (Gene Codes, Ann Arbor, MI, USA).

Identification of virus isolated in Vero E6 cells – Nucleic acids were isolated from spent cell-culture medium and from scraped cells using the QIAamp viral RNA purification kit and tested

for SARS CoV-2 vRNA by rtRT-PCR. Because the samples tested negative for SARS CoV-2 vRNA, it was assumed that other respiratory viruses may have been isolated since air-sampling was performed at the respiratory ward of a clinic. Therefore, an attempt to identify viruses was undertaken using a BioFire FilmArray Respiratory 2 Panel (BioMérieux Inc., Durham, North Carolina), following the manufacturer's instructions.

Phylogenetic inference and compartmentalization test – The GISAID database (https://www.gisaid.org/) was accessed 11 April 2020 and 6203 full genome sequences of approx. 30,000 bp in size were downloaded. Genomes were aligned with Mafft (Katoh and Standley, 2016), and to be able to handle such information, we extracted variable sites using an in-house script developed in R.

Evaluation of the presence of phylogenetic signal satisfying resolved phylogenetic relationships among sequences was carried out with IQ-TREE, allowing the software to search for all possible quartets using the best-fitting nucleotide substitution model (Schmidt et al., 2002). ML tree reconstruction was performed in IQ-TREE based on the best-fit model chosen according to Bayesian Information Criterion (BIC) (Nguyen et al., 2014; Trifinopoulos et al., 2016).

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