Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
SARS-CoV-2 in residential rooms of two self-isolating persons with COVID-19

Sripriya Nannu Shankar\textsuperscript{a}, Chiran T. Witanachchi\textsuperscript{a}, Alyssa F. Morea\textsuperscript{a}, John A. Lednicky\textsuperscript{b,c}, Julia C. Loeb\textsuperscript{b,c}, Md. Mahbubul Alam\textsuperscript{b,c}, Z. Hugh Fan\textsuperscript{d,e}, Arantzazu Eiguren-Fernandez\textsuperscript{f}, Chang-Yu Wu\textsuperscript{a,*}

\textsuperscript{a} Department of Environmental Engineering Sciences, College of Engineering, University of Florida, Gainesville, FL, 32611, USA
\textsuperscript{b} Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida, Gainesville, FL, 32610, USA
\textsuperscript{c} Emerging Pathogens Institute, University of Florida, Gainesville, FL, 32610, USA
\textsuperscript{d} Department of Mechanical & Aerospace Engineering, University of Florida, Gainesville, FL, 32611, USA
\textsuperscript{e} J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville, FL, 32611, USA
\textsuperscript{f} Aerosol Dynamics Inc., Berkeley, CA, 94710, USA

ARTICLE INFO

Keywords:
Self-isolation rooms
Residential setting
Size-fractionated aerosols
Aerosol transmission
Human adenovirus B3

ABSTRACT

Individuals with COVID-19 are advised to self-isolate at their residences unless they require hospitalization. Persons sharing a dwelling with someone who has COVID-19 have a substantial risk of being exposed to the virus. However, environmental monitoring for the detection of virus in such settings is limited. We present a pilot study on environmental sampling for SARS-CoV-2 virions in the residential rooms of two volunteers with COVID-19 who self-quarantined. Apart from standard surface swab sampling, based on availability, four air samplers positioned 0.3–2.2 m from the volunteers were used: a Viable Virus Aerosol Sampler (VIVAS), an inline air sampler that traps particles on polytetrafluoroethylene (PTFE) filters, a NIOSH 2-stage cyclone sampler (BC-251), and a Sioutas personal cascade impactor sampler (PCIS). The latter two selectively collect particles of specific size ranges. SARS-CoV-2 RNA was detected by real-time Reverse-Transcription quantitative Polymerase Chain Reaction (rRT-qPCR) analyses of particles in one air sample from the room of volunteer A and in various air and surface samples from that of volunteer B. The one positive sample collected by the NIOSH sampler from volunteer A’s room had a quantitation cycle (Cq) of 38.21 for the N-gene, indicating a low amount of airborne virus [5.69E-02 SARS-CoV-2 genome equivalents (GE)/cm\textsuperscript{3} of air]. In contrast, air samples and surface samples collected off the mobile phone in volunteer B’s room yielded Cq values ranging from 14.58 to 24.73 and 21.01 to 24.74, respectively, on the first day of sampling, indicating that this volunteer was actively shedding relatively high amounts of SARS-CoV-2 at that time. The SARS-CoV-2 GE/cm\textsuperscript{3} of air for the air samples collected by the PCIS was in the range 6.84E+04 to 3.04E+05 using the LED-N primer system, the highest being from the stage 4 filter, and similarly, ranged from 2.54E+03 to 1.68E+05 GE/cm\textsuperscript{3} in air collected by the NIOSH sampler. Attempts to isolate the virus in cell culture from the samples from volunteer B’s room with the aforementioned Cq values were unsuccessful due to out-competition by a co-infecting Human adenovirus B3 (HAdV B3) that killed the Vero E6 cell cultures within 4 days of their inoculation, although Cq

* Corresponding author. Department of Environmental Engineering Sciences, Engineering School of Sustainable Infrastructure and Environment, University of Florida, P.O. Box 116450, Gainesville, FL, 32611-6450, USA.
E-mail address: cywu@ufl.edu (C.-Y. Wu).

https://doi.org/10.1016/j.jaerosci.2021.105870
Received 26 July 2021; Received in revised form 21 August 2021; Accepted 23 August 2021
Available online 28 August 2021
0021-8502/© 2021 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license
values of 34.56–37.32 were measured upon rRT-qPCR analyses of vRNA purified from the cell culture medium. The size distribution of SARS-CoV-2-laden aerosol particles collected from the air of volunteer B’s room was >0.25 μm and >0.1 μm as recorded by the PCIS and the NIOSH sampler, respectively, suggesting a risk of aerosol transmission since these particles can remain suspended in air for an extended time and travel over long distances. The detection of virus in surface samples also underscores the potential for fomite transmission of SARS-CoV-2 in indoor settings.

1. Introduction

Caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the coronavirus disease 2019 (COVID-19) pandemic has resulted in more than 191 million cases and over 4.1 million deaths as of July 22, 2021 (WHO, 2021). To contain the spread of the virus, it is critical to understand how the virus is transmitted from person to person. Current epidemiological evidence suggests SARS-CoV-2 is spread among people by two main routes of infection: (a) direct or indirect contact of a recipient with virus-laden coughed or sneezed droplets produced by a COVID-19 patient situated nearby, and (b) inhalation of aerosol particles containing the virus. Aerosol transmission of infectious microorganisms can occur when they are inhaled in the form of droplet nuclei or small particles bearing these agents in aerosols. By definition, aerosols are colloidal suspensions of small solid or liquid particles suspended in air or another gas. As a result of their small sizes, particles in aerosols can remain suspended in air for prolonged times and travel long distances from the emitting source (Pan et al., 2019). The World Health Organization (WHO) originally stated that airborne transmission of SARS-CoV-2 might occur during aerosol generating procedures such as tracheal intubation, non-invasive ventilation, tracheotomy, cardiopulmonary resuscitation, bronchoscopy, sputum induction, and dentistry and autopsy procedures (WHO, 2020a).

Since these procedures are carried out in hospital settings and due to the exposure risk for healthcare personnel attending to COVID-19 patients, previous studies wherein infectious virus was isolated, were focused on COVID-19 patient isolation rooms in hospital settings (Ahn et al., 2020; Lednicky, Lauzardo, et al., 2020; Santarpia et al., 2020). Airborne transmission of SARS-CoV-2 outside of healthcare facilities, particularly in indoor settings and/or in crowded and inadequately ventilated spaces requires further study (WHO, 2020b) since infectious microorganisms in small droplets or droplet nuclei in aerosols can linger in air within closed, air-conditioned spaces, for several hours (Carbone et al., 2021). Additionally, superspreading events are reported to have occurred in such settings (Lewis, 2021). With stay-at-home orders issued across the world and most of the infected individuals advised to self-isolate unless they require medical attention, household transmission becomes a concern. Moreover, in contrast to the isolation rooms in hospitals, residential settings do not have negative pressure isolation rooms, and disinfection processes differ between these two settings.

Based on epidemiological investigations and contact tracing, airborne transmission of SARS-CoV-2 has been reported among household contacts (Hwang et al., 2021; Lewis et al., 2020; Wu et al., 2020). The secondary infection rate increases in such settings (Grijalva et al., 2020; Qian et al., 2020), implicating household transmissions as a major source of new infections (Metlay et al., 2021). In a residential setting, people share common spaces and are hence prone to substantial risk of infection while residing with SARS-CoV-2-infected individuals (Fung et al., 2020). Monitoring the environments of individuals who self-isolate or quarantine in their residences is important for assessing the risk of exposure to SARS-CoV-2 for other inhabitants of the same domiciles. According to an environmental sampling conducted by Maestre et al. (2021), detection of SARS-CoV-2 RNA in samples collected from the surface of a carpet in a room unused by self-isolating COVID-19 individuals suggests the dispersal and deposition of virus-laden aerosols shed by the infected individuals. In another study monitoring the household of quarantining individuals with COVID-19, SARS-CoV-2 RNA was detected in samples collected from surfaces and wastewater, but not in the air samples (Dohla et al., 2020). Because the authors used only one type of sampler (Coriolis micro, based on cyclonic technology) to collect airborne particles, they recommended the use of different air samplers to exclude method-related false negative results. In both the studies mentioned above (Dohla et al., 2020; Maestre et al., 2021), environmental monitoring of the households of quarantining individuals with COVID-19 detected SARS-CoV-2 RNA but did not prove the infectivity of the virus. Demonstration that the sampled SARS-CoV-2 is infectious is exceptionally important: non-infectious SARS-CoV-2 cannot cause COVID-19, and thus detection alone does not equate with inhalation risk. Thus, current literature reveals a need for studies in residential settings to: (1) determine if self-isolating individuals with varying degrees of symptoms shed infectious virus as people with severe COVID-19 symptoms are considered to have high viral load and a long period of virus shedding (Liu et al., 2020a); and (2) ascertain the size range of virus-laden particles shed by such infected individuals. The latter is important because large droplets can be deposited onto surfaces in close proximity to the infected individual while smaller droplets and droplet nuclei can remain suspended in air for a long time in most indoor settings (Fennelly, 2020; Pan et al., 2019).

In this pilot study, we collected (1) the entirety of airborne particles (without size classification) using polytetrafluoroethylene (PTFE) filters and a Viable Virus Aerosol Sampler (VIVAS), (2) size-fractionated particles in aerosols according to aerodynamic size using a 2-stage cyclone aerosol sampler (NIOSH bioaerosol sampler, BC-251) and a Sioutas personal cascade impactor sampler (PCIS), and (3) high-touch surface swab samples in the residential rooms of two self-isolating individuals with COVID-19. We report that SARS-CoV-2 was present in some of the samples collected from the air and surface samples in the rooms of the volunteers.

2. Methods

This work was IRB exempt as it does not involve research with human subjects or collect information that may reveal the identity of
the volunteers. Informed consent was obtained from the volunteers to position the air samplers in their rooms and to collect surface samples from frequently touched areas.

2.1. Sampling sites

The bedrooms of two 20-year-old persons with symptomatic COVID-19 were sampled during their self-isolation periods within their apartments. After their diagnoses, both persons volunteered to have their rooms tested for the virus through environmental sampling. Volunteer A resided in a single-occupancy room (3.05 m × 2.96 m) of a 4 bedroom-4 bath apartment, while volunteer B resided in a single-occupancy room (4.69 m × 3.35 m) of a 2 bedroom-1 bath apartment. The air conditioners were set at 24 °C and 23 °C in volunteer A’s and B’s apartments, respectively. The relative humidity in the rooms was not measured in this study.

2.2. Case descriptions

Volunteer A was asymptomatic when a nasopharyngeal (NP) swab specimen was collected for a SARS-CoV-2 RT-PCR test on September 17, 2020 and was notified the test was positive the next day. The individual self-reported that symptoms were not apparent until September 20, 2020, when loss of sense of smell occurred and minor, nonproductive cough developed. A follow-up rRT-qPCR test on September 29, 2020 was negative, yet this person continued to endure a minor cough and a dull headache until October 9, 2020.

Starting September 24, 2020, volunteer B experienced symptoms of COVID-19 including breathlessness, chills, fever, headache, loss of appetite, mild cough, nausea, sore throat, tiredness, and whole-body ache. A NP swab was collected for a SARS-CoV-2 rRT-qPCR test on September 27, 2020, and the volunteer was informed the result was positive on September 30, 2020. This individual experienced persistent headaches and tiredness until October 11, 2020, when another NP sample was taken, which tested negative for SARS-CoV-2.

2.3. Samplers

At each testing interval, air samplers were set at fixed points in the rooms with their air inlets ~1.5 m from the ground, a height that approximates the distance from the ground to the nose of a seated person. Air samplings were carried out in volunteer A’s room on September 22, 23 and 25, 2020 with the PTFE filter, NIOSH bioaerosol sampler (BC-251), and the VIVAS. On September 22, 2020, two samplers were set up in the bedroom – one at a distance of ~0.3 m and the other ~1.8 m from volunteer A. The samplers were ~1.8 m away on September 23 and 25, 2020. Volunteer B was ~2.2 m away from the samplers on the days sampled (October 2, 2020 and October 6, 2020). A PCIS was used in place of the VIVAS in volunteer B’s room. The surfaces of frequently touched areas as reported by the volunteers were swabbed with sterile nylon swabs. The surfaces swabbed were on a mobile phone (~80 cm away on September 22, 23 and 25, 2020 with the PTFE filter, NIOSH bioaerosol sampler (BC-251), and the VIVAS. On September 22, 2020, two

PTFE filters (47 mm, 5 μm pore, Millipore Sigma, US) placed in an inline filter holder (47 mm, stainless steel, XX5004710, Millipore Sigma, US or 47 mm, stainless steel, Product ID: 2220, Pall Corporation, US) were run at 3 L per minute (LPM) with AirChek sampler pump (Model 224-PCXR4, SKC Inc., US or Escort ELF pump (Item Number: 805560, Zefon International, US). This sampler collects all particles without size classification. The 2-stage cyclone aerosol sampler (NIOSH bioaerosol sampler, BC-251) developed by Lindsey et al. (2006) was run at 3 LPM with AirChek PCXR4 pump. This sampler comprises of a 15 mL tube (Falcon conical centrifuge tubes, catalog no. 14-959-53A, Fisher Scientific, US), 1.5 mL Eppendorf tube (Microcentrifuge tubes, Fisher scientific, catalog no. 02-681-373) and a filter cassette containing a PTFE filter (37 mm, 2 μm pore, catalog no. 225–1709, SKC Inc., US), which collects particles of aerodynamic diameter >4.4 μm, 1.1–4.4 μm and <1.1 μm, respectively. The calculation for cut-off size of each stage is described in the supplementary material.

The VIVAS (Aerosol Dynamics Inc., US) functions through a laminar air flow, water vapor condensation mechanism, and has been proven to collect airborne SARS-CoV-2 (Lednický et al., 2020a, 2020b). The operating principle and efficiency of this sampler in collecting viruses has been documented elsewhere (Lednický et al., 2016; Pan et al., 2017). Briefly, as airborne particles are collected by the VIVAS, a shell of water molecules deposits onto their surfaces, and that shell progressively enlarges through sequential water molecule condensation as the particle travels along three distinct zones of the growth tubes inside the VIVAS: viz., conditioner (4 °C), moderator (31 °C) and initiator (40 °C), and moderator (31 °C). In this study, the VIVAS was operated for 3 h at a flow rate of 8 LPM. The particles were subsequently collected by gentle impaction onto 1.5 mL of sterile Virus Transport Medium (VTM) maintained at 25 °C in a 35 mm Petri dish. The VTM used in an earlier study (Lednický, Lauzardo, et al., 2020) was not used. Instead, a different VTM that we have validated (Lednický, unpublished) for coronaviruses that we prepared and distributed for use in COVID-19 diagnostic tests was used, as a large surplus was available. The VTM used for this study was formulated with cryoprotectants in the form of additional protein and sucrose to preserve infectivity of the virus. It is comprised of 1 × brain heart infusion broth (Difco, US) supplemented with 0.4 mg/mL neomycin sulfate (Cell culture grade, CAS Number: 1405-10-3, Sigma Aldrich, US), 2.5 μg/mL gentamycin (Cell culture, CAS no. 1405-41-0, Sigma Aldrich, US), 2.5 μg/mL neomycin sulfate (Cell culture grade, CAS Number: 1405-10-3, Sigma Aldrich, US), 0.2 M sucrose (Cell culture grade, CAS no. 57-50-1, Sigma Aldrich, US) and 4 g/L of bovine serum albumin (BSA) fraction V (Lyophilized, catalog no. 15260037, Sigma Aldrich, US).

The PCIS (catalog no. 225–370, SKC Inc., US) was used with a Leland Legacy pump (catalog no. 100–3002, SKC Inc., US) and operated at a flow rate of 9 L/min for 90 min. PTFE filters (25 mm, 0.5 μm pore, catalog no. 225–2708, SKC Inc., US) were used to collect particles of size >2.5 μm, 1–2.5 μm, 0.5–1 μm and 0.25–0.5 μm in the 4 collection stages. 37 mm PTFE filters (2 μm pore, catalog no. 225–1709, SKC Inc., US) were used for the 5th stage, considered the ‘after-filter’ stage (SKC, 2021).
Calibration of the pumps used for PTFE filters, NIOSH bioaerosol sampler and VIVAS was performed with a Gilian Gilibrator-2 NIOSH primary standard air flow calibrator (Sensidyne, US). The operating flow rate of PCIS was calibrated by a Defender Primary Standard Calibrator (catalog no. 717-510H, SKC Inc., US).

2.4. Collection and processing of samples

The PCIS and NIOSH sampler were preloaded with filters/vials, their tubing connected to the pump and sealed in sterile zip lock bags in a BSL2 laboratory. Similarly, 1.5 mL of sterile collection medium was added to a Petri dish and sealed with parafilm. Sterile swabs and vials containing VTM for transport and storage of surface swab samples were delivered along with the air samplers on a cart to the doorstep of the volunteer’s isolation room. Thereafter, the volunteers moved the cart into the room, then positioned and turned on the air sampling devices. After sampling intervals, the volunteers disinfected the surfaces of the cart, sampling devices, zip lock bags, etc., with 70% ethanol before returning the surface-decontaminated materials to the doorstep of the isolation rooms. The researchers again disinfected the exterior of all the surfaces with 70% ethanol and H2O2 wipes when the cart was outside the isolation room. All the sample containers and samplers were placed in secondary polypropylene bags prior to transport to the laboratory. The volunteers wore gloves while operating and returning the devices, and the researchers used N95 masks and gloves while handling the devices and samples. The researchers did not have in-person contact with the volunteers or their co-inhabitants. Positioning and operation of the samplers were pre-discussed with the volunteers virtually.

The samples were processed and concentrated in a BSL-2+ laboratory at the University of Florida (UF) within 30 min of air sampling. After their removal from the sampler, 1.0 mL and 0.5 mL of VTM were added to stages 1 and 2 tubes of the NIOSH bioaerosol sampler, respectively, while the after-filter was immersed in 5 mL of the medium. Swabs used for surface samplings were immersed in 1 mL of the VTM. The filters from PCIS were individually immersed in 1 mL of recovery solution [1 × Phosphate buffered saline (PBS) with 0.5% w/v BSA fraction V] for 30 min at room temperature to rehydrate materials collected on their surfaces and to help dislodge virions stuck on the filter surfaces (Lednicky & Loeb, 2013). The filters and fluid were then transferred to a sterile 100 × 15 mm plastic Petri dish (Fisher Scientific, Cat. No. S33580A), and the filters scraped with flocked swabs pre-wetted with the recovery solution. The residual fluid in each swab was extruded into the corresponding recovery solution. The solutions were further concentrated by centrifugation in Amicon Ultra-15 centrifugal filter units with UltraCel-100 membranes with a molecular mass cutoff of 100 kDa (Millipore, Bedford, MA) at 4000 × g for 12 min, and the concentrates were adjusted to 400 μL by addition of recovery solution. They were then aseptically transferred to sterile plastic cryotubes with silicone gaskets (Fisher Scientific, Cat. No. 10-500-26), and the tubes were thereafter transported in a Styrofoam container with wet ice to a BSL2-enhanced laboratory at UF’s Emerging Pathogens Institute (UF-EPI) where they were stored in a locked –80 °C freezer until further analysis (Dzung et al., 2021; FDA, 2021).

2.5. Detection of SARS-CoV-2 genomic RNA (vRNA)

Virus genomic RNA (vRNA) for preliminary rRT-PCR SARS-CoV-2 screens was extracted from thawed samples in a BSL2-enhanced laboratory as described previously (Lednicky, Lauzardo, et al., 2020b). Briefly, vRNAs were purified using a QIAamp viral RNA mini kit (Qiagen, Valencia, CA, US) and rRT-PCR tests were performed in a BioRad CFX96 Touch Real-Time PCR detection system using the SARS-CoV-2 N-gene detection primers and probe and rRT-qPCR parameters described in Lednicky et al. (2020a, 2020b). The primers and probe for detection of the virus’ RdRp gene (Carbone et al., 2021) are given in Table 1 and were used with the following parameters: 400 nM final concentration of forward and reverse primers and 100 nM final concentration of probe using SuperScript III One-Step RT-PCR system with Platinum Taq DNA Polymerase (Thermo Fisher Scientific). Cycling conditions were 2 min at 50 °C for the reverse transcription step, followed by 2 min at 95 °C for the Taq polymerase activation step, then 44 cycles of 15 s at 95 °C of denaturing, 30 s at 45 °C for annealing, and 20 s at 68 °C for extension. The Cq values were recorded from rRT-qPCR analysis.

2.6. Quantification of GE

SARS-CoV-2 genome equivalents (GE) per cm3 of air sampled was estimated from the Cq values by the method described by Lednicky et al. (2020a). The process used to calculate GE/cm3 of air and GE/cm3 of air per Δlog(Dp, i.e., particle diameter) is described in the supplementary information section. In this study, the lower size limit cannot be inferred for NIOSH bioaerosol sampler since stage 3 of the sampler collects particles <1.1 μm. Thus, the lower limit of 0.1 μm based on the size of a SARS-CoV-2 virion as evidenced by electron microscopy of SARS-CoV-2 cultured on Vero E6 cells (e.g., a median size of 100 nm shown in Lau et al. (2021)) was taken into consideration. While it is possible to detect SARS-CoV-2 RNA in aerosol particles smaller than the size of a virion, such RNA is either incompletely packaged virus genetic material or degraded virus RNA. This cannot be used as proof that infectious virions are present. An upper limit of 10 μm for PCIS and NIOSH samplers as used for graphing purposes (Fig. 1) was adopted from prior studies.

### Table 1

| Primer/probe name | Description | Oligonucleotide sequence (5’ to 3’) | Label |
|-------------------|-------------|-----------------------------------|-------|
| Led-RdRp-F | Forward Primer | GGTGGAGACCTCCTAGGAGATGC | None |
| Led-RdRp-R | Reverse Primer | CCATCAGTAGGATTAAGTGCTTTA | None |
| Led-RdRp-Probe | Probe | FAM-CTGCCTTATGCTAATAGTGGTTTAAAC-BHQ1 | FAM, BHQ1 |
since aerosol particles can also deposit on surfaces, SARS-CoV-2 GE that could be deposited on the sampled surfaces during the sampling event was estimated from GE/cm$^3$ of air calculated for air samples. As we did not measure the size distribution with a particle counter, we estimated this GE based on the deposition velocity of the midpoint diameter of size range in each stage of PCIS and NIOSH bioaerosol sampler (supplementary information). The deposition velocities reported by Hinds (1999) was used for the estimation (values on Table S1).

### 2.7. Sanger sequencing

Sanger sequencing was performed on vRNA extracted from the stage 4 filter of the PCIS, since this sample had the lowest Cq value. A gene walking approach with non-overlapping primers was used for sequencing (Lednicky et al., 2020b), details of which are provided in the supplementary material. Briefly, cDNA produced using AccuScript high-fidelity reverse transcriptase (Agilent Technologies, Santa Clara, CA, US) was amplified with Q5 polymerase (New England BioLabs, US) and gene-specific primers. The 5' and 3' ends of the genome were obtained using a Rapid Amplification of cDNA Ends (RACE) kit (Life Technologies, Inc., Carlsbad, CA, US). The sequences were assembled with Sequencher DNA sequence analysis software version 2.1 (Gene Codes, Ann Arbor, MI, US) and the completed genome sequence, designated as SARS-CoV-2 UF-30, submitted to GenBank.

### 2.8. Isolation of virus in cell culture

For samples that tested positive by rRT-PCR, virus isolation was attempted in a BSL-3 laboratory by analysts wearing powered-air purifying respirators. Vero E6 cells (African green monkey kidney cells, ATCC CRL-1586), procured from the American Type Culture Collection (ATCC), were used as the host cells. The cells were grown and maintained as monolayers and were inoculated with aliquots of material collected at volunteer B’s room once they attained 80% confluence in T-25 flasks. Briefly, the samples that had been concentrated using centrifugal filters were passed through sterile 0.45 μm pore size PVDF syringe-tip filters to remove large particulates and contaminating bacteria and fungi. The spent cell culture medium was removed from the T-25 flasks with Vero E6 cells and replaced with 1 mL of sterile cell culture medium and 50 μL of the filtered samples, and the flasks with inoculated cells incubated for 1 h at 37 °C. Following that, 2 mL of complete media was added to the flasks, which were reincubated at 37 °C. The cells were periodically observed for cytopathic effects (CPE). An aliquot of the nucleic acids was also tested using a GenMarkDx multiplex PCR eSensor XT-8 respiratory viral panel (eSensor RVP; GenMark Diagnostics, Inc., Carlsbad, CA). The GenMark sensor RVP detects the genomic material of common human respiratory viruses (Pan et al., 2017).

![Fig. 1. Estimated SARS-CoV-2 GE per cm$^3$ of air per Δlog(particle diameter) recorded for (a–b) PCIS and (c–d) NIOSH bioaerosol sampler [The GE per cm$^3$ of air per Δlog(Dp) is marked in each size bin].](image-url)
3. Results

Volunteer A developed mild COVID-19 symptoms starting September 21, 2020, which was 4 days after a positive NP swab test. All the air and surface samples collected from volunteer A’s room on September 22 and 23, 2020 were rRT-qPCR negative for SARS-CoV-2 vRNA. On September 25, 2020, a Cq value of 38.21 was produced in a rRT-qPCR test of material collected by stage 1 of the NIOSH bioaerosol sampler, revealing the presence of 5.69E-02 SARS-CoV-2 GE per cm$^3$ of air (Table 2).

SARS-CoV-2 vRNA was detected in the samples collected from volunteer B’s room on October 2, 2020 (day 9 from onset of symptoms), for which the corresponding Cq values resulting from rRT-qPCR analyses and SARS-CoV-2 GE/cm$^3$ of air are presented in Table 3a. The positive air samples included those from stages 1–4 of the PCIS and all the stages of the NIOSH bioaerosol sampler, implying the size range of the aerosol particles containing SARS-CoV-2 to be >0.25 μm and >0.1 μm as recorded by respective samplers. From Table 3a, it is evident that the highest concentration was recorded in stage 4 filter of the PCIS, i.e., >0.25–0.5 μm with both the primer sets used (3.04E+05 GE/cm$^3$ of air for LED-N and 1.19E+05 GE/cm$^3$ of air for LED-RdRp) and the lowest was from stage 2, which collects 1–2.5 μm particles. The samples collected from the NIOSH bioaerosol sampler were estimated to have 2.9E+04, 1.68E+05 and 2.54E+03 GE per cm$^3$ air in stages 1, 2 and 3 respectively, for LED-N primers. The estimated SARS-CoV-2 GE per cm$^3$ of air per Δlog(D$_p$) for the samples collected at volunteer B’s room by PCIS and NIOSH bioaerosol sampler on October 2, 2020 are presented in Fig. 1. The Cq values of surface samples were lower than the air samples, and the corresponding GE estimated from the front and back screens of the volunteer’s mobile phone were 1.48E+09 and 3.2E+09, respectively, for LED-N primer system.

Among the air and surface samples collected from volunteer B’s room on October 6, 2020 (day 13 from onset of symptoms), SARS-CoV-2 was detected only in stage 2 of the NIOSH bioaerosol sampler (Table 3b). The GE corresponding to this sample was 1.62E-01 per cm$^3$ air, which is ~10$^9$ lower than that recorded on October 2, 2020 for stage 2 of the NIOSH sampler. Even after 14 days of inoculation onto Vero E6 cells, CPE were not observed, suggesting the absence of infectious SARS-CoV-2 in the samples collected on October 6, 2020. This was confirmed by rRT-qPCR tests of the cell culture media that were performed 7- and 14-days post-inoculation of the cells.

The GE estimated for surface samples collected from volunteer B’s mobile phone on October 2, 2020 were 3.24E+09 and 5.63E+08 for the back cover and front screen, respectively (Table 3c). Considering particles in aerosols deposit onto surfaces at deposition velocities corresponding to their size, the GE that would be deposited onto sampled surfaces was estimated from the GE/cm$^3$ of air recorded for each size range by the air samplers. From Table S1, it is evident that the GE recorded for surface samples is of the same order of magnitude (i.e., 10$^9$) as that of the GE estimated to deposit from air onto sampled surfaces.

Sanger sequencing was used to obtain the nucleotide sequence of the SARS-CoV-2 genome in the sample collected on October 2, 2020 by stage 4 filter of the PCIS. The genome sequence was deposited in GenBank (Accession no. MW261767.1) and is also available through GISAID (Accession no. EPI_ISL_641318). Genome sequence alignment of SARS-CoV-2 UF-30 with that of the SARS-CoV-2 reference strain, Wuhan-Hu-1, reveals 99% identity (29879/29895 rnt) and no sequence gaps. The deduced amino acid substitutions in UF-30 compared to those of Wuhan-Hu-1 are: SPIKE D614G, SPIKE F220L, N G212C, NS3 Q57H, NS8 S24L, NSP2 L501F, NSP4 T189I, NSP5 T45I, NSP12 P323L, NSP13 T96I, NSP15 M271V. SARS-CoV-2 UF-30 has the D614G mutation, which was dominant in circulating SARS-CoV-2 strains around the world (Korber et al., 2020; Plante et al., 2021). It has nucleotide (Nt) markers C241T, C3037T, A23403G, G25563T and amino acid markers S-D614G.

Virus-induced CPE were observed in Vero E6 cells inoculated with air and surface samples collected from volunteer B’s room within

### Table 2

rRT-qPCR results of environmental samples from volunteer A’s room.

| Date of sampling      | Sampler          | Flow rate (LPM) | Sampling time (min) | Volume of air collected (m$^3$) | Distance from volunteer (m) | Cq value | SARS-CoV-2 GE/cm$^3$ of air |
|-----------------------|------------------|-----------------|---------------------|---------------------------------|-----------------------------|---------|-----------------------------|
| September 22, 2020    | PTFE filters     | 3               | 120                 | 0.36                            | 0.3                         | –       | –                           |
|                       | PTFE filters     | 3               | 120                 | 0.36                            | 1.8                         | –       | –                           |
| September 23, 2020    | Surface sample a. Arm-elbow | –               | –                   | –                               | –                           | –       | –                           |
|                       | Surface sample a. Mobile phone | –               | –                   | –                               | –                           | –       | –                           |
|                       | Surface sample a. Laptop touch pad | –               | –                   | –                               | –                           | –       | –                           |
| September 25, 2020    | NIOSH bioaerosol sampler – stage 1 | 3               | 180                 | 0.54                            | 1.8                         | –       | 38.21 0.0569                |
| Positive template (SARS-CoV-2 RNA) | N/A          |     |                     |                                 |                             |         |                             |
| Positive template (N-plasmid) | N/A          |     |                     |                                 |                             |         |                             |
| No-template (negative)                              | N/A            |                     |                           |                                 |                             |         |                             |
Since the Cq value was high (>34) when nucleic acids extracted from the cell growth media of the cell cultures were tested by RT-qPCR for SARS-CoV-2, we suspected an additional respiratory virus was present, as we have previously observed in other studies (Lednicky et al., 2020b; Pan et al., 2017). The GenMark sensor RVP produced a preliminary identification of human adenovirus. To gain insights on the type of adenovirus, we interrogated the sample using the primer systems of Kuo et al. (2009), which produced a specific amplicon of approx. 280 bp using primer pair HAdV ABCDEF-hexon25f and B-hexon308r, presumptively identifying the virus as a B species human adenovirus. For proof, primer pair HAdV B hex-for: 5′-ccgccgcagcagaggagaaag-3′.

4 days of their inoculation. Since the Cq value was high (>34) when nucleic acids extracted from the cell growth media of the cell cultures were tested by RT-qPCR for SARS-CoV-2, we suspected an additional respiratory virus was present, as we have previously observed in other studies (Lednicky et al., 2020b; Pan et al., 2017). The GenMark sensor RVP produced a preliminary identification of human adenovirus. To gain insights on the type of adenovirus, we interrogated the sample using the primer systems of Kuo et al. (2009), which produced a specific amplicon of approx. 280 bp using primer pair HAdV ABCDEF-hexon25f and B-hexon308r, presumptively identifying the virus as a B species human adenovirus. For proof, primer pair HAdV B hex-for: 5′-ccgccgcagcagaggagaaag-3′.
and HAdV B hex-rev: 5’T-cgcagctgctgtggcaagaagc-3’ were synthesized and used in PCR to obtain the entire hexon gene sequence. The resulting amplicon was sequenced and informed that volunteer B was co-infected with HAdV species B type 3. The hexon gene sequence that was obtained is provided in the supplementary material.

**4. Discussion**

We detected SARS-CoV-2 in air-conditioned indoor residential settings, with the air samplers placed ~0.3–2.2 m from volunteers who self-isolated following diagnoses of COVID-19. Only one SARS-CoV-2 rRT-qPCR positive test resulted among the samples taken on three separate days from volunteer A’s room. The positive sample was recovered on September 25, 2020 from the NIOSH bioaerosol sampler’s stage 1 tube, and registered a high Cq value of 38.21 using primer set LED-N. In contrast, the air and surface samples collected from volunteer B’s room on October 2, 2020 had low Cq values of 15.97–24.73 using the LED-RdRp and 15.9–23.33 with the LED-N gene primer pairs. Information regarding the size fractions of particles in the aerosols were provided by data generated using the PCIS and NIOSH bioaerosol sampler, which showed the virus-associated particles were >0.25 μm and >0.1 μm respectively. This is consistent with the findings of previous studies on the collection of airborne SARS-CoV-2 in different settings (Chia et al., 2020; Feng et al., 2021; Lednicky, Lauzardo, et al., 2021; Liu et al., 2020b; Ong et al., 2021). In general, particles in the respirable size range, i.e., particles of size <4 μm can reach the alveoli upon inhalation (Brown et al., 2013). The WHO defines the size of particles in aerosols as <5 μm (WHO, 2020a). This definition arises from the fact that small droplets (<5 μm) and droplet nuclei can remain suspended in air for a long time while droplets (>5 μm) can settle down near the source. Thus, particle size is an important determinant of aerosol behavior and size fractionation of aerosols is helpful in implementing suitable infection control measures (Fennelly, 2020). Our study shows the presence of SARS-CoV-2 in aerosol particles of size <4 μm in some samples collected by the PCIS and NIOSH bioaerosol samplers, suggesting the possibility of aerosol transmission in a residential self-isolation room. Cq values lower than 25 were recorded by rRT-PCR analysis for all the air and surface samples collected, with the lowest being 14.58, from stage 4 filter of PCIS operated in air for a long time while droplets (>5 μm) can remain suspended in air show that stage 4 of PCIS which collects particles of 0.25–0.5 μm had the highest (3.04E-05), while the lowest was from stage 2, which collects 1–2.5 μm particles. Previously, 1–40 copies of SARS-CoV-2 RNA per m³ of air were detected using a PCIS in a hospital setting (Liu et al., 2020b). The infectivity of the virus was not proven in that study, which is likely due to inactivation of the virus owing to long sampling duration (260 min–7 days). With lower sampling duration (135 min) and immediate hydration after air sampling, infectious SARS-CoV-2 was isolated using a PCIS around a minimally symptomatic COVID-19 individual (Lednicky, Lauzardo, et al., 2021). Our attempts to preserve the infectivity of the virus with much lower sampling time (90 min) and immediate hydration of the filters post-sampling were unsuccessful because volunteer B was apparently co-infected with HAdV B3, which outgrew SARS-CoV-2 in our Vero E6 cells. Adenovirus B3 causes acute respiratory infections (Lynch & Kajon, 2016), and likely contributed to the respiratory symptoms experienced by volunteer B. Although a major objective of our environmental study was to determine whether SARS-CoV-2 was infectious in the air and on environmental surfaces, we were unable to make that determination as it was outgrown by the adenovirus. Indeed, this is a challenging issue when multiple viruses that can grow in the same type of host cell are present in the environment (Lednicky et al., 2020b, 2021b). The efficiency of collecting infectious virus also differs corresponding to the air sampler deployed for the study. For example, the low efficiency of the NIOSH bioaerosol sampler for collecting infectious viruses (~26%) is well documented and a consequence often attributed to drying of the virus along the walls of the tube and filter (Cao et al., 2011; Leung et al., 2016; Verreault et al., 2008). This may explain why we were unable to isolate SARS-CoV-2 from the samples collected by the NIOSH bioaerosol sampler. Herein, isolation in virology means the ability to separate virions from host cells inoculated with samples potentially containing the target virus to demonstrate the infectivity of the virus rather than merely RNA determined from samples.

Detection of vRNA on surface samples suggests deposition of particles exhaled by the infected individual onto surfaces through settling of airborne particles or through direct contact. In this study, we recorded Cq values between 21 and 24 for the samples swabbed over volunteer B’s mobile phone on October 2, 2020. This demonstrates the deposition of SARS-CoV-2 on high touch surfaces and the possibility of fomite transmission in a residential setting. Unfortunately, our attempts to isolate SARS-CoV-2 in cell culture were unsuccessful as volunteer B was co-infected with HAdV B3 and this virus outgrew SARS-CoV-2 in cell culture. Döhl et al. (2020) detected SARS-CoV-2 on the surfaces of different areas in households occupied by quarantined individuals, though all the air samples were negative. The surfaces that tested positive for rRT-qPCR analyses in their study were an electronic device (remote control), two
metallic doorknobs and a wooden stove overlay. A household of 2 self-isolating individuals with COVID-19 had high amounts of vRNA in dust samples ($10^5$–$10^6$ copies/g of floor dust and $10^4$–$10^5$ copies/g of HVAC filter dust); while ~100 SARS-CoV-2 N2 gene copies/swab were detected in surface samples (such as child’s toy, toilet handle and doorknob of primary bedroom) and 20 copies/cm$^2$ in the carpet of primary bedroom (Maestre et al., 2021). Interestingly, the authors detected SARS-CoV-2 vRNA in the carpet of an unused bedroom (4.8 N2 copies/cm$^2$), which they attribute to the dispersal and deposition of virus-laden aerosols shed by the infected individuals. Mouchtouri et al. (2020) detected SARS-CoV-2 RNA in surface swabs collected from doorknobs on the outer side of a toilet door in a ferry boat and a patient room in a nursing home, with Cq values of 37 and 32, respectively. In the same study, the virus was not detected in other samples collected from the doorknobs of a nursing home and a hospital. Because people in contact with infectious surfaces may also have contact with infected individuals, it is hard to distinguish between airborne and fomite transmission in a given setting.

Recently, Eilts et al. (2021) demonstrated that airborne particles can deposit onto surfaces depending on the velocity and particle size, in a mock work environment. While aerosol particles have longer lifetimes in the air, infectious viral particles may still possibly deposit and later spread by fomite transmission. The equivalent GE deposit on the phone surface from measured GE in aerosol is calculated (supplementary information) for comparison with the measured GE on phone surface. The result shows the same order of magnitude as that of the surface samples (Table S1 vs. Table 3c), implying that the deposited material can come from contaminated room air. The comparison points out that aerosol transmission may not mean infection solely spread by inhalation, i.e., inhalation is not mutually exclusive with deposition and contact transfer or deposition onto an individual. While Port et al. (2021) showed higher SARS-CoV-2 disease severity and transmission efficiency for airborne compared to fomite exposure in Syrian hamsters, positive passive surface sample results are equally important to quantify as active aerosol sampler results.

Another major factor impacting the transmission and thus collection of airborne SARS-CoV-2 are the dynamics of COVID-19 (He et al., 2020), i.e., the events that occur from start to end of an infection within an individual. Generally, peak SARS-CoV-2 load in the respiratory tract is observed at the time of symptom onset or in the first week of illness, with subsequent decline thereafter, which indicates the highest infection potential just before or within the first five days of symptom onset (Cevik et al., 2021). Other studies indicate that infectious virus is not produced by a COVID-19 patient after a week (Wölfel et al., 2020) or two (Sohn et al., 2020) of onset of symptoms, though vRNA is persistently detected by RT-PCR analysis. This may be related to the production of neutralizing antibodies in COVID-19 patients after 5–10 days of infection (van Kampen et al., 2021). In our study, the onset of infection in volunteer A is unknown since the patient was asymptomatic initially. The volunteer reported mild symptoms and was active throughout the isolation period. While SARS-CoV-2 was detected in the air of volunteer B’s room by the NIOSH bioaerosol sampler on October 6, 2020, estimates of SARS-CoV-2 GE in air show decline from that collected on October 2, 2020. The high Cq values recorded in PCR analysis for the samples collected on September 25, 2020 and declining SARS-CoV-2 GE in air estimated for samples collected on October 6, 2020 suggests that the concentration of virions shed by the volunteers was low, consistent with expectations during recovery from COVID-19.

For American healthcare settings that deal with COVID-19 patients, guidelines regarding stringent infection control practices such as the use of personal protective equipment by the caregiver and the patient, limited in-person visitors, periodical disinfection of the patient rooms, optimized air-handling system, and limited movement of the patient outside of the room, are available (CDC, 2021a). However, these measures may not be followed rigorously in a residential setting. It is thus recommended that CDC guidelines be followed regarding: (a) limiting direct contact with the patient, (b) living in separate areas without sharing personal items, (c) using mask and gloves as applicable when using common spaces, and (d) maintaining self-hygiene practices (CDC, 2021b) to reduce the risk of exposure to the virus, while sharing a residence with an infected individual.

Our study has a few limitations such as a small sample size, inconsistent use of samplers and no measurements on aerosol size distribution. Firstly, because infected individuals prefer to reduce social interactions due to the overall emotional burden following SARS-CoV-2 infection (Verberk et al., 2021), knowledge of who is typically infected is restricted to limited numbers of family members and close friends. Thus, gaining access to conduct experiments in their residence is a challenge, at least in the US. Indeed, our study was an opportunistic sampling event. Ideally, a larger cohort, including individuals with varying degrees of symptoms, asymptomatic and pre-symptomatic COVID-19 patients, such as in quarantine dorms, is warranted to explain the differences in the virus transmission dynamics among these individuals. Secondly, with no standard samplers for collecting airborne viruses (Pan et al., 2019) and limited knowledge on SARS-CoV-2 transmission in a household setting, defining a standard sampling protocol was not feasible, especially for such a new virus in the community. Thus, we deployed different samplers with an aim to collect suspended particles with and without size fractionation in this opportunistic sampling event. A systematic study to compare various air samplers, such as that by Ratnesar-Shumate et al. (2021), and to establish standard protocols to collect airborne viruses is warranted. Thirdly, we did not measure the size distribution of particles in the self-isolation rooms. Consequently, we fixed limits for calculations correlating active samplers and passive samples. Future studies with aerosol measurements will provide information on the relation between passive surface sample results and active aerosol sampler results to better understand the dominating exposure route.

5. Conclusions

In this study, SARS-CoV-2 was detected in air with air samplers placed at ~1.8 m from an infected person with mild symptoms (volunteer A), and ~2.2 m from an infected person with severe symptoms (volunteer B). In vitro isolation of SARS-CoV-2 detected in volunteer B’s room was challenging since the volunteer was co-infected with another respiratory virus that outgrew SARS-CoV-2 in cell culture. Cytopathic effects were observed within 4 days post inoculation of the samples, and subsequent partial sequencing of this virus revealed it was HAdV B3. Results of rRT-PCR analyses of the cell culture with SARS-CoV-2 primers revealed Cq values of SARS-CoV-2...
RNA were >34, indicating either low-level replication or presence of residual virions. With our study consistent with the possibility of SARS-CoV-2 transmission through aerosols and fomites in a residential setting, it is recommended that infected individuals undergo self-isolation in their own residence and the household members (1) wear masks when sharing common spaces; (2) regularly disinfect self or objects; and (3) have sufficient air circulation through heating, ventilation, and air conditioning (HVAC) systems with high air change rates or natural ventilation through open windows, as the weather permits. Such measures can reduce the risk of being exposed to infectious virions released by an infected individual to the environment.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The study was funded by National Science Foundation (Grant No. 2030844) and National Institutes of Health (Grants No. R44ES030649 and R01AI158868). The authors thank Laksmi Arroyo for assisting in conducting the study; Dr. William G. Lindsley of the National Institute for Occupational Safety and Health for the free loan of NIOSH BC-251 samplers; Dr. David Kaplan, University of Florida, for providing access to the state vehicle and Dr. Katherine Deliz Quinones, University of Florida, for the access to her laboratory’s biosafety hood.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaerosci.2021.105870.

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.

References

Ahn, J. Y., An, S., Sohn, Y., Cho, Y., Hyun, J. H., Baek, Y. J., Kim, M. H., Jeong, S. J., Kim, J. H., Ku, N. S., Yeom, J.-S., Smith, D. M., Lee, H., Yong, D., Lee, Y.-J., Kim, J. W., Kim, H. R., Hwang, J., & Choi, J. Y. (2020). Environmental contamination in the isolation rooms of COVID-19 patients with severe pneumonia requiring mechanical ventilation or high-flow oxygen therapy. Journal of Hospital Infection, 106(3), 570–576. https://doi.org/10.1016/j.jhin.2020.08.014

Brown, J. S., Gordon, T., Price, O., & Asgharian, B. (2013). Thoracic and respirable particle definitions for human health risk assessment. Particle and Fibre Toxicology, 10. https://doi.org/10.1186/1743-8977-10-12. Article 12.

Cao, G., Noti, J. D., Blachere, F. M., Lindsley, W. G., & Beezhold, D. H. (2011). Development of an improved methodology to detect infectious airborne influenza virus using the NIOSH bioaerosol sampler. Journal of Environmental Monitoring, 13(2), 3321–3328. https://doi.org/10.1039/c1em10607d

Carbone, M., Lednicky, J., Xiao, S. Y., Venditti, M., & Bucci, E. (2021). Coronavirus 2019 infectious disease epidemic: Where we are, what can be done and hope for. Journal of Thoracic Oncology, 16(4), 546–571. https://doi.org/10.1016/j.jto.2020.12.014

CDC. (2021a). Interim infection prevention and control recommendations for healthcare personnel during the coronavirus disease 2019 (COVID-19) Pandemic. Retrieved from https://www.cdc.gov/coronavirus/2019-ncov/hcp/infection-control-recommendations.html# print (Accessed 1 March 2021). Accessed.

CDC. (2021b). Caring for someone sick at home: Advice for caregivers in non-healthcare settings. Retrieved from https://www.cdc.gov/coronavirus/2019-ncov/if-you-are-sick/care-for-someone.html. (Accessed 20 April 2021).

Cevik, M., Tate, M., Lloyd, O., Maroalo, A. E., Schafers, J., & Ho, A. (2021). SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectionness: A systematic review and meta-analysis. The Lancet Microbe, 2(1), e13–e22. https://doi.org/10.1016/S2666-5247(20)30172-5

Chia, P. Y., Coleman, K. K., Tan, Y. K., Du, S. W. X., Gun, M., Lau, S. K., Lim, X. F., Lim, A. S., Sutjipto, S., Lee, P. H., Son, T. T., Young, B. E., Milton, D. K., Gray, G. C., Schuster, S., Barkham, T., De, P. P., Vasoo, S., Chan, M., & Marimuthu, K. (2020). Detection of air and surface contamination by SARS-CoV-2 in hospital rooms of infected patients. Nature Communications, 11. https://doi.org/10.1038/s41467-020-16670-2. Article 2800.

Dohla, M., Wilbring, G., Schulte, B., Kümmerer, B. M., Diegmann, C., Sib, E., Richter, E., Haag, A., Engelhart, S., Eis-Hübinger, A. M., & Exner, M. (2020). SARS-CoV-2 in environmental samples of quarantined households. The Lancet Respiratory Medicine, 8, Article 12800. https://doi.org/10.1016/S2213-2600(20)30323-4

Dzung, A., Cheng, P. F., Stoffel, C., Tastanova, A., Turko, P., Levesque, M. P., & Bosshard, P. P. (2021). Prolonged unfrozen storage and repeated freeze-thawing of SARS-CoV-2 patient samples have minor effects on SARS-CoV-2 detectability by RT-PCR. Journal of Molecular Diagnostics, 23(6), 691–697. https://doi.org/10.1016/j.jmoldx.2021.03.003

Eilts, S. M., Li, L., Pope, Z. C., & Hogan, C. J. (2021). Characterization of exhaled particle deposition and ventilation in an indoor setting. Atmospheric Environment, 262. https://doi.org/10.1016/j.atmosenv.2021.118602. Article 118602.

FDA. (2021). Real-time fluorescent RT-PCR kit for detecting SARS-CoV-2. FDA. Retrieved from https://www.fda.gov/media/136472/download. (Accessed 10 August 2021).

Feng, B., Xu, K., Gu, S., Zheng, S., Zou, Q., Xu, Y., Yu, L., Lou, F., Yu, F., Jin, T., Li, Y., Sheng, J., Yen, H. I., Zhong, Z., Wei, J., & Chen, Y. (2021). Multi-route transmission potential of SARS-CoV-2 in healthcare facilities. Journal of Hazardous Materials, 402, 123771. https://doi.org/10.1016/j.jhazmat.2020.123771

Fennelly, K. P. (2020). Particle sizes of infectious aerosols: Implications for infection control. The Lancet Respiratory Medicine, 8(9), 914–924. https://doi.org/10.1016/S2213-2600(20)30323-4

Fung, H. F., Martinez, L., Alarid-Escudero, F., Salomon, J. A., Studdert, D. M., Andrews, J. R., Goldhaber-Fiebert, J. D., Chin, E. T., Claypool, A. L., Fernandez, M., et al. (2020). Transmission of SARS-COV-2 infections in households—Tennessee and Wisconsin, April–September 2020. Morbidity and Mortality Weekly Report, 69(44), 1631–1634. https://doi.org/10.15585/mmwr.mm6944e1
S. Nunnia Shankar et al.
Journal of Aerosol Science 159 (2022) 105870

He, X., Lai, E. H. Y., Wu, P., Deng, X., Wang, J., Hao, X., Lai, Y. C., Wong, J. Y., Guan, Y., Tan, X., Mo, X., Chen, Y., Liao, W., Chen, W., Hu, F., Zhang, Q., Zhong, M., Wu, Y., Zhao, L., & Leung, G. M. (2020). Temporal dynamics in viral shedding and transmissibility of COVID-19. Nature Medicine, 26, 672–675. https://doi.org/10.1038/s41591-020-0865-9

Hinds, W. C. (1999). Aerosol technology: Properties, behavior, and measurement of airborne particles (2nd ed.). John Wiley & Sons (Appendix A1b).

Hwang, S. E., Chang, J. H., Oh, B., & Heo, J. (2021). Possible aerosol transmission of COVID-19 associated with an outbreak in an apartment in Seoul, South Korea, 2020. Journal of Aerosol Science, 159, 105870. https://doi.org/10.1016/j.jaerosci.2020.105870

van Kampen, J. A., van de Vijver, D. A. M. C., Fraaij, P. L. A., Haagmans, B. L., Lamers, M. M., Okba, N., van den Akker, J. P. C., Endeman, H., Gommers, D. A. M. P. J., Cornelissen, J. J., Hoek, R. A. S., van der Eerden, M. M., Hesselink, D. A., Melselaar, H. J., Verbon, A., de Steenwinkel, J. E. A., Arons, M. J. C., van Boeijen, F. A., & van Rolleghem, J. A. (2021). Duration and key determinants of infectious virus shedding in hospitalized patients with coronavirus disease-19 (COVID-19). Nature Communications, 12. https://doi.org/10.1038/s41467-020-20568-4. Article 267

Korber, B., Fischer, W. M., Gnanakaran, S., Yoon, H., Theiler, J., Abfalterer, W., Hengartner, N., Giorgi, E. E., Bhattacharya, T., Foley, B., Hastei, K. M., Parker, M. D., Zhou, J., Chu, D. K. W., Yu, H., Lindsley, W. G., Beezhold, D. H., Yen, H. L., Li, Y., Seto, W. H., Peiris, J. S. M., & Cowling, B. J. (2020). Quantification of influenza virus RNA copies in airborne particles from two Wuhan hospitals. The Science of the Total Environment, 778, 146201. https://doi.org/10.1016/j.scitotenv.2021.146201. Article 146201.

Misra, C., Singh, M., Shen, S., Sioutas, C., & Hall, P. M. (2002). Development and evaluation of a personal cascade impactor sampler (PCIS). International Journal of Infectious Diseases, 6(8), 975–980. https://doi.org/10.1016/S1201-9712(05)80019-7. Article 407

Metlay, J. P., Haas, J. S., Soltoff, A. E., & Armstrong, K. A. (2021). Household transmission of SARS-CoV-2. JAMA Network Open, 4(2), Article e0245352. https://doi.org/10.1001/jama networkopen.2021.45352

Mouhat, V., Krivitsy, M., Vountas, I., Kourentis, L., Sapounas, S., Rigakos, G., Petinaki, E., Tsiodras, S., & Hadjichristodoulou, C. (2020). Environmental contamination of SARS-CoV-2 on surfaces, air-conditioner and ventilation systems. International Journal of Hygiene and Environmental Health, 230, 68–78. https://doi.org/10.1016/j.ijheh.2020.113599. Article 113599.

Ong, S. W. X., Tan, Y. K., Coleman, K. K., Tan, B. H., Leo, Y. S., Wang, D. L., Ng, C. G., Ng, O. T., Wong, M. S. Y., & Marimuthu, K. (2021). Lack of viable SARS-CoV-2 among PCR-positive air samples from hospital rooms and community isolation facilities. Infection Control and Hospital Epidemiology, 1–6. https://doi.org/10.1017/ice.2021.146021

Pan, M., Bonny, T. S., Loeb, J., Jiang, X., Lodwick, A. E., Kernaghan, E., Hering, S., Fan, Z. H., & Wu, C. Y. (2021). Collection of viable aerosolized influenza virus from PCR-positive air samples from a hospital room with COVID-19 patients. International Journal of Infectious Diseases, 108, 212–216. https://doi.org/10.1016/j.ijid.2021.04.063

Quan, H., Miao, T., Liu, L., Zheng, X., Luo, D., & Li, Y. (2020). Indoor transmission of SARS-CoV-2. Indoor Air, 31, 639–645. https://doi.org/10.1111/ina.12766

Ratnesar-Shumate, S., Bhannoman, K., Williams, G., Holland, B., Krause, M., Green, B., Freeburger, D., & Dabisch, P. (2021). Comparison of the performance of aerosol sampling devices for measuring infectious SARS-CoV-2 aerosols. Aerosol Science and Technology, 55(8), 975–986. https://doi.org/10.1080/02786826.2021.1910137

Secor, R. (2021). Sioutas five-stage cascade impactor. Retrieved from https://www.skcinc.com/products/sioutas-five-stage-cascade-impactor. (Accessed 2 August 2021)
Sohn, Y., Jeong, S. J., Chung, W. S., Hyun, J. H., Baek, Y. J., Cho, Y., Kim, J. H., Ahn, J. Y., Choi, J. Y., & Yeom, J. S. (2020). Assessing viral shedding and infectivity of asymptomatic or mildly symptomatic patients with COVID-19 in a Later Phase. *Journal of Clinical Medicine, 9*. https://doi.org/10.3390/jcm9092924. Article 2924.

Verberk, J. D., Anthierens, S. A., Tonkin-Crine, S., Goossens, H., Kinsman, J., de Hoog, M. L., Bielicki, J. A., Verhagen, P. C. J. L. B., & Gobat, N. H. (2021). Experiences and needs of persons living with a household member infected with SARS-CoV-2: A mixed method study. *PloS One, 16*(3), Article e0249391. https://doi.org/10.1371/journal.pone.0249391.

Verreault, D., Moineau, S., & Duchaine, C. (2008). Methods for sampling of airborne viruses. *Microbiology and Molecular Biology Reviews, 72*(3), 413–444. https://doi.org/10.1128/MMBR.00002-08.

WHO. (2020a). *Advice on the use of masks in the context of COVID-19: Interim guidance*. Retrieved from https://apps.who.int/iris/handle/10665/331693. (Accessed 1 March 2021).

WHO. (2020b). *Transmission of SARS-CoV-2: Implications for infection prevention precautions-scientific brief*. https://www.who.int/publications/i/item/modes-of-transmission-of-virus-causing-covid-19-implications-for-ipc-precaution-recommendations. (Accessed 1 March 2021).

WHO. (2021). *WHO Coronavirus (COVID-19) dashboard*. Retrieved from https://covid19.who.int/. (Accessed 22 July 2021).

Wölfel, R., Corman, V. M., Guggemos, W., Seilmaier, M., Zange, S., Müller, M. A., Niemeyer, D., Jones, T. C., Vollmar, P., Rothe, C., Hoelscher, M., Bleicker, T., Brünink, S., Schneider, J., Ehmann, R., Zwirglmaier, K., Drosten, C., & Wendtner, C. (2020). Virological assessment of hospitalized patients with COVID-19. *Nature, 581*, 465–469. https://doi.org/10.1038/s41586-020-2196-x.

Wu, J., Huang, Y., Tu, C., Bi, C., Chen, Z., Luo, L., Huang, M., Chen, M., Tan, C., Wang, Z., Wang, K., Liang, Y. J., Huang, J., Zheng, X., & Liu, J. (2020). Household transmission of SARS-CoV-2, Zhuhai, China, 2020. *Clinical Infectious Diseases, 71*(16). https://doi.org/10.1093/cid/ciaa557.