Environmental and Aerosolized SARS-CoV-2 Among Hospitalized COVID-19 Patients

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Summary:

In studying 20 hospitalized COVID-19 patients, we found the highest prevalence of SARS-CoV-2 RNA among patients' nasopharyngeal and saliva samples (high correlation), but also RNA on fomites and in room air. However, only two nasopharyngeal swabs were culture-positive.

Abstract

During April and May 2020, we studied 20 hospitalized COVID-19 patients, their hospital rooms (fomites and aerosols), and their close contacts for molecular and culture evidence of SARS-CoV-2 virus. Among the more than 400 samples, we found molecular evidence of virus in most sample types, especially the nasopharygeal (NP), saliva, and fecal samples, but the prevalence of molecular positivity among fomites and aerosols was low. The agreement between NP swab and saliva positivity was high (89.5%, Kappa 0.79). Two NP swabs collected from patients on one and seven days post-symptom onset had evidence of infectious virus (2 passages over 14 days in Vero E6 cells). In summary, the low molecular prevalence and lack of viable SARS-CoV-2 virus in fomites and air samples implied low nosocomial risk SARS-CoV-2 transmission through inanimate objects or aerosols.

Key words: COVID-19, SARS-CoV-2, aerosol, transmission, epidemiology

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly spread across the globe since its likely origins in China during late 2019.[1] Many infection control and physical distancing efforts have been employed to stem this transmission.[1] SARS-CoV-2 transmission by fomites has been assumed, based on experimental and observational studies, alongside assumptions that SARS-CoV-2 behaves similarly to other respiratory viruses.[2-5] Similarly, an existing body of observational coronavirus disease 2019 (COVID-19) studies and related experimental research work suggests that SARS-CoV-2 is transmitted by aerosols and may travel greater distances than two meters, similar to SARS-CoV-1 in 2003/04.[6-11] Further, fecal-oral transmission has been deemed possible as well.[12]

However, more information is needed to clearly define the above mentioned transmission pathways, particularly in clinical settings where the protection of health care professionals is critical while personal protective gear is in shortage. Towards this end, we collected, screened and cultured biological, environmental and aerosol samples from hospitalized COVID-19 patients and their close contacts.

Methods

Study Population

This study was approved by Duke University IRB (Protocol 00105055). Between April 10th and May 22nd, 2020, hospitalized patients at Duke University Hospital (DUH) with either a positive SARS-CoV-2 molecular test or COVID-19 symptomatology (n=20) were invited to participate in the study (Figure S1). Close contacts of COVID-19 patients were also encouraged to participate. COVID-19 patients and close contacts were asked to permit the collection of a

nasopharyngeal (NP) swab, complete a brief questionnaire and to provide a saliva and a self-collected rectal swab sample. The same biological samples and a brief symptom questionnaire were again collected 14 and 28 days post-enrollment from COVID-19 patients, and 21 days post-enrollment from their close contacts. Environmental swabs and aerosol samples were collected in COVID-19 patient rooms.

Environmental conditions

The COVID-19 patient rooms sampled during this study were all single occupant rooms without a connected bathroom or toilet. Instead, in-room portable toilets (commodes) were available for patients (S2). Measurements performed in a representative empty DUH patient room on the COVID-19 ward determined that air inside the room was exchanged ~14 times every hour. This is higher than the American Society of Heating, Refrigerating and Air-Conditioning Engineers recommended ventilation (6 air exchanges per hour for recovery rooms or 12 air exchanges per hour for airborne infection isolation). There was no scheduled cleaning performed in rooms while COVID-19 patients were occupying them. Deep cleaning of the rooms and sanitation procedures such as disinfecting floors and surfaces with bleach solution and UV light emitter treatment for 45mins were performed between patients. However, there was likely minimal surface cleaning performed by health care providers as needed during visits and procedures.

Aerosol sampling

Detailed descriptions of the molecular and viral culture methods are provided in the supplementary appendix. Briefly, eight National Institute for Occupational Safety and Health (NIOSH) BC 251 Aerosol Samplers (Figure S3) were placed 1.5m from the ground, at ~1 meter, ~1.4 meters, ~2.2 meters, and ~3.2 meters from the SARS-CoV-2 patient's head (Figure 1), and subsequently run for ~4 hours.[13] Per our previous publications,[14-16] NIOSH samplers were calibrated to a flow-rate of 3.5 L_{air}/min before the sampling event, each sampling approximately 840 L_{air} during the 4 hours. The selected flow-rate represents typical human inhalation rates, ranging between 3-13 L_{air}/min during rest or light intensity activities.[17] The NIOSH samplers separated particles by three sizes: >4 µm, 1-4 µm, and <1 µm in 15 mL tubes, 1.5 mL centrifuge tubes, and polytetrafluoroethylene (PTFE) filters, respectively (Figure S3).

Biological and environmental swab sample collection

Detailed descriptions of the molecular and viral culture methods are provided in the supplementary appendix. Briefly, NP swabs were collected with BD™ Universal Viral Transport Standard Kits (BD, Franklin Lakes, NJ) containing a 3 mL VTM vial with scored and spun sterile regular polyester-tipped plastic swabs using standard clinical procedures. Study participants were asked to collect ~2 mL passive drool into a 50 mL conical tube, which was then diluted 2:1 with PBS (with 0.5% BSA) in order to preserve potential virus contained in the sample and to facilitate further processing. When possible, a self-collected rectal swab was obtained from study subjects by providing either a sterile FLOQSwab® (Copan, Avenue Murrieta, CA) or sterile BD™ swab and 1.5 mL VTM (Redoxica, Little Rock, Arkansas). The

following environmental swab samples from patient rooms were collected with pre-wetted FLOQSwabs® and placed in 1.5 mL VTM: toilet seat and interior of toilet bowl, TV remote control, cell phone, bed railing (left and right) and bed tray (Figure S2).

Environmental sampling controls

Fomite and aerosol sampling was conducted in an empty hospital room (no patient contact for four days) in the DUH COVID-19 ward which had been disinfected by bleach solution wipe downs and UV light treatment for 45 minutes. We also performed aerosol sampling in the COVID-19 hospital ward hallway with two aerosol samplers outside of an occupied patient room and two samplers outside of an empty hospital room that had been disinfected, both 1.5 meters from the ground. On the same day, environmental swabs from the hospital ward break room and head nurse and physician workstation were collected.

SARS-CoV-2 RT-PCR

Detailed descriptions of the molecular and viral culture methods are provided in the supplementary appendix. Briefly, viral RNA was extracted from clinical samples using QIAamp Viral RNA Mini Kits (QIAGEN, Hilden, Germany). We adapted the US Centers for Disease Control and Prevention's (CDC) 2019-nCoV real-time RT-PCR assay[18] protocol targeting the viral nucleocapsid (N) gene. For quality control, we ran all RT-PCR assays twice, used positive and negative controls, and a separate laboratory replicated a subset of results.

Vero E6 Cell Inoculation

Detailed descriptions of the culture methods are provided in the supplementary appendix. Briefly, SARS-CoV-2 virus culture work was performed in a biosafety level 3 laboratory at the Duke Regional Biocontainment Laboratory. Specimens with molecular evidence of SARS-CoV-2 infection were inoculated onto Vero E6 cells in two passages by transferring 250 µl of supernatant at 7 days post-inoculation for a total 14 days of incubation. Cells were monitored for cytopathic effect (CPE) every 48 hours. The cells and supernatant harvested 14 days post-inoculation were screened for SARS-CoV-2 by molecular assay.

Infectious SARS-CoV-2 was confirmed when (i) CPE was detected in inoculated wells and (ii) SARS-CoV-2 was detected in inoculated wells by real-time RT-PCR, at least two Cts below the original sample.

The SARS-CoV-2 isolate USA-WA1/2020 (BEI Resources, Manassas, VA) was used as the positive control in these passages. Mock infections, performed with media only, were maintained in parallel throughout the observation period.

Statistical Methods

Detailed descriptions of the statistical methods are provided in the supplementary appendix. All analyses were conducted in R, version 4.0.2.

Results

Study population

Among the 20 SARS-CoV-2 hospitalized patients enrolled, 10 (50.0%) were female, eight (40.0%) were Caucasian, eight (40.0%) were African American, three (15.0%) were Hispanic, and one (5.0%) was Native American. Ages ranged from 29 to 91 years, while the median age was 50 years. The majority (n=15, 75.0%) had self-reported pre-existing illnesses such as chronic respiratory conditions

(asthma, chronic bronchitis or emphysema), hypertension, diabetes, cancer, or liver disease (Table S1). Six (30.0%) were living in institutions prior to hospitalization: four in prison and two in a nursing facility. All others lived in private homes. On average, the COVID-19 patients were enrolled 10 days post symptom onset (DPSO; STDV 7.6 days, range 1-34 days). Among the 20 hospitalized patients, six (30.0%) completed both follow-up visits (14 and 28 days post-enrollment) and two (10.0%) completed one follow-up visit (14 days post-enrollment).

Six close contacts (four females and two males) were enrolled. All six were Caucasian and two were minors. All completed their follow-up visit 21 days post-enrollment (Figure S1).

Molecular results

A total of 109 biological, 112 fomite and 195 aerosol samples were collected and screened for SARS-CoV-2 RNA from the 20 hospitalized patients and their six close contacts during the enrollment visit and subsequent follow-up visits, along with the samples collected on the hospital ward.

Among the 20 COVID-19 patients, all (100%) provided a saliva specimen, 19 (95.0%) permitted a NP swab collection, and 12 (60.0%) provided a rectal swab during the enrollment visit. Of those, 12 (60.0%) saliva, 11 (57.9%) NP swabs, and 3 (25.0%) rectal swabs were SARS-CoV-2 RNA-positive by real-time RT-PCR (Table 1).

The agreement between NP swab and saliva positivity was substantial (89.5%, Kappa: 0.79). The agreement between NP swab and rectal swab positivity was moderate (81.8%, Kappa: 0.49). Similarly, the agreement between rectal swab and

saliva positivity was moderate (81.8%, Kappa: 0.49), see Tables S2, S3 and S4.

Among the environmental swabs taken from the 19 patient rooms, five rooms (26.3%) had one or more positive fomite such as toilet bowl, bed railing (twice), TV remote (twice), bed tray and cell phone (Cts ranged from 36.4 - 39.8), see Table 1 and Table S5. The SARS-CoV-2 RNA-positive toilet corresponded to a patient with a positive rectal swab from the same visit. Overall, each positive fomite corresponded to a patient with a positive saliva sample. However, not every patient with positive saliva also had a positive fomite. Further, a univariable logistic regression model indicated that the predicted probability of having any positive sample (biological, fomite, or aerosol) at enrollment decreased from 0.78 (95% C.I.: 0.37 – 0.96) at 1 DPSO to 0.05 (95% C.I.: 0.001 – 0.77) at 28 DPSO, corresponding to a per-day odds ratio of 0.86 (95% C.I.: 0.70 – 1.05), see Figure S4.

Among the aerosol samples collected in 20 patient rooms during enrollment, three patients (15.0%) had one positive aerosol sample (Table 1 and Table S5). Patient number six was four DPSO (runny nose, headache and fever) when SARS-CoV-2 RNA was detected in aerosols particle size <4 µm at ~1.4 meters from the head of her bed. The concomitantly collected NP swab and saliva samples were SARS-CoV-2-positive as well. No SARS-CoV-2 RNA was detected on the fomites that were sampled in her room. She was not receiving remdesevir upon enrollment.

Patient number nine was 10 DPSO (cough, difficulty breathing, fatigue, loss of smell and gastrointestinal symptoms) when SARS-CoV-2 RNA was detected in aerosols particle size <4 µm at ~2.2m from the head of her bed. The concomitantly collected saliva sample was SARS-CoV-2 positive. SARS-CoV-2 RNA was also detected on her bed tray. She had received one treatment of remdesivir upon

enrollment.

Patient number 16 was six DPSO (cough, difficulty breathing, fatigue) when SARS-CoV-2 RNA was detected in aerosols particle size >4 µm at ~2.2m from the head of her bed. The concomitantly collected NP swab and saliva samples were SARS-CoV-2 positive. No SARS-CoV-2 RNA was detected on the fomites that were sampled in this room. She was participating in a blinded randomized clinical trial and had therefore received antivirals.

Cell culture results

All SARS-CoV-2 RNA-positive biological, fomite, and aerosol samples collected from COVID-19 patient enrollment were screened for infectious virus through cell culture (Cts ranged from 19.4 to 39.8). Among these, the two samples with the lowest Ct counts demonstrated viable virus in culture (Table S5). Hence, two enrollment NP swabs showed CPE during the second passage (Figure 2 and Figure 3) and the harvested inoculates were SARS-CoV-2 real-time RT-PCR-positive (the Cts were on average 6 counts lower than the original samples), indicating viral growth in culture. The enrollment NP swabs had been collected from COVID-19 patient number two and 19 at one and seven DPSO, respectively (Table S5).

Among the 20 COVID-19 patients, 33 follow-up samples were collected (14 NP; 14 saliva; five rectal swabs). One sample (saliva) tested positive for SARS-CoV-2 at the second follow-up visit (25 DPSO) but failed to yield evidence of infectious virus.

Close contacts

All close contacts (family members of two COVID-19 patients) had NP and saliva samples collected during the enrollment and the follow-up visit (21 days post-enrollment). One close contact was confirmed for SARS-CoV-2 infection by NP swab (6 DPSO, saliva from the same visit was negative) during enrollment while displaying COVID-19 symptoms. This SARS-CoV-2-positive NP sample was inoculated and failed to yield evidence of infectious virus. Four close contacts developed COVID19-like symptoms but did not test positive for SARS-CoV-2 during enrollment or the follow-up visit.

Control samples

COVID-19 ward (nurses break room, head nurse workstation, physician work stations, and aerosol samples from the hospital ward hallway) and similar samples collected from a clean hospital room (no patient for four days and disinfected by bleach solution wipe downs and UV light emitter treatment for 45mins) were SARS-CoV-2 real-time RT-PCR negative.

Discussion

In contrast to several previously published reports,[4,19-21] we found sparse evidence of SARS-CoV-2 among fomite and aerosol samples collected from COVID-19 patients at the DUH hospital ward, despite relatively high SARS-CoV-2 prevalence among biological samples. Where molecular evidence of virus was detected, the Ct values were high (median 35.3 Ct), suggesting that while specific SARS-CoV-2 nucleic acid was present, the quantities were low. Furthermore, no

infectious virus was cultured from fomite or aerosol samples. Importantly, 10 (50.0%) of our study participants were enrolled eight days or more into their illness, a cut point after which others have found it difficult to culture viable virus from patient samples and after which viral shedding may be cleared.[22-25,23,26]. Interestingly, the agreement between NP swab and saliva positivity was high, indicating that passive drool may be a valuable non-invasive sample for SARS-CoV-2 detection.

There has been considerable debate regarding the risk of SARS-CoV-2 transmission through contact with fomites and inhalation of aerosols.[27,28] SARS-CoV-1, genomically almost identical to SARS-CoV-2,[29] is thought to be transmissible through aerosols,[30] and SARS-CoV-2 has been shown to remain viable for up to 16 hours in aerosols,[31] supporting potential airborne transmission. Interestingly, cats may be infected by aerosolized SARS-CoV-2,[32] but not humanized ACE2 mice.[33] Moreover, observational studies support potential aerosol transmission of SARS-CoV-2, as exemplified by transmission during a choir practice outbreak in Washington[34] and a restaurant in China.[35] Finally, several other studies conducted in clinical settings found high viral RNA positivity in fomite and aerosol samples and evidence of infectious virus has been implied in aerosol samples.[4,6,9-11,19,20] In contrast, only three out of 195 (1.5%) aerosol samples had detectable SARS-CoV-2 RNA in our clinical setting, and none showed evidence of viable virus. Similarly, a hospital study conducted in Wuhan, China observed no SARS-CoV-2 RNA in 44 aerosol samples and a strikingly low positivity among fomites (1.3%).[36] A hospital study including three patients in Singapore also failed to detect SARS-CoV-2 RNA in aerosols,[21] although a follow up study later detected it in two COVID-19 patient rooms in the same facility.[6] As

for captured droplet size, the NIOSH sampler has roughly a 95% collection efficiency for aerosols with a diameter of 7 micrometers or less, which decreases to approximately 40% efficiency for aerosols ~80 micrometers in diameter. Hence, smaller aerosolized particles were more likely to be captured by our study as compared to larger ballistic droplets. However, surfaces, likely contaminated by larger droplets near the patient, were sampled via the surface swabbing. Importantly, temperature, humidity and airflow affect the spread of the virus and likely also infectivity. Hence, additional bioaerosol and viability studies should be conducted in order to determine which factors affect the viability and infectivity of SARS-CoV-2 in clinical versus non-clinical settings in order to extrapolate our findings to other clinical or non-clinical settings.

Interestingly, infectious virus was isolated from NP swabs up to seven DPSO, confirming potential transmission beyond a week. While SARS-CoV-2 RNA was detected in rectal swab samples from patients at one, 11 and 20 DPSO, none of these samples had evidence of infectious virus in culture.

As for the longitudinal arm of the study, only one saliva sample was SARS-CoV-2-positive at 25 DPSO. Interestingly, this patient's saliva was SARS-CoV-2-negative during enrollment two weeks earlier. While most patients were caught late during symptom onset, the agreement between NP swab and saliva positivity was high, making passive drool samples a valuable non-invasive alternative for SARS-CoV-2 diagnostics.[37]

The study had a number of limitations. As has been previously documented, viral loads are highest shortly after symptom onset and the median length of time from infection until viral clearance is estimated to be nine days.[22-25] Hence, it is plausible that a number of the studied COVID-19 patients were not shedding

virus as readily as they were early in their disease course. It is also possible that therapeutic interventions may have reduced virus transmission as eight (40.0%) of subjects were co-enrolled in clinical trials. We also acknowledge that we used dry cyclone aerosol samplers, which are not as well-suited for viable virus collection when compared to liquid collection medium-based bioaerosol samplers as exemplified by a study capturing infectious SARS-CoV-2 2 and 4.8 meters from patients with the VIVAS air samplers that operate on a gentle water-vapor condensation principle.[10] Further, while the long bioaerosol sampling time (4 hours) intended to capture the temporal variability of viral emissions from the patients, it may also have decreased the viability of infectious virus.[13] However, we have previously captured viable virus using the NIOSH samplers, albeit in settings with high levels of viral contamination, such as live bird markets in Vietnam and China.[38,39] We also acknowledge that the study has a relatively small sample size and the patients were not randomly selected. Hence patients may not be representative, with regard to risk of transmission, in comparison to hospitalizations worldwide. Finally, patients' rooms had relatively high air exchanges. The engineering controls (i.e. high ventilation rates) may have diluted the virus below the limit of detection and may not represent the environmental conditions of other medical centers.

While our findings support the position that fomite and aerosol transmission were of relatively low risk among these COVID-19 patients, our results may also be consistent with aerosol transmission occurring early on in the course of disease, well before COVID-19 patients are ill enough to require hospitalization. We further conclude that fomite and aerosol transmission should be assessed soon after disease onset.

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Table 1. Real-time RT-PCR Results at Enrollment Among Hospitalized COVID-19 Patients, sorted by days post-symptom onset.

	Patient Characteristics			Biological Samples*			Fomites*					Aerosol Samples* (approximate distance from head of bed over aerosol particle size)							
ID	DPSO	Gender	Age (yrs)	NP	Rectal	Saliva	Toilet†	Tray	Bed Rails‡	TV remote	Cell Phone	1m <4µm	1m >4µm	1.4m <4µm	1.4m >4µm	2.2m <4µm	2.2m >4µm	3.2m <4µm	3.2m >4µm
19	1	M	66	+	+	+	.	–	–	–	.	–	–	–	–	–	–	–	–
12	2	M	61	+	.	+	–	–	–	–	.	–	–	–	–	–	.	.	.
6	4	F	60	+	–	+	–	–	–	–	.	–	–	+	–	–	–	–	–
11	5	M	48	–	–	–	.	–	–	–	.	–	.	.	–	–	–	–	–
18	5	F	52	+	.	+	–	–	–	–	+	–	–	–	–	–	–	–	–
14	6	F	30	–	–	–	–	–	–	–	–	–	–	–	–	–	–	.	–
16	6	F	56	+	.	+	.	–	–	–	.	.	–	–	–	–	+	–	.
2	7	F	91	+	.	+	–	–	+	+	.	–	–	–	–	–	–	–	–
8	7	M	81	+	.	+	–	–	+	+	.	.	–	.	–	–	–	–	.
15	7	F	46	–	.	–	–	–	–	–	.	–	–	–	–	–	–	–	–
17	8	M	43	+	–	+	–	–	–	–	–	–	–	.	–	–	–	.	–
9	10	F	44	.	.	+	–	+	–	–	–	.	–	–	–	+	–	–	–

1	11	F	29	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
3	11	M	33	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5	11	M	52	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
10	11	F	36	–	.	–	.	.	.	–	.	–	–	–	–	–	–	–
13	12	F	60	–	–	–	–	–	–	–	.	–	.	.	–	–	–	–
4	20	M	48	+	+	+	.	.	.	.	.	–	–	–	–	–	–	–
7	21	M	47	–	–	+	–	–	–	–	.	–	–	–	–	–	–	.
20	34	M	52	–	.	–	–	–	–	–	.	–	–	–	–	–	–	–

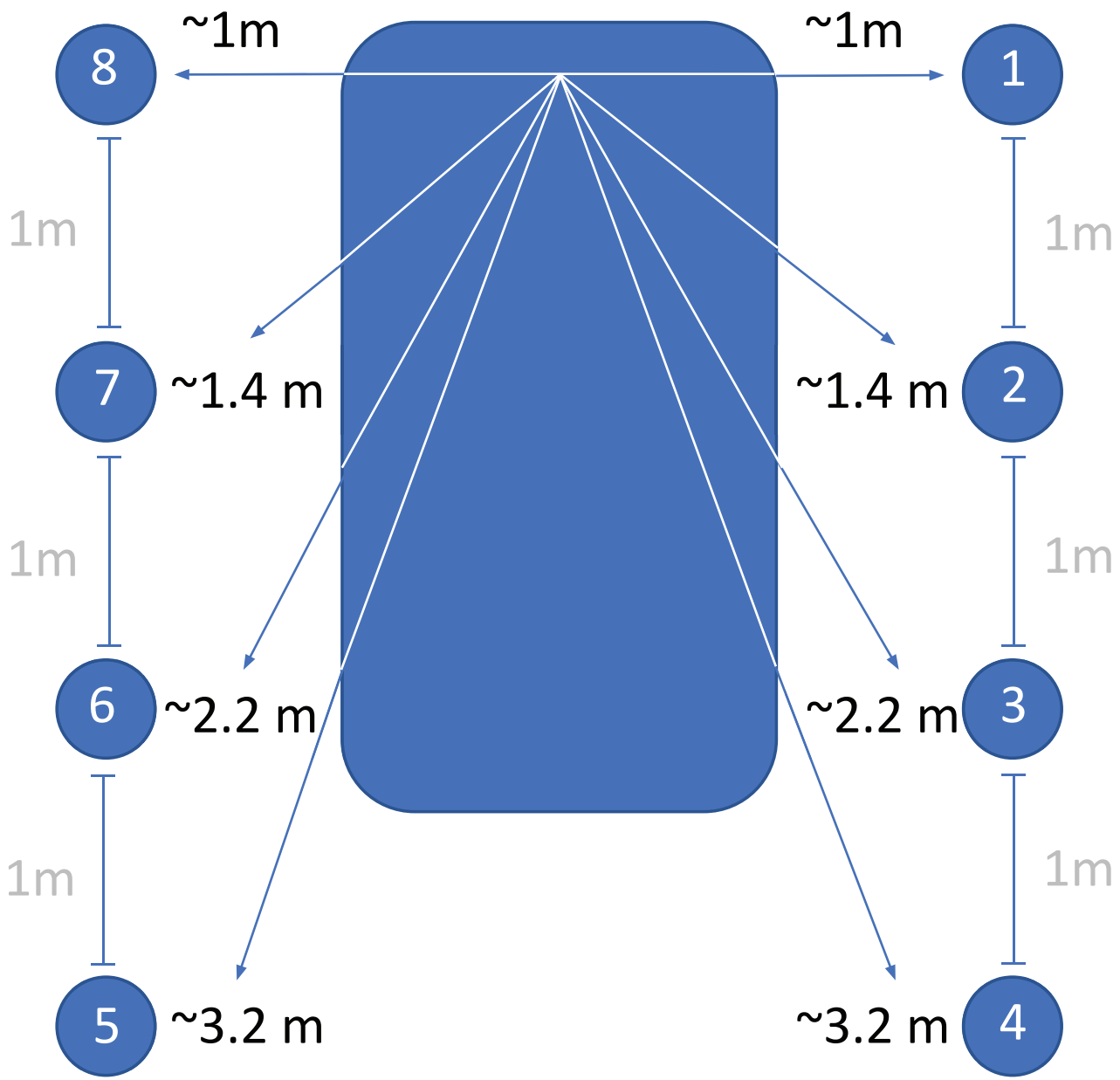
ID = patient number; [*](-) negative sample; (+) positive sample; and (.) sample not available. DPSO = days post-symptom onset [†] Positive when either seat or toilet or both are SARS-CoV-2 real-time RT-PCR-positive. [‡] Positive when either left or right or both bed rails are SARS-CoV-2 real-time RT-PCR positive.

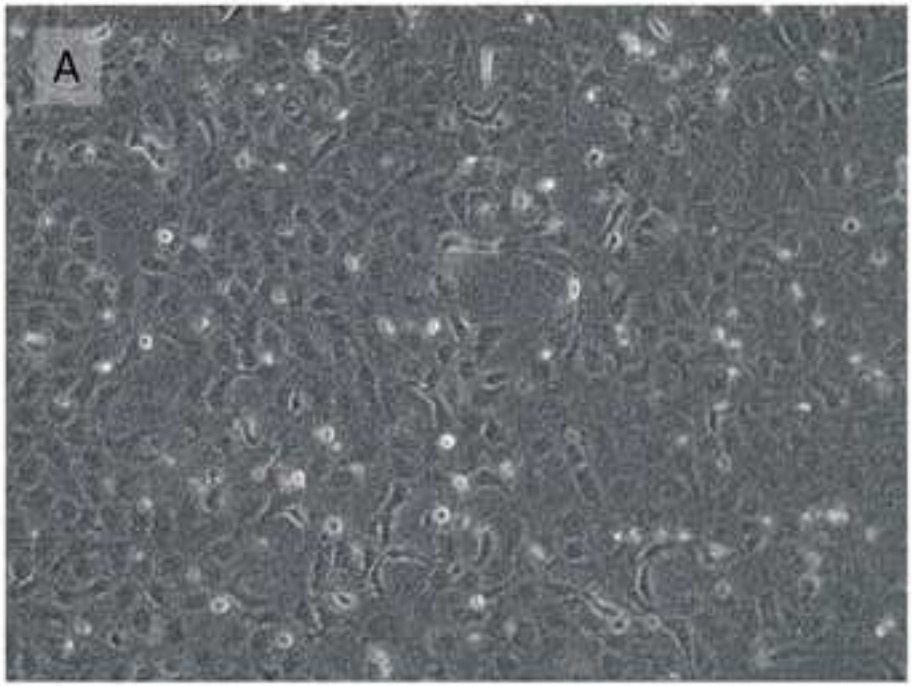
Figure Legends

Figure 1. Diagram of aerosol sampler placement around patient bead.

Figure 2 Vero E6 cells inoculated with SARS-CoV-2 real-time RT-PCR-positive samples. (A) Vero E6 cells inoculated with SARS-CoV-2 isolate USA-WA1/2020 (positive control), 200x. (B) Mock-infected Vero E6 cells, 200x. (C) Vero E6 cells inoculated with nasopharyngeal (NP) swab sample from patient number 2 (N1 Ct =20.2, N2 Ct =20.9, 7 DPSO), 200x. 168 hours post-infection, second passage.

Figure 3. Vero E6 cells inoculated with SARS-CoV-2 real-time RT-PCR-positive samples Vero E6 cells inoculated with SARS-CoV-2 isolate USA-WA1/2020 (positive control), 200x. (B) Mock-infected Vero E6 cells, 100x. (C) Vero E6 cells inoculated with nasopharyngeal (NP) swab sample from patient number 19 (N1 Ct =19.9, N2 Ct =19.4, 1 DPSO), 200x. 168 hours post-infection, second passage.


8
~1m
~1m
1
1m
1m
7
~1.4 m
~1.4 m
2
1m
1m
6
~2.2 m
~2.2 m
3
1m
1m
5
~3.2 m
~3.2 m
4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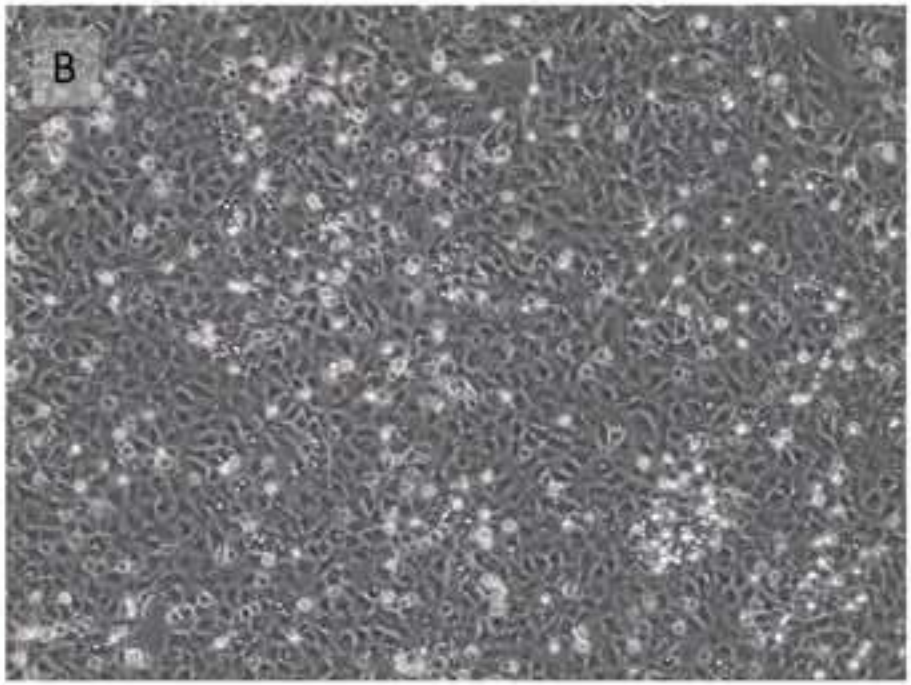
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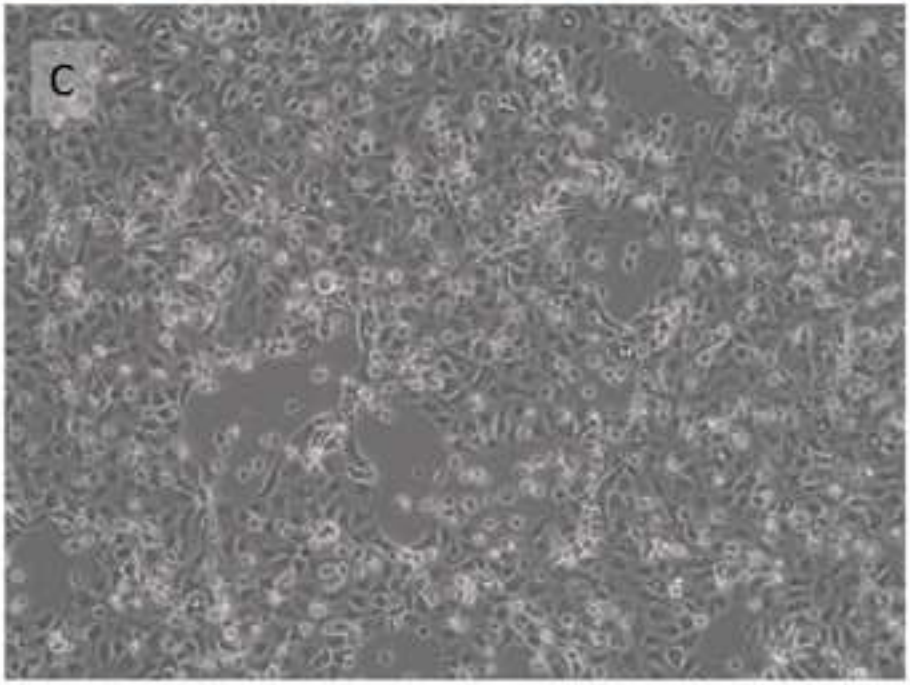
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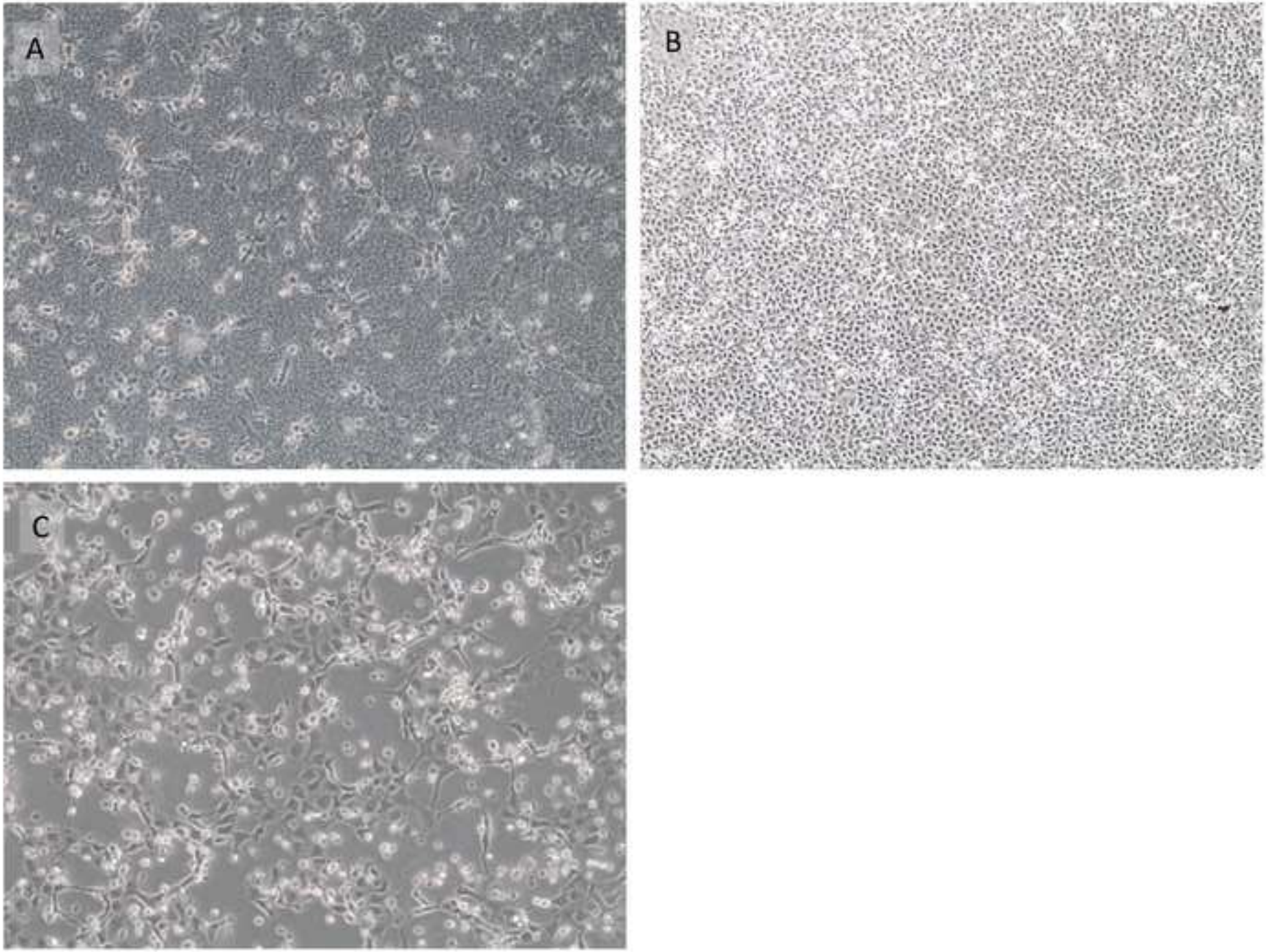
A
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