

Bioaerosol Sampling for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in a Referral Center with Critically Ill Coronavirus Disease 2019 (COVID-19) Patients March–May 2020

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Background. Previous research has shown that rooms of patients with coronavirus disease 2019 (COVID-19) present the potential for healthcare-associated transmission through aerosols containing severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). However, data on the presence of these aerosols outside of patient rooms are limited. We investigated whether virus-containing aerosols were present in nursing stations and patient room hallways in a referral center with critically ill COVID-19 patients.

Methods. Eight National Institute for Occupational Safety and Health BC 251 2-stage cyclone samplers were set up throughout 6 units, including nursing stations and visitor corridors in intensive care units and general medical units, for 6 h each sampling period. Samplers were placed on tripods which held 2 samplers positioned 102 cm and 152 cm above the floor. Units were sampled for 3 days. Extracted samples underwent reverse transcription polymerase chain reaction for selected gene regions of the SARS-CoV-2 virus nucleocapsid and the housekeeping gene human RNase P as an internal control.

Results. The units sampled varied in the number of laboratory-confirmed COVID-19 patients present on the days of sampling. Some of the units included patient rooms under negative pressure, while most were maintained at a neutral pressure. Of 528 aerosol samples collected, none were positive for SARS-CoV-2 RNA by the estimated limit of detection of 8 viral copies/m³ of air.

Conclusions. Aerosolized SARS-CoV-2 outside of patient rooms was undetectable. While healthcare personnel should avoid unmasked close contact with each other, these findings may provide reassurance for the use of alternatives to tight-fitting respirators in areas outside of patient rooms during the current pandemic.

Keywords. COVID-19; airborne transmission; aerosols; hospital.

As coronavirus disease 2019 (COVID-19) continues to surge in the United States and around the globe, healthcare personnel (HCP) remain at risk of contracting the disease through occupational exposure from both their patients and co-workers. Reports as of September 28, 2020 from the Centers for Disease Control and Prevention (CDC) estimate around 275 000 COVID-19 cases among HCP in the United States [1]. While infection prevention and control recommendations have been based on available data, there is still more to learn about severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) transmission in healthcare facilities [2].

It is thought that SARS-CoV-2, the virus that causes COVID-19, is spread primarily through droplets and possibly by smaller aerosols, and may be spread by fomites and contact transmission to the respiratory tract and mucous membranes [3–6]. SARS-CoV-2 has been detected in air samples and on surfaces in COVID-19 patients' rooms and in areas where personal protective equipment (PPE) is removed, although these studies have primarily identified SARS-CoV-2 RNA, and virus infectivity has been estimated to be very low based upon viral culture [6–15]. Ong et al. (2020) found that disinfection of patient rooms, specifically daily disinfection of the floors using 1000 ppm of sodium dichloroisocyanurate and twice-daily disinfection of high-touch surfaces using 5000 ppm of the same cleaner, reduced SARS-CoV-2 RNA in aerosols and on surfaces in patient rooms [16, 17]. Li et al. (2020) similarly found that only 2% of surface and air samples from the sampled hospital were positive when tested 1 h after routine cleaning, which included twice-daily disinfection of high-touch surfaces (using 500 mg/L

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sodium dichloroisocyanurate) and floors (using 1000 mg/L sodium dichloroisocyanurate), as well as 4-times-daily air disinfection using a plasma air sterilizer (Laoken Medical Technology Co.) [17]. Two other recent publications indicated that patient room hallways had low detection of SARS-CoV-2 in air samples [10, 13]. HCP working in areas outside of patient rooms may not always be wearing the PPE recommended for in-room patient care, depending on the infection prevention and control protocols of their facility and the availability of supplies.

Given the number of HCP diagnosed with COVID-19 in the United States and worldwide, a better understanding of HCP exposures to SARS-CoV-2 in healthcare facilities is needed to inform infection prevention and control protocols. This study sought to further investigate the presence of SARS-CoV-2 in hospital nursing stations, COVID-19 patient hallways, and visitor corridors throughout a tertiary referral hospital in Atlanta, GA, USA.

METHODS

Bioaerosol Sampling

Eight National Institute for Occupational Safety and Health (NIOSH) BC 251 2-stage cyclone samplers were used for each day of sampling (see [Supplementary Figure 1](#) for photograph and diagram) [18]. The NIOSH samplers separate particles into 3 size fractions, which are collected in a 15 mL centrifuge tube ($<4\ \mu\text{m}$), a 1.5 mL centrifuge tube ($1\text{--}4\ \mu\text{m}$), and on a filter cassette containing a 37-mm diameter, polytetrafluoroethylene filter with $2\ \mu\text{m}$ pore size ($<1\ \mu\text{m}$). Each sampler was connected with a 6.35-mm Tygon tubing to a sampling pump (PCXR-4, SKC, Eighty Four, PA) at a 3.5 L/min flow rate. We obtained positive control samples from 2 patient rooms, which contained individuals infected with SARS-CoV-2, utilizing the same sampling methodology employed throughout the rest of the study.

Two samplers were placed 102 cm and 152 cm above the floor on each of 4 tripods. Throughout the sampling period, we sampled nursing stations in intensive care units (ICUs), family/visitor corridors outside of ICUs, and medical unit patient room hallways. All of the units sampled were designated for patients with COVID-19 or those with symptoms awaiting results. The family/visitor corridors were used as a “warm zone” or “bridging zone” and were not allowing visitors at the time; HCP were using them to move between rooms in some of the units. These units are composed of between 7 and 20 single patient rooms that were staffed in a 2:1 ratio if an ICU, and a 4:1 ratio if a medical/surgical unit. All nurses were required to wear face masks, either procedural or cloth masks (homemade, hospital-issued, etc.), in the areas that were sampled. Patients were not required to wear face masks in their rooms, but were required to wear them if they entered the hallway. No other PPE was required outside of patient rooms. Rooms in two of the six units were maintained at a negative pressure ([Supplementary Figures 5 and 7](#)). One of the negative

pressure units also has 3 rooms with anterooms in between the patient room and the hallway where the samplers were placed ([Supplementary Figure 5](#)). Most units were sampled for 3 days, with the samplers placed in the same locations each day ([Table 1](#)). We collected data on the days since illness occurred within a range of 1–50 days onset for each of the patients in the rooms nearest to the air samplers. Some units were not sampled for the full 3-day period because they were no longer accepting COVID-19 patients or were inaccessible at the time of sampling, and one was sampled for 4 days due to other activities occurring in the hospital, preventing other units from being sampled. The samplers were run for 6 h each sampling period, which was always at the same time of day. The sampling period began around the time of the morning shift change and ended before the next shift change.

Over 22 days, we sampled 6 units—in either the nursing station, patient room hallway, or visitor corridor ([Supplementary Figures 2–9](#)). Notes on the physical characteristics of the patient care units (location of samplers, presence of negative pressure rooms) are listed in the figure legends. Due to a shortage of supplies for COVID-19 nucleic acid amplification testing during the pandemic, no sampling took place for a 2-week period between sampling days 10 and 11.

Laboratory Methods

At the end of the 6 h sampling period, all aerosol samples were immediately stored at $-80\ ^\circ\text{C}$ in the laboratory where the samples were processed. Collected samples were processed in a BSL Class II biosafety cabinet as follows: 1) 1000 μL viral transport medium (Copan UTM) or phosphate buffered saline (PBS) was added to the 15 mL tube, the tube was vortexed, inverted, and vortexed again, then frozen at $-80\ ^\circ\text{C}$; 2) 400 μL viral transport medium or PBS was added to the 1.5 mL tube, the tube was vortexed, inverted, and vortexed again and frozen at $-80\ ^\circ\text{C}$; 3) sterile forceps were used to remove the filter from its cassette and place it in a 15 mL tube; 1000 μL of viral transport medium or PBS was added to wet the entire filter, and the tube was vortexed and stored at $-80\ ^\circ\text{C}$. RNA extraction occurred on either 1) the *m2000* (Abbott Molecular, Abbott Park, IL) with 600 μL of input sample volume, and sample elute of 50 μL , or 2) an *eMag* instrument (bioMerieux, Durham, NC) with 1000 μL of input sample volume and a sample elute of 50 μL . The extracts were then frozen at $-20\ ^\circ\text{C}$ or colder until amplification.

The extracted samples were thawed and then underwent reverse transcriptase polymerase chain reaction (rRT-PCR) for selected gene regions of the SARS-CoV-2 virus nucleocapsid (N1, N2, N3) and human RNase P gene [19] using a protocol adapted from CDC [20, 21] ($n = 309$) or by a triplex laboratory developed test (LDT) targeting N2 and the envelope (E) gene of SARS-CoV-2 and the RNase P gene (PMC7323516) ($n = 219$) based on reagent availability. Because we were not likely to consistently detect human cellular material in this study, and therefore the RNase P gene was not needed for this study, the results of

Table 1. Sampling Location, Sampling Day, Patient Characterization and Results of NIOSH Samplers

Sampled Unit	Sampling Day #	SARS-CoV-2 Detected (\pm total)	Number of Positive Patients in Unit/ Number of Rooms in Unit	Patients Waiting on SARS-CoV-2 Results	Number of SARS-CoV-2 Positive Patients in Hospital
ICU 1 Nursing Station	1	0/24	12/20	1	55
	4	0/24	13/20	2	53
ICU 1 Visitor Corridor	2	0/24	14/20	1	49
	3	0/24	13/20	1	41
	11	0/24	1/20	0	30
	12	0/24	0/20	0	30
Medical Unit 1 Hallway	5	0/24	8/11	2	40
	6 ^a	0/24	10/11	2	43
	13	0/24	8/11	0	28
Medical Unit 2 Hallway (Neg Pressure Rooms)	7	0/24	4/8	2	43
	8	0/24	5/8	2	44
	14	0/24	6/8	1	26
ICU 2 Hallway	9	0/24	12/24	2	43
	10	0/24	11/24	2	42
	19	0/24	4/24	0	23
ICU 3 Nursing Station	15	0/24	3/7	3	29
	16 ^a	0/24	4/7	3	25
	17 ^a	0/24	3/7	4	26
ICU 3 Visitor Corridor	18	0/24	3/7	4	23
ICU 4 Nursing Station	20	0/24	0/7	6	19
	21	0/24	1/7	4	17
	22	0/24	1/7	5	20

Abbreviations: ICU, intensive care unit; Neg, negative; NIOSH, National Institute for Occupational Safety and Health; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

^aFlow fault experienced on 2 samplers on sampling day 6 (1 mins), 2 samplers on day 16 (89 min) and 1 sampler on day 17 (2 min).

this test are not included in the results. Briefly, for samples tested by the modified CDC protocol, following RNA extraction, 20 μ L reactions were set up containing 5 μ L of sample RNA and 1 of 2 reaction mixes, based on reagent availability. The first reaction mixture was prepared with 8.5 μ L of nuclease-free water, 1.5 μ L of combined primer/probe mix, and 5 μ L of TaqPath™ 1-Step RT-qPCR Master Mix (Thermo Fisher, Waltham, MA). Thermal cycling was performed at 25 °C for 2 min, followed by 50 °C for 15 min, followed by an initial denaturation at 95 °C for 2 min, followed by 45 cycles of amplification at 95 °C for 3 s and 55.0 °C for 30 s (Thermo Fisher). The triplex LDT was performed as described (PMC7323516). A previously characterized SARS-CoV-2 sample was extracted and tested concurrently as a positive control on all runs. Exponential growth curves that crossed the threshold line within 40 cycles ($C_t < 40$) were considered positive. The limit of detection for the samples was 10 viral copies/mL.

RESULTS

Sampling was conducted during a period from March to May 2020. Over 22 days, 528 aerosol samples were collected. The samples were collected from locations within 6 COVID-19 units (medical units ($n = 2$) and ICUs ($n = 4$)), including patient hallways ($n = 3$), nursing stations ($n = 3$) and visitor corridors ($n = 2$). Each unit sampled varied in the number of hospitalized SARS-CoV-2-positive

patients or suspected COVID-19 patients waiting on confirmatory testing results on the day of sampling, from 0 to 15 (median = 4, $SD = 3$) (Table 1). Samplers were placed outside of doorways of patient rooms where patients who were positive for SARS-CoV-2, or who were awaiting confirmatory testing results, were located. The days since symptom onset for the patients in the rooms nearest to the samplers varied throughout the sampling. Throughout the 6 h sampling periods, there were approximately 5–10 nurses present in the ICUs and 3–5 present in the medical units. No samples were positive for SARS-CoV-2 RNA.

DISCUSSION

The current study sought to investigate the presence of SARS-CoV-2 in areas that have not yet been sufficiently researched. We sampled for aerosols containing the virus in nursing stations, patient room hallways, and visitor corridors throughout a referral center with critically ill COVID-19 patients. Over the 22 day period, all 528 aerosol samples collected were negative by rRT-PCR for SARS-CoV-2. Throughout this sampling period, the number of patients with COVID-19 or with suspected COVID-19 waiting for results in each of 6 units was changing daily. These results indicate that the amount of airborne SARS-CoV-2 outside of patient rooms was undetectable by a sensitive sampling technique in all of these cases.

In this study, any viral aerosols that were present inside of patient rooms were not escaping in large enough quantities to be detected in the hallways and nursing stations surrounding the rooms. Two of the units sampled included negative pressure patient rooms with some also having anterooms with negative pressure relative to the hallway, which were intended to prevent aerosols from within the room escaping into the hallway. Given the lack of viral aerosols detected in the units without negative pressure rooms, it appears that negative pressure may not have been necessary to prevent SARS-CoV-2 aerosols from escaping into the hallway. Alternatively, it is also possible that environmental cleaning combined with engineering controls was sufficient to reduce the dispersion of viral aerosols outside of patient rooms. Some of the sampling was conducted in ICUs, in which a large number of patients were mechanically ventilated. Given the results of our previous study, in which aerosols were not detected in an airborne infection isolation room of a ventilated patient, viral aerosols may not have even been present in the rooms of patients on mechanical ventilation and therefore were not detected outside of the room [22]. However, recent studies in the rooms of nonventilated patients have detected SARS-CoV-2 aerosols, so it is likely that they were present in the rooms of nonventilated patients [8, 11, 15]. Our positive control samples also detected viral aerosols in patient rooms within one of the units sampled in this study. It is also possible that the amount of viral aerosol within the patient rooms varied. The patients in the rooms nearest to the aerosol samplers varied in their duration of illness on the day of sampling, which may indicate that the amount of virus being shed by these patients was different [23]. Future studies will address these gaps in understanding.

Few aerosol sampling studies have been conducted outside of patient rooms during the COVID-19 pandemic, and those that were conducted present conflicting results. Liu et al. (2020) found that nonpatient room public areas did not have detectable levels of SARS-CoV-2 in aerosols as compared to inside patient rooms and in areas where PPE is doffed [9]. However, Santarpi et al. (2020) found that 58% of aerosol samples collected in patient room hallways were SARS-CoV-2 RNA positive [8]. Ding et al. (2020) sampled patient rooms, a nursing station, a corridor, an air-conditioning system, and other spaces of an isolation unit and found that all but 1 of the 46 samples were negative for SARS-CoV-2 [13]. The one “weakly” SARS-CoV-2 RNA positive sample, defined by the researchers to be a cycle threshold (CT) value between 37–38 as opposed to a “positive” sample with CT < 38, in that study was located in the corridor. Guo et al. (2020) sampled units next to air outlets and inlets, as well as in the doctor’s office areas, and found that aerosols with detectable SARS-CoV-2 RNA were present throughout these spaces, but that their presence decreased with increasing distance from the patient [24]. It is important to note that these studies conducted air sampling with a variety of air

samplers, including those that collect at a higher flow rate, using different collection methods and filters than the samplers used in the current study. Air samplers used in the other studies may therefore have a higher sensitivity for detection. Our air samplers were run for 6 h to account for the lower collection flow rate as compared to high-volume samplers; however, other differences between the samplers used may explain some of the differences in results.

It is important to note the limitations of our study. First, we cannot comment on the possibility of SARS-CoV-2 transmission within the areas sampled, because this study only measured aerosols by air sampling and did not examine other potential pathways for virus transmission. Further, the sample may have been too dilute, and the amount of SARS-CoV-2 RNA may have been below the limit of detection of 10 viral copies/mL. Aerosol concentrations within rooms and larger spaces can vary tremendously from location to location and are greatly affected by airflow patterns, which tend to be complex. An aerosol sampler only collects aerosols from the immediate vicinity of the sampler and thus indicates the aerosol concentration at a specific place but does not necessarily indicate the aerosol concentration at other locations. The extractions and PCR testing were also conducted using 2 different tests due to limited availability of supplies, but we do not believe this resulted in any false negatives because all tests have been validated for use with SARS-CoV-2 and were both modified from the same CDC protocol. It is also possible that some of the RNA may have degraded within the sampler during the 6-h collection period. However, a recent comparison study conducted by the National Biodefense Analysis and Countermeasures Center found that SARS-CoV-2 RNA did not degrade after being collected in the NIOSH BC 251 samplers [25]. Further chamber studies for aerosol sampling of SARS-CoV-2 could investigate the limit of detection, precision, and recovery efficiency for aerosol sampling methods. This will provide a better understanding of negative air sampling results.

Lastly, for practical reasons, we chose to use a convenience sample of sampling locations to minimize the burden on hospital staff. We received data on the number of SARS-CoV-2 positive patients in the units after the completion of sampling, which meant that units on 2 of the days we sampled did not have any patients with SARS-CoV-2 infection. We also do not have data on where patients were in their course of illness, so we do not know how much shedding was occurring in the rooms near the samplers. However, sampling in these units was still useful for understanding whether SARS-CoV-2 containing aerosols were present from other sources (ie, asymptomatic HCP, hospital ventilation).

While we are unable to comment on the possibility of SARS-CoV-2 transmission within patient room hallways and nursing stations, the lack of viral detection in aerosol samples has implications for policies surrounding the use of PPE in these areas. Throughout the sampling period, HCP in the

patient room hallways and nursing stations were wearing face masks—either surgical, procedural, or cloth masks. Research is ongoing as to the effectiveness of different types of masks for source control and as protection for the wearer. There are alternative options, such as N95s and other respirators that are known to provide more protection for the wearer [26]. However, given the results of this and some of the other aerosol sampling studies, it may not be necessary to turn to those alternatives outside of patient rooms.

The results of this study point to the effectiveness and importance of environmental controls (ie, ventilation, air exchange rate, cleaning) and PPE in controlling the presence of aerosols containing SARS-CoV-2 outside of patient-care areas. If aerosols containing the virus were present in the patient rooms, they were not detected in the hallways, and if anyone in the hallways was shedding asymptotically or pre-symptomatically, aerosols containing SARS-CoV-2 were not detected. However, given that we are continuing to learn about SARS-CoV-2, further work is needed to better define exposures to HCP and determine the optimal ways of reducing hospital-associated transmission risks. We are contributing to that work through studies aiming at other areas of the hospital, such as operating rooms, and using purposive sampling of high viral load, nonventilated patients to provide clearer context for sampling results.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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