

Indoor Air Quality and Environmental Sampling as Support Tools to Detect SARS-CoV-2 in the Healthcare Setting

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Objectives: To evaluate severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread inside the healthcare setting using environmental sampling and indoor air quality (IAQ) parameters. **Methods:** Ward/ICU rooms had IAQ parameters monitored in real-time, including volatile organic compounds and particulate matter. Surface and three air samples with different exposure times were collected in each room and tested for SARS-CoV-2 using quantitative Rt-PCR. Environmental sampling and IAQ data were compared to provide information about viral spread. **Results:** SARS-CoV-2 RNA was detected in 6/10 rooms and 9/30 air samples, which is proportionally higher than previous studies. Sampling time confirmed to be crucial to viral detection. No correlations between IAQ parameters could be associated with positive/negative samples even when aerosol-generating procedures were performed. **Conclusion:** Environmental sampling of SARS-CoV-2 RNA may be used as an indicator of occupational safety. IAQ is also a potential tool but requires further research.

Keywords: aerosol-generating procedures, environmental sampling, indoor air quality, particulate matter, viral spread

The potential for transmission of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is controversial, resulting in the adoption of different policies to stop viral transmission across the world.¹ The safety of health care workers has been a continuous worry, especially regarding poor ventilation indoors and aerosol-generating procedures (AGPs), which may contribute to viral spread.²

Multiple strategies to mitigate the risks of transmission have been adopted globally, but there is still a paucity of evidence addressing key questions, such as airborne transmissibility,^{3,4} that may be a consequence of the difficulty in analyzing a virus that is very sensitive to small environmental changes. In addition, before the coronavirus disease (COVID-19) outbreak, this subject was not studied deeply, with just a few authors pointing to different findings and with heterogeneous methodologies.³

Currently there is a need to comprehend aerosol dynamics and indoor air quality (IAQ) inside the healthcare setting, due to the

demand of producing appropriate data to guide medical practices in the current and future pandemics. Therefore, we aimed to evaluate IAQ parameters and the detection of SARS-CoV-2 ribonucleic acid (RNA) on air and surface samples collected in a COVID-19 dedicated hospital.

MATERIALS AND METHODS

This study was conducted in a 600-bed COVID-19 dedicated hospital in Brazil in 2020. Ward and ICU rooms had their environment sampled for SARS-CoV-2 RNA and were monitored with an IAQ device. The purpose was to the results in isolation and, also, to find possible relations between them (eg, levels of particulate matter versus positivity of air samples).

All units inside the institution have a ventilation system that operates with a ventilation rate between 162 m³/hour and 774 m³/hour, except for the ones with negative pressure rooms which were not considered for this experiment.

The following prerequisites were considered for room selection:

- The presence of a patient with positive Reverse Transcriptase-Polymerase Chain Reaction (Rt-PCR) for SARS-CoV-2, independently of the number of days since the last positive test or onset of symptoms;
- Evaluated rooms could not be physically adjacent;
- One bed only;
- Windows and doors had to be closed throughout the day, except for when the staff entered and left the space according to each patient's necessity

ICU rooms were selected based on the execution of any of the following AGPs that COVID-19 patients commonly need during their hospitalization including tracheostomy, bronchoscopy, chest tube, and the use of high-flow nasal cannula. Ward rooms were chosen randomly, despite any specific procedure.

IAQ Monitoring

A laser-based sensor (Spiri – Omni Electronica – São Paulo, Brazil) was positioned at a height of 1.2 m, close to the bed headboard, and was used to do real-time monitoring of the following IAQ parameters: temperature (°C), relative humidity (RH) (%), carbon dioxide (CO₂) (ppm), Total Volatile Organic Compounds (TVOC) (ppb), and particulate matter (PM) smaller than 1 μm (PM1.0), 2.5 μm (PM2.5), and 10 μm (PM10.0). Since aerosols are considered particles smaller than 5 μm, PM2.5 was used as the standard parameter for correlations considering the particulate matter. This device only analyses the environment physically and/or chemically, thus it cannot assess the air microbiologically. Other specifications are available in Supplemental Materials, <http://links.lww.com/JOM/A936>.

Air and Surface Sampling

Air sampling was performed using an instrument that was assembled as follows: a small vacuum pump was placed inside a plastic case and connected to a calibrated rotameter; the case had one vent, which had the side facing the interior of the case connected

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Clinical significance: using a simple device, SARS-CoV-2 RNA was detected in more than a half of the rooms; sampling/exposure time seems to be a crucial element in positive air samples; associations between indoor air quality and positive samples could not be found.

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to the pump and the side facing to the exterior connected to a silicon hose; on the other end of the hose it was attached to a 37-mm disposable cassette (Merck Millipore M000037A0 – Burlington, Massachusetts) loaded with a filter pad and a 37-mm hydrophobic polytetrafluoroethylene membrane with 0.45- μ m pores (Merck Millipore FHLP03700 – Burlington, Massachusetts). When the device was turned on, the air was suctioned from the room, passing through the cassette/membrane on the end of the rose at the rate set on the rotameter (5L/min).

Every part of the experimental instrument was disinfected with ethanol 70% before and after each sampling session and the membranes of the disposable cassettes and the swabs were stored individually to avoid contamination.

To verify if a room with virus-laden aerosol would result in contaminated surfaces inside those places, an area of 25 cm² (5 cm × 5 cm) over the cabinet next to the patient’s bed was swabbed at the end of each monitoring/air sampling.

Each sample was sent to molecular analysis using Rt-PCR for SARS-CoV-2. Results in this study are shown in copies/ μ L and in copies/L of air sampled (eg, flow rate of 5L/min during 2 hours = 600 L).

To test if different exposure times could result in different viral loads, three air samples were collected in the following

fashion: a long-exposition sample (LES) which was collected throughout the whole session (16 hours approximately); an intermediate-exposure sample (IES) that was installed in the beginning of the session and removed 8 hours later or before any planned AGP; a short-exposition sample (SES) which was installed just after the IES was removed for 2 hours. In ICU rooms, the SES was always performed simultaneously with the AGP to check if it could be a specific source of environmental contamination.

The sampling cassette was always positioned at a height of 1.2 m, close to the IAQ sensor, except for when the SES was collected from a room where there was a patient with a chest tube. In this scenario, the cassette of the SES was positioned close the vent of chest tube bottle, to check if the air coming from its interior was contaminated.

The environmental sampling scheme, timeline, and device positioning is shown in Figure 1.

Quantitative Rt-PCR

Quantitative Rt-PCR was carried out using SuperScript III One-Step Rt-PCR System with Platinum Taq DNA-Polymerase (Thermo Fisher Scientific – Waltham, Massachusetts). Each Rt-PCR reaction consisted of a total volume of 20 μ L containing 1 μ L of 10 μ M forward/reverse primers and 5 μ M of specific probe, 5 μ L

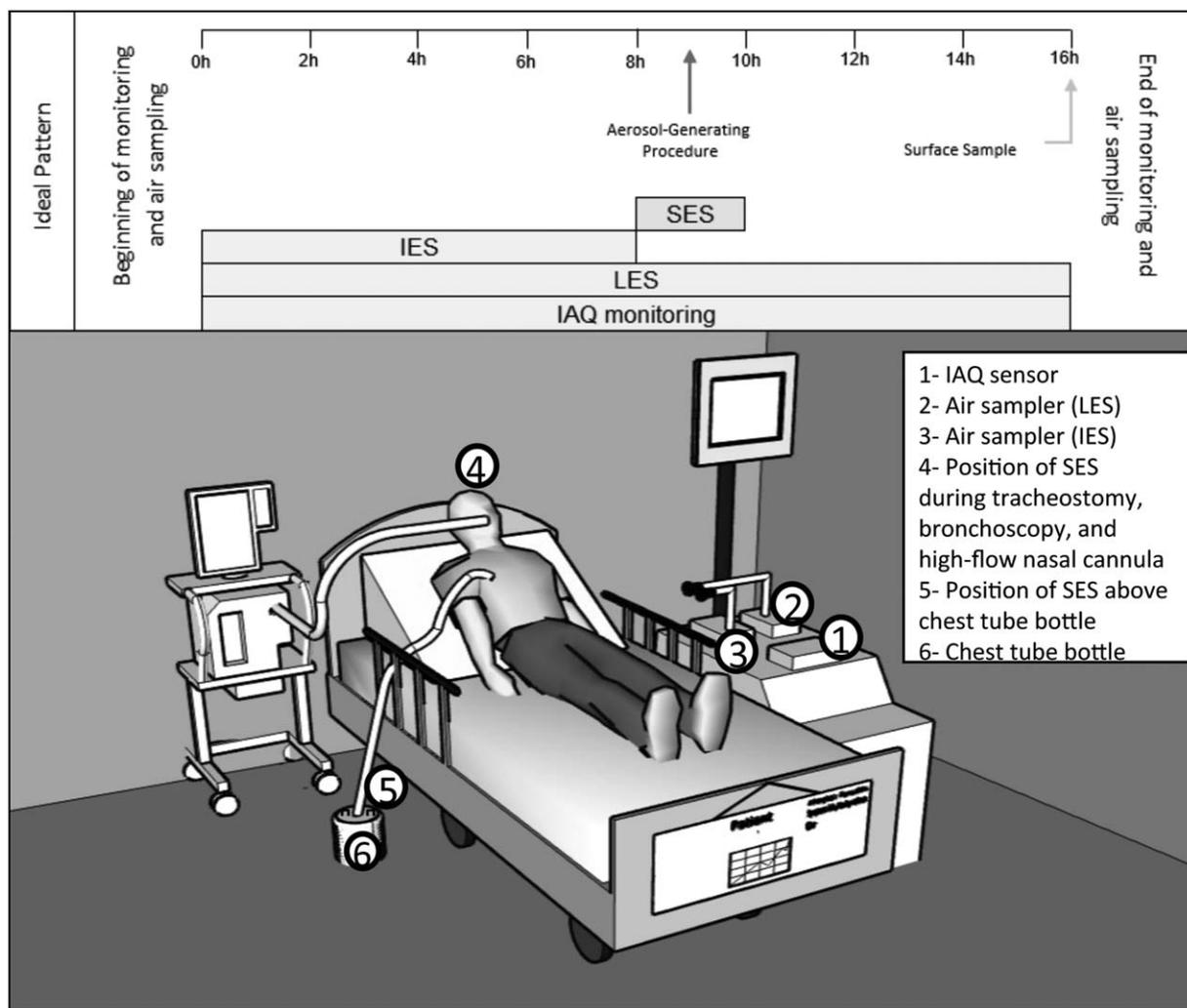


FIGURE 1. Room evaluation plan and setup according to each aerosol-generating procedure. IAQ, indoor air quality; IES, intermediate-exposure sample; LES, long-exposure sample; SES, short-exposure sample.

TABLE 1. Room Individual Data

Room ID	Days Between Room Monitoring and Patient		Aerosol-Generating Procedure/Scenario	Respiratory Support Device
	Onset of Symptoms	Last Positive Swab-PCR		
Ward				
#1	10	2	No	Nasal cannula
#2	8	2	No	Nasal cannula
#3	7	1	No	Nasal cannula
ICU				
#4	9	6	HFNC	HFNC
#5	18	8	Chest tube with air-leak	ETT
#6	58	47	Chest tube	ETT
#7	18	3	Tracheostomy	ETT/tracheostomy
#8	18	8	Tracheostomy	ETT/tracheostomy
#9	58	47	Tracheostomy	ETT/tracheostomy
#10	20	13	Bronchoscopy	ETT

ETT, endotracheal tube; HFNC, high-flow nasal cannula; ICU, intensive care unit; SWAB-PCR, coronavirus reverse transcriptase-polymerase chain reaction of nasopharyngeal swab or tracheal aspirate.

of Total RNA, 10 μ L of 2 \times Reaction Mix, 0.8 μ L Enzyme Mix and 3.2 μ L of DNase/RNase-free water (Merck – Kenilworth). All samples were done in duplicate and each Rt-qPCR run included a positive and a negative control. Quantitative positive results were considered for cycle threshold values lower than 38.00 for the SARS-CoV-2 marker N1. RT-qPCR runs were performed with CFX96 Real-Time PCR Detection System (BioRad – Hercules). Cycle threshold value was calculated using CFX Manager Software (BioRad – Hercules). Qualitative Rt-PCR reactions were performed as multiplex reactions containing all primers and probes sets from CDC (N1, N3, and RNase P).⁵ A detailed protocol is available in the Supplemental Materials.

Data Analysis

Normal and non-normal data are presented using mean \pm standard deviation (SD) and median/interquartile range (IQR) respectively. Mann–Whitney *U* test was used to compare non-normal data. Correlation between IAQ variables were made using the Spearman correlation coefficient with a Rho (ρ) ≤ 0.5 being considered as weak, >0.5 and ≤ 0.8 moderate, and >0.8 strong. A *P*-

value ≤ 0.05 was considered statistically significant. All tests were made using Stata 14 (StataCorp, College Station, Texas).

Patient Data and Informed Consent

Date of the onset of symptoms, last positive SARS-CoV-2 PCR, and type of respiratory support device were the only data recorded from patients. Since no sensitive personal data was used in this study together with the trouble in obtaining consent forms during the pandemic, the need for an informed consent form was dismissed. The research was approved by the local ethics committee board (CAAE 33433120.2.0000.0068).

RESULTS

A total of three ward and seven ICU rooms were evaluated. The mean (\pm SD) number of days between unit evaluation versus the onset of symptoms and last positive Rt-PCR were 22.4 (± 13.7) and 13.7 (± 17.9), respectively. Generally, ICU had patients with a higher number of days between the onset of symptoms (28.4 ± 20.5 vs 8.3 ± 1.5 ; *P* = 0.01) and last positive Rt-PCR (18.8 ± 19.4 vs 1.6 ± 0.5 ; *P* = 0.01) when compared to ward ones.

TABLE 2. Indoor Air Quality Data

Room ID	Procedure	Temperature	RH (%)	TVOC (ppb)	CO ₂ (ppm)	PM1.0 (μ g/m ³)	PM2.5 (μ g/m ³)	PM10 (μ g/m ³)
		(°C)						
		Mean (\pm Standard Deviation)	Median (Interquartile Range)					
Ward		24.3 (± 1.5)	48.3 (± 4.1)	34 (60)	783 (143.5)	13 (6)	16 (10)	17 (11)
#1	No	25.1 (± 0.8)	51.7 (± 2.3)	312 (293)	516 (63)	19 (5)	28 (9)	29 (11)
#2	No	25.3 (± 1.4)	50.0 (± 3.6)	22 (46)	852 (156)	15 (5)	19 (7)	19 (7)
#3	No	23.4 (± 0.8)	46.4 (± 3.8)	38.5 (53)	776 (142)	10 (4)	11 (6)	12 (8)
ICU		25.9 (± 3.4)	37.3 (± 6.7)	136 (188)	714 (147)	17 (10)	21 (14)	23 (15)
#4*	No	–	–	–	–	–	–	–
#5	Chest tube with air-leak	24.9 (± 5.1)	38.5 (± 10.1)	223 (173)	769.5 (119)	25 (10.5)	34 (19)	39 (25)
#6	Chest tube	22.6 (± 0.7)	41.5 (± 2.4)	182 (139)	629 (81)	21 (3)	29 (5)	32 (7)
#7	Tracheostomy	29.1 (± 1.8)	31.6 (± 5.0)	203 (197)	763 (255)	20 (6)	22 (7)	23 (8)
#8	Tracheostomy	26.4 (± 3.0)	39.4 (± 4.2)	53 (65)	728 (62)	16 (4)	21 (7)	23 (8)
#9	Tracheostomy	25.7 (± 1.79)	37.5 (± 5.1)	55 (62)	649 (84)	10 (7)	12 (9)	13 (11)
#10	Bronchoscopy	27.9 (± 2.0)	34.7 (± 5.5)	177 (365)	802 (129)	11 (7)	15 (12)	16 (13)
All rooms		25.4 (± 3.0)	40.7 (± 7.9)	94 (171)	737 (165)	16 (9)	19 (13)	20 (14)

CO₂, carbon dioxide; ICU, intensive care unit; ppb, parts per billion; ppm, parts per million; RH, relative humidity; TVOC, total volatile organic compounds. PM1.0, particulate matter $< 1.0 \mu$ m; PM10.0, particulate matter $< 10 \mu$ m; PM2.5, particulate matter $< 2.5 \mu$ m.

*Data could not be retrieved.

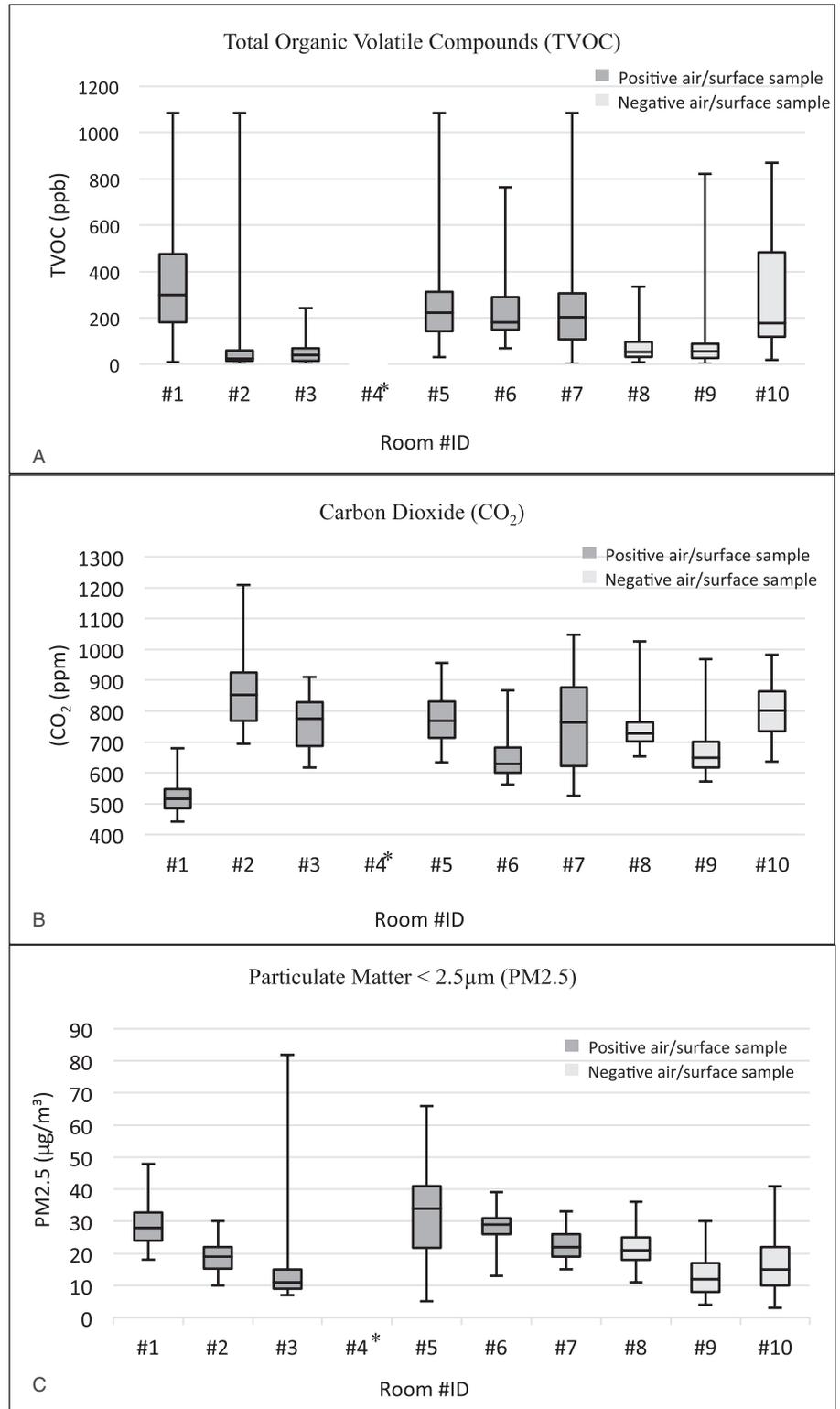


FIGURE 2. Box-and-whisker plots of Total Volatile Organic Compounds (A), carbon dioxide (B), and particulate matter <2.5 µm, (C) according to each room. *No data available for this unit.

All data concerning patient characteristics, type of ventilation support, and associated AGPs are available in Table 1.

IAQ data were retrieved for all units except for room #4, where the sensor was accidentally turned off, but the room was still kept in the study considering that the air and surface sampling were performed properly. Individual IAQ results and box-and-whisker

plots for TVOC, CO₂, and PM_{2.5} are shown individually in Table 2 and Figure 2, respectively.

The highest medians of TVOC, PM_{1.0}, PM_{2.5}, and PM_{10.0} were registered in room #5 which was the ICU room with the greatest number of RNA copies on environmental sampling. The highest levels of CO₂ were registered in room #2. Correlations between

TABLE 3. Air and Surface Sampling Results

Room ID	Procedure	Air Sampling (Copies/ μ L)/(copies/L of air)			Surface Swab
		SES	IES	LES	
Ward rooms					
#1	No	–	45.9/19.1	–	52.8
#2	No	–	–	1527.2/285.4	7861.5
#3	No	94.5/157.5	–	196.2/40.8	144.0
ICU rooms					
#4	No	–	–	–	–
#5	Chest tube with air-leak	36.4/60.7	–	104.2/19.5	175.9
#6	Chest tube	–	58.9/25.6	53.8/10.5	71.9
#7	Tracheostomy	–	–	32.8/6.8	133.9
#8	Tracheostomy	–	–	–	–
#9	Tracheostomy	–	–	–	–
#10	Bronchoscopy	–	–	–	–

ICU, intensive care unit; IES, intermediate-exposure sample; LES, long-exposure sample; SES, short-exposure sample.

PM and TVOC/CO₂ were done for all rooms (Table 3) and, majorly, only weak correlations were found between those variables.

A total of 40 molecular analyses, 30 air, and 10 surface samples, were performed (Table 4). Six rooms had at least one positive air sample and except for room #1, all of them had an isolated or a concomitant positive LES. It is also worth pointing that all rooms with a positive air sample had a positive surface sample as well as a higher number of copies per μ L.

Generally, the occurrence of AGPs could not be associated with a simultaneous and substantial change in the levels of ventilation indicators (TVOC and CO₂) or PM_{2.5}. Charts (Fig. 3) with the timeline of each session and levels of TVOC, CO₂, and PM_{2.5} were done for rooms #2 (ward room with the highest number of copies per μ L in 16-hour and surface sampling), #5 (chest tube with continuous air-leak), #7 (tracheostomy), and #10 (bronchoscopy).

DISCUSSION

Experiment Rationale

Environmental transmission of SARS-CoV-2 is still not deciphered and may be one of the keys to achieve COVID-19 control⁴; however, a high heterogeneity is usually seen among experiments concerning this theme.⁶ Besides using different sampling instruments and strategies, they are frequently conducted in different places (eg, hospital toilet, corridor), what hinders data

extrapolation since each of those spots has different ventilation dynamics. Additionally, parameters such as temperature and air humidity play an important role in SARS-CoV-2 viability,⁷ but only a few studies performing air sampling have specified its underlying environmental conditions.⁸

In view of those issues, the purpose of using an IAQ sensor was to better establish the conditions in which environmental sampling was being conducted and to investigate possible relationships between them. To allow further comparison and reproduction of this experiment, the sampling process was simplified as much as possible, what is characterized by the sampling device that only assembled a vacuum pump, a rotameter, and a hose to connect to a polytetrafluoroethylene membrane; the flow rate was set to 5 L/min to simulate the standard respiratory minute ventilation; rooms with similar characteristics and typical routines were chosen. Even with those simplifications, we were able to detect SARS-CoV-2 RNA in 6 out of 10 rooms or 9 out of 30 air samples what is proportionally higher than most similar experiments that frequently do not detect or have a low positivity rate as pointed in a recent systematic review that analyzed 11 experimental studies concerning airborne transmission of COVID-19.⁹

Environmental Sampling and Indoor Air Quality

Duration of the exposure is considered a crucial factor for disease transmission,¹⁰ but this has been essentially demonstrated in

TABLE 4. Spearman Correlation Between Indoor Air Quality Parameters

Room ID	TVOC vs PM _{2.5}	TVOC vs PM _{10.0}	CO ₂ vs PM _{2.5}	CO ₂ vs PM _{10.0}
Ward rooms				
#1	0.37	0.43	0.37	0.39
#2	–0.58	–0.57	–0.13	–0.12*
#3	0.16	0.18	–0.48*	–0.46*
ICU rooms				
#4	–	–	–	–
#5	–0.2*	–0.19*	0.37*	0.38*
#6	0.08	0.13*	0.28*	0.24*
#7	0.01	0.03	0.03	0.04
#8	–0.33*	–0.32*	0.1	0.1
#9	–0.44*	–0.43*	–0.44*	–0.44*
#10	–0.05	0.002	0.22*	0.25*

CO₂, carbon dioxide; ICU, intensive care unit; PM_{10.0}, particulate matter < 10 μ m; PM_{2.5}, particulate matter < 2.5 μ m; TVOC, total volatile organic compounds.

* $P \leq 0.05$.

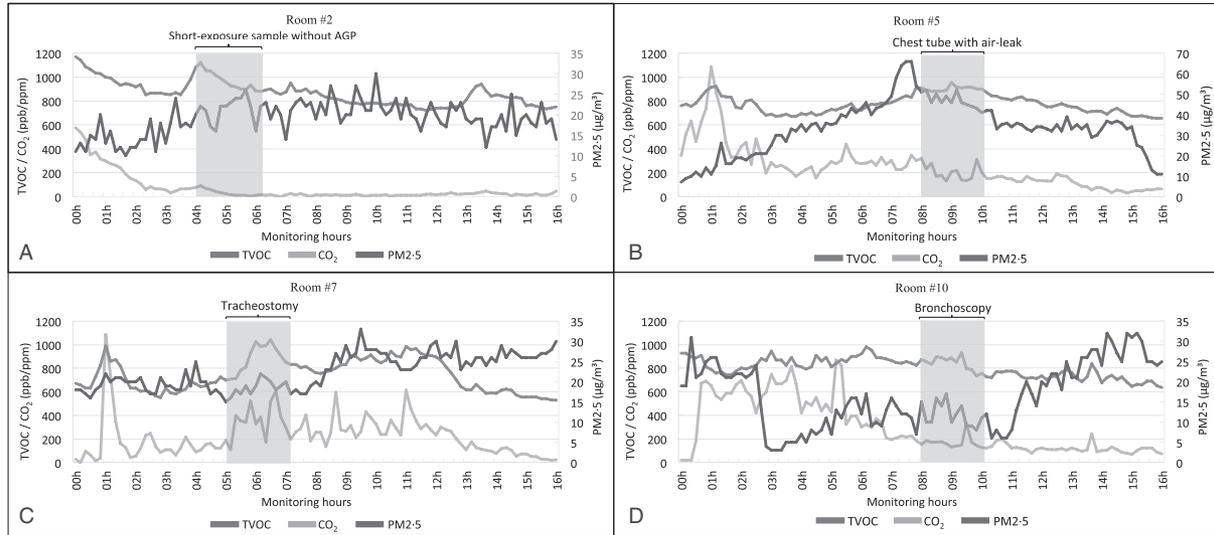


FIGURE 3. Charts showing the timeline of TVOC, CO₂, and PM_{2.5} levels in rooms #2 (A), #5 (B), #7 (C), and #10 (D) during each monitoring. *Grey boxes show the period in which short-exposure samples were collected and/or AGPs were performed. AGP, aerosol-generating procedure; CO₂, carbon dioxide; PM_{2.5}, particulate matter < 2.5; TVOC, total organic volatile compounds.

close contacts of patients with COVID-19, like Hu et al which observed an increase of 0.15% in attack rate per hour of cotravel among train passengers.¹¹ Previous studies concerning air sampling did not test different exposure times,^{12–14} and, thus, the finding that only one out of the six rooms with positive air samples did not have a concomitant or isolated positive LES, adds more evidence to the principle that the duration of exposure is a cornerstone to COVID-19 transmissibility.

The timeframe after the onset of symptoms that a positive swab Rt-PCR may be collected is controversial, but at least 2 or 4 weeks should be considered.^{15,16} Across the evaluated rooms, positive samples were obtained from units where patients had more than 50 days since the beginning of the symptoms, suggesting that not only swab-tests may be positive for longer periods, but also the surrounding environment.¹⁷ The finding that all ward rooms, where only individuals with respiratory manifestations initiated in the previous 10 days at the latest were staying in them, had positive air/surface samples is in accordance with Chia et al that found a higher positive rate of environmental sampling in rooms with patients in the first 7 days of the illness.¹⁸

Another important finding is that positive surface samples were only obtained where the air sample was positive as well. Considering that the evaluated rooms had their door and windows closed and were not shared with other patients, this result suggests that aerosol deposition may be one of the elements to have an environment with contaminated surfaces. Moreover, this could be clinically relevant for the safety of healthcare workers inside those facilities since surface swab together with IAQ parameters, which are much easier to be done than air sampling, could be used as an indicator of an environment with potential risk of transmission, triggering disinfection measures, and adjustments in IAQ such as temperature and air humidity which play a crucial role in the survival of several viruses.^{19,20}

IAQ evaluation was focused on the parameters that may be classified as ventilation indicators (TVOC and CO₂) and total suspended particles (PM_{1.0}, PM_{2.5}, and PM_{10.0}). Low TVOC and CO₂ concentrations have been associated with proper ventilation inside buildings, and it is reasonable to infer that decreased levels would also result in low levels of PM in the air, an association that has already been hypothesized elsewhere.²¹ However, in this

study, while the levels of TVOC/CO₂ did not surpass the recommended standards, such as 1000ppm for CO₂,²² using them as indicators of good ventilation was not straightforward, since correlations between PM and TVOC/CO₂ could not be found in any room.

No obvious correlation between PM and RNA concentration could also be identified, what may be a consequence of the several factors affecting those results. The use of PM as an indicator of microbiological safety has been investigated in the literature but attained negative results.^{23–25} However, those studies were done majorly in operating theatres and considered only bacterial contamination. Moreover, airborne transmission of infectious agents inside buildings is not properly investigated as demonstrated by a recent study that could only find 10 studies over 45 years with conclusive results regarding this topic.²⁶ Considering that fine particulate matter is thought to be one of the carriers of coronavirus particles across the environment, the reported findings point to the need for further investigations of IAQ inside healthcare facilities.

Aerosol-Generating Procedures

UCI rooms were chosen based on the execution of common clinical practice procedures to check if those scenarios would be related to variations in IAQ or environmental sampling. As exemplified in the timelines in Figure 2, no substantial changes of TVOC, CO₂, and PM_{2.5} levels were detected simultaneously with those events, what may be a consequence of the rigorous actions adopted to prevent aerosol generation across the institution. Together with the negative samples collected during those AGPs, the lack of variation in the IAQ parameters denotes the effectiveness of those precaution measures.

The evaluation of Room #5 evaluation has drawn attention because it had the highest concentration of PM_{2.5} and coincidentally there was patient with a chest tube presenting continuous and severe air-leak. Also, it was the only unit with a positive SES where an AGP occurred, and, distinctly, the sample cassette was closely attached to the vent of the bottle, in such a way that the only source of air flowing through it was coming from the bottle inside. The potential of aerosol generation from chest tubes has been in debate since the beginning of the pandemic and has been recently demonstrated experimentally by Duffy and colleagues that detected

increased concentrations of PM in a closed environment when a filter was not attached to the vent of the bottle.^{27(p19)} Even if only one experiment in such scenario was performed, our results add evidence to the transmission risks surrounding chest tubes in COVID-19 patients.

Limitations

This study has several limitations that have to be addressed. A larger sample could generate more robust information. Although no final conclusions may be drawn from this study, it may give insights for further research and help to standardize future experiments. Another drawback was that viral culture was not performed and, thus, valuable information about the risks of transmission could not be evaluated, since Rt-PCR only indicates the presence of genetic material but no direct evidence of its infectious potential.²⁸

The use of the National Institute for Occupational Safety and Health cyclone sampler,²⁹ that size-fractionates aerosols into >4 µm, 1 to 4 µm, and <1 µm would allow separate molecular analysis of them. While this could provide relevant information such as if the virus is carried over specific particle sizes and, then, to better understand airborne transmission, it would also increase the complexity the experiment.

CONCLUSION

SARS-CoV-2 RNA airborne transmission is gradually being understood but due the complexity of this theme several steps still need to be accomplished. Although associations between air or surface sampling and environmental parameters could not be found in this research, we believe that IAQ is a cornerstone for the standardization of similar studies. Even with the use of a simple device, a good detection rate was achieved in environmental sampling and the experiment design suggests that longer expositions and places with patients with recent onset of symptoms may pose an increased risk of contact with viral genetic material. Larger scale experiments may help to fulfill the current gaps in the knowledge concerning SARS-CoV-2 RNA transmissibility inside healthcare settings.

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