

Environmental Contamination by SARS-CoV-2 During Noninvasive Ventilation in COVID-19

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BACKGROUND: Environmental contamination by SARS-CoV-2 from patients with COVID-19 undergoing noninvasive ventilation (NIV) in the ICU is still under investigation. This study set out to investigate the presence of SARS-CoV-2 on surfaces near subjects receiving NIV in the ICU under controlled conditions (ie, use of dual-limb circuits, filters, adequate room ventilation). **METHODS:** This was a single-center, prospective, observational study in the ICU of a tertiary teaching hospital. Four surface sampling areas, at increasing distance from subject's face, were identified; and each one was sampled at fixed intervals: 6, 12, and 24 h. The presence of SARS-CoV-2 was detected with real-time reverse transcriptase–polymerase-chain-reaction (RT-PCR) test on environmental swabs; the RT-PCR assay targeted the SARS-CoV-2 virus nucleocapsid N1 and N2 genes and the human RNase P gene as internal control. **RESULTS:** In a total of 256 collected samples, none were positive for SARS-CoV-2 genetic material, whereas 21 samples (8.2%) tested positive for RNase P, thus demonstrating the presence of genetic material unrelated to SARS-CoV-2. **CONCLUSIONS:** Our data show that application of NIV in an appropriate environment and with correct precautions leads to no sign of surface environmental contamination. Accordingly, our data support the idea that use of NIV in the ICU is safe both for health care workers and for other patients. *Key words:* COVID-19; infection control; critical care; patient safety; noninvasive ventilation; aerosol-generating procedures. [Respir Care 2023;68(1):1–7. © 2023 Daedalus Enterprises]

Introduction

The COVID-19 pandemic still affects millions of people worldwide. Although vaccinations and virus variants have progressively lowered its burden on health care systems, COVID-19 remains a leading cause of hospital and ICU admission¹; hospitals worldwide are settling in organizational models^{2,4} to allow “coexistence” of COVID-19 and non-COVID-19 pathways with the target of providing the highest possible levels of care to both “positive” and “negative” patients, especially in time-critical cases, such as elective surgery.⁵ Yet the recent decision of Canadian health authorities to allow COVID-positive patients to share the same rooms with vaccinated negative patients sparked widespread debate in the media.⁶

Although the primary method of transmission is direct exposure through droplets from the airways,⁷ evidence still indicates the possible role of indirect transmission by means of contamination of ambient air and/or environment surfaces.^{7,8} Environmental contamination by SARS-CoV-2

in the ICU and in the medical ward settings ranges between 5–86% of collected samples, depending on hospital, type of ward, and type of sampled surfaces.^{8–11}

Several studies performed before the COVID-19 pandemic showed that noninvasive ventilation (NIV) may be a relevant aerosol-generating procedure.¹² Hui and co-workers¹³ showed that during NIV, droplets and airborne particles from the respiratory system spread widely (150–230 cm) in the environment in a short period of time. However, Strand-Amundsen and colleagues¹⁴ did not confirm these data and showed that neither high-flow nasal cannula (HFNC) nor NIV led to an increase in aerosol dispersal compared to the use of low-flow nasal cannula oxygen.

One of the major concerns for using NIV to treat acute respiratory failure due to COVID-19 was the risk of generating bioaerosol that could expose health care workers (HCWs) and other patients to SARS-CoV-2 infection.¹⁵ The World Health Organization advocates using NIV for the management of respiratory failure in patients with COVID-19 provided that stringent procedures for personnel

protection are implemented.¹⁶ Franco and colleagues¹⁷ found that approximately 10% of HCWs involved in the management of subjects with COVID-19 treated with NIV contracted the infection. Although use of NIV has widely increased during the first 2 pandemic years,^{18,19} little is still

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known about viral spread and surface contamination from NIV use, leaving unanswered the question of safety for HCWs and other patients.

This study set out to test the hypothesis that adequate room ventilation and use of total face masks, dual-limb circuit, and appropriate filters lead to no sign of presence of SARS-CoV-2 on surfaces near subjects during NIV.

Methods

This prospective, observational, single-center study was conducted in one COVID-19–dedicated ICU of a quaternary care university hospital between April 2020–March 2021. Sampling was performed on inanimate surfaces and did not require institutional review board approval. The only subject-related data (positivity for SARS-CoV-2 and ventilatory pressures) were collected anonymously for another observational study.¹⁷

The ICU is composed of 4 rooms equipped with negative pressure with 10 air changes per hour. Each room houses 2 patients and is connected to a corridor that serves as the central nursing station (Fig. 1). Patient beds were the same model (TotalCare Model 1900, Hill-Rom, Chicago, Illinois). Stations were tested if allocated subjects matched the following criteria: (1) recent diagnosis of COVID-19 (within the past 72 h), (2) radiological evidence of bilateral interstitial pneumonia, and (3) NIV treatment through

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QUICK LOOK

Current knowledge

Although direct transmission remains the main way of transmission of SARS-CoV-2 among humans, indirect transmission (air or surfaces contamination) may play a role; but the real entity is still debated, especially in relation to noninvasive respiratory support.

What this paper contributes to our knowledge

The use of noninvasive ventilation (NIV) with a total face mask, using appropriate precautions in a controlled environment (such as the ICU), had a rate of environmental contamination on patient-related surfaces of 0%. The results imply that NIV performed in the ICU does not increase risks for health care workers or other patients; this may have important implications for planning and designing future ICUs.

a total face mask (Respironics PerforMax, Philips, Amsterdam, the Netherlands). All ventilators used dual-limb circuits, and appropriate filters were applied between both ventilator ports and the ventilator-side limbs of the circuit (Eco Maxi Pleat, P/N 4244/701; GVS Filter Technology UK, Morecambe, United Kingdom).

Sampling Procedure

Four sampling areas were identified, and each one was placed at different distance from the source (subject's mouth): area A (head lateral bed rail, 50 cm), area B (body lateral bed rail, 80 cm), area C (table bed rail, 150cm), and area D (1-m-high upside-down bin on the floor at 200 cm from the subject's mouth). Each sampling point was marked with adhesive tape delimiting an area of 3 × 3 cm. Figure 1 shows the distance from the subject's mouth to each sampling area. A specific order was followed when sampling

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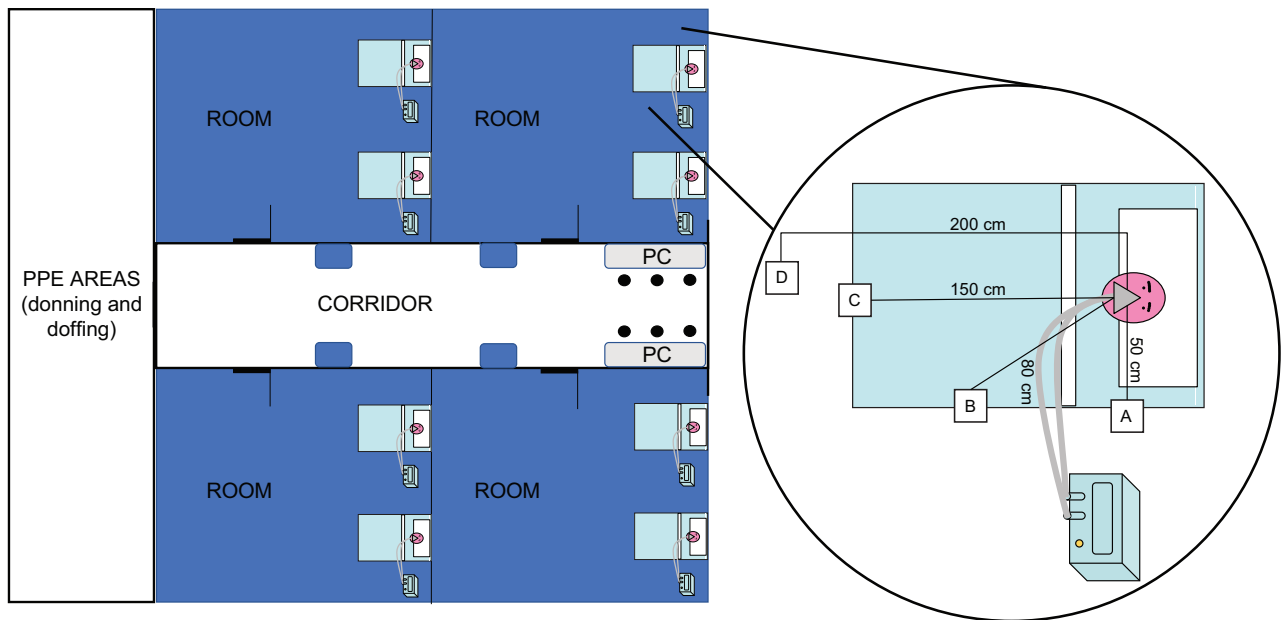


Fig. 1. Layout of the COVID-19 dedicated unit. Personal protective equipment (PPE) area indicates areas dedicated to doffing and donning of PPE for health care workers. PC indicates workstations for health care workers. Details of patient bed stations and positions of sampling locations are also shown.

the subjects' bed stations, working from point A (higher contamination risk) to point D (lower contamination risk).

Accurate disinfection (71% isopropyl alcohol or 0.05% sodium hypochlorite) of the same sampling areas was carried out to remove any previously deposited viral material. The timeline started at T_0 , defined as the baseline after disinfection (negative control), when the first 4 samples were collected. Sampling took place by rubbing a swab on all identified surfaces in the delimited area. Labels indicating bed number, collection date, collection time, and surface area were immediately applied to the collected samples, which were subsequently sent to the laboratory. Sampling after T_0 was made respectively at +6 h (T_1), +12 h (T_2), and +24 h (T_3). A total of 16 samples for each bed was screened. All researchers, HCWs, and cleaners were instructed not to touch the collecting surfaces. Sanitation and disinfection procedures on collecting surfaces were suspended over the 24-h period of sampling. Once the collection had started, a "No Touch" reminder was attached on each bed station.

Based on individual subject nursing care needs, NIV masks were removed only after switching off the ventilator. After disconnection, subjects were supported by oxygen treatment for time necessary to perform nursing tasks.

Microbiological Procedures

Environmental samples from different surfaces were obtained using flexible nasopharyngeal nylon flocked swabs dipped in 3 mL universal transport medium (UTM,

Copan Italia, Brescia, Italy). As a positive control, in a subset of cases (4 of 16), also the inner surface of the NIV mask was sampled.

The presence of SARS-CoV-2 was detected with a real-time reverse transcriptase–polymerase-chain-reaction (RT-PCR) test on environmental swabs. The samples were immediately tested or stored at -80°C until processed. Total genomic DNA/RNA was extracted from 500 μL of the sample and diluted in 50 μL using a NucliSENS easyMAG system (bioMérieux, Marcy-l'Étoile, France) in accordance with the manufacturer's instructions. Detection of SARS-CoV-2 virus was performed by RT-PCR following the Centers for Disease Control and Prevention protocols.²⁰ This RT-PCR assay targets the SARS-CoV-2 virus nucleocapsid N1 and N2 genes and the human RNase P gene. Three separate master mix sets were prepared for N1, N2, and RNase P. The PCR reaction was performed using 15 μL of each master mix and 5 μL of extracted sample. Amplification was performed on the Applied Biosystems QuantStudio 5 Real-Time PCR System (QNS-5; Thermo Fisher Scientific, Waltham, Massachusetts). The sequences of primers and probes are showed in Table 1.

Statistical Methods

Continuous variables were expressed as medians and interquartile ranges (IQRs). Categorical variables were summarized as counts and percentages. Analyses were conducted using R version 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria).

SARS-CoV-2 ENVIRONMENTAL CONTAMINATION DURING NIV

Table 1. Primers and Probes²⁰

Description	Oligonucleotide Sequence (5' > 3')
2019-nCoV_N1 forward primer	GAC CCC AAA ATC AGC GAA AT
2019-nCoV_N1 reverse primer	TCT GGT TAC TGC CAG TTG AAT CTG
2019-nCoV_N1 probe	FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1
RNase P forward primer	AGA TTT GGA CCT GCG AGC G
RNase P reverse primer	GAG CGG CTG TCT CCA CAA GT
RNase P probe	FAM – TTC TGA CCT GAA GGC TCT GCG CG – BHQ-1

FAM = 6-carboxyfluorescein
BHQ-1 = black hole quencher 1

Table 2. SARS-CoV-2 Genome Persistence at COVID-19 in the Bed Stations

	Sampling Time							
	T ₀		T ₁		T ₂		T ₃	
	RNase	SARS-Cov-2	RNase	SARS-Cov-2	RNase	SARS-Cov-2	RNase	SARS-Cov-2
Point A: head bed rail	-	-	-/+	-	-	-	-/+	-
Point B: main bed rail	-	-	-/+	-	-/+	-	-/+	-
Point C: bedside table	-	-	-/+	-	-/+	-	-/+	-
Point D: bin on the floor	-	-	-/+	-	-/+	-	-/+	-

Absolute presence of SARS-CoV-2 genome or RNase in each evaluation is qualitatively expressed as negative (-) if all samples resulted negative, positive (+) if all samples resulted positive, or negative/positive (-/+) if at least one sample resulted positive.

Results

From April 28, 2020–March 28, 2021, a total of 16 bed stations was analyzed for the presence of SARS-CoV-2 across 4 sampling time points. A total of 16 samples for each bed station was collected, for a total of 256 environmental swabs.

None of the subjects included into the study underwent aerosol-generating procedures other than NIV during the study period. All subjects were ventilated on pressure support ventilation with double-limb circuits, and the ventilators were set in NIV mode; the median value of inspiratory pressure delivered during NIV was 16 cm H₂O (IQR 16–19). Subjects were disconnected at least once during the 24-h period of observation and supported with HFNC during nursing care. Subjects were unable to leave their beds during their ICU stay, and all nursing care was delivered at the subjects' bedside. The environment in which samples were collected was under negative pressure, and temperature was kept constant at 23.0 ± 1.0°C. The median time from diagnosis to the first environmental sampling (T₀ or negative control) was 48 h (IQR 24–48). Median cycle threshold of diagnostic nasopharyngeal swabs was 27.3 cycles (IQR 24.0–30.3).

None of the 256 collected samples were positive for SARS-CoV-2. All samples were analyzed for RNase to detect the presence of other genetic material on surfaces.

Only 21 (8.2%) samples resulted positive for genomic material, indicating the presence of environmental contamination unrelated to a SARS-CoV-2 virus source. In relation to time, RNase was found on 8/64 (12.5%), 5/64 (7.8%), and 8/64 (12.5%) at T₁, T₂, and T₃, respectively. All samples resulted negative for RNase at T₀. Area A (24%) and area B (38%) were more frequently contaminated than areas C (14%) and D (24%). A synthetic representation of these results is presented in Table 2. All samples collected inside the NIV mask (positive control) resulted positive for both SARS-CoV-2 and RNase.

Discussion

Our data demonstrate the complete absence of SARS-CoV-2 genome in the totality of samples collected on the surfaces of 16 ICU bed stations allocated to subjects undergoing NIV with total face mask for COVID-19-related respiratory failure.

To date, studies on environmental contamination in the clinical settings^{8-10,21-26} show that (1) the prevalence of contaminated surfaces by SARS-CoV-2 is widely variable among studies, from < 5% to > 80% of collected samples;⁸⁻¹⁰ (2) environmental contamination and viral loads (quantitative PCR) in the ICU seem to be lower than in general wards;²¹ (3) use of CPAP or HFNC does not appear to increase levels

of surface viral contamination;^{9,26} and (4) SARS-CoV-2 is usually not detected by air sampling.²⁷

Although in the early phases of the pandemic the use of NIV was discouraged for the risk of so-called patient self-inflicted lung injury²⁸ and for the fear of environmental contamination, the number of patients treated with these techniques widely increased and became a cornerstone treatment for the following reasons: (1) These techniques have shown to be safe and effective for the patients, even if applied outside ICU settings;^{18,29} (2) nearly 50% of patients with COVID-19 may avoid intubation, receiving only noninvasive respiratory treatment;³⁰ and (3) reported risk of high contamination associated with NIV treatment is low,^{17,31} to the point that some authors are proposing removal of NIV from the lists of aerosol-generating procedures.^{32,33}

Contrary to other studies conducted mostly during the first pandemic wave,^{9,11} our data clearly show that application of NIV in an appropriate environment and with correct precautions is safe for HCWs and other patients nearby. Indeed, we can assume that all the samples collected in the present study were negative for the following reasons: First, we used total face masks, which act as hermetic systems, forming an effective barrier against droplet spread; second, we used double-limb circuits and applied filters on both limbs; third, we limited every other aerosol-generating procedure as much as possible; lastly, although the ventilation system of the ICU provided 10 air changes per hour, alternative measures, such as opening windows and vents connected to the outside (> 160 L/s/subject), were taken in order to achieve a safer environment. Moreover, it should be also noted that, contrary to bench studies³⁴ showing that infectious titers of SARS-CoV-2 are found for up to 3 h in air, studies performed in real-life clinical settings (such as the present study) demonstrated that even air samples exposed to air around hospitalized subjects with COVID-19 were negative for SARS-CoV-2.

The pragmatic design and robust methodology are major strengths of the present study. To minimize the risk of false negatives, we included only bed stations where the subjects had a recent microbiological diagnosis of COVID-19 (first positive PCR on nasopharyngeal swab up to 72 h prior to inclusion in the study). Standardized selection of sampling spots and time points was accurately enforced, and disinfection of sampling spots before protocol start was always applied. A further strength of the study is represented by the period over which the study was conducted, namely between the end of the first pandemic wave and the end of the third wave, thus focusing on a period when organizational issues in the ICU (such as lack of beds, personnel, and devices) had mostly been solved. Another strength of this study is the high total number of collected environmental samples (256 samples in total).

Our results may provide hospital administrators, clinicians, and nurses with evidence to plan for the future

evolution of ICUs. In the present time, hospitalizations due to COVID-19 are decreasing but will certainly not disappear in the near future; meanwhile, there is increasing pressure on the health systems to make up “lost time” during the early pandemic stages, when many patients were practically left behind because of lack of beds, equipment, and personnel that were substantially absorbed by the treatment of patients with COVID-19. There is an urgent need for new strategies and for new ICU models able to cope with patients with COVID-19 and non-COVID patients at the same time, warranting safety for all patients and HCWs. Our results imply that this coexistence may be safely implemented provided that well-known precautions, such as total face masks, double-limb ventilatory circuits with filters, adequate room ventilation, and air changes, are strictly enforced.

This study has some limitations. First, our end point was the contamination of surfaces, whereas the main way of contagion is direct transmission through droplets from the airways; however, since microbiological air sampling requires the use of sophisticated experimental techniques, in line with other studies,⁸⁻¹¹ we used surface contamination as a robust proxy of airborne contamination. Second, although all staff were instructed to avoid touching and/or cleaning the delimited sampling areas over the 24-h sampling period, a very small chance remains that some cleaning activity or inadvertent touching could have possibly taken place, unbeknownst to investigators. To assess the presence of genomic materials on surfaces, all samples were tested for the presence of RNase; 21 samples (8%) were positive for RNase. In relation to time, the RNase was detected at T₁ (8/64), T₂ (5/64), and T₃ (8/64), respectively, whereas all samples were negative for RNase at T₀. Of note, with respect to time, most of the contaminated spots were found at T₃ and T₄ (ie, sampled at 12 h and 24 h, respectively). Another finding to consider, the most distant surface point D (200 cm) showed more contamination than point C (150 cm). From the results obtained on RNases, it is highly probable that these surfaces were contaminated via fomites; but despite this, the results were still negative for presence of SARS-CoV-2 genetic material. This information further highlights the relatively low risk of environmental contamination. Moreover, only small areas (3 × 3 cm) were sampled, potentially excluding some areas contaminated by SARS-CoV-2; we are also aware that longer observation periods (ie, > 24 h) may have possibly yielded higher prevalence of SARS-CoV-2 presence on surfaces,¹⁰ but at the same time, this could have increased probability of bias by accidental contamination or unwanted disinfection. It is also worth noting that viral shedding has been reported to peak in the first week of illness and to decrease thereafter,³⁵ and most patients are admitted to the ICU after ~ 1 week since the onset of symptoms,³⁶ so patients admitted to the ICU may be far from their peak in viral shedding; to mitigate this factor, we limited enrollment to bed stations where subjects had a

microbiological diagnosis < 72 h before. Finally, our results may not be generalizable to resource-limited settings, since our study was conducted under optimal conditions (ie, double-limb ventilators and ICU rooms with negative pressure and air exchange frequency of 10 times per hour).

Conclusions

Our findings are relevant for further planning of ICUs in the present post-pandemic era, when patients with COVID-19 are still being hospitalized, but there is an urgent need to bring health care systems back to speed with all non-COVID-related activities. NIV, once considered one of the most significant aerosol-generating procedures, seems to present a very low risk of environmental contamination in real-life clinical conditions.

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