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# The Aerosolization of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): Phase I

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## ABSTRACT

**Introduction:** The degree to which Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is aerosolized has yet to be determined. The aim of this study is to prove methods of detection of aerosolization of SARS-CoV-2 in hospitalized patients in anticipation of testing for aerosolization in procedural and operative settings.

**Methods:** In this prospective study, inpatients with SARS-CoV-2 were identified. Demographic information was obtained, and a symptom questionnaire was completed. Polytetrafluoroethylene (PTFE) filters, which were attached to an air pump, were used to detect viral aerosolization and placed in four locations in each patient's room. The filters were left in the rooms for a three-hour period.

**Results:** There were 10 patients who enrolled in the study, none of whom were vaccinated. Only two patients were more than a week from the onset of symptoms, and half of the patients received treatment for COVID with antivirals and steroids. Among ten RT-PCR positive and hospitalized patients, and four filters per patient, there was only one positive SARS-CoV-2 aerosol sample, and it was directly attached to one of the patients. Overall, there was no correlation between symptoms or symptom onset and aerosolized test result.

**Conclusions:** The results of this suggest that there is limited aerosolization of SARS-CoV-2 and provided proof of concept for this filter sampling technique. Further studies with increased sample size should be performed in a procedural and operative setting to provide more information about SARS-CoV-2 aerosolization.

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## Introduction

Since the start of the coronavirus disease 2019 (COVID-19) pandemic, there have been over 165 million confirmed cases of COVID-19 worldwide and nearly 3.5 million associated deaths.<sup>1</sup> Patients with an illness severity that warrants hospitalization have particularly grim outcomes with an

in-hospital mortality rate of 28.3%.<sup>2</sup> The mortality rate of patients in intensive care units (ICUs) has been reported to be nearly double the overall mortality rate with the worst outcomes for patients who are mechanically ventilated.<sup>3</sup> ICUs and general hospital wards are a high-risk location for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) transmission.<sup>4</sup> Aerosolizing procedures, such as breathing

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treatments or bronchoscopy, are performed in the acute care setting and place hospital staff members at high risk for disease contraction.<sup>5</sup> Because of this risk, it has become common practice for nurses, doctors, and respiratory therapists to wear respirators and N-95 masks in an attempt to minimize their risk for transmission.<sup>6</sup> Especially during the beginning of the pandemic, shortages of personal protective equipment were common as healthcare workers sought to protect themselves from a virus with an unknown degree of aerosolization.

While there is still much to be learned, the amount of knowledge that we now have regarding the SARS-CoV-2 virus is significant in comparison to the start of the pandemic. For instance, it is now known that masking and social distancing are both required in order to reduce the exponential spread of COVID-19 within a population.<sup>7</sup> Airborne transmission of SARS-CoV-2 has been identified as the predominant route of viral transmission, which consists of aerosols and droplets.<sup>8</sup> Aerosols are smaller particles (<5  $\mu\text{m}$ ) that rapidly evaporate and disseminate in the air, while droplets are larger particles (>5  $\mu\text{m}$ ) that are affected by gravity and accumulate on the floor or other surfaces.<sup>9</sup> Viruses that are able to be transmitted readily through aerosols, such as measles, are considered highly infectious.<sup>10</sup> SARS-CoV-2 has been documented to remain viable in aerosols for several hours, but this finding alone does not alone indicate infectivity.<sup>11</sup> SARS-CoV-2 has a reproduction number ( $R_0$ ) that is generally quoted to be between two and three, although this has been higher in recent variants, meaning that there are two to three people infected for every person with COVID-19.<sup>12,13</sup> The  $R_0$  for SARS-CoV-2 is comparable to viruses that are spread predominantly by droplet transmission, such as influenza, and not aerosols.<sup>9</sup>

The degree by which SARS-CoV-2 is aerosolized and to what extent that correlates with infectivity is unclear. Studies looking at aerosolization of viral particles in the ambient air have had success in collecting aerosolized particles using a filter, which is then plated onto viral culture media to assess the presence and viability.<sup>14,15</sup> This technique was successfully performed in China in hospitals and public areas to detect the concentrations of viral RNA.<sup>16</sup> The ability for this technique to be employed in other areas in healthcare seems promising. The aims of this study are to determine if SARS-CoV-2 viral particles are aerosolized in an acute care, non-procedural setting and to determine proof-of-concept for a sampling technique. The importance and impact of this study is to inform healthcare workers if viral particles are aerosolized and detectable. If so, these methods can be utilized to better understand exposure in procedural and operative settings.

## Methods

### Patient selection

Following Institutional Board Review approval, patients were prospectively identified in medical and surgical ICUs and the general hospital wards at an urban, 515-bed teaching hospital in the northeastern United States. The electronic medical record was used to determine the patients who had a positive Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

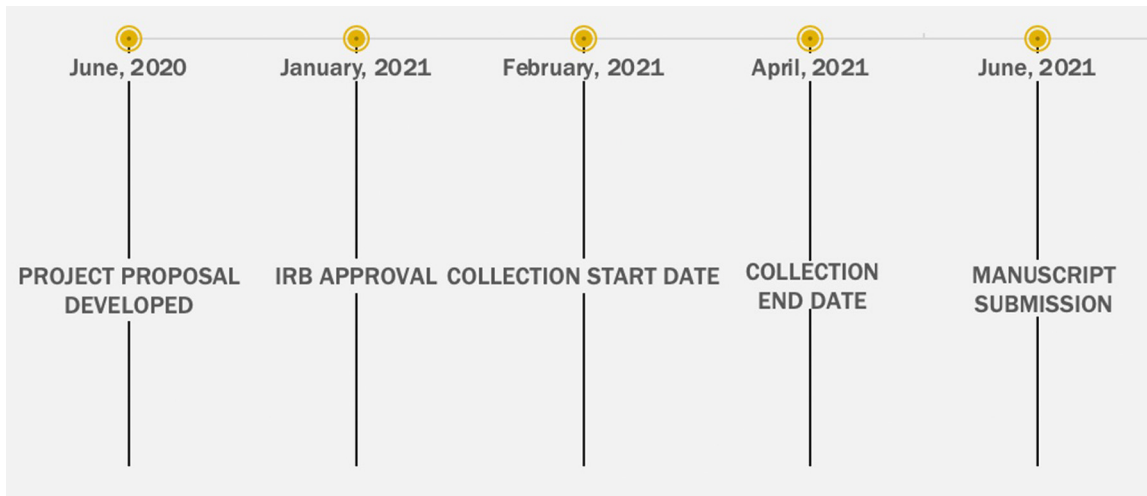
Covid-19 test and were over the age of 18. All efforts were made to recruit patients who were within 10 d of onset of symptoms, considering this is when the level of infectivity appears to be the highest.<sup>17</sup> Informed consent was obtained from each patient prior to study participation. Ventilated patients were excluded, as were those who did not wish to consent to participation in the study. The target sample size was 10 patients, which would allow the research team to determine if there was enough data to verify their collection process with positive tests. Sample collection took place from February 23, 2021 to April 12, 2021 prior to wide circulation of new variants of the virus. The timeline for the study is shown in [Figure 1](#).

### Patient characteristics and outcomes

Once patients were identified, patient demographics were obtained, which included patient age, ethnicity, gender, vaccination history, and day of symptom onset. Vital signs were documented at the time of data collection, and information was also attained about treatment with antibiotics, steroids, or antivirals (i.e., Remdesivir). A symptom questionnaire was completed by each patient to try to correlate symptoms with the aerosolized test result. The symptom questionnaire asked patients about cough, shortness of breath, fever, gastrointestinal symptoms, loss of taste or smell, and 'other' symptoms. The primary outcome for the study was the presence or absence of aerosolized SARS-CoV-2.

### Collection of samples

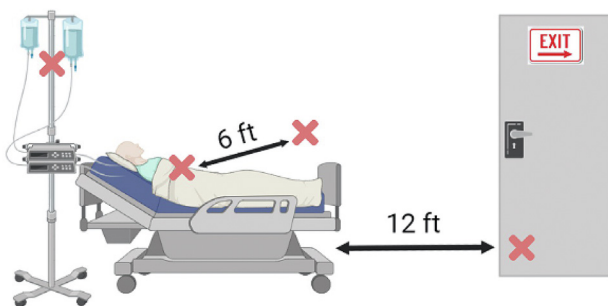
All persons collecting samples from the COVID-19 positive patients adhered to strict personal protective equipment (PPE) protocols that were in line with current recommended national and local guidelines. This included, at minimum, protective goggles, an N-95 mask, full-body protective covering, and gloves. The equipment that was utilized for aerosol sampling was supplied by SKC Inc. (Eighty Four, PA). Specifically, a 37-mm cassette preloaded polytetrafluoroethylene (PTFE) filter was used that was attached to an AirChek TOUCH pump (5 to 5000 mL/min) and a Chek-Mate Calibrator. The PTFE filter was designed to detect particles <5  $\mu\text{m}$ . The sealed cassette pumps were placed in four locations within the patient's room at a time when no aerosolizing procedures were being performed. The rooms were standard hospital rooms and were not negative pressure rooms or airborne isolation rooms. The cassette pumps were placed in the following locations: attached to the patient at the top of their hospital gown, suspended on the intravenous (IV) pole at the head of the bed, 6 feet away from the patient (social distancing guidelines), and 12 feet away from the patient ([Fig. 2](#)). The AirChek TOUCH pump was calibrated prior to the start sample collection with the Chek-Mate Calibrator set to cycle air at 5 L/min. In addition to the patient samples, a positive control trial was performed in which a COVID-19 positive control sample was placed directly onto a PTFE filter. This was a thawed version of the control PCR sample; because it came directly from a test tube, no air pump device was used. The positive control served to ensure the viral particles would



**Fig. 1 – Figure 1 shows a timeline for the study.**

adhere to the PTFE filter and be able to be extracted from the filter during the PCR test.

Patients did not wear masks during the collection process. The sample collector left the room after setting up the cassette devices and then returned at the end of the collection process. Cassettes remained at their designated locations for a total of 3 h.<sup>11</sup> After 3 h elapsed, the cassettes were taken to a designated laboratory space by a member of the research team. The filters were removed from the 37-mm cassette and placed into test tubes under a laboratory hood where they were mixed with normal saline. The sample was then shaken, vortexed down, and placed in a Simplexa (Boca Raton, FL) RT-PCR machine. A cycle threshold (Ct) value was determined, which was defined as the number of cycles for a signal during PCR analysis to cross a threshold value. Based on existing evidence, a Ct of <40 was used as a marker for infection and a surrogate for viral load.<sup>18</sup> The RT-PCR machine cycled each sample 40 times and measured the fluorescence at each cycle to determine whether or not the COVID-19 S gene or ORF1ab gene could be detected above its baseline level. An RNA internal control (RNA 1C) was used to determine PCR failure or inhibition, which ensured the integrity of the PCR process.



**Fig. 2 – A typical room setup is shown in which cassettes (represented by Xs) are attached to the patient, on the intravenous pole at the head of the bed, 6 feet from the patient, and 12 feet from the patient.**

### Data analysis

Data were analyzed using Microsoft Excel (Redmond, WA) and SAS program version 9.4 (SAS, Cary, NC). Categorical variables were reported as percentages, and continuous variables were reported as means with corresponding standard deviations when appropriate. There were no statistical comparisons between groups, so there was no level of significance set for the study.

### Results

A total of 10 patients were recruited to participate in the study (Table 1). Many patients declined participation or did not meet criteria when approached for consent. Specifically, the investigators had difficulty identifying patients who within 1 wk of symptom onset at the time of recruitment for the study as many patients presented to the hospital further along in their clinical course. None of the patients in the study were vaccinated prior to participation. The patients had a mean age of  $57.2 \pm 16.7$  y and were 50% female. The mean time to onset of symptoms was  $5.4 \pm 2.1$  d (range: 4–9). Only two patients (20%) were included who had onset of symptoms greater than 1 wk prior to sample collection. The mean temperature, heart rate, oxygen saturation, and white blood cell count were  $37.6 \pm 0.6$  Celsius,  $98.0 \pm 17.4$  beats per minute,  $89.1 \pm 10.1\%$ , and  $6.1 \pm 2.0 \times 10^9/L$ , respectively. Hypoxia (70%) and tachycardia (40%) were the most common vital sign abnormalities. Half of the patients required nasal cannula with another patient on high flow nasal cannula (HFNC). Only one (10%) of these patients went on to require intubation (patient on HFNC), and this patient ultimately died in the hospital.

Treatments for COVID-19 with antivirals and steroids were prescribed for 50% of patients (Table 2). Two patients (20%) received antibiotics for presumed bacterial pneumonia but did not receive the COVID-19 specific treatments with antivirals and steroids. In the symptom questionnaire completed, cough was endorsed by every patient. Shortness of breath and fever were

**Table 1 – Patient demographics and admission symptoms.**

Patient	1	2	3	4	5	6	7	8	9	10
Age (y)	52	60	53	<b>66</b>	53	23	86	73	59	47
Gender	M	M	M	F	M	F	F	M	F	F
Temperature (Celsius)	37.7	38.1	38.9	<b>37.3</b>	36.7	37.7	37.2	37.9	36.9	37.9
Heart rate (beats per minute)	91	118	125	<b>95</b>	95	101	67	99	78	111
Oxygen saturation (%)	87	92	92	<b>86</b>	90	97	94	63	100	90
White blood cell count	6.0	8.4	6.5	<b>3.7</b>	5.3	5.0	8.8	4.3	9.0	4.4
Symptom onset (d)	6	4	9	<b>5</b>	6	2	6	8	4	4
Oxygen required	3L NC	RA	2L NC	<b>3.5 L NC</b>	2L NC	RA	2L NC	HFNC 55 L/ 100%	RA	2L NC

NC = Nasal Cannula, RA = Room Air, HFNC = High Flow Nasal Cannula.

Patient 4: Positive aerosol test result.

Bold = patient with positive aerosol test result.

the second and third most common symptoms, which were each endorsed by 70% of patients. The ‘other’ symptoms were not specified by study participants. Only one patient was pan-positive and affirmed every symptom on the questionnaire.

### Outcomes

There were no issues with sample collection, and all cassettes were left in place for 3 h. None of the patients had nasogastric tubes, and therefore, gastrointestinal samples were not collected. Only one patient had a positive aerosol test result (S gene or ORF1ab gene with amplified fluorescence), which was from the sample attached to the patient. All other patients did not have either SARS-CoV-2 gene detected from any of the four filters. As expected, the positive control did have the SARS-CoV-2 genes detected. Along with a representative negative sample, the RT-PCR results for the positive control are depicted in [Figure 3](#).

### Characteristics of patients with positive aerosol sample

The one patient who had a positive aerosol test was 5 d from the onset of symptoms ([Table 2](#)). The patient was a 66-year-old female who had no pulmonary comorbidities and

endorsed all symptoms on the questionnaire besides from fever. She was hemodynamically stable at time of the study and did not have leukocytosis or other significant laboratory abnormalities. The only cassette that detected a positive result was the one that was attached to the patient. The other three cassettes (IV pole, 6 feet, and 12 ft) were all negative. On the day following sample collection, the patient displayed clinical improvement and was discharged home from the hospital without the need for supplemental oxygen.

### Discussion

The amount of information that has been learned about SARS-CoV-2 has exponentially increased within the last year. SARS-CoV-2 is known to be spread primarily through airborne transmission, although the degree to which aerosols contribute to transmission is debated.<sup>8</sup> In this study, aerosol samples were collected using a PTFE filter attached to a calibrated air pump, which cycled air at a set rate of 5 L/min. The sampling technique was confirmed using a positive COVID-19 control and then further validated with a positive aerosolized test from one of the samples collected that was attached to a patient. While this study examined a small number of

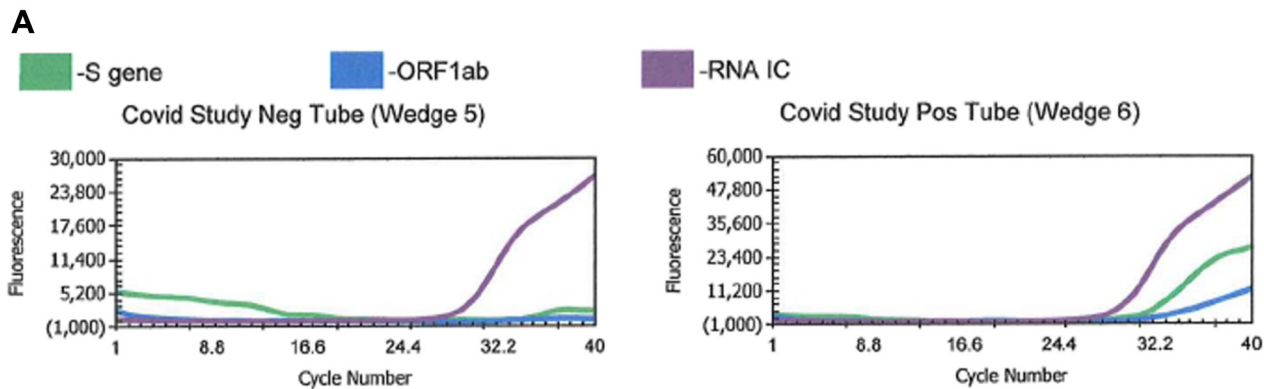
**Table 2 – COVID-19 treatment and patient symptoms.**

Patient	1	2	3	4	5	6	7	8	9	10
Remdesivir	x		x	<b>x</b>			x	x		x
Steroids	x		x	<b>x</b>			x	x		x
Antibiotics		x					x			
Cough	x	x	x	<b>x</b>	x	x	x	x	x	x
Shortness of breath	x	x	x	<b>x</b>	x			x		x
Fever	x	x	x		x	x		x		x
Gastrointestinal symptoms	x	x	x	<b>x</b>				x		x
Loss of taste/Smell	x	x		<b>x</b>						
‘Other’		x	x	<b>x</b>	x	x	x		x	x

Patient 4: Positive aerosol test result.

Bold = patient with positive aerosol test result.





**Fig. 3 – (A) A negative aerosol sample is shown on the left, and the positive control aerosol sample is shown on the right. In the positive sample, there is increased fluorescence in the S and ORF1ab genes prior to cycle number 40. (B) A positive aerosol sample from patient 4 compared to three negative samples collected with a corresponding Ct value of 33.9. Other samples from the same patient were negative.**

patients, it suggests that there is only a limited degree of aerosolization of SARS-CoV-2, which is independent from droplet transmission. It is also encouraging that the sample positive was also the sample closest to the airway of an unmasked patient. There were no clinical factors that were predictive for the positive test result. Since this sampling technique has demonstrated the ability to detect aerosolized SARS-CoV-2, it can be utilized in larger studies and in different healthcare contexts, such as the operating room and the intensive care unit.

The results from this study are consistent with the study by Liu *et al.* who were the first group to demonstrate the viability of a filter collection technique for SARS-CoV-2.<sup>16</sup> In that study, a gelatin filter was used to detect the concentration (copies  $m^{-3}$ ) of SARS-CoV-2 using digital PCR in isolated patient rooms, as well as work areas and public spaces. Although there was no defined relationship between viral concentration and infectivity, the concentration of SARS-CoV-2 was reported as either low or non-detectable in patient rooms, where the filters were placed 2-3 m from the patient. A further up study by Chia *et al.* looked at air and surface sampling in ICU and infection isolation rooms and found that viral detection may be more prevalent than previously suggested. Chia *et al.* reported an ability to detect aerosolized SARS-CoV-2 particles that were 1-5  $\mu m$  in two of three patient rooms despite the rooms having multiple air changes per hour.<sup>19</sup> In addition, Chia *et al.* found that 56.7% of the total rooms that were sampled had at least one source of surface contamination, which raises concern that aerosolization may also contribute to surface deposition.

The present study is unique in a number of ways. Firstly, it detects droplet transmission or possible aerosolization at designated distances in relation to the patient. These distances were strategically chosen because they help quantify the risk for members of the healthcare team and the risk in a social setting. For example, the cassette placed on the IV pole could represent the risk of a bedside nurse or an anesthesiologist. Similarly, the cassettes that were placed 6

feet and 12 feet from the patient represent the recommendations for social distancing (and double the distance of social distancing). Provided that the only positive sample came from the cassette that was attached to a patient, it would appear that the risk is limited when distanced. This risk would likely be further reduced if patients were wearing a mask to reduce droplet spread, which seems to be the main contributor to transmission. Based on these findings, wearing an N-95 around all patients, including COVID-19 positive patients, in the hospital may not be necessary. Another difference between this study and other similar studies is that it was conducted in patients in non-isolated rooms, not ventilated, or undergoing an aerosolizing procedure. Therefore, this study is applicable for the aerosolization of SARS-CoV-2 in noncritically ill, symptomatic COVID patients and can be generalized and more widely applicable to other more common social settings. Additionally, in this prospective study, patients were identified based on symptom duration and tested for a set duration of exposure, and provided more specific information on transmission and characteristics of SARS-CoV2. This study did not detect the exact concentration of SARS-CoV-2 or define an exact particle size but relied on a relative increase in fluorescence at a designated Ct count.

There was no decipherable correlation between patient symptoms and test results seen in this study. All ten patients experienced a cough, which could mechanically enhance the aerosolization of SARS-CoV-2 but still did not result in frequent particle aerosolization. The one patient who had a positive aerosol test endorsed all symptoms besides fever and was otherwise stable in terms of vitals and laboratory values. This patient was weaned off of oxygen and discharged home the day after the sample was collected. Conversely, the sickest patient who was on HFNC in the ICU did not have any positive samples. The timing of sample collection from onset of symptoms was performed in a narrowly defined range, mostly within 1 wk of symptom onset, which should be the group of patients who have the highest viral load (Table 1).<sup>20</sup> The

**B**

**Ct Values**

Wedge	Sample Id	Sample Type	S gene (FAM)	ORF1ab (JOE)	RNA IC (Q670)	QC Statement/Notes
1	PT sample	Unknown	33.9	0	29.9	
2	IV Pole	Unknown	0	0	29.6	
3	6FT	Unknown	0	0	29.8	
4	12FT	Unknown	0	0	29.8	

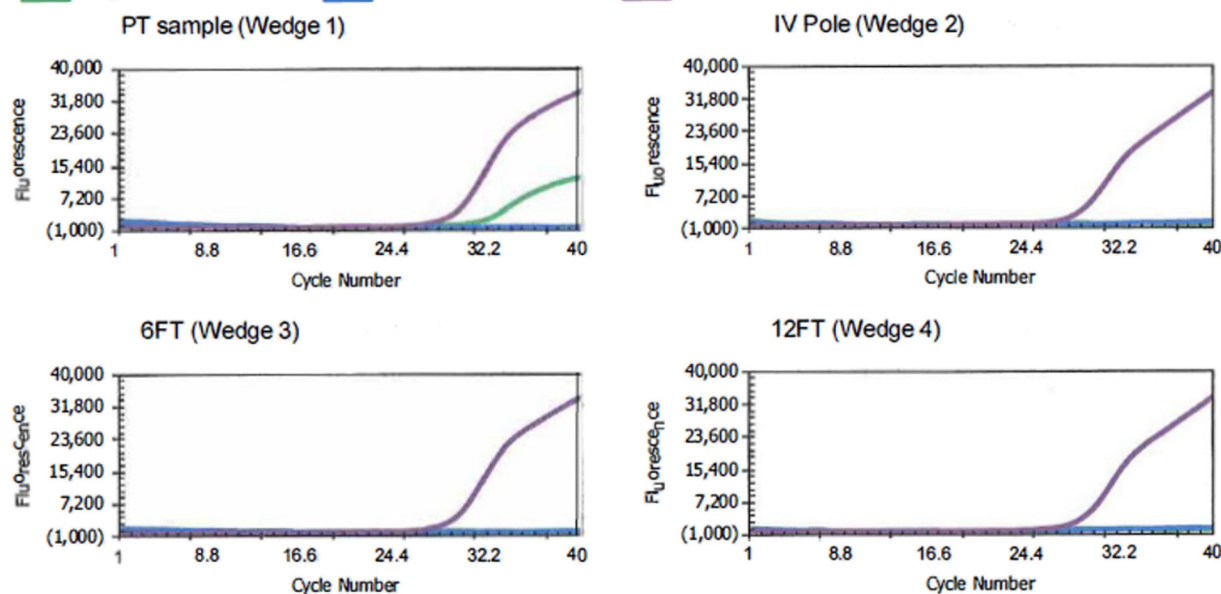
**Results**

Wedge	Sample Id	Sample Type	S gene (FAM)	ORF1ab (JOE)	RNA IC (Q670)	QC Statement / Notes
1	PT sample	Unknown	S gene: Detected	ORF1ab: Not Detected	RNA IC: Valid	Positive
2	IV Pole	Unknown	S gene: Not Detected	ORF1ab: Not Detected	RNA IC: Valid	Negative
3	6FT	Unknown	S gene: Not Detected	ORF1ab: Not Detected	RNA IC: Valid	Negative
4	12FT	Unknown	S gene: Not Detected	ORF1ab: Not Detected	RNA IC: Valid	Negative

-S gene

-ORF1ab

-RNA IC



**Fig. 3 – Continued.**

present study focused only on patients who were symptomatic but it is likely that asymptomatic carriers aerosolize SARS-CoV-2 as well. The asymptomatic patient population is known to shed SARS-CoV-2 particles from their oropharynx and transmit the virus.<sup>21</sup>

None of the patients in this study were vaccinated prior to participation. Since this study was performed, vaccination to COVID-19 has increased drastically. Four in ten Americans

are now vaccinated with vaccination rates varying from 28% to 53% depending on the state.<sup>22</sup> With the widespread availability of the vaccine for all demographics, this number will be expected to increase and needs to increase in order to achieve population-level immunity.<sup>23</sup> There have been no studies examining aerosolization levels since the rollout of the vaccine. Preliminary evidence does suggest that since the advent of the vaccine, the viral load for COVID-positive

patients has been reduced when compared to the viral load prevaccine.<sup>24</sup> It can be inferred from these findings that the COVID vaccine may further diminish aerosolization and transmission. Other studies using this or similar techniques to determine SARS-CoV-2 aerosolization should compare the viral aerosolization between vaccinated and non-vaccinated patients.

There are limitations to this study that should be acknowledged. The study was limited predominantly by the small sample size and enrolled only 10 patients. However, the purpose of the study was to serve as a pilot study and provide proof of concept for a sampling technique. By confirming a positive test result for the COVID-19 control and in an additional patient, the sampling technique was validated as a way to detect COVID-19 particles. There still exists the possibility that not all viral particles are detected in this technique, but a low level of the virus may prove negligible long-term regarding infectious risk. An increase in the number of patients enrolled may have helped to elucidate other relationships, such as a correlation between patient symptoms and the aerosolized test result. Other limitations to the study included a lack of patients who were even earlier in the onset of symptoms (<4 d) or patients who had received one or both doses of the COVID-19 vaccine. The research team found it challenging to identify patients who presented to the hospital immediately after the onset of symptoms as most people are hospitalized later in their clinical course due to COVID-19 complications. Although a positive result was detected in these ten patients, it does not necessarily correlate with infectivity, as that has not been proven based on positive samples alone. It does, however, make it more likely that SARS-CoV2 will be transmitted if no viral particles are detected. Lastly, the dates of collection for the study were February 2021 to April 2021. The authors recognize that these dates fall before the widespread outbreak of the Delta and Omicron variants. In Phase II of the study, we will inevitably include patients who are infected with a variant form of the virus, which may result in increased transmissibility of the virus, and lead to higher rates of positive samples detected.

The future direction of study will aim to apply this sampling technique to large study populations and in other healthcare settings. To date, this is one of the only studies examining viral sampling within an inpatient healthcare setting to ascertain aerosolization and transmission rates for COVID-19. The authors of this study all specialize in general or minimally invasive surgery and plan on exploring the aerosolization SARS-CoV-2 in the operating room (Phase II). Although it has become commonplace to do things in the operating room like wear N-95 protection and use a smoke evacuator, there is relatively little evidence to support the aerosolization of SARS-CoV-2 in surgical smoke.<sup>25</sup> Whether laparotomy or laparoscopy provides a safety advantage is a question that is currently unanswered.<sup>26</sup> Additional information regarding aerosolization during operative procedures will be paramount in determining safety in the operating room and decrease potential transmission. Identifying risks of aerosolized hazards in the hospital and the procedural setting will help inform future studies and allow healthcare providers to be better equipped and prepared against other potential pathogens.

## Conclusions

The results of this study suggest limited viral aerosolization of SARS-CoV-2 in an inpatient setting. In this proof-of-concept study, only one in ten of the aerosolized samples attached to the patient resulted in a positive test after 3 h, which indicated the little overall risk of transmission from aerosolization and was likely due to droplet transmission. None of the samples remote from the patient resulted in a positive test. Due to the limited sample size, there was no detectable correlation between COVID-19 symptoms or duration of symptoms and the aerosolized test result. Larger studies are needed to evaluate viral aerosolization as it significantly affects healthcare providers. Methods for studying mechanisms, safety, and recommendations should be clearly defined based on data.

## Author Contributions

Study Design: All; Data Acquisition: Ayuso, Shao; Statistical Analyses: Ayuso, Shao; Interpretation of Data: All; Draft of Manuscript: Ayuso, Shao; Revisional and Final Approval: All.

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## Disclosure

Dr Ayuso has no disclosures to report. Dr Soriano is a proctor for Intuitive Surgical. Dr Augenstein receives speaking honoraria from Medtronic, Allergan, Intuitive, Acelyty, and Bard. Dr Shao receives research grant funding from the Intuitive Foundation.

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