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Abstract

Objectives: This study assessed the concentration of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the air of hospital rooms occupied by coronavirus disease (COVID-19) patients who had viable SARS-CoV-2 in nasopharyngeal samples in early infection.

Methods: Between July and October 2021, nasopharyngeal swabs were collected from 20 inpatients with early SARS-CoV-2 infection admitted to a tertiary hospital in Japan. Air samples were collected from their rooms, tested for SARS-CoV-2 RNA, and cultured to determine potential infectivity.

Results: The nasopharyngeal swab samples of 18 patients were positive for viable SARS-CoV-2 (median concentration: 4.0×10^5 TCID50/mL). In the air samples, viral RNA (median concentration: 1.1×10^5 copies/m^3) was detected in 12/18 (67%) patients and viable virus (median concentration: 8.9×10^2 TCID50/m^3) was detected in 5/18 (28%) patients. The median time between illness onset and sampling was 3 days. The RNA concentration was significantly higher in samples wherein viable SARS-CoV-2 was detected than in samples in which viable virus was not detected ($p = 0.027$).

Conclusions: Viable SARS-CoV-2 can be detected in the air surrounding patients with early SARS-CoV-2 infection. Healthcare workers should pay attention to infection control when caring for patients with early SARS-CoV-2 infection.

Keywords: SARS-CoV-2, aerosol, air sampling, airborne transmission, Delta SARS-CoV-2 variant

Introduction

Coronavirus disease (COVID-19) is caused by the highly transmissible severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Understanding the role of surface and air contamination near patients with COVID-19 in the transmission of SARS-CoV-2 is essential to ensure the prevention of SARS-CoV-2 transmission. SARS-CoV-2 RNA has been detected on surfaces and in the air of hospitals (Dinoi et al., 2022; Gonçalves et al., 2021). However, only a few studies have recovered viable SARS-CoV-2 from air collected near the patients with COVID-19 (Lednicky et al., 2020; Lednicky et al., 2021; Vass et al., 2022).

Patients with COVID-19 have higher SARS-CoV-2 viral loads in clinical samples in the early phase of the illness (Kim et al., 2021). A previous study showed that surface samples that yielded viable SARS-CoV-2 were collected from patients' environments within 5 days of illness onset (Kotwa et al., 2022). However, few studies have investigated the load of viable SARS-CoV-2 in air samples from hospitalized patients. Therefore, we hypothesized that viable SARS-CoV-2 may be detectable in the air surrounding patients with COVID-19 during the early phase of infection. This study aimed to determine the concentration of SARS-CoV-2 in the air of hospital rooms occupied by patients with COVID-19 who had viable SARS-CoV-2 in nasopharyngeal samples.

Material and methods

Study protocol

This prospective study was conducted from July 20 to October 31, 2021, in Hiroshima University Hospital, Hiroshima, Japan. We included adult patients who were hospitalized in general wards during working hours and who had laboratory-confirmed COVID-19 based on a positive reverse-transcription quantitative polymerase chain reaction (RT-qPCR) SARS-CoV-2 test result performed on a nasopharyngeal swab. Patients who have had COVID-19 symptoms for ≤7 days, admitted to a private or multi-patient room with no other patients who were not under negative pressure, and have not received treatment for COVID-19 prior to the time of sampling were included. The rooms of the patients were air-conditioned and maintained at a temperature of approximately 26°C. Relative humidity was not controlled or measured.

Nasopharyngeal swab samples were collected before air sampling on the day of air sampling, using 1 mL of sterile saline. Demographic and clinical data were collected through participant interviews and chart reviews. The data collected included the Charlson Comorbidity Index (CCI) (Charlson et al., 1987), the interval between illness onset and sample collection, presence or absence of symptoms (fever [temperature >37.5°C], cough, sore throat, runny nose, and diarrhea), and the requirement for supplemental oxygen at the time of air sampling. We also recorded whether patients were wearing surgical masks at the time of air sampling.

This study was approved by the Ethics Committee for Epidemiology of Hiroshima University (approval number: E-2157). Written informed consent was obtained from each participant.

Air sampling

Air samples were collected using an MD8 Airport (Sartorius AG, Göttingen, Germany) with a water-soluble gelatin membrane filter (80 mm in diameter and 3 μm pore size) at a fixed rate of 50 L/min for 40 minutes, corresponding to a final sample volume of 2 m^3 of ambient air. In the patient's rooms, samplers were placed approximately 1 m from the patient's head and anterior to the mouth and nose. The windows of the room were closed during the air sampling.

Laboratory procedures

All samples were transported and processed immediately at Hiroshima University. All work with SARS-CoV-2 was conducted in a biosafety level 3 laboratory. Nasopharyngeal swab samples were vortexed for 30 seconds before aliquoting. The gelatin membrane filters used for air sampling were placed in 5 mL of sterile saline and vortexed for 30 seconds before aliquoting.

RT-qPCR

RNA extraction and RT-qPCR were performed as previously described (Nomura et al., 2022). Before RNA extraction, the air samples were pre-warmed to 37°C until the gelatin had dissolved completely. RNA extraction was performed using the Maxwell Viral Total Nucleic Acid Purification Kit (Promega Corporation, Madison, WI, USA), and RT-qPCR was performed using the One-Step PrimeScript III RT-qPCR mix (Takara Bio Inc., Shiga, Japan), which targets the SARS-CoV-2 nucleocapsid (N) gene. Samples with cycle threshold (Ct) values of ≤40.0 were considered positive. A known quantity of SARS-CoV-2 RNA was used as a positive control, and the viral load was calculated from the Ct values.

Cell and viral culture

Vero cells expressing TMRPSS2 (VeroE6/TMRPSS2 cells, procured from the JCRB Cell Bank, Japan) were used to culture SARS-CoV-2 (Matsuyama et al., 2020). The viral culture methods were performed as described previously (Kitagawa et al., 2021). The cells were observed daily for 5 days to check for a cytopathic effect (CPE), and the infection titer was determined using the median tissue culture infectious dose (TCID50) method. If no CPE was observed within 5 days, the culture was considered negative. The RT-qPCR assay described above was used to confirm SARS-CoV-2 isolation from the supernatant.

Mutant strain test and whole-genome sequencing

Primer/Probe N501Y (SARS-CoV-2) and Primer/Probe L452R (SARS-CoV-2) Ver.2 (Takara Bio Inc., Japan) were used to detect the N501Y mutation in the Alpha variant of concern (VOC) and the L452R mutation in the Delta VOC in the nasopharyngeal and air samples. These tests were performed using the same procedure as that used for RT-qPCR, according to the manufacturer's instructions. Whole-genome sequencing for SARS-CoV-2 isolated from nasopharyngeal samples was performed as described previously (Ko et al., 2021). The identification of SARS-CoV-2 variants in the isolates was performed using the Global Initiative on Sharing All Influenza Data (GISAID) database (https://www.gisaid.org).

Statistical analysis

The Wilcoxon rank-sum test was used to test for statistically significant differences in continuous variables between groups, and χ^2 tests or Fisher's exact test were used to test for statistically significant differences in categorical variables between groups. All statistical analyses were performed using JMP 16.0 (SAS Institute Inc., Cary, NC, USA). P values of <0.05 were considered statistically significant.

Results

Twenty patients were enrolled in the study. Nasopharyngeal swabs were collected from each patient and air samples were collected from each patient's room. The virus cultures of the nasopharyngeal swab samples from two patients were negative. Therefore, their data were excluded from the analysis. No viral RNA or viable virus was detected in the air samples of these two patients.

Demographic, clinical, and virological data from the 18 participants are shown in Table 1. The participant characteristics and the detailed results of the nasopharyngeal and air samples collected are shown in Supplemental Table 1. Of the 18 participants, five (28%) were male and the median age was 61 years (interquartile range [IQR]: 46–78 years). The median interval between the onset of illness and sampling was 3 days (IQR: 2–4 days). The median RNA concentration in the nasopharyngeal swabs was 2.3×10^7 copies/mL (IQR: 1.0×10^7–2.4×10^8

copies/mL), and the median viable virus concentration was 4.0×10^{5} TCID50/mL (IQR: 3.5×10^{4}–3.0×10^{6} TCID50/mL). Viral RNA was detected in 12 of the 18 air samples (67%), and viable virus was detected in five air samples (28%). The median RNA concentration in the air samples was 1.1×10^{5} copies/m^{3} (IQR: 1.5×10^{2}–4.7×10^{5} copies/m^{3}) and the median viable virus concentration in the air samples was 8.9×10^{2} TCID50/m^{3} (range: 5.8×10^{2}–1.0×10^{4} TCID50/m^{3}).

Age, sex, CCI, the interval between the onset of illness and sampling (median days [range] 3 [2–4] vs. 3 [1–7], $p = 0.68$), symptoms, mask wearing, the Ct value of the nasopharyngeal swab samples (Ct >20 vs. Ct ≤20), and the SARS-CoV-2 RNA and viable SARS-CoV-2 concentrations of the nasopharyngeal swab samples did not differ significantly between patients, with and without detectable viable SARS-CoV-2 in the air samples. At the sampling, only one patient with detected viable SARS-CoV-2 in the air samples required oxygen by face mask. Other patients did not require oxygen. In the air samples in which SARS-CoV-2 RNA was detected, the RNA concentration was significantly higher in samples in which viable SARS-CoV-2 was detected than in samples in which viable virus was not detected (median RNA concentration 5.5×10^{5} copy/m^{3} and 2.4×10^{2} copy/m^{3}, respectively; $p = 0.027$).

The relationship between SARS-CoV-2 RNA concentration in nasopharyngeal swabs and air samples is shown in Figure 1, while that of viable SARS-CoV-2 concentration in nasopharyngeal swabs and SARS-CoV-2 RNA concentration in air samples is shown in Figure 2. The relationship between SARS-CoV-2 RNA concentration and viable SARS-CoV-2 concentration in air samples is shown in Figure 3. Viable SARS-CoV-2 in air samples was associated with a high concentration of viral RNA in air samples; however, it was not associated with the concentration of nasopharyngeal viral RNA or viable virus.

All SARS-CoV-2 strains isolated from the nasopharyngeal swab samples and air samples were negative for the N501Y mutation and positive for the L452R mutation, suggesting that they were the Delta variant. Whole-genome sequencing was performed on 12 of 18 SARS-CoV-2 strains isolated from nasopharyngeal swab samples (Supplemental Table 2), which confirmed that all strains belonged to the B.1.617.2 lineage (Delta variant).

Discussion

In our study, SARS-CoV-2 RNA and viable SARS-CoV-2 were detected in 12/18 (67%) and 5/18 (28%) air samples, respectively. The viable virus concentration in the air was 2.8×10^{2} to 1.5×10^{4} TCID50/m^3, which is similar to the results of a previous study showing viral concentrations in air ranging from 6.0×10^{3} TCID50/m^3 to 7.4×10^{4} TCID50/m^3 (Lednicky et al., 2020). To our knowledge, the study by Lednicky et al. (2020) is the only other study that has isolated viable SARS-CoV-2 from air samples in a hospital setting. In the study, the air samplers collected airborne particles using a water vapor condensation method, and the samplers were stationed 2.0 to 4.8 m away from the patients. In other settings, viable Delta variant SARS-CoV-2 has been detected in air collected by air samplers positioned 3 m away from an infected individual experiencing mild symptoms in a self-isolating room (Vass et al. 2022). In the study, the same air samplers as that in the study of Lednicky et al. (2020) were used. Viable SARS-CoV-2 was also detected in the air of a car driven by an individual with COVID-19 (Lednicky et al., 2021). In our study, an air sampler with a gelatin membrane filter (3 μm pore size) was used. Since previous reports showed that viable SARS-CoV2 was detected from samples collected by air samplers, which can collect airborne particles even smaller than 3 μm (Lednicky et al., 2020; Lednicky et al., 2021; Vass et al., 2022)., the actual concentration of SARS-CoV-2 in the air around patients with COVID-19 may be even higher than that found in our study.

Several previous studies, which used different methodologies to measure airborne SARS-CoV-2, detected SARS-CoV-2 RNA from the air around patients with COVID-19 but failed to detect viable SARS-CoV-2 (Dinoi et al., 2022). To the best of our knowledge, our study is the first to detect viable SARS-CoV-2 from air samples collected by air samplers with gelatin filters. However, our methodology, including the use of air sampler with gelatin filters, the sampled air volumes, and the distance from the air sampler to the patient was not specific.

The relatively high proportion of positive air samples in our study may be attributable to the short interval between symptom onset and sampling and the high nasopharyngeal viral load of the participants (Kim et al., 2021). However, the detection of viable SARS-CoV-2 and SARS-CoV-2 RNA in air samples was not necessarily associated with the concentration of nasopharyngeal viral RNA or viable virus (Figures 1, 2). These results suggest that the detection of viable SARS-CoV-2 and SARS-CoV-2 RNA in air samples is also associated with a multitude of factors other than patient's viral load. These include patient's behaviors such as coughing, sneezing, talking, and mask wearing, and environmental circumstances. Previous studies reported that the highest number of SARS-CoV-2 RNA was emitted by singing, followed by talking, and the least was from breathing (Alsved M, et al., 2022; Coleman KK, et al., 2022). In addition, mask wearing reduces emitted airborne SARS-CoV-2 RNA (Adenaiye OO, et al., 2022). In this study, the median interval between the onset of illness and sampling was 3 days, which is shorter than that in previous reports (Kotwa et al., 2022; Lebreil et al., 2022; Zhou et al., 2021). A previous study found that viable SARS-CoV-2 could be detected in samples from surfaces near patients within 5 days of illness onset, but viable virus in air samples could not be detected (Kotwa et al., 2022). Our results suggest that the risk of SARS-CoV-2 transmission in hospitalized patients early during their disease course may be high, and attention should be paid in controlling transmission.

In addition, we used Vero E6 cells expressing transmembrane serine protease 2 (TMPRSS2), which can bind and cleave the SARS-CoV-2 spike protein more efficiently and facilitate early surface-mediated cell entry and viral fusion. Vero E6 cells expressing TMPRSS2 have been shown to enhance SARS-CoV-2 isolation (Hoffmann et al., 2020; Matsuyama et al., 2020). Therefore, a more sensitive culture assay using Vero E6 TMPRSS2 cells may facilitate a higher rate of positive viral culture in air samples.

Previous studies have suggested that individuals infected with the Delta variant have a higher viral load than those infected with the wild-type strain (Li et al., 2022; Ong et al., 2021; Teyssou et al., 2021). Based on the whole-genome sequencing, the timing of the study period, and the mutations identified, the SARS-CoV-2 isolates in the nasopharyngeal swabs and air samples are all likely Delta (B.1.617.2) variant.

This study showed viable SARS-CoV-2 in air samples was associated with a high viral RNA concentration in air samples; however, it was not associated with the concentration of nasopharyngeal viral RNA or viable virus. This study included only patients with early SARS-CoV-2 infection who had viable SARS-CoV-2 in nasopharyngeal samples. Therefore, these results suggest that the presence of viable SARS-CoV-2 in a patient's nasopharyngeal sample is not the only condition for detecting viable SARS-CoV-2 from the air surrounding the patient. Two air samples in which SARS-CoV-2 RNA was detected and viable SARS-CoV-2 was not detected contained 1.8×10^5 and 2.3×10^5 copies/m^3 of SARS-CoV-2 RNA, respectively. The concentration of SARS-CoV-2 in these samples was higher than that in some air samples containing viable SARS-CoV-2. We were unable to determine whether the failure to culture the virus from air samples was due to the low level of viable SARS-CoV-2 in the air samples, below the sensitivity of the virus culture; viable virus being inactivated during the air sampling and viral culture process; or whether only nonviable viral RNA was collected. Virus particles collected by various air sampling devices can become inactivated during the air sampling process (Pan et al., 2019).

Our study had several limitations. First, owing to the small sample size, we were unable to identify risk factors associated with the detection of viable SARS-CoV-2 in air samples based on participant characteristics or behavior. Among the five patients who did not wear a mask during air sampling, five (100%) and three (60%) were positive for SARS-CoV-2 RNA and viable SARS-CoV-2, respectively. However, in our study, we did not formally investigate whether masks can prevent the shedding of viable SARS-CoV-2 virus. In addition, during the air sampling, patients were not monitored to see if they continued to wear a mask. The severity of coughing and sneezing as well as the behavior of the COVID-19 patients, such as talking, were not assessed. Viral emission may depend on a multitude of factors, such as viral load, patient's behaviors such as coughing, sneezing, talking, shouting, and mask wearing, and environmental circumstances. Therefore, further studies are needed to determine the conditions under which viable SARS-CoV-2 can be isolated from the air. Second, air samplers were placed adjacent to participants, 1 m in front of their heads. Therefore, the air sampler likely harvested both droplets and aerosols. However, our air sampling method did not differentiate particle size, and we were unable to distinguish droplets from aerosols (<5 µm). Third, we did not investigate the association between the presence or quantity of viral RNA or viable viruses in air samples and the risk of transmission. Fourth, we did not perform whole-genome sequencing on all SARS-CoV-2 isolates to determine the types of variants. Moreover, the genetic relationship between SARS-CoV-2 strains isolated from the patient and air sampling was not investigated as in a previous study (Kotwa et al., 2022). However, based on the timing of the study period and the mutations identified, the SARS-CoV-2 isolates are all likely Delta variant. The possibility that emission may differ for other more contagious SARS-CoV-2 variants, such as omicron, is likely, but further studies are required.

In conclusion, viable SARS-CoV-2 can be detected in the air surrounding COVID-19 patients with a high viral load. Healthcare workers should pay attention to droplet and airborne infection control when caring for patients admitted shortly after the onset of symptoms. Further studies are needed to investigate the conditions under which viable SARS-CoV-2 is frequently and best isolated from air samples and what factors determine its emission efficacy.

Conflict of interest

The authors have no conflicts of interest to declare.

Funding source

The authors received no funding for this study.

Ethical approval statement

This study was approved by the Ethics Committee for Epidemiology of Hiroshima University (approval number: E-2157).

Acknowledgments

None.

Data availability

The characteristics of the participants and the detailed results of the collected samples are shown in Supplemental Table 1. The 12 full genomes sequence data of SARS-CoV-2 included in this study are deposited at GISAID (https://www.gisaid.org) and GenBank (https://www.ncbi.nlm.nih.gov/genbank/). The accession numbers are provided in Supplemental Table 2.

References

Adenaiye OO, Lai J, de Mesquita PJB, Hong F, Youssefi S, German J, et al. Infectious Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in Exhaled Aerosols and Efficacy of Masks During Early Mild Infection. Clin Infect Dis 2022;75:241–8. https://doi.org/10.1093/cid/ciab797.

Alsved M, Nygren D, Thuresson S, Medstrand P, Fraenkel CJ, Löndahl J. SARS-CoV-2 in Exhaled Aerosol Particles from COVID-19 Cases and Its Association to Household Transmission. Clin Infect Dis 2022;75:50–6. https://doi.org/10.1093/cid/ciac202.

Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987;40:373–83. https//doi.org/10.1016/0021-9681(87)90171–8.

Coleman KK, Tay DJW, Tan KS, Ong SWX, Than TS, Koh MH, et al. Viral Load of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in Respiratory Aerosols Emitted by Patients With Coronavirus Disease 2019 (COVID-19) While Breathing, Talking, and Singing. Clin Infect Dis 2022;74:1722–8. https://doi.org/10.1093/cid/ciab691.

Dinoi A, Feltracco M, Chirizzi D, Trabucco S, Conte M, Gregoris E, et al. A review on measurements of SARS-CoV-2 genetic material in air in outdoor and indoor environments: implication for airborne transmission. Sci Total Environ 2022;89:151137. https//doi.org/10.1016/j.scitotenv.2021.151137.

Gonçalves J, da Silva PG, Reis L, Nascimento MSJ, Koritnik T, Paragi M, et al. Surface contamination with SARS-CoV-2: a systematic review. Sci Total Environ 2021;798:149231. https//doi.org/10.1016/j.scitotenv.2021.149231.

Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020;181:271–80.e8. https//doi.org/10.1016/j.cell.2020.02.052.

Kim MC, Cui C, Shin KR, Bae JY, Kweon OJ, Lee MK, et al. Duration of culturable SARS-CoV-2 in hospitalized patients with Covid-19. N Engl J Med 2021;384:671–3. https//doi.org/10.1056/NEJMc2027040.

Kitagawa H, Nomura T, Nazmul T, Omori K, Shigemoto N, Sakaguchi T, et al. Effectiveness of 222-nm ultraviolet light on disinfecting SARS-CoV-2 surface contamination. Am J Infect Control 2021;49:299–301. https//doi.org/10.1016/j.ajic.2020.08.022.

Ko K, Nagashima S, Bunthen E, Ouoba S, Akita T, Sugiyama A, et al. Molecular characterization and the mutation pattern of SARS-CoV-2 during first and second wave outbreaks in Hiroshima, Japan. PLoS One 2021;16:e0246383. https//doi.org/10.1371/journal.pone.0246383.

Kotwa JD, Jamal AJ, Mbareche H, Yip L, Aftanas P, Barati S, et al. Surface and air contamination with severe acute respiratory syndrome coronavirus 2 from hospitalized coronavirus disease 2019 patients in Toronto, Canada, March–May 2020. J Infect Dis 2022;225:768–76. https//doi.org/10.1093/infdis/jiab578.

Lebreil AL, Greux V, Glenet M, Huguenin A, N'Guyen Y, Berri F, et al. Surfaces and air contamination by severe acute respiratory syndrome coronavirus 2 using high-flow nasal oxygenation or assisted mechanical ventilation in intensive care unit rooms of patients with coronavirus disease 2019. J Infect Dis 2022;225:385–91. https//doi.org/10.1093/infdis/jiab564.

Lednicky JA, Lauzard M, Fan ZH, Jutla A, Tilly TB, Gangwar M, et al. Viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients. Int J Infect Dis 2020;100:476–82. https//doi.org/10.1016/j.ijid.2020.09.025.

Lednicky JA, Lauzardo M, Alam MM, Elbadry MA, Stephenson CJ, Gibson JC, et al.

Isolation of SARS-CoV-2 from the air in a car driven by a COVID patient with mild illness. Int J Infect Dis 2021;108:212–6. https//doi.org/10.1016/j.ijid.2021.04.063.

Li B, Deng A, Li K, Hu Y, Li Z, Shi Y, et al. Viral infection and transmission in a large, well-traced outbreak caused by the SARS-CoV-2 Delta variant. Nat Commun 2022;13:460. https//doi.org/10.1038/s41467-022-28089-y.

Matsuyama S, Nao N, Shirato K, Kawase M, Saito S, Takayama I, et al. Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. Proc Nat Acad Sci U S A 2020;117:7001–3. https//doi.org/10.1073/pnas.2002589117.

Nomura T, Kitagawa H, Omori K, Shigemoto N, Kakimoto M, Nazmul T, et al. Duration of infectious virus shedding in patients with severe coronavirus disease 2019 who required mechanical ventilation. J Infect Chemother 2022;28:19–23. https//doi.org/10.1016/j.jiac.2021.09.006.

Ong SWX, Chiew CJ, Ang LW, Mak TM, Cui L, Toh MPHS, et al. Clinical and virological features of SARS-CoV-2 variants of concern: a retrospective cohort study comparing B.1.1.7 (Alpha), B.1.315 (Beta), and B.1.617.2 (Delta). Clin Infect Dis 2021;ciab721. https://doi.org/10.1093/cid/ciab721. Online ahead of print.

Pan M, Lednicky JA, Wu CY. Collection, particle sizing and detection of airborne viruses. J Appl Microbiol 2019;127:1595–1611. https://doi.org/10.1111/jam.14278.

Teyssou E, Delagrèverie H, Visseaux B, Lambert-Niclot S, Brichler S, Ferre V. The Delta SARS-CoV-2 variant has a higher viral load than the Beta and the historical variants in nasopharyngeal samples from newly diagnosed COVID-19 patients. J Infect 2021;83:e1–3. https://doi.org/10.1016/j.jinf.2021.08.027.

Vass WB, Lednicky JA, Shankar SN, Fan ZH, Eiguren-Fernandez A, Wu CY. Viable SARS-CoV-2 Delta variant detected in aerosols in a residential setting with a self-isolating college student with COVID-19. J Aerosol Sci 2022;165:106038.

https://doi.org/10.1016/j.jaerosci.2022.106038.

Zhou J, Otter JA, Price JR, Cimpeanu C, Meno Garcia D, Kinross J, et al. Investigating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) surface and air contamination in an acute healthcare setting during the peak of the coronavirus disease 2019 (COVID-19) pandemic in London. Clin Infect Dis 2021;73:e1870–7. https://doi.org/10.1093/cid/ciaa905.

Figure captions

Figure 1. Relationship between SARS-CoV-2 RNA concentration in nasopharyngeal swabs and air samples of 18 participants

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TCID50, median tissue culture infectious dose

Figure 2. Relationship between viable SARS-CoV-2 concentration in nasopharyngeal swabs and SARS-CoV-2 RNA concentration in air samples of 18 participants

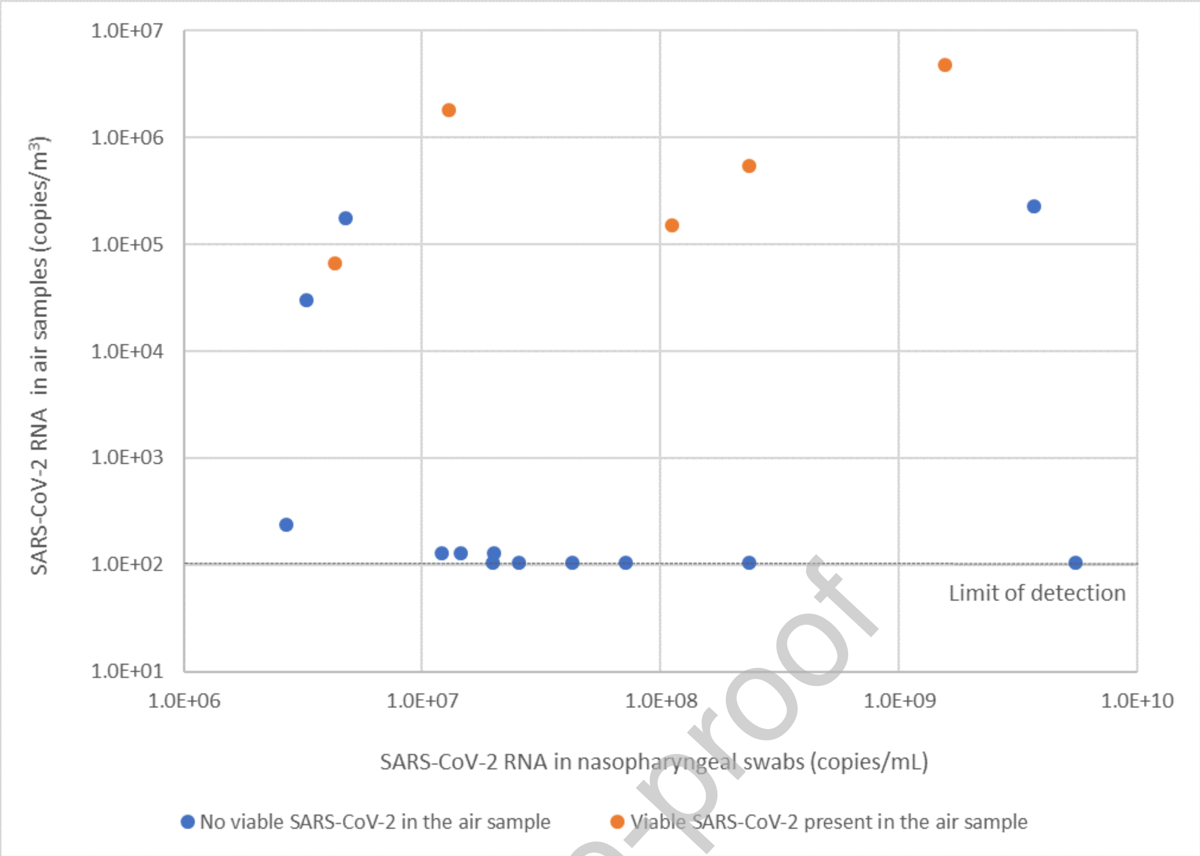
SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TCID50, median tissue culture infectious dose

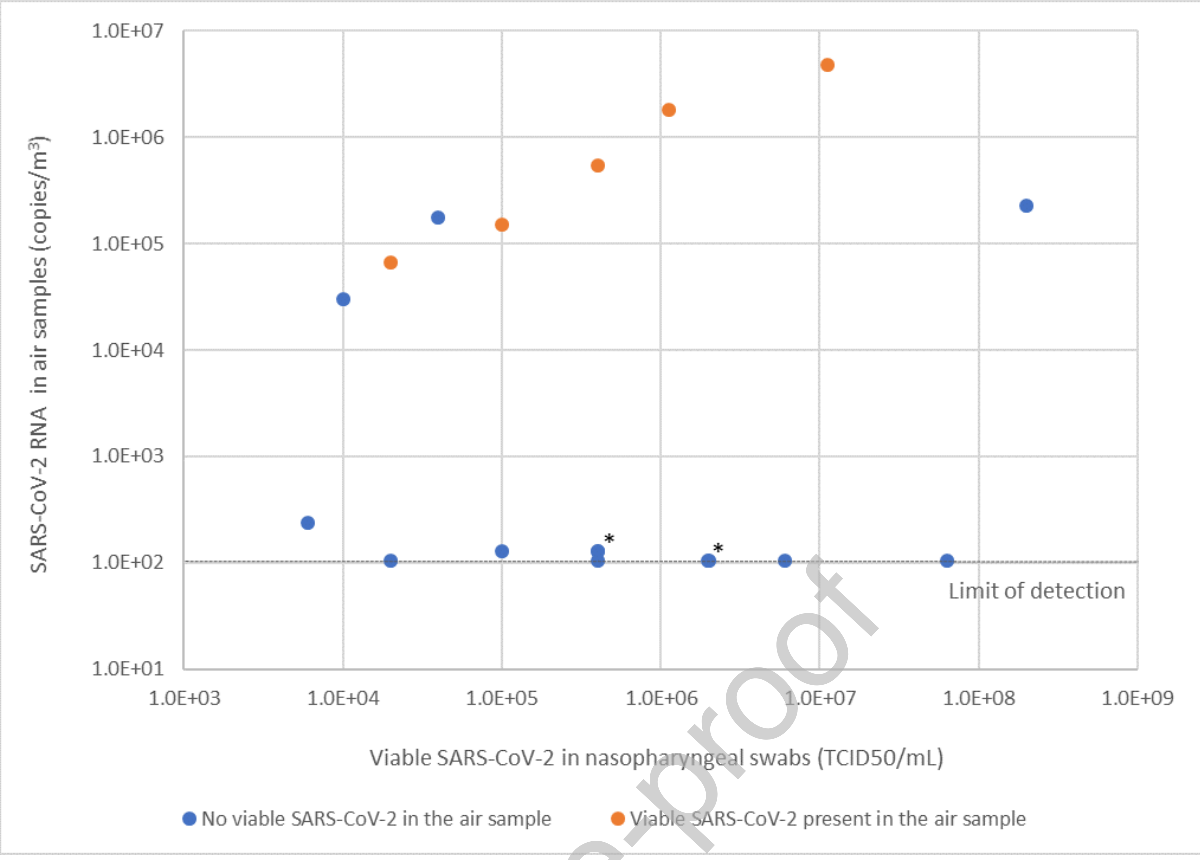
*This dot includes the data of two samples.

Figure 3. Relationship between SARS-CoV-2 RNA concentration and viable SARS-CoV-2 concentration in air samples of 18 participants

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TCID50, median tissue culture infectious dose

*This dot includes the data of three samples.


1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
SARS-CoV-2 RNA in air samples (copies/m³)
1.0E+06
1.0E+07
1.0E+08
1.0E+09
1.0E+10
SARS-CoV-2 RNA in nasopharyngeal swabs (copies/mL)
Limit of detection
No viable SARS-CoV-2 in the air sample
Viable SARS-CoV-2 present in the air sample


1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
SARS-CoV-2 RNA in air samples (copies/m³)
1.0E+03
1.0E+04
1.0E+05
1.0E+06
1.0E+07
1.0E+08
1.0E+09
Limit of detection
*
*
Viable SARS-CoV-2 in nasopharyngeal swabs (TCID50/mL)
No viable SARS-CoV-2 in the air sample
Viable SARS-CoV-2 present in the air sample

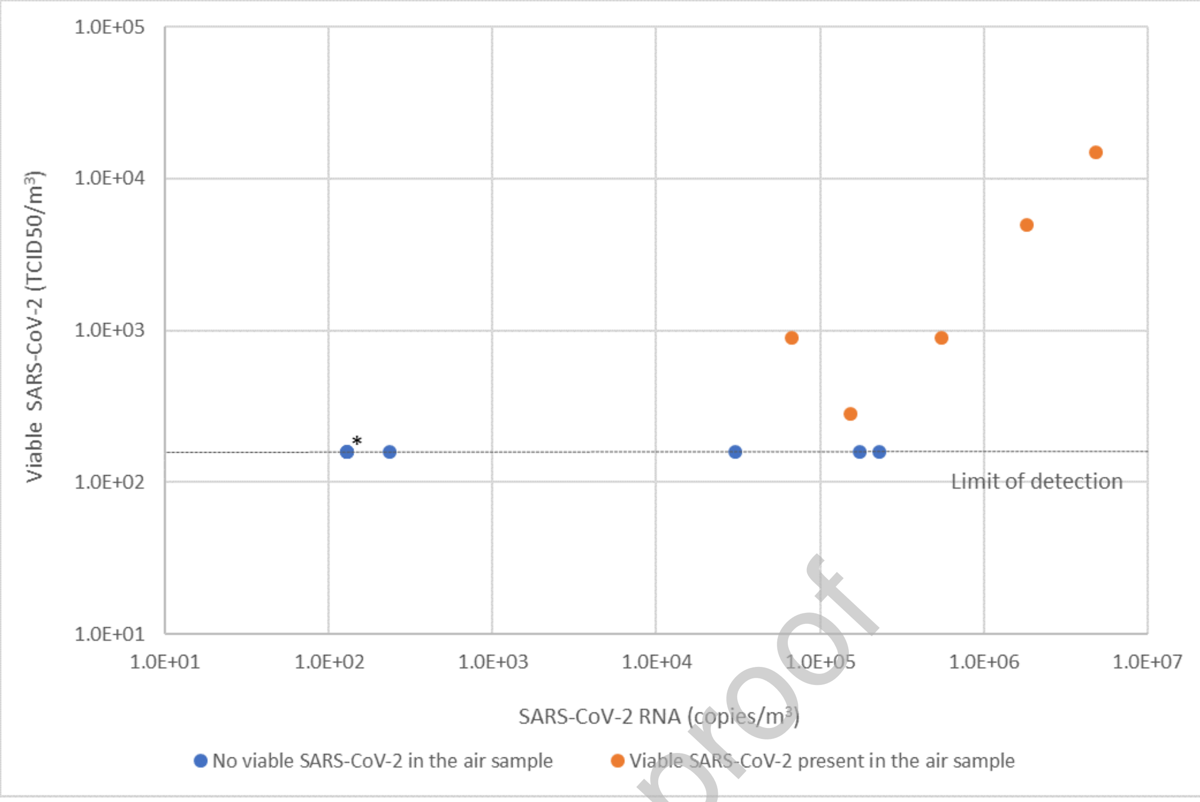

1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
Viable SARS-CoV-2 (TCID50/m³)
*
Limit of detection
1.0E+01
1.0E+02
1.0E+03
1.0E+04
1.0E+05
1.0E+06
1.0E+07
SARS-CoV-2 RNA (copies/m³)
No viable SARS-CoV-2 in the air sample
Viable SARS-CoV-2 present in the air sample

Table 1. Demographic, clinical, and virological data of patients with COVID-19

Patient characteristics	Patients with viable SARS-CoV-2 in air samples (n = 5)	Patients without viable SARS-CoV-2 in air samples (n = 13)	P value
Age (years), median (range)	65 (56–94)	56 (26–92)	0.14
Sex, number, (%)			0.58
Male	2 (40)	3 (23)	
Female	3 (60)	10 (77)	
Charlson Comorbidity Index, n (%)			
Moderate–severe (≥3)	2 (40)	3 (23)	0.58
Symptoms at time of air sampling, n (%)			
Fever (>37.5℃)	5 (100)	9 (69)	0.28
Cough	5 (100)	8 (62)	0.25
Sore throat	1 (20)	2 (15)	>0.99
Runny nose	3 (60)	4 (31)	0.32
Diarrhea	0 (0)	3 (23)	0.52
Wearing mask at the time of air sampling, n (%)	2 (40)	11 (85)	0.10
Nasopharyngeal swab samples			
Ct value, median (range)	19.9 (15.9–23.5)	21.2 (14.0–24.7)	0.55
Ct value ≤20	3 (60)	3 (23)	0.27
SARS-CoV-2 RNA (copies/mL), median (range)	1.1×10^{8} (4.3×10^{6}–1.6×10^{9})	2.0×10^{7} (2.7×10^{6}–5.5×10^{9})	0.62
Viable SARS-CoV-2 (TCID50/mL), median (range)	4.0×10^{5} (2.0×10^{4}–1.1×10^{7})	4.0×10^{5} (6.0×10^{3}–2.0×10^{8})	>0.99
Air samples			
Ct value, median (range)	27.9 (25.0–31.0)	39.1 (29.2–40.0)[a]	0.027
SARS-CoV-2 RNA (copies/m^3), median (range)	5.5×10^{5} (6.7×10^{4}–4.8×10^{6})	2.4×10^{2} (1.3×10^{2}–2.3×10^{5})[a]	0.027
Viable SARS-CoV-2 (TCID50/m^3), median (range)	8.9×10^{2} (2.8×10^{2}–1.5×10^{4})	N/A	

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Ct, cycle threshold; RNA, ribonucleic acid; TCID50, median tissue culture infectious dose; N/A, not applicable.

[a] Only samples with Ct values ≤40.0 (n=7) were analyzed.