LiCellMo™

Product in Development

*LiCellMo — Paving the Way for Metabolic Research and Cell & Gene Therapy**

Live cell metabolic analysis paves the way not only for metabolic research, but also the manufacturing of significant CGT products.

The investigation and understanding of cellular metabolism has become increasingly important in recent years. In the expanding fields of cancer immunotherapy, stem cell research, and commercial biological manufacturing process development, greater metabolic control is resulting in more effective outcomes and higher quality end products. In the field of cancer immunotherapy — such as CAR-T and TCR-T therapy, stem cell research including embryonic stem (ES) and induced pluripotent stem (iPS) cells and commercial cell and gene therapy (CGT) manufacturing process development — all require investigating and understanding the metabolic activities of cells which are critical.

Learn more: https://www.phchd.com/us/biomedical/live-cell-metabolic-analyzer

* *Product not yet commercially available*

PHCbi

Received: 8 February 2023 | Accepted: 10 April 2023

DOI: 10.1002/jmv.28748

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

Airborne virus shedding of the alpha, delta, omicron SARS-CoV-2 variants and influenza virus in hospitalized patients

David S. Y. Ong[1,2] | Peter de Man[1] | Tim Verhagen[3] | Gerda Doejaaren[1] | Marloes A. Dallinga[4] | Esmee Alibux[1] | Matthijs L. Janssen[3,5,6] | Evert-Jan Wils[3,6]

[1]Department of Medical Microbiology and Infection Control, Franciscus Gasthuis & Vlietland, Rotterdam, The Netherlands

[2]Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands

[3]Department of Intensive Care, Franciscus Gasthuis & Vlietland, Rotterdam, The Netherlands

[4]Department of Pulmonary Medicine, Franciscus Gasthuis & Vlietland, Rotterdam, The Netherlands

[5]Department of Pulmonary Medicine, Erasmus MC, Rotterdam, The Netherlands

[6]Department of Intensive Care, Erasmus MC, Rotterdam, The Netherlands

Correspondence
David S. Y. Ong, Department of Medical Microbiology and Infection Control, Franciscus Gasthuis & Vlietland, Kleiweg 500, 3045 PM Rotterdam, The Netherlands.
Email: davidsyong@gmail.com

Funding information
Stichting Bevordering Onderzoek Franciscus

Abstract

Airborne transmission is an important transmission route for the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Epidemiological data indicate that certain SARS-CoV-2 variants, like the omicron variant, are associated with higher transmissibility. We compared virus detection in air samples between hospitalized patients infected with different SARS-CoV-2 variants or influenza virus. The study was performed during three separate time periods in which subsequently the alpha, delta, and omicron SARS-CoV-2 variants were predominant. In total, 79 patients with coronavirus disease 2019 (COVID-19) and 22 patients with influenza A virus infection were included. Collected air samples were positive in 55% of patients infected with the omicron variant in comparison to 15% of those infected with the delta variant ($p < 0.01$). In multivariable analysis, the SARS-CoV-2 omicron BA.1/BA.2 variant (as compared to the delta variant) and the viral load in nasopharynx were both independently associated with air sample positivity, but the alpha variant and COVID-19 vaccination were not. The proportion of positive air samples patients infected with the influenza A virus was 18%. In conclusion, the higher air sample positivity rate of the omicron variant compared to previous SARS-CoV-2 variants may partially explain the higher transmission rates seen in epidemiological trends.

KEYWORDS
air sampling, airborne, COVID-19, influenza, PCR, SARS-CoV-2

1 | INTRODUCTION

Airborne transmission is an important transmission route for the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] Previously, we compared the presence of airborne SARS-CoV-2 RNA in air samples among recently infected individuals at home, and different groups of hospitalized patients with coronavirus disease 2019 (COVID-19) on various oxygen delivery systems during a period when the SARS-CoV-2 D614G and alpha variant were predominant.[2,3]

Epidemiological data indicate that SARS-CoV-2 variants vary in their transmissibility potential with estimated secondary attack rates of 43% for omicron, 36% for alpha, 30% for delta, and 19% during the early phase of the pandemic according to a meta-analysis.[4] Secondary attack rates for the SARS-CoV-2 omicron variant were higher in comparison to the delta variant in household settings.[5] Virus

transmissibility is multifactorial, which includes the infectivity of the pathogen, the contagiousness of the infected individual, the susceptibility of the exposed individual, the contact patterns between the infected individual and the exposed individual, and environmental determinants (e.g., temperature and humidity).[6]

Because airborne virus shedding is one of the mechanisms contributing to higher transmissibility rates, we addressed the question whether the SARS-CoV-2 omicron variant is independently associated with more airborne virus shedding in comparison to previous variants. In this prospective observational study, we compared the extent of air sample positivity, as measure of airborne virus shedding between patients infected with different SARS-CoV-2 variants of concern in hospitalized patients. In addition, we explored whether airborne shedding of the influenza virus can also be detected with our air sampling methodology.

2 | METHODS

We included hospitalized patients with COVID-19 between August 16, 2021 and September 29, 2021 (period with delta variant predominance), and between January 9, 2022 and April 19, 2022 (period with omicron variant predominance), who received 2–6 L/min oxygen support by nasal cannula and had positive quantitative reverse transcription polymerase chain reaction (RT-qPCR) results on SARS-CoV-2 RNA in their nasopharyngeal swabs. Data were also compared to patients from an earlier studied cohort with similar inclusion criteria, who were included between February 22, 2021 and April 17, 2021 (period with alpha variant predominance).[3] We also included patients who were infected with influenza A or B virus between March 30, 2022 and May 12, 2022. The Institutional Review Board (IRB) approved the study protocol (IRB protocol number 2021-134) and declared that this study does not fall within the scope of the Dutch Medical Research Involving Human Subjects Act, since no specific instructions or other behavior requirements were given to patients and only environmental air samples were collected. Patients were informed about the study and were asked oral consent. The study was performed in accordance with the Helsinki Declaration as revised in 2013.

Previously, we developed a vacuum cleaner-based air sampling method combined with RT-qPCR detection of viral RNA.[2] Air sampling was performed within 48 h after diagnostic testing with nasopharyngeal swab, which was usually obtained at hospital admission. The SARS-CoV-2 variant in the diagnostic samples were confirmed by multiplex variant RT-PCR using melting curve analyses, which was implemented in the routine practice of our hospital.[7]

The collection of clinical data and detailed methodology of air sampling and harvesting viral RNA from the sample filters was performed as previously described.[2,3] In short, an IIR type surgical face mask (Romed Holland, type MASK-L) was used as a sample filter placed on the hose inlet of a vacuum cleaner (Nilfisk household vacuum cleaner, with high-efficiency particulate air filter). Air was sampled for 2.5 min at two separate locations sequentially: 50 cm behind and 30 cm above the patient's head (i.e., dorsal sample) and 50 cm in front and 30 cm below (i.e., ventral sample). RNA was extracted from the sample filters using the Roche MagNa Pure large volume total nucleic acid extraction kit. Sample filters were analyzed on our validated in-house RT-qPCR assay on the presence of viral RNA. In the last part of our study during the omicron predominance and influenza virus season, we adapted our RNA extraction method to decrease workload and time of analysis. The manual vortex action with the MagNA Lyser (Roche Diagnostics) benchtop device was replaced by prefilling the samples tubes with glass beads and tissue lysis buffer (Buffer ATL; Qiagen) before rotation to disrupt and extract the RNA from the sample filters. To assess comparability between the two extraction methods, we analyzed a series of random air samples with both methods simultaneously. Finally, 500 µL of the extraction was used for RNA extraction using the MagNA Pure Total Nucleic Acid Isolating Large Volume Kit (Roche). Demographic, clinical, laboratory, and RT-qPCR data were recorded as previously described.[3]

All data were analyzed using Microsoft Excel, R version 3.3.2 (R Foundation for Statistical Computing), and GraphPad Prism v9 (GraphPad Software). Proportions were compared by using χ^2 test or Fisher's exact test as appropriate. Multivariable analysis with adjustment for cycle threshold (C_t) value in positive diagnostic nasopharyngeal swab and COVID-19 vaccination status was performed to assess the associations between the SARS-CoV-2 variant and air sample positivity. The C_t value reflects the viral load in the nasopharynx, which is assumed to be an important determinant of virus shedding, although the C_t value is also affected by other factors such as the execution of the nasopharyngeal sampling procedure. Moreover, we included vaccination status due to its possible association with decreased viral shedding. As a sensitivity analysis, we built a second multivariable model which included all variables with $p < 0.20$ according to univariable comparison between airborne positive and negative samples. For all analyses, values of p that were <0.05 were considered to be statistically significant.

3 | RESULTS

In total, 79 patients with COVID-19 were included for analyses, of whom 38 and 26 patients infected with the omicron and delta variant, respectively, and 15 patients of the previous cohort infected with the alpha variant that fulfilled the inclusion criteria. Thirty (38%) of those patients had at least one positive air sample. Collected air samples were more frequently positive in patients infected with the omicron variant in comparison to those infected with the delta variant: 55% versus 15%, respectively ($p < 0.01$; Table 1). In contrast, no significant differences were detected between the omicron and the alpha variant (55% vs. 33%; $p = 0.26$), and between the alpha and the delta variant (33% vs. 15%; $p = 0.34$).

In univariable analysis SARS-CoV-2 positive air samples were associated with higher age, higher 4C mortality score and higher cough severity (Table 2).[8,9] The median C_t-value of diagnostic

TABLE 1 Positive air samples according to virus variant and sampling position.

	Positive air sample in ventral and/or dorsal position	Positive air sample in ventral position	Positive air sample in dorsal position
SARS-CoV-2 omicron (n = 38)	21 (55)	18 (47)	17 (45)
SARS-CoV-2 delta (n = 26)	4 (15)	4 (15)	2 (8)
SARS-CoV-2 alpha (n = 15)	5 (33)	3 (20)	4 (27)
Influenza A virus (n = 22)	4 (18)	3 (14)	3 (14)

Note: Data are reported as absolute number (percentage). Data from the 15 patients with the alpha variant were derived from our previous cohort study.[3]

Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

TABLE 2 Characteristics of patients with SARS-CoV-2 positive and negative air samples.

	Positive air sample (n = 30)	Negative air sample (n = 49)	p Value
Age	73 (63–80)	64 (49–71)	<0.01
Gender (male)	20 (67)	29 (59)	0.67
Hypertension	15 (50)	22 (45)	0.83
Diabetes mellitus	11 (37)	15 (31)	0.19
Asthma	4 (13)	4 (8)	0.72
COPD	8 (27)	8 (16)	0.41
Body mass index	28 (23–34)	29 (25–34)	0.67
CRP (mg/L)	63 (34–129)	77 (41–146)	0.27
4C mortality score	12 (9–14)	7 (5–12)	0.01
Fisman cough severity score during sampling	1 (0–2)	0 (0–1)	0.05
COVID-19 vaccination	15 (50)	13 (27)	0.06
Symptom duration until day of sampling (days)	6 (4–10)	8 (5–13)	0.73
C_t value of diagnostic PCR	21 (16–24)	24 (21–28)	<0.01

Note: Continuous data are presented as median (interquartile range). Categorical variables are reported as absolute number (percentage).

Abbreviations: COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; C_t, cycle threshold; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

RT-qPCR was lower (i.e., higher viral load) in patients with positive air samples compared to those with negative air samples (median 21 [interquartile range [IQR]: 16–24] vs. 25 [IQR: 22–29]; $p < 0.01$). Patients with positive air samples were sampled earlier after onset of symptoms than those with negative air samples: median 6 (IQR: 3.5–9.5) versus 9 days (IQR: 6–13) ($p = 0.03$), respectively. Moreover, there was a significant correlation between symptom duration at the time of sampling and the viral load in the nasopharynx (Supporting Information: Figure S1).

Compared to the delta variant, patients infected with the omicron variant were older, had higher 4C mortality score, were more frequently vaccinated and their diagnostic RT-qPCR results had lower C_t values (Table 3). In contrast, patients' characteristics were not significantly different between those infected with the alpha variant versus those infected with the delta variant.

In multivariable analysis, the SARS-CoV-2 omicron variant (compared to the delta variant) and the viral load in nasopharynx were both independently associated with air sample positivity, but the alpha variant (compared to the delta variant) and previous COVID-19 vaccination were not significantly associated (Table 4). In the sensitivity analysis, which included SARS-CoV-2 variant type, vaccination status, C_t-value of the nasopharyngeal swab, age, diabetes mellitus, 4C mortality score, and Fisman cough severity as variables, the SARS-CoV-2 omicron variant type and the viral load in nasopharynx were the only independent variables associated with air sample positivity (Supporting Information: Table S1).

TABLE 3 Characteristics of patients according to SARS-CoV-2 variant.

	Omicron SARS-CoV-2 (n = 38)	Delta SARS-CoV-2 (n = 26)	p Value (omicron vs. delta)	Alpha SARS-CoV-2 (n = 15)	p Value (alpha vs. delta)
Age	72 (67–80)	58 (45–67)	<0.01	63 (51–68)	0.39
Gender (male)	24 (63)	16 (62)	1.00	9 (60)	1.00
Hypertension	22 (58)	10 (39)	0.20	5 (33)	1.00
Diabetes mellitus	15 (40)	5 (19)	0.15	6 (40)	0.28
Asthma	3 (8)	4 (15)	0.59	1 (7)	0.74
COPD	11 (29)	3 (12)	0.18	2 (13)	1.00
Body mass index	28 (23–33)	30 (26–35)	0.30	29 (27–31)	0.77
CRP	74 (34–228)	90 (58–247)	0.20	74 (41–140)	0.80
4C mortality score	13 (9–14)	7 (5–11)	<0.01	6 (5–11)	0.98
Fisman cough severity score during sampling	1 (0–2)	0 (0–1)	0.13	0 (0–1)	0.83
COVID-19 vaccination	24 (63)	4 (15)	<0.01	0 (0)	0.29
Symptom duration until day of sampling (days)	6 (3–11)	9 (6–11)	0.08	10 (6–13)	0.60
C_t value of diagnostic PCR	22 (18–25)	25 (21–27)	0.05	22 (20–27)	0.29

Note: Continuous data are presented as median (interquartile range). Categorical variables are reported as absolute number (percentage).
Data from the 15 patients with the alpha variant were derived from our previous cohort study.[3]
Abbreviations: COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; C_t, cycle threshold; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

TABLE 4 Association between SARS-CoV-2 variant and air sample positivity.

	Univariable		Multivariable	
Variables	Unadjusted odds ratio	p Value	Adjusted odds ratio	p Value
SARS-CoV-2 variant (ref = delta)				
Omicron	6.79 (1.9–24.1)	<0.01	5.73 (1.40–23.39)	0.01
Alpha	2.75 (0.6–12.9)	0.19	2.39 (0.5–12.0)	0.28
C_t value of diagnostic PCR	0.87 (0.78–0.96)	<0.01	0.88 (0.8–0.99)	0.03
Vaccination status	2.77 (1.04–7.35)	0.04	0.98 (0.3–3.6)	0.97

Note: Data from the 15 patients with the alpha variant were derived from our previous cohort study.[3]
Abbreviations: PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Forty air samples were analyzed according to both the manual vortex method and the MagNA Lyser method: 10 (25%) and 10 (25%) samples were positive for SARS-CoV-2 RNA, respectively (p = 1.00).

Additionally, 22 patients infected with influenza A virus were sampled. No patients infected with influenza B virus were detected. Patient's characteristics such as age, gender, and comorbidities were similar to those infected with SARS-CoV-2 (Supporting Information: Table S2). However, the patients with influenza A virus were sampled earlier after the onset of symptoms than those with SARS-CoV-2 (i.e., median 4 vs. 8 days, respectively) and had a lower body mass index (i.e., with a median of 25 vs. 29, respectively). The proportion of positive air samples among those infected with influenza A virus was 18% versus 38% for SARS-CoV-2 (p = 0.14). The proportion of positive air samples was significantly higher among those infected with the omicron variant in comparison to the influenza A virus (p = 0.01).

4 | DISCUSSION

This prospective observational study shows that in recently admitted patients the omicron variant is associated with more frequent SARS-CoV-2 RNA in air samples in comparison to the delta variant after adjustment for viral load in the nasopharyngeal sample and COVID-19 vaccination status. More exhalation of the airborne virus by individuals infected with the omicron variant compared to the

delta variant or variants not associated with increased transmissibility was also observed in two observational studies which studied viral shedding in eight omicron- and five delta-infected hospitalized patients,[10] and mildly symptomatic individuals of whom 29 were infected with omicron and 57 were infected with variants not associated with increased transmissibility, respectively.[11]

In this study, low C_t values (i.e., high viral loads) in diagnostic nasopharyngeal samples were associated with shorter durations between symptom onset and time of sampling, and positive air samples, which is in line with previous findings.[2,3] Also, in our multivariable analysis, the nasopharyngeal viral load was independently associated with positive air samples after adjustment for other variables.

Although we did not find a significant difference in airborne SARS-CoV-2 RNA positivity between vaccinated and unvaccinated patients, we cannot exclude that other factors may have confounded this comparison. In our study, omicron-infected individuals were older, more often vaccinated and had a higher viral load detected in the nasopharynx in comparison to those infected with the delta variant. Nevertheless, COVID-19 vaccination was also not associated with air sample positivity in our multivariable sensitivity analysis including age, viral load in the nasopharynx, and other factors. Yet, selection bias may have contributed to the selection of a distinct group of omicron-infected individuals requiring hospitalization despite previous vaccinations. Previous studies suggested that fully vaccinated individuals shed viable virus during shorter durations and were associated with lower secondary attack rates as compared to unvaccinated individuals, while the initial viral load was similar between groups.[12,13]

Detection of airborne influenza virus RNA in exhaled breath has been reported in up to 76% of symptomatic influenza-infected nonhospitalized individuals early after symptom onset.[14,15] In our study the proportion of positive influenza A virus air samples (18%) was lower, which might be partially explained by late sampling after a median of 4 days after symptom onset (i.e., upon hospital admission instead during onset at home) and differences in sampling methodology (i.e., type of sampler and duration of sampling). Indeed, influenza A virus shedding peaked on the first 2 days of clinical illness and decreased gradually to undetectable levels after 6 days in a community-based observational study.[16] It remains to be investigated whether this air sampling method is able to detect other respiratory viruses, and how it compares head-to-head with more labor-intensive air samplers.

To our knowledge, this is the largest air sampling study that compared multiple different SARS-CoV-2 variants of concern. Moreover, we included only patients on low-flow oxygenation modality, because air sample positivity was more frequent in these patients as compared to patients on high-flow nasal cannula oxygen therapy.[3] The current results are in line with previous findings that were derived using the same air sampling method and consistently showing the frequent detection of SARS-CoV-2 RNA in air samples that were taken in repetition or from different locations for every individual participant.[2,3] This study also has important limitations to consider. First, the observed C_t-value of the positive air sample filters was high (median: 36.1, IQR: 34.8–36.9). Detection of very low viral loads in air is challenging due to the detection limit of RT-qPCR and could lead to variability in diagnostic sensitivity. However, at this moment molecular detection methods remain more sensitive than viral culture-based air sampling methods. Second, the timing of air sampling was after hospital admission, which is considered to be a less contagious phase. Indeed, we previously showed higher proportions of SARS-CoV-2 positive air samples taken near recently infected individuals at home in comparison to hospitalized patients.[2] As most virus transmission occurs during the first days after symptom onset, it is possible that differences in airborne shedding between SARS-CoV-2 variants are even more pronounced during the early phase of infection. Third, in the study period omicron BA.1 and BA.2 infections were included but not BA.5 and newer omicron subvariants. Fourth, the statistically nonsignificant difference in air sample positivity between the omicron and alpha variant could be related to the low number of alpha variant-infected included individuals, who originated from the previous study and fulfilled the current study inclusion criteria. Fifth, higher omicron infection rates do not have to be the result of only higher airborne infectivity or higher viral loads present in the nasopharynx, because virus transmissibility is multifactorial and not all possible factors were assessed.[6]

5 | CONCLUSION

The higher air sample positivity rate for patients infected with the omicron variant compared to those infected with previous variants may explain, at least partially, the differences in transmission rates between SARS-CoV-2 variants as observed in epidemiological data. Furthermore, we showed that our vacuum cleaner-based air sampling method is able to detect influenza virus RNA in the air.

AUTHOR CONTRIBUTIONS

David S. Y. Ong, Peter de Man, and Evert-Jan Wils contributed to the conception and design of the study. Tim Verhagen, Gerda Doejaaren, Marloes A. Dallinga, Esmee Alibux, and Matthijs L. JanssenL acquired the data. David S. Y. Ong analyzed the data. All authors contributed to the interpretation of the data. David S. Y. Ong drafted the first manuscript and all authors revised it critically for important intellectual content. All authors approved this manuscript version to be submitted.

ACKNOWLEDGMENTS

The authors would like to thank Margo Nieberg and Elise de Graaf for their assistance during air sampling, and Dick Wille and Han Veltman for their technical assistance of analyzing the sample filters. This research was funded by the Stichting Bevordering Onderzoek Franciscus (project 2021-11) and did not receive any additional funding from agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Most data generated during this study are included in this article. Additional data are available from the corresponding author on reasonable request.

ORCID

David S. Y. Ong http://orcid.org/0000-0001-5688-6443
Evert-Jan Wils https://orcid.org/0000-0002-2868-0920

REFERENCES

1. Greenhalgh T, Jimenez JL, Prather KA, Tufekci Z, Fisman D, Schooley R. Ten scientific reasons in support of airborne transmission of SARS-CoV-2. *Lancet*. 2021;397:1603-1605. doi:10.1016/S0140
2. de Man P, Ortiz MA, Bluyssen PM, et al. Airborne SARS-CoV-2 in home and hospital environments investigated with a high-powered air sampler. *J Hosp Infect*. 2022;119:126-131. doi:10.1016/j.jhin.2021.10.018
3. Janssen ML, Klazen YP, de Man P, Hanselaar W, Ong DSY, Wils EJ. Airborne SARS-CoV-2 RNA excretion by patients with COVID-19 on different oxygen-delivery systems: a prospective observational study. *J Hosp Infect*. 2022;123:87-91. doi:10.1016/j.jhin.2022.03.001
4. Madewell ZJ, Yang Y, Longini IM, Halloran ME, Dean NE. Household secondary attack rates of SARS-CoV-2 by variant and vaccination status: an updated systematic review and meta-analysis. *JAMA Netw Open*. 2022;5(4):e229317. doi:10.1001/jamanetworkopen.2022.9317
5. Jørgensen SB, Nygård K, Kacelnik O, Telle K. Secondary attack rates for omicron and delta variants of SARS-CoV-2 in Norwegian households. *JAMA*. 2022;327(16):1610-1611. doi:10.1001/jama.2022.3780
6. Leung NHL. Transmissibility and transmission of respiratory viruses. *Nat Rev Microbiol*. 2021;19(8):528-545. doi:10.1038/s41579-021-00535-6
7. Ong DSY, Koeleman JGM, Vaessen N, Breijer S, Paltansing S, de Man P. Rapid screening method for the detection of SARS-CoV-2 variants of concern. *J Clin Virol*. 2021;141:104903. doi:10.1016/j.jcv.2021.104903
8. Knight SR, Ho A, Pius R, et al. Risk stratification of patients admitted to hospital with covid-19 using the ISARIC WHO clinical characterisation protocol: development and validation of the 4C mortality score. *BMJ*. 2020;370:m3339. doi:10.1136/bmj.m3339
9. Fisman EZ, Shapira I, Motro M, Pines A, Tenenbaum A. The combined cough frequency/severity scoring: a new approach to cough evaluation in clinical settings. *J Med*. 2001;32(3-4):181-187.
10. Lina L, Zhendong G, Sevalie S, et al. Comparison of the amount of SARS-CoV-2 exhaled by delta and omicron patients. *J Infect*. 2022;85(5):573-607. doi:10.1016/j.jinf.2022.08.028
11. Lai J, Coleman KK, Tai SHS, et al. Exhaled breath aerosol shedding by highly transmissible versus prior SARS-CoV-2 variants. *Clin Infect Dis*. 2023;76(5)786-794. doi:10.1093/cid/ciac846
12. Singanayagam A, Hakki S, Dunning J, et al. Community transmission and viral load kinetics of the SARS-CoV-2 delta (B.1.617.2) variant in vaccinated and unvaccinated individuals in the UK: a prospective, longitudinal, cohort study. *Lancet Infect Dis*. 2022;22(2):183-195. doi:10.1016/S1473-3099(21)00648-4
13. Jung J, Kim JY, Park H, et al. Transmission and infectious SARS-CoV-2 shedding kinetics in vaccinated and unvaccinated individuals. *JAMA Netw Open*. 2022;5(5):e2213606. doi:10.1001/jamanetworkopen.2022.13606
14. Leung NHL, Chu DKW, Shiu EYC, et al. Respiratory virus shedding in exhaled breath and efficacy of face masks. *Nat Med*. 2020;26(5):676-680. doi:10.1038/s41591-020-0843-2
15. Yan J, Grantham M, Pantelic J, et al. Infectious virus in exhaled breath of symptomatic seasonal influenza cases from a college community. *Proc Natl Acad Sci USA*. 2018;115(5):1081-1086. doi:10.1073/pnas.1716561115
16. Ip DKM, Lau LLH, Chan KH, et al. The dynamic relationship between clinical symptomatology and viral shedding in naturally acquired seasonal and pandemic influenza virus infections. *Clin Infect Dis*. 2016;62(4):431-437. doi:10.1093/cid/civ909

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Ong DSY, de Man P, Verhagen T, et al. Airborne virus shedding of the alpha, delta, omicron SARS-CoV-2 variants and influenza virus in hospitalized patients. *J Med Virol*. 2023;95:e28748. doi:10.1002/jmv.28748