

# Dispersion of SARS-CoV-2 in air surrounding COVID-19-infected individuals with mild symptoms

Jostein Gohli<sup>1</sup>  | Ane Marie Anderson<sup>2</sup> | Arne Broch Brantsæter<sup>3</sup> | Kari Oline Bøifot<sup>1,4</sup> | Carola Grub<sup>5</sup> | Cathrine Lund Hadley<sup>2,6</sup> | Andreas Lind<sup>2</sup> | Ellen Susanne Pettersen<sup>2</sup> | Arne Vasli Lund Søråas<sup>2,6</sup> | Marius Dybwad<sup>1,4</sup>

<sup>1</sup>Norwegian Defence Research Establishment, Kjeller, Norway

<sup>2</sup>Oslo University Hospital, Nydalen, Norway

<sup>3</sup>Department of Infectious Diseases, Norwegian National Unit for CBRNE Medicine, Oslo University Hospital, Nydalen, Norway

<sup>4</sup>Department of Analytics, Environmental & Forensic Sciences, King's College London, London, UK

<sup>5</sup>Institute of microbiology, Norwegian Armed Forces Joint Medical Services, Kjeller, Norway

<sup>6</sup>AGE LABS AS, Oslo, Norway

## Correspondence

Jostein Gohli, Norwegian Defence Research Establishment, PO Box 25, NO-2027 Kjeller, Norway.  
Email: Jostein.Gohli@gmail.com

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## Abstract

Since the beginning of the pandemic, the transmission modes of SARS-CoV-2—particularly the role of aerosol transmission—have been much debated. Accumulating evidence suggests that SARS-CoV-2 can be transmitted by aerosols, and not only via larger respiratory droplets. In this study, we quantified SARS-CoV-2 in air surrounding 14 test subjects in a controlled setting. All subjects had SARS-CoV-2 infection confirmed by a recent positive PCR test and had mild symptoms when included in the study. RT-PCR and cell culture analyses were performed on air samples collected at distances of one, two, and four meters from test subjects. Oronasopharyngeal samples were taken from consenting test subjects and analyzed by RT-PCR. Additionally, total aerosol particles were quantified during air sampling trials. Air viral concentrations at one-meter distance were significantly correlated with both viral loads in the upper airways, mild coughing, and fever. One sample collected at four-meter distance was RT-PCR positive. No samples were successfully cultured. The results reported here have potential application for SARS-CoV-2 detection and monitoring schemes, and for increasing our understanding of SARS-CoV-2 transmission dynamics.

## Practical implications.

In this study, quantification of SARS-CoV-2 in air was performed around infected persons with mild symptoms. Such persons may go longer before they are diagnosed and may thus be a disproportionately important epidemiological group. By correlating viral concentrations in air with behavior and symptoms, we identify potential risk factors for viral dissemination in indoor environments. We also show that quantification of total aerosol particles is not a useful strategy for monitoring SARS-CoV-2 in indoor environments.

## KEYWORDS

aerosol, cell culture, coronavirus, COVID-19, RT-PCR, SARS-CoV-2

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## 1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported from an outbreak in Wuhan, China December 31, 2019.<sup>1</sup> On March 11, 2020, The World Health Organization (WHO) declared coronavirus disease 2019 (COVID-19) a global pandemic.<sup>2</sup> The case-fatality rate of COVID-19 varies among countries,<sup>3</sup> but is generally low (~1%)<sup>4</sup> compared to severe acute respiratory syndrome (SARS) caused by SARS-CoV-1 (~11%)<sup>5</sup> and Middle East respiratory syndrome (MERS) caused by MERS-CoV (~30%).<sup>6</sup> However, unlike SARS and MERS, COVID-19 has developed into a true pandemic with the number of SARS-CoV-2-infected people currently estimated to be 245 million, and the death toll estimated at 4.98 million.<sup>4</sup>

Since the beginning of the pandemic, the mode of transmission of SARS-CoV-2 has been highly controversial and the topic of much research and debate.<sup>7-14</sup> The dominating view during the early phase of the pandemic was that SARS-CoV-2 was mainly transmitted by respiratory droplets and contact. Due to their size, large droplets cannot stay suspended in air for longer than a few seconds, and will normally travel distances of less than two meters when projected from a person, for example when talking, singing, coughing, or vomiting.<sup>15</sup> Aerosols, on the other hand, are smaller particles that can stay suspended in air, cover larger distances, and may be a pathway of transmission for certain infectious diseases. Aerosol has traditionally been defined as simply being smaller than 5  $\mu\text{m}$  in size.<sup>16</sup> However, recent evidence indicates that much larger particles can stay suspended in air and cover considerable distances under certain conditions.<sup>17</sup> There is an ongoing debate on both the definition of aerosol in terms of size, and to what extent aerosols play a role in the transmission of SARS-CoV-2 and other respiratory viruses.<sup>15</sup> Accumulating evidence suggests that aerosol transmission of SARS-CoV-2 is an important factor that needs to be accounted for when designing transmission prevention strategies,<sup>18</sup> such as social distancing. Hospital environments are high-risk environments for SARS-CoV-2 transmission, due to the proximity to infected individuals.<sup>19</sup> The risk appears to be strongly dependent on ventilation condition,<sup>19</sup> which is intuitive given its importance for diluting virus concentrations in indoor air.<sup>20</sup> Understanding how virus particles travel through air and infect individuals is of great importance for the purpose of infection control and prevention. However, this is only one part of the puzzle; determining the effects of physicochemical properties of aerosol particles,<sup>21</sup> viral loads in different tissues,<sup>22</sup> infectious dose,<sup>23</sup> human behavior,<sup>24</sup> and the effect of ventilation<sup>19,20</sup>—to name only a few—are all important if the transmission rates are to be minimized.

In this study, we quantified viral concentration in air surrounding 14 SARS-CoV-2-infected test subjects with mild disease at distances of one-, two-, and four meters. We investigated to what extent different SARS-CoV2 PCR markers (gene targets) used in patient diagnostics are useful for detecting SARS-CoV-2 in air samples. Lastly, we evaluated whether test subject behavior, self-reported and observed symptoms, or viral load in the upper airways were significant predictors of viral concentration in the surrounding air.

## 2 | METHODS

### 2.1 | Study design

The study was designed as an observational study nested within the Norwegian Corona Cohort Study (ClinicalTrials.gov Identifier: NCT04320732), which is an ongoing prospective observational study established in March 2020. All adults testing positive for SARS-CoV-2 RT-PCR at either Oslo University Hospital or Frst Medical Laboratory within five days of the planned trial sessions (October 14<sup>th</sup>, December 6<sup>th</sup>, January 17<sup>th</sup>, and March 14<sup>th</sup>) were invited to participate in the Norwegian Corona Cohort Study by SMS, before receiving a follow-up phone call inviting them to the air trial session.

### 2.2 | Sample collection

Fourteen subjects that had recently tested positive for SARS-CoV-2 consented to participate in the air sampling trials. The study included two men and twelve women. The two men were 30 and 36 years old, while the females had a median age of 34 (range 23–54). On the air sampling date, the subjects filled out an electronic form registering the presence of symptoms, including rhinorrhea; cough; fever; chest pain; loss of smell/taste; and dyspnea, the duration of the symptoms, the reason the SARS-CoV-2 test was performed, the test date and comorbidities. The subjects self-reported symptoms ranged from none to mild/moderate respiratory symptoms. The duration of symptoms prior to air sampling was between 2 and 15 days (average = 6 days, median = 5 days, data missing for 3 subjects). None of the test subjects were or had been hospitalized.

The subjects entered a testing room (3.45 m  $\times$  5.3 m, ceiling height of 2.7 m) through an outside entrance, were seated in front of five air samplers, and had a 15-minute conversation with a physician. The room was naturally ventilated with a single ventilation shaft, and by briefly opening the outside entrance between trials. During sampling both the test subject and physician were stationary. The physician recorded the behavior of the subjects during the sampling, which included talking, mild and severe coughing, laughing, and sneezing. Talking was recorded in minutes (maximum 15), while the other variables were counted.

After the 15-minute air sampling trials, oronasopharyngeal samples were taken from consenting test subjects. A single specimen swab was used to sample both sides of the oropharynx, and subsequently, the nasopharynx by insertion and rotation for 10 s. Samples were stored in virus transport medium (UTM™ viral transport media, Copan Diagnostics) and sent to the Department of Microbiology, Oslo University Hospital (OUH) for analysis.

Air samples were collected using electret filters with SASS 3100 air samplers (Research International, Monroe, WA, USA) for 15 min, at 300 L of air per minute. The inlet was positioned at the height of the test subjects' face, and at a 45° downward angle to avoid direct deposition of larger respiratory droplets. The SASS inlet was cleaned with ethanol wipes before filters were mounted. For each

test subject, five SASS air samplers were used—two in parallel at one-meter distance, two in parallel at two-meter distance, and a single air sampler at four-meter distance. Distances were measured from the face of the test subjects, who were seated during the trials (Figure 1). The SASS air sampler at four-meter distance collected particles continuously during the test sessions, while the other samplers were fitted with new filters for each new test subject. At no point were test subjects closer than four meters to the four-meter sampler. Trials were conducted on October 14th, December 6th, 2020, January 17th, and March 14, 2021—with two, two, two, and eight test subjects, respectively.

The parallel samples collected at one- and two-meter distance were analyzed with RT-PCR and cell culture assays. The sample collected at four-meter distance on the 6th of December was sent only for cell culture, while the other three four-meter samples were analyzed only with RT-PCR. Air filters for RT-PCR were placed in 50 ml sterile vials containing 10 ml of NucliSENS lysis buffer (BioMérieux, Marcy-l'Étoile, France) using sterile forceps. Following transport on ice packs back to the laboratory, the air filters were stored at  $-80^{\circ}\text{C}$  until further processing. Air filters for cell culture were placed in 50 ml sterile vials containing 10 ml of Dulcos Modified Essential Medium (DMEM, Sigma) with 1% penicillin/streptomycin/amphotericin B (PSA, Gibco) using sterile forceps and transported to the laboratory on ice packs.

Total particle concentrations were quantified using an Aerotrak 8220 (TSI, Shoreview, MN, US) optical particle counter (Model: 1300102), which was placed directly behind the two SASS samplers at one-meter distance (Figure 1). The Aerotrak particle counter



**FIGURE 1** Setup used in preliminary testing, with two SASS3100 air samplers in parallel and an Aerotrak 8220 optical particle counter

binned particles by the following low end size limits: 0.3, 0.5, 3, 5, and  $10\ \mu\text{m}$ .

## 2.3 | RNA isolation and RT-PCR

Air samples were thawed and vortexed, and filters were removed with sterile forceps from the lysis buffer and placed in sterile syringes to extract the remaining liquid back into the lysis buffer vial before discarding the filter. Before RNA isolation with NucliSENS Magnetic Extraction Reagents (BioMérieux), an internal control (LightMix Modular EAV RNA Extraction Control, TIB-MOLBIOL, Germany) was added ( $1\ \mu\text{l}$ ) to each sample. RNA isolation was performed with  $90\ \mu\text{l}$  silica suspension, otherwise according to the manufacturer's protocol. RNA was eluted in  $100\ \mu\text{l}$  NucliSENS elution buffer before analysis with SARS-CoV-2 RT-PCR assays.

The LightCycler Multiplex RNA Virus Master (Roche Diagnostics, Norway) was used to quantify SARS-CoV-2 RNA in air samples by RT-PCR using the following primers and probes (Eurogentec, Belgium): RdRp nCoV IP2 and IP4<sup>25</sup> from Pasteur in duplex, and HKU (ORF1b-nsp14) from Chu et al.<sup>26</sup> in duplex with the internal control. Probes for IP2 and IP4 used BHQ-1 quencher instead of BBQ. Each reaction contained  $4\ \mu\text{l}$  5X reaction mixture,  $0.1\ \mu\text{l}$  200X enzyme solution,  $25\ \mu\text{g}$  BSA,  $0.5\ \mu\text{M}$  of each primer,  $0.25\ \mu\text{M}$  probe, and  $5\ \mu\text{l}$  sample. For the HKU assays,  $0.5\ \mu\text{l}$  primer/probe mixture for internal control was included in the master mix. PCR-grade water was used to reach a final reaction volume of  $20\ \mu\text{l}$ . SARS-CoV-2 Synthetic RNA Control 1 (Twist Bioscience, CA, USA) was used as a positive control, and PCR-grade water was used as a negative control for RT-PCR. Samples were analyzed in duplicates with a LightCycler 96 (Roche Diagnostics, Norway) using the following conditions: reverse transcription at  $55^{\circ}\text{C}$  for 10 min, initial denaturation at  $95^{\circ}\text{C}$  for 30 s, and, finally, 45 cycles with a two-step amplification, starting with denaturation at  $95^{\circ}\text{C}$  for 5s and annealing/extension at  $58^{\circ}\text{C}$  for 30 s.

Tests of assay sensitivity were performed. A Ct value of 32 was equivalent to a concentration of  $10^2$  copies/ $\mu\text{l}$  for the RdRp genes. HKU was less sensitive with a Ct value of 35 at the same concentration.  $10^2$  copies/ $\mu\text{l}$ —which amounts to a concentration of 2.2 virus copies per liter of air in our sampling setup—were also the limit of detection (LoD) for these assays, which is defined as the lowest concentration where both duplets are detected consistently. Both assays were able to detect lower concentration in a less stable manner, with RdPp being more stable than HKU.

## 2.4 | Cell culture

Samples with a Ct value of 33 or less were chosen for cell culture. In the laboratory, vials with air filters were vortexed for 30 s before removal of the filters with sterile forceps, and fluid was extracted as described in the RT-PCR procedure. African green monkey kidney cells (Vero E6, ATCC: CRL-1586) were used to culture the samples. Samples were incubated for 1 h at  $37^{\circ}\text{C}$  and 5%  $\text{CO}_2$  atmosphere,

before the samples were removed, and the cells were maintained in DMEM (Sigma) supplemented with 5% heat-inactivated fetal bovine (FBS, Gibco) serum and 1% PSA (Gibco). The cells were propagated in a humidified 37 °C incubator in an atmosphere of 5% CO<sub>2</sub> for 6 days. 500 µl of supernatant was collected on day 3 and 6. After RNA isolation (NucliSENS Magnetic Extraction Reagents), RT-PCR was performed as described above.

## 2.5 | Oronasopharyngeal sample RT-PCR and sequencing

Oronasopharyngeal samples were sent to The Department of Microbiology, OUH, for detection of SARS-CoV-2 RNA by routine PCR-based diagnostic protocols. Primary analyses were performed with the Cobas<sup>®</sup> SARS-CoV-2 kit on the Cobas<sup>®</sup> 6800 system (Roche Diagnostics GmbH, Mannheim, Germany). All initially positive samples were reanalyzed with an RT-PCR assay based on the protocol by Vogel et al., detecting probable variant strains and targeting the N-gene as a pan-SARS-CoV-2-positive control.<sup>27,28</sup> Reported Ct values reflect the N-gene target for all samples. Positive samples were sequenced to confirm viral strain if possible.<sup>27</sup>

## 2.6 | Analyses

Negative RT-PCR samples, both from air and oronasopharyngeal samples, were set to a Ct value of 45 for the purpose of statistical analysis and data visualization. Ct values (N-gene) from oronasopharyngeal samples were used as a surrogate for viral load in upper airways and regressed on air sample Ct values (IP2, IP4, and HKU at one- and two-meter distance), testing the hypothesis that viral load in the upper airways is associated with viral concentration in the surrounding air. A multivariable regression model was used to assess associations between air sample Ct values and behavior, that is, talking, mild coughing, and laughing (no severe coughing or sneezing was recorded during the trials). A stepwise Akaike information criterion (AIC) model selection scheme, that is, iteratively dropping the variable with the lowest significance score, was performed. A  $\Delta$ AIC  $\geq 2$  criterion,<sup>29</sup> along with likelihood ratio tests and a preference for more parsimonious models were used to determine the optimal model specification. To investigate causal relationships, this analysis was also performed with oronasopharyngeal sample Ct values as the dependent variable, and with a subsample of air sample Ct values that matched that of the oronasopharyngeal data set. Multivariable regression analyses, using the same AIC model selection scheme, were also used to test for association between symptoms reported by the test subjects and Ct values from air samples. Lastly, we tested for associations between air sample Ct values and particle concentration recorded by the Aerotrack particle counter. Mean particle counts for each size bin (per minute count average) from each trial were used in these models.

Due to differences in data coverage, including all predictors in single multivariable tests for each marker would have resulted in highly reduced data sets. Hence, separate models were ran as described above. All statistical models and visualizations were produced in R v.4.1.1.<sup>30</sup> All models were checked for normality of the residuals, linearity, and heteroscedasticity using model diagnostic plots.

## 3 | RESULTS

Of the 14 persons included in the trials, seven had positive air sample RT-PCR results for one or more markers at either one- or two-meter distance (Figure 2). Eleven of the 42 RT-PCR assays (14 test subject and three PCR markers) performed on samples collected at one-meter distance, and 6 of the 42 RT-PCR assays at two-meter distance were positive. HKU was positive in only two air samples at one-meter (Ct values both under 35).

Of the three air samples collected at four-meter distance that were analyzed with RT-PCR, one (with two test subjects) was negative for all three PCR markers, one (with two test subjects) was positive for IP4 only (Ct = 37.71), and the sample collected on March 14, 2021—when eight subjects were tested—was positive on all three markers (IP2 Ct = 31.60, IP4 Ct = 32.09, HKU Ct = 33.63).

The RT-PCR from the cell culture assay was negative for all samples.

Ten of the 14 test subjects consented to give oronasopharyngeal samples. Two of these were negative, and eight were positive. Linear models of RT-PCR Ct values from oronasopharyngeal samples (N-gene) as a predictor of air sample RT-PCR Ct values at one- and two-meter distance (Table 1) revealed a significant association for IP4 at one-meter distance ( $p = 0.02$ ). IP2 at one meter was not significant with a  $P$ -value of 0.08, and HKU at one-meter and all three PCR markers at two-meter distance were non-significant ( $p > 0.22$ ). The models predicted that oronasopharyngeal sample Ct values of 40 and 30 would correspond to 44.76 (95% CI: 39.43 – 50.09) and 41.27 (95% CI: 37.53 – 45.02) for IP2 and 44.81 (95% CI: 40.45 – 49.17) and 40.66 (95% CI: 37.60 – 43.73) for IP4, both at one-meter distance. A visualization of the relationship between mean Ct values from the three markers at one-meter distance and Ct values from oronasopharyngeal samples is shown in Figure 3. No SARS-CoV-2 was detected in air around the two persons that had negative oronasopharyngeal samples.

The model selection scheme performed on the multivariable regression model which included talking, mild coughing, and laughing (data shown in Figure S2) during trials as predictors of air sample RT-PCR Ct values indicated that a model which included only mild coughing was optimal. Mild coughing was a significant predictor of the Ct values from all three markers at one-meter distance ( $p < 0.04$ ). At one-meter distance, the models predicted that an increase from one to five mild coughs corresponded to a decrease in air sample Ct value of 41.99 (95% CI: 39.65 – 44.33) to 39.60 (95% CI: 36.56 – 42.64) for IP2, from 40.50 (95% CI: 37.84 – 43.16)

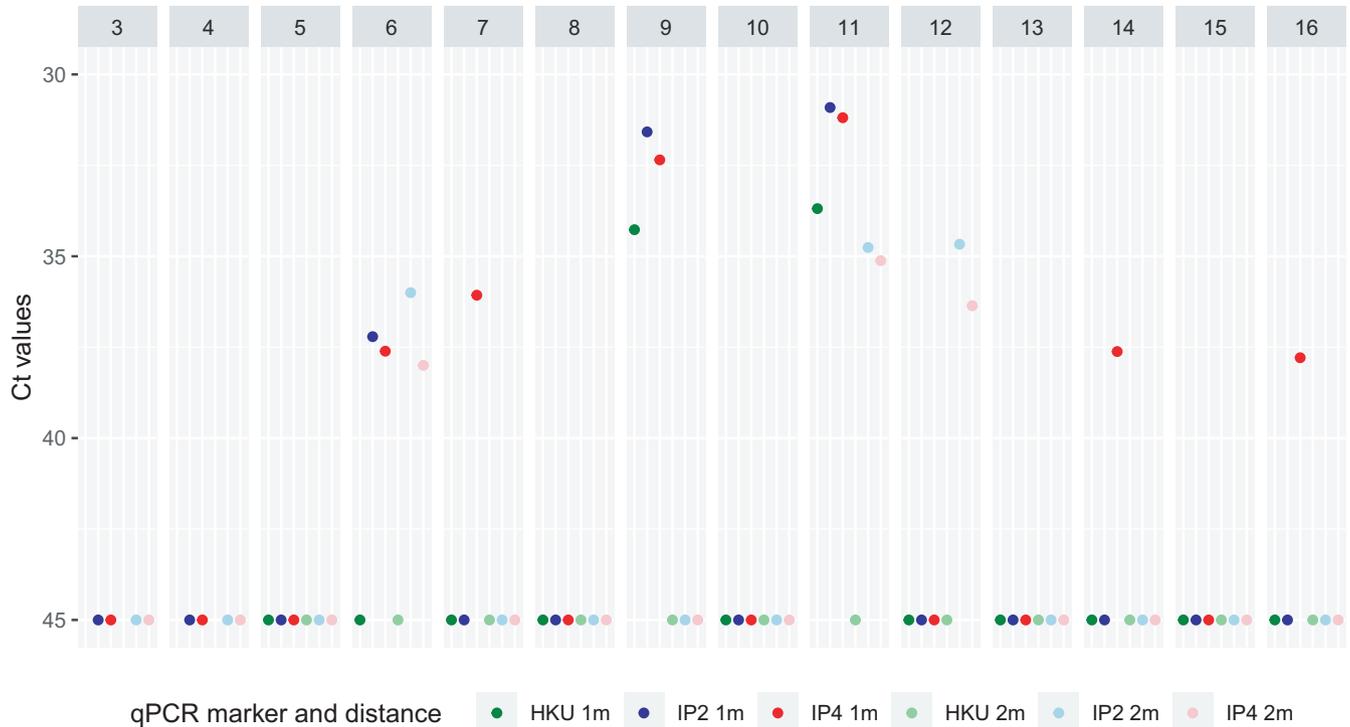


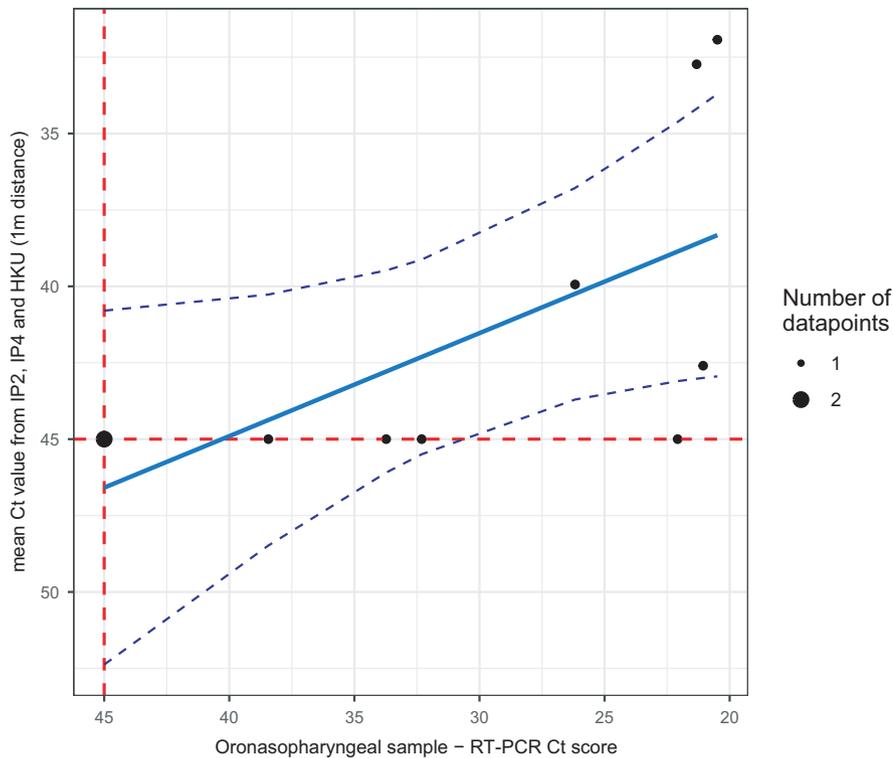
FIGURE 2 Air sample RT-PCR Ct values from three markers (IP2, IP4, and HKU), at one- and two-meter distance from the 14 test subjects. Negative samples were set to a Ct value of 45

TABLE 1 RT-PCR Ct values from oronasopharyngeal samples (N-gene) as a predictor of RT-PCR Ct values from air samples (IP2, IP4, and HKU), at one- and two-meter distance from the test subjects

		Intercept	Ct N-gene (oronasopharyngeal sample)	N	R <sup>2</sup> / R <sup>2</sup> adjusted
Ct IP2 1m	<i>Estimates</i>	30.81	0.35	10	0.332 / 0.249
	<i>CI</i>	17.95 – 43.68	–0.05 – 0.75		
	<i>p</i>		0.081		
Ct IP4 1m	<i>Estimates</i>	28.22	0.41	10	0.513 / 0.452
	<i>CI</i>	17.69 – 38.75	0.09 – 0.74		
	<i>p</i>		0.020		
Ct HKU 1m	<i>Estimates</i>	35.35	0.24	8	0.237 / 0.109
	<i>CI</i>	22.30 – 48.40	–0.19 – 0.66		
	<i>p</i>		0.222		
Ct IP2 2m	<i>Estimates</i>	37.62	0.14	10	0.088 / –0.026
	<i>CI</i>	25.48 – 49.76	–0.24 – 0.52		
	<i>p</i>		0.406		
Ct IP4 2m	<i>Estimates</i>	38.36	0.13	10	0.099 / –0.014
	<i>CI</i>	27.83 – 48.89	–0.20 – 0.46		
	<i>p</i>		0.377		
Ct HKU 2m	<i>Estimates</i>	45.00	0.00	8	0.367 / 0.261
	<i>CI</i>	45.00 – 45.00	–0.00 – 0.00		
	<i>p</i>		0.786		

to 38.50 (95% CI: 35.05–41.96) for IP4, and from 43.05 (95% CI: 40.84 –45.27) to 41.26 (95% CI: 38.54 – 43.98) for HKU. All tests of Ct values from two-meter distance were non-significant

( $p > 0.25$ ). Results from all linear models including mild coughing and Ct values are given in Table 2, and a visualization of the relationship between mean RT-PCR Ct value from IP2, IP4, and HKU



**FIGURE 3** Mean air sample RT-PCR Ct values from IP2, IP4, and HKU at one-meter distance plotted against the RT-PCR Ct values from oronasopharyngeal samples (N-gene). The dashed blue lines correspond to the upper and lower limits of the 95% CI. Negative samples were set to a Ct value of 45, which is indicated by dashed red lines

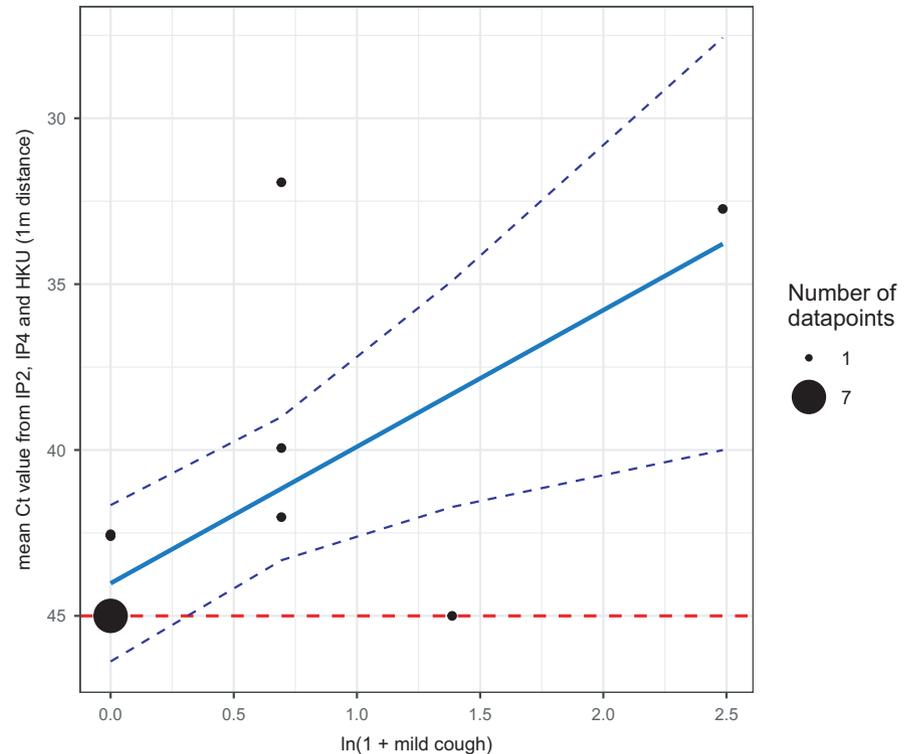
		Intercept	1 + mild cough [log]	N	R <sup>2</sup> /R <sup>2</sup> adjusted
Ct IP2 1m	Estimates	44.51	-4.78	14	0.457/0.412
	CI	41.81 - 47.21	-8.06 - -1.51		
	p		0.008		
Ct IP4 1m	Estimates	42.60	-3.99	14	0.312/0.255
	CI	39.53 - 45.67	-7.71 - -0.27		
	p		0.038		
Ct HKU 1m	Estimates	44.94	-3.58	12	0.416/0.358
	CI	42.28 - 47.60	-6.56 - -0.59		
	p		0.023		
Ct IP2 2m	Estimates	42.93	-0.11	14	0.000/-0.083
	CI	39.96 - 45.91	-3.71 - 3.49		
	p		0.948		
Ct IP4 2m	Estimates	43.23	-0.12	14	0.001/-0.083
	CI	40.64 - 45.82	-3.26 - 3.02		
	p		0.934		
Ct HKU 2m	Estimates	45.00	0.00	12	0.511/0.462
	CI	45.00 - 45.00	-0.00 - 0.00		
	p		0.247		

**TABLE 2** Mild cough as a predictor of RT-PCR Ct values from air samples (IP2, IP4, and HKU), at one- and two-meter distance from the test subjects

at one-meter distance and mild coughing is shown in Figure 4. The regression analysis of mild coughing as a predictor oronasopharyngeal sample Ct values ( $N = 10$ ) was not significant ( $est. = -5.85$ ,  $Std. Err. = 3.85$ ,  $T = -1.52$ ,  $p = 0.17$ ), while the model with one-meter air sample Ct values and mild coughing—using the same ten test subjects—retained significance (all three markers  $p > 0.03$ ).

Multivariable regression models with reported symptoms as predictors of air sample Ct values revealed that fever and high fever were significant across all three air sample PCR markers at one-meter distance, and rhinorrhea was significant for IP2 and HKU at one-meter distance (Table 3; Figure 5). No significant associations were found between symptoms and air sample Ct values in the

**FIGURE 4** Mean air sample RT-PCR Ct value from IP2, IP4, and HKU at one-meter distance plotted against number of mild coughs during the sampling duration. The dashed blue lines correspond to the upper and lower limits of the 95% CI. Negative samples were set to a Ct value of 45, which is indicated by the dashed red line



samples collected at two-meter distance (all predictors in all tests had  $p > 0.14$ ).

None of the linear models examining associations between RT-PCR Ct values from air samples and particle concentration in different size bins were significant ( $p > 0.32$ , Bonferroni corrected  $p = 1$ ), nor did the effect sizes indicate any consistent pattern of association (Figure S1). HKU Ct values from samples collected at two meters were significantly associated with particle concentrations, but only due to all RT-PCR tests being negative, resulting in perfect model fit.

Visualizations of particle concentrations in the testing room (Figures S3–S5), indicated that particle concentrations ( $< 1\mu\text{m}$ ) peaked between trials. Due to equipment failure, no particle data was collected on January 17, 2021.

## 4 | DISCUSSION

While SARS-CoV-2 has been identified and quantified around hospitalized patients,<sup>31</sup> we are unaware of previous studies that have quantified SARS-CoV-2 in air around infected individuals with mild symptoms in a controlled setting. Since infected individuals with mild symptoms may be diagnosed late<sup>32</sup>—giving them more time to interact with non-infected individuals—they may be a disproportionately important epidemiological group.

The three different SARS-CoV2 PCR markers used to analyze air samples mirrored the sensitivity reported for patient samples.<sup>33</sup> Although there was no appreciable difference in the sensitivity of IP2 and IP4, our results underline that it is necessary to include several markers to increase the chance of getting a signal, especially when expected results are close to the limit of the assays' performance.

The LoD in our study was 2.2 virus copies per liter of air, which is higher than some other SARS-CoV-2 air studies.<sup>34,35</sup> Many studies do not report LoD in terms of viral concentration in air, but the lowest reported values across several SARS-CoV-2 air sampling studies indicate large differences in LoD.<sup>36</sup>

It is important to highlight that none of the samples—one four-meter sample and parallel samples at one- and two-meter distance—were culture positive. Although the Ct values in the RT-PCR samples were all above what is considered feasible for cell culture, we cannot exclude the possibility that the air sampling was too harsh for viral particles to retain viability; Lednicky et al.<sup>37</sup> sampled air at 2 and 4.8 meters from a hospitalized COVID patient, using a more gentle condensation growth air sampling method, and successfully cultured SARS-CoV-2. It is also possible that no viable virus particles were sampled as most test subjects were at the end of the first week of symptoms, when the probability to propagate the virus in culture decreases.<sup>38</sup>

An important finding in this study was the detection of a relatively strong RT-PCR signal from air sampled at four-meter distance from SARS-CoV-2-infected individuals with mild symptoms. The positive sample was collected during a session where eight test subjects were inside the testing room for approximately 15–20 min each (15 min of air sampling followed by oronasopharyngeal sampling). The Ct value from this four-meter sample was in range with the lowest Ct value among all the one-meter, 15-min, single subject samples. There was also a weak signal—on one of the three PCR gene targets—for one other four-meter distance sample, collected during a shorter time span when only two subjects visited the testing room. Due to the nature of the sampling strategy, the four-meter data are quite limited; however, the results do support the notion of air

**TABLE 3** Reported symptoms as predictors of RT-PCR Ct values from air samples (IP2, IP4, and HKU) at one-meter distance from the test subjects. Reference levels were set to the “no symptom” category for each predictor variable, that is, *estimates* show the effect size of a given symptom on Ct values

Ct IP2 1m			
Predictors	Estimates	CI	p
(Intercept)	46.62	42.85 – 50.40	
Fever	–8.12	–13.46 – –2.79	0.007
High fever	10.51	1.04 – 19.97	0.033
Rhinorrhea	–4.01	–8.62 – 0.61	0.082
Observations	14		
R <sup>2</sup> / R <sup>2</sup> adjusted	0.624 / 0.511		
Ct IP4 1m			
Predictors	Estimates	CI	p
(Intercept)	42.65	40.04 – 45.26	
Fever	–8.93	–14.36 – –3.50	0.004
High fever	11.28	1.76 – 20.80	0.024
Observations	14		
R <sup>2</sup> / R <sup>2</sup> adjusted	0.567 / 0.488		
Ct HKU 1m			
Predictors	Estimates	CI	p
(Intercept)	45	42.25 – 47.75	
Fever	–5.51	–10.27 – –0.75	0.027
Observations	12		
R <sup>2</sup> / R <sup>2</sup> adjusted	0.400 / 0.340		

transmission of SARS-CoV-2 in unventilated spaces,<sup>39</sup> even during normal conversation from an infected person with mild symptoms.

While several RT-PCR samples were positive at two-meter distance there were, as expected, more positives and generally lower Ct values at one-meter distance. No analyses where RT-PCR results were compared with behavior, self-reported symptoms, or oronasopharynx RT-PCR results were significant for two-meter samples. This is as expected as viral concentration in air decreases as a function of distance to the infected individual; however, it is also possible that the SASS air samplers at one-meter distance (Figure 1; which collected air at 2\*300L/minute) affected the normal flow of particles to the samplers at two-meter distance. In this study, it was considered important to collect directly comparable data for each individual at different distances; however, quantification of SARS-CoV-2 at different distances, with no disturbing elements between the test subject and sampler, would be recommended for future study.

A significant association ( $p = 0.02$ ) between air sample IP4 Ct values and oronasopharyngeal Ct values at one-meter distance indicate that the viral load in the upper airways to some extent mirrors concentration in air immediately surrounding infected subjects (Table 1; Figure 3). It should be noted that oronasopharyngeal samples may be affected by technique and patient compliance as well

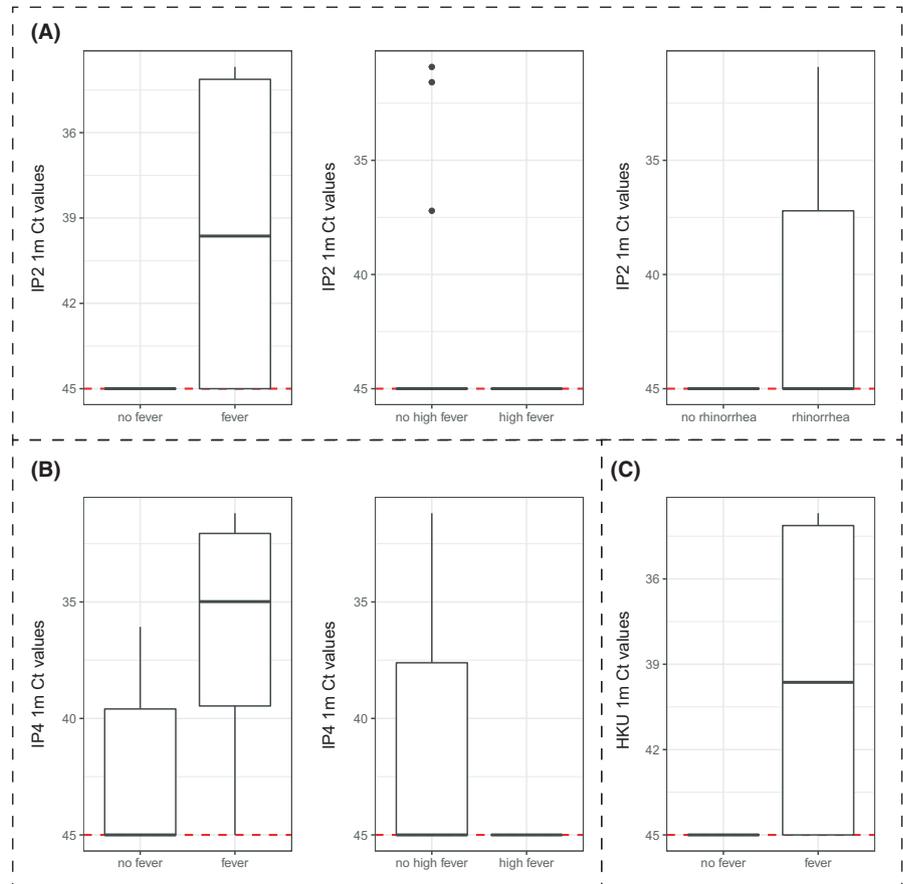
as true viral load in the upper airways. IP2 and KHU Ct values were not significant' with  $P$ -values of 0.08 and 0.22, respectively. A similar study that compared oronasopharyngeal- and exhaled breath samples (the latter collected using an electret filter mounted to a mouth-piece) found no such correlation.<sup>40</sup> These diverging results might stem from different distributions of virus in the upper and lower airways in non-hospitalized vs. hospitalized patients, time of sampling, as well as different dynamics of particle formation in expiration vs. regular conversation.<sup>41</sup> Larger studies are warranted to further understand these findings.

Coughing has been shown to produce a large amount of respiratory droplets, as many as 3000 per cough.<sup>42</sup> Droplet/particle sizes emanating from coughs have been reported to lie in the size range of 0.62 – 15.9  $\mu\text{m}$ <sup>43</sup> and are indeed important for transmission of pathogens.<sup>42</sup> Here, we report a significant association between mild coughing and air sample Ct values. To evaluate whether coughing itself increased viral concentration in air, or if coughing was positively associated with viral load in the airways but did not directly contribute to viral concentration in air, we regressed mild coughing on oronasopharyngeal sample Ct values and found no significant association ( $p = 0.17$ ). Only 10 of 14 test subjects consented to give oronasopharyngeal samples, so the dataset is smaller than that reported in Table 2 (air sample Ct values as response variables). To account for this, we reanalyzed mild cough as a predictor of the Ct values from air samples at one-meter distance with only test subjects that provided oronasopharyngeal samples ( $N = 10$ ) and observed that all three markers retained their significance ( $p > 0.03$ ). These results collectively indicate that mild coughing actively contributes to viral concentration in air at distances  $\leq 1$  meter. However, the analyzed data contained an outlier—one test subject coughed 11 times during the sampling trial (Figure S2). Removing this person from the dataset rendered the tests non-significant (IP2 1m,  $p = 0.18$ ; IP4 1 m,  $p = 0.25$ ; HKU 1m,  $p = 0.43$ ). Given the small dataset and this outlier, we recommend that the results (Table 2; Figure 4) are reproduced.

Self-reported fever and high fever were significant predictors of air sample Ct values (Table 3). The effect size for fever was in the predicted direction—the presence of mild fever was associated with lower Ct values, that is, higher viral concentrations in air. For high fever the opposite pattern was observed; however, only one individual reported high fever, and this person had negative RT-PCR results for both oronasopharyngeal and air samples.

We found no significant associations between air particle concentration and viral concentration in air samples (Figure S1). Visualizations of particle concentrations in the testing room (Figures S3–S5) indicated that particle concentration peaked prior to the 15-minute sample sessions and showed a steady decline during the actual sampling. This indicates that the physician preparing for the next test subject and the subject entering the room produced larger concentrations of particles than test subjects simply sitting down and talking. The results show that if particle concentration and viral concentration are to be compared appropriately, a different sampling design will have to be implemented.

**FIGURE 5** Self-reported symptoms that were significant predictors of air sample RT-PCR Ct values from IP2 (A), IP4 (B), and HKU (C) at one-meter distance (Table 3). Negative samples were set to a Ct value of 45, which is indicated by the dashed red line



We identified several statistically significant associations; however, given the size of the data set, these results should be interpreted with care. Particularly the RT-PCR positive at four-meter distance—which is a single data point consisting of eight test subjects—should be reproduced before strong conclusions are drawn with respect to air transmission beyond two meters. It is also worth noting that the non-significant results presented here do not constitute support for no association, particularly given the size of the data set.

In this study, we have shown that SARS-CoV-2 RNA can be detected in air at a distance up to four meters from an infected individual presenting with mild symptoms, and that viral concentration in air within one-meter distance of infected individuals can be predicted by viral concentration in the upper airways, coughing, and mild fever. The study was carried out in a testing room with natural ventilation, and the results are not likely to be representative for indoor areas with more efficient ventilation.<sup>35</sup> We were not able to successfully propagate virus from air samples; hence, it is possible that the detected virus RNA did not come from viable virus. Since samples taken from patients with mild symptoms are unlikely to be successfully cultured after ten days of symptoms,<sup>44</sup> and since infected individuals appear to be most contagious around symptom onset,<sup>45</sup> the negative cell culture results from air samples reported here is not surprising, and our results cannot be used to rule out air transmission of viable SARS-Cov-2. This study should be useful for both detection and monitoring applications in air and for increasing our understanding of SARS-CoV-2 transmission dynamics.

#### ETHICS STATEMENT

The study was approved by the Regional Committees for Medical and Health Research Ethics (REK) in Norway (reference number 2020/124170) and conducted in accordance with the Helsinki Declaration.

#### PATIENT CONSENT STATEMENT

Inclusion was based on written, informed consent.

#### PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

Not applicable.

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#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

#### AUTHORS' CONTRIBUTIONS

JG designed and led the study, performed the data analyses, and drafted the paper. KOB performed the RT-PCR analyses, contributed to the data management and writing. CG established the RT-PCR (HKU, RdRp), and cell culture assays, contributed to data

management and writing. AMA contributed to study design, data collection, sampling coordination, and writing. ESP contributed to data collection and sampling coordination. AL performed the analyses of oronasopharyngeal samples and contributed to data management. CLH and AVLS facilitated sampling coordination and data handling. ABB contributed to study design and coordination. MD contributed to study design and coordination and supervised the study. All authors contributed to the writing of the manuscript and approved the final version.

## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/ina.13001>.

## DATA AVAILABILITY STATEMENT

The data has been submitted to dryad and has been given a doi:<https://doi.org/10.5061/dryad.r4xgxd2f6>.

## ORCID

Jostein Gohli  <https://orcid.org/0000-0002-7273-1860>

## REFERENCES

- World Health Organization. Pneumonia of unknown cause – China. 2020; <https://www.who.int/emergencies/disease-outbreak-news/item/2020-DON229>
- Cucinotta D, Vanelli M. WHO declares COVID-19 a pandemic. *Acta Biomed*. 2020;91(1):157-160.
- Cifuentes-Faura J. Factors influencing the Covid-19 mortality rate in the European Union: importance of medical professionals. *Public Health*. 2021;200:1-3.
- Worldometers. COVID-19 Coronavirus pandemic. 2021; <https://www.worldometers.info/coronavirus/> Accessed 26th, 2021
- Chan-Yeung M, Xu R-H. SARS: epidemiology. *Respirology*. 2003;8(s1):S9-S14.
- Ahmed AE. The predictors of 3- and 30-day mortality in 660 MERS-CoV patients. *BMC Infect Dis*. 2017;17(1):615.
- Ahmad T, Khan M, Musa TH, et al. COVID-19: Zoonotic aspects. *Travel Med Infect Dis*. 2020;36:101607.
- Pollock AM, Lancaster J. Asymptomatic transmission of covid-19. *BMJ*. 2020;371: m4851.
- Zheng R, Xu Y, Wang W, Ning G, Bi Y. Spatial transmission of COVID-19 via public and private transportation in China. *Travel Med Infect Dis*. 2020;34:101626.
- Hindson J. COVID-19: faecal-oral transmission? *Nat Rev Gastroenterol Hepatol*. 2020;17(5):259.
- Davies NG, Klepac P, Liu Y, et al. Age-dependent effects in the transmission and control of COVID-19 epidemics. *Nat Med*. 2020;26(8):1205-1211.
- Moghadas SM, Fitzpatrick MC, Sah P, et al. The implications of silent transmission for the control of COVID-19 outbreaks. *Proc Natl Acad Sci*. 2020;117(30):17513-17515.
- Tobías A, Molina T. Is temperature reducing the transmission of COVID-19? *Environ Res*. 2020;186:109553.
- Li W, Zhang B, Lu J, et al. Characteristics of household transmission of COVID-19. *Clin Infect Dis*. 2020;71(8):1943-1946.
- Wang CC, Prather KA, Sznitman J, et al. Airborne transmission of respiratory viruses. *Science*. 2021;373:6558.
- Leung NHL. Transmissibility and transmission of respiratory viruses. *Nat Rev Microbiol*. 2021;19(8):528-545.
- Milton DK. A rosetta stone for understanding infectious drops and aerosols. *Journal of the Pediatric Infectious Diseases Society*. 2020;9(4):413-415.
- Greenhalgh T, Jimenez JL, Prather KA, Tufekci Z, Fisman D, Schooley R. Ten scientific reasons in support of airborne transmission of SARS-CoV-2. *The Lancet*. 2021;397(10285):1603-1605.
- Dinoi A, Feltracco M, Chirizzi D, 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;809: 151137.
- Fadaei A. Ventilation Systems and COVID-19 Spread: Evidence from a Systematic Review Study. *Eur J Sustain Dev Res*. 2021;5(2):em0157.
- Pöhlker ML, Krüger OO, Förster J-D, et al. Respiratory aerosols and droplets in the transmission of infectious diseases. *arXiv preprint arXiv:210301188*. 2021.
- Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature*. 2020;581(7809):465-469.
- Karimzadeh S, Bhopal R, Nguyen TH. Review of infective dose, routes of transmission and outcome of COVID-19 caused by the SARS-COV-2: comparison with other respiratory viruses. *Epidemiol Infect*. 2021;149:e96.
- West R, Michie S, Rubin GJ, Amlôt R. Applying principles of behaviour change to reduce SARS-CoV-2 transmission. *Nature Human Behaviour*. 2020;4(5):451-459.
- World Health Organization. Protocol: real-time RT-PCR assays for the detection of SARS-CoV-2, Institut Pasteur, Paris. 2020; <https://www.who.int/docs/default-source/coronaviruse/whoinhouseassays.pdf>
- Chu DKW, Pan Y, Cheng SMS, et al. Molecular diagnosis of a novel coronavirus (2019-nCoV) causing an outbreak of pneumonia. *Clin Chem*. 2020;66(4):549-555.
- Lind A, Barlinn R, Landaas ET, et al. Rapid SARS-CoV-2 variant monitoring using PCR confirmed by whole genome sequencing in a high-volume diagnostic laboratory. *J Clin Virol*. 2021;141:104906.
- Vogels CBF, Breban MI, Alpert T, et al. PCR assay to enhance global surveillance for SARS-CoV-2 variants of concern. *medRxiv*. 2021.
- Burnham KP, Anderson DR. Multimodel Inference: Understanding AIC and BIC in Model Selection. *Sociological Methods & Research*. 2004;33(2):261-304.
- R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria: <https://www.R-project.org/> [computer program]. 2021.
- Birgand G, Peiffer-Smadja N, Fournier S, Kerneis S, Lescure F-X, Lucet J-C. Assessment of air contamination by SARS-CoV-2 in hospital settings. *JAMA*. 2020;3(12):e2033232.
- Santarpia JL, Rivera DN, Herrera VL, et al. Aerosol and surface contamination of SARS-CoV-2 observed in quarantine and isolation care. *Sci Rep*. 2020;10(1):12732.
- Etievant S, Bal A, Escuret V, et al. Performance Assessment of SARS-CoV-2 PCR Assays Developed by WHO Referral Laboratories. *Journal of Clinical Medicine*. 2020;9(6):1871.
- Ang AXY, Luhung I, Ahidjo BA, et al. Airborne SARS-CoV-2 surveillance in hospital environment using high-flowrate air samplers and its comparison to surface sampling. *Indoor Air*. 2022;n/a(n/a).
- Conte M, Feltracco M, Chirizzi D, et al. Airborne concentrations of SARS-CoV-2 in indoor community environments in Italy. *Environ Sci Pollut Res*. 2021.
- Cherrie JW, Cherrie MPC, Davis A, et al. Contamination of air and surfaces in workplaces with SARS-CoV-2 virus: a systematic review. *medRxiv*. 2021;65(8):879-892.
- Lednický JA, Lazard M, Fan ZH, et al. Viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients. *Int J Infect Dis*. 2020;100:476-482.
- Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of

- viral shedding, and infectiousness: a systematic review and meta-analysis. *The Lancet Microbe*. 2021;2(1):e13-e22.
39. Shah Y, Kurelek JW, Peterson SD, Yarusevych S. Experimental investigation of indoor aerosol dispersion and accumulation in the context of COVID-19: Effects of masks and ventilation. *Phys Fluids*. 2021;33(7):073315.
  40. Malik M, Kunze A-C, Bahmer T, Herget-Rosenthal S, Kunze T. SARS-CoV-2: Viral loads of exhaled breath and oronasopharyngeal specimens in hospitalized patients with COVID-19. *Int J Infect Dis*. 2021;110:105-110.
  41. Johnson GR, Morawska L, Ristovski ZD, et al. Modality of human expired aerosol size distributions. *J Aerosol Sci*. 2011;42(12):839-851.
  42. Dhand R, Li J. Coughs and sneezes: Their role in transmission of respiratory viral infections, including SARS-CoV-2. *Am J Respir Crit Care Med*. 2020;202(5):651-659.
  43. Yang S, Lee GW, Chen C-M, Wu C-C, Yu K-P. The size and concentration of droplets generated by coughing in human subjects. *J Aerosol Sci*. 2007;20(4):484-494.
  44. Walsh KA, Spillane S, Comber L, et al. The duration of infectiousness of individuals infected with SARS-CoV-2. *J Infect*. 2020;81(6):847-856.
  45. Casey-Bryars M, Griffin J, McAloon C, et al. Presymptomatic transmission of SARS-CoV-2 infection: a secondary analysis using published data. *BMJ Open*. 2021;11(6):e041240.

## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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