

 Open access • Posted Content • DOI:10.1101/2020.08.03.20167395

## Viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients

— [Source link](#) 

John A. Lednicky, Michael Lauzardo, Z. Hugh Fan, Antarpreet Jutla ...+15 more authors

**Institutions:** University of Florida

**Published on:** 04 Aug 2020 - medRxiv (Cold Spring Harbor Laboratory Press)

**Topics:** Respiratory infection, Viral culture and Room air distribution

Related papers:

- [Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1.](#)
- [Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals.](#)
- [Aerosol and surface contamination of SARS-CoV-2 observed in quarantine and isolation care.](#)
- [Air, Surface Environmental, and Personal Protective Equipment Contamination by Severe Acute Respiratory Syndrome Coronavirus 2 \(SARS-CoV-2\) From a Symptomatic Patient.](#)
- [Aerosol and Surface Distribution of Severe Acute Respiratory Syndrome Coronavirus 2 in Hospital Wards, Wuhan, China, 2020.](#)

Share this paper:    

View more about this paper here: <https://typeset.io/papers/viable-sars-cov-2-in-the-air-of-a-hospital-room-with-covid-44o891e2hg>

1 **Viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients**

2  
3 John A. Lednicky, PhD<sup>1,2\*</sup>, Michael Lauzardo, MD<sup>2,3</sup>, Z. Hugh Fan, PhD<sup>4,5</sup>, Antarpreet Jutla, PhD,<sup>6</sup>  
4 Trevor B. Tilly, PhD,<sup>6</sup> Mayank Gangwar<sup>6</sup>, Moiz Usmani<sup>6</sup>, Sripriya Nannu Shankar<sup>6</sup>, Karim Mohamed<sup>5</sup>,  
5 Arantza Eiguren-Fernandez, PhD<sup>7</sup>, Caroline J. Stephenson<sup>1,2</sup>, Md. Mahbubul Alam<sup>1,2</sup>, Maha A. Elbadry,  
6 PhD<sup>1,2</sup>, Julia C. Loeb<sup>1,2</sup>, Kuttinchantran Subramaniam, PhD<sup>2,8</sup>, Thomas B. Waltzek, PhD<sup>2,8</sup>, Kartikeya  
7 Cherabuddi, MD<sup>3</sup>, J. Glenn Morris, Jr., MD<sup>2,3</sup>, and Chang-Yu Wu, PhD<sup>6</sup>

8  
9 <sup>1</sup>Department of Environmental and Global Health, College of Public Health and Health Professions,  
10 University of Florida, USA

11 <sup>2</sup>Emerging Pathogens Institute, University of Florida, USA

12 <sup>3</sup>Division of Infectious Diseases and Global Medicine, Department of Medicine, College of Medicine,  
13 University of Florida, USA

14 <sup>4</sup>Department of Mechanical & Aerospace Engineering, College of Engineering, University of Florida,  
15 USA

16 <sup>5</sup>J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, USA

17 <sup>6</sup>Department of Environmental Engineering Sciences, College of Engineering, University of Florida, USA

18 <sup>7</sup>Aerosol Dynamics Inc., Berkeley, California, USA

19 <sup>8</sup>Department of Infectious Diseases and Immunology, College of Veterinary Medicine, University of  
20 Florida, USA

21  
22 \*Corresponding author: John A. Lednicky, PhD

23 Dept. of Environmental and Global Health, College of Public Health and Health Professions

24 University of Florida – Gainesville, Box 100188, Gainesville, FL, USA 32610-0188

25 Tel: (1) 352-273-9204; Fax: (1) 352-273-6070; Email: [jlednicky@php.ufl.edu](mailto:jlednicky@php.ufl.edu)

26

27 **Summary**

28 **Background** There currently is substantial controversy about the role played by SARS-CoV-2 in aerosols  
29 in disease transmission, due in part to detections of viral RNA but failures to isolate viable virus from  
30 clinically generated aerosols.

31 **Methods** Air samples were collected in the room of two COVID-19 patients, one of whom had an active  
32 respiratory infection with a nasopharyngeal (NP) swab positive for SARS-CoV-2 by RT-qPCR. By using  
33 VIVAS air samplers that operate on a gentle water-vapor condensation principle, material was collected  
34 from room air and subjected to RT-qPCR and virus culture. The genomes of the SARS-CoV-2 collected  
35 from the air and of virus isolated in cell culture from air sampling and from a NP swab from a newly  
36 admitted patient in the room were sequenced.

37 **Findings** Viable virus was isolated from air samples collected 2 to 4.8m away from the patients. The  
38 genome sequence of the SARS-CoV-2 strain isolated from the material collected by the air samplers was  
39 identical to that isolated from the NP swab from the patient with an active infection. Estimates of viable  
40 viral concentrations ranged from 6 to 74 TCID<sub>50</sub> units/L of air.

41 **Interpretation** Patients with respiratory manifestations of COVID-19 produce aerosols in the absence of  
42 aerosol-generating procedures that contain viable SARS-CoV-2, and these aerosols may serve as a source  
43 of transmission of the virus.

44 **Funding** Partly funded by Grant No. 2030844 from the National Science Foundation and by award  
45 1R43ES030649 from the National Institute of Environmental Health Sciences of the National Institutes of  
46 Health, and by funds made available by the University of Florida Emerging Pathogens Institute and the  
47 Office of the Dean, University of Florida College of Medicine.

48

49

50

51

52

53 **Research in context**

54 **Evidence before this study**

55 Various studies report detection of SARS-CoV-2 in material collected by air samplers positioned in  
56 clinics and in some public spaces. For those studies, detection of SARS-CoV-2 has been by indirect  
57 means; instead of virus isolation, the presence of the virus in material collected by air samplers has been  
58 through RT-PCR detection of SARS-CoV-2 RNA. However, questions have been raised about the clinical  
59 significance of detection of SARS-CoV-2 RNA, particularly as airborne viruses are often inactivated by  
60 exposure to UV light, drying, and other environmental conditions, and inactivated SARS-CoV-2 cannot  
61 cause COVID-19.

62 **Added value of this study**

63 Our virus isolation work provides direct evidence that SARS-CoV-2 in aerosols can be viable and thus  
64 pose a risk for transmission of the virus. Furthermore, we show a clear progression of virus-induced  
65 cytopathic effects in cell culture, and demonstrate that the recovered virus can be serially propagated.  
66 Moreover, we demonstrate an essential link: the viruses we isolated in material collected in four air  
67 sampling runs and the virus in a newly admitted symptomatic patient in the room were identical. These  
68 findings strengthen the notion that airborne transmission of viable SARS-CoV-2 is likely and plays a  
69 critical role in the spread of COVID-19.

70 **Implications of all the available evidence**

71 Scientific information on the mode of transmission should guide best practices Current best practices for  
72 limiting the spread of COVID-19. Transmission secondary to aerosols, without the need for an aerosol-  
73 generating procedure, especially in closed spaces and gatherings, has been epidemiologically linked to  
74 exposures and outbreaks. For aerosol-based transmission, measures such as physical distancing by 6 feet  
75 would not be helpful in an indoor setting and would provide a false-sense of security. With the current  
76 surges of cases, to help stem the COVID-19 pandemic, clear guidance on control measures against SARS-  
77 CoV-2 aerosols are needed.

78

79 **Introduction**

80 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), genus *Betacoronavirus*, subgenus  
81 *Sarbecovirus*, family *Coronaviridae*, is a positive-polarity single-stranded RNA virus that probably  
82 originated in bats<sup>1-3</sup> and is the causative agent of coronavirus disease of 2019 (COVID-19).<sup>4</sup> The  
83 dynamics of the COVID-19 pandemic have proven to be complex. Many challenges remain pertaining to  
84 a better understanding of the epidemiology, pathology, and transmission of COVID-19. For example, the  
85 clinical manifestations of COVID-19 range from an asymptomatic infection, mild respiratory illness to  
86 pneumonia, respiratory failure, multi-organ failure, and death.<sup>5-7</sup> Diarrhea due to gastro-intestinal  
87 infection can also occur, and *in vitro* modeling suggests that the virus infects human gut enterocytes.<sup>8</sup>  
88 SARS-CoV-2 RNA can be found in rectal swabs and fecal aerosols, even after nasal-pharyngeal testing  
89 has turned negative,<sup>9-12</sup> suggesting that a fecal–oral transmission route may be possible.

90 To-date, there has been a strong emphasis on the role of respiratory droplets and fomites in the  
91 transmission of SARS-CoV-2.<sup>13,14</sup> Yet SARS-CoV-2 does not appear to be exclusively inhaled as a  
92 droplet, and epidemiologic data are consistent with aerosol transmission of SARS-CoV-2.<sup>15-19</sup>  
93 Furthermore, SARS-CoV-2 genomic RNA has been detected in airborne material collected by air  
94 samplers positioned distal to COVID-19 patients.<sup>9, 20-23</sup> Any respiratory virus that can survive  
95 aerosolization poses an inhalation biohazard risk, and van Doremalen *et al.*<sup>24</sup> experimentally generated  
96 aerosol particles with SARS-CoV-2 and found that the virus remained viable during a three-hour testing  
97 period. More recently, Fears *et al.*<sup>25</sup> reported that the virus retained infectivity and integrity for up to 16  
98 hours in laboratory-created respirable-sized aerosols. Nevertheless, finding virus RNA in material  
99 collected by an air sampler may not correlate with risk. Indeed, the air we breathe is full of viruses  
100 (animal, plant, bacterial, human, etc.), yet a large proportion of the viruses in air are non-viable due to  
101 UV-inactivation, drying, etc., and non-viable viruses cannot cause illnesses. Because efforts to isolate  
102 virus in cell cultures in the aforementioned air sampling studies in hospital wards were not made,<sup>20,22</sup> or  
103 failed when they were attempted due to overgrowth by faster replicating respiratory viruses,<sup>23</sup> or provided

104 weak evidence of virus isolation,<sup>21</sup> uncertainties about the role of aerosols in COVID-19 transmission  
105 remain.

106 It is well known that virus particles collected by various air samplers become inactivated during the air  
107 sampling process,<sup>26</sup> and if such is the case for SARS-CoV-2, this partly explains why it has been difficult  
108 to prove that SARS-CoV-2 collected from aerosols is viable. Because we previously collected SARS-  
109 CoV-2 from the air of a respiratory illness ward within a clinic but were unable to isolate the virus in cell  
110 cultures due to out-competition by other respiratory viruses,<sup>23</sup> we sought to perform air sampling tests in a  
111 hospital room reserved for COVID-19 patients, to lessen the probability of collecting other airborne  
112 human respiratory viruses. We thus collected aerosols containing SARS-CoV-2 in a room housing  
113 COVID-19 patients using our VIVAS air samplers that collect virus particles without damaging them,  
114 thus conserving their viability. These samplers operate using a water-vapor condensation mechanism.<sup>27,28</sup>  
115 Air samplings were performed at the University of Florida Health (UF Health) Shands Hospital, which is  
116 a 1,050-bed teaching hospital situated in Gainesville, Florida. As of 10 July 2020, > 200 patients have  
117 been treated at the hospital for COVID-19. The current study was conducted as part of ongoing  
118 environmental investigations by the UF Health infection control group to assess possible healthcare  
119 worker exposure to SARS-CoV-2.

120

## 121 **Methods**

122 Detailed methods are provided in a Technical Appendix. An abbreviated summary of methods is  
123 provided below:

### 124 **Institutional Review Board (IRB) approval and patients**

125 The study protocol was approved by the UF IRB (study IRB202002102). Patient 1 was a person with  
126 coronary artery disease and other co-morbidities who had been transferred from a long-term care facility  
127 for COVID-19 treatment the evening before our air sampling tests were initiated; he had a positive NP  
128 swab test on admission that was positive for SARS-CoV-2 by RT-PCR. Patient 2 had been admitted four  
129 days before the air sampling tests with a mid-brain stroke; the patient had a positive NP swab test for

130 SARS-CoV-2 on admission, but a repeat test was negative, and the patient was in the process of being  
131 discharged at the time the air sampling was being done.

### 132 **Hospital room**

133 Air samples were collected in a room that was part of a designated COVID-19 ward (Figure 1). The room  
134 had six air changes per hour and the exhaust air underwent triple filter treatment (minimum efficiency  
135 reporting value [MERV] 14, 75%-85% efficiency for 0.3  $\mu\text{m}$  particles), coil condensation (to remove  
136 moisture), and UV-C irradiation prior to recycling 90% of the treated air back to the room.

### 137 **Air samplers and sampling parameters**

138 Three serial 3-hr air samplings were performed using our prototype VIVAS air sampler,<sup>23, 27, 28</sup> as well as  
139 a BioSpot-VIVAS BSS300P, which is a commercial version of the VIVAS (available from Aerosol  
140 Devices Inc., Ft. Collins, CO). These samplers collect airborne particles using a water-vapor  
141 condensation method.<sup>23, 27, 28</sup> Two samplers were used so that air could be collected/sampled at different  
142 sites of the same room during a given air sampling period. For each sampler, the second of the three  
143 samplings was performed with a high efficiency particulate arrestance (HEPA) filter affixed to the inlet  
144 tube, a process we implement to reveal whether virus detected in consecutive samplings reflect true  
145 collection and not detection of residual virus within the collector. The air-samplers were stationed from 2  
146 to 4.8 m away from the patients (Figure 1).

### 147 **Detection of SARS-CoV-2 genomic RNA (vRNA) in collection media**

148 vRNA was extracted from virions in collection media and purified by using a QIAamp Viral RNA Mini  
149 Kit (Qiagen, Valencia, CA, USA). Twenty-five  $\mu\text{L}$  (final volume) real-time reverse-transcription  
150 polymerase chain reaction (rtRT-PCR) tests were performed in a BioRad CFX96 Touch Real-Time PCR  
151 Detection System using 5  $\mu\text{L}$  of purified vRNA and rtRT-PCR primers and the probe listed in Table 1  
152 that detect a section of the SARS-CoV-2 N-gene.<sup>23</sup> The N-gene rRT-PCR assay that was used was part of  
153 a dual (N- and RdRp-gene) rRT-PCR assay designed by J. Lednicky and does not detect common human  
154 alpha- or beta-coronaviruses. Using this particular N-gene rRT-PCR detection system, the limit of  
155 detection is about 1.5 SARS-CoV-2 genome equivalents per 25  $\mu\text{L}$  rRT-PCR assay.

156 **Cell lines for virus isolation**

157 Cell lines used for the isolation of SARS-CoV-2 were obtained from the American Type Culture  
158 Collection (ATCC) and consisted of LLC-MK2 (Rhesus monkey kidney cells, catalog no. ATCC CCL-7)  
159 and Vero E6 cells (African green monkey kidney cells, catalog no. ATCC CRL-1586).

160 **Isolation of virus in cultured cells**

161 Cells grown as monolayers in a T-25 flask (growing surface 25 cm<sup>2</sup>) were inoculated when they were at  
162 80% of confluency. First, aliquots (100 µL) of the concentrated air sampler collection media were filtered  
163 through a sterile 0.45 µm pore-size PVDV syringe-tip filter to remove bacterial and fungal cells and  
164 spores. Next, the spent LLC-MK2 and Vero E6 cell culture medium was removed and replaced with 1 mL  
165 of cell culture medium, and the cells inoculated with 50 µL of cell filtrate. When virus-induced cytopathic  
166 effects (CPE) were evident, the presence of SARS-CoV-2 was determined by rRT-PCR.

167 **Quantification of SARS-CoV-2 genomes in sampled air**

168 The number of viral genome equivalents present in each sample was estimated from the measured  
169 quantification cycle (Cq) values. To do so, a 6-log standard curve was run using 10-fold dilutions of a  
170 calibrated plasmid containing an insert of the SARS-CoV-2 N-gene that had been obtained from IDT  
171 Technologies, Inc. (Coralville, Iowa). The data was fit using equation (eq.) 1:

172 Eq. 1.  $y = (\log_{10}GE)(a) + b$ , where  $y = Cq$  value,  $a =$  slope of the regression line,  $\log_{10}GE$  is the base  
173 10 log genome equivalents, and  $b$  is the intercept of the regression line.

174 **Sanger sequencing of SARS-CoV-2 genomes in material collected by air samplers**

175 To obtain the virus consensus sequence prior to possible changes that might occur during isolation of the  
176 virus in cell cultures, a direct sequencing approach was used. Because the amount of virus present in the  
177 samples was low and thus unsuitable for common next-generation sequencing approaches, Sanger  
178 sequencing based on a gene-walking approach with over-lapping primers was used to obtain the virus  
179 sequence.<sup>23</sup>

180 **Next-generation sequencing the genome of SARS-CoV-2 isolated from NP swab**



181 The vRNA extracted from virions in spent Vero E6 cell culture medium served as a template to generate a  
182 cDNA library using a NEBNext Ultra II RNA Library Prep kit (New England Biolabs, Inc.). Sequencing  
183 was performed on an Illumina MiSeq sequencer using a 600-cycle v3 MiSeq Reagent kit. Following the  
184 removal of host sequences (*Chlorocebus sabaesus*; GenBank assembly accession number  
185 GCA\_000409795.2) using Kraken 2,<sup>29</sup> *de novo* assembly of paired-end reads was performed in SPAdes  
186 v3.13.0 with default parameters.<sup>30</sup>

## 187 **Results**

188 SARS-CoV-2 genomic RNA (vRNA) was detected by real-time reverse transcriptase quantitative  
189 polymerase chain reaction (rRT-qPCR) in material collected by air samplings 1-1, 1-3, 2-1, and 2-3,  
190 which had been performed without a HEPA filter covering the inlet tube. In contrast, in the presence of a  
191 HEPA filter, no SARS-CoV-2 genomes were detected in air samplings 1-2 and 2-2 (Table 1).

192 Virus-induced CPE were observed in LLC-MK2 and Vero E6 cells inoculated with material extruded  
193 from the NP specimen of patient 1 and from liquid collection media from air samples 1-1, 1-3, 2-1, and 2-  
194 3. Early CPE in both LLC-MK2 and Vero E6 cells consisted of the formation of cytoplasmic vacuoles  
195 that were apparent within 2 days post-inoculation (dpi) of the cells with material extruded from the NP  
196 swab and 4 to 6 dpi with aliquots of the liquid collection media from the air samplers. At later times (4  
197 days onwards after inoculation of cell cultures with material from the NP swab, and 6 – 11 dpi of the cells  
198 with material collected by air samplers), rounding of the cells occurred in foci, followed by detachment of  
199 the cells from the growing surface. Some of the rounded cells detached in clumps, and occasional small  
200 syncytia with 3 -5 nuclei were observed. Apoptotic and necrotic cells were also observed. A  
201 representative collage showing the progressive development of CPE in Vero E6 cells inoculated with  
202 material collected during air sampling 1-1 is shown in Figure 2. Cytopathic effects were not observed and  
203 virus was not detected or isolated from the culture medium of samples 1-2 and 2-2, wherein HEPA filters  
204 had been affixed to the inlet nozzles of the air samplers, and were not observed in mock-inoculated cells  
205 which were maintained in parallel with the inoculated cell cultures.

206 SARS-CoV-2-specific rRT-PCR tests were performed and the results indicated that the LLC-MK2 and  
207 Vero E6 cultures inoculated with collection media from air samplings 1-1, 1-3, 2-1, and 2-3 contained  
208 SARS-CoV-2 (data not shown). No other respiratory virus was identified in the samples using a BioFire  
209 FilmArray Respiratory 2 Panel (BioMérieux Inc., Durham, North Carolina), following the manufacturer's  
210 instructions.

211 Whereas the concentration of SARS-CoV-2 genome equivalents per liter of air were estimated (Table  
212 2), determination of the specific infectivity (ratio of SARS-CoV-2 genome equivalents present for every  
213 one able to infect a cell in culture) required performance of a plaque assay or a standard 50% endpoint  
214 dilution assay (TCID<sub>50</sub> assay). Plaque assays could not be performed due to a nationwide non-availability  
215 of some critical media components (due to COVID-19 pandemic-related temporary lockdown of  
216 production facilities), so TCID<sub>50</sub> assays were performed in Vero E6 cells to estimate the percentage of the  
217 collected virus particles that were viable. Estimates ranged from 2 to 74 TCID<sub>50</sub> units/L of air (Table 3).

218 A nearly complete SARS-CoV-2 genome sequence was obtained by next-generation sequencing (NGS)  
219 of RNA purified from cell culture medium of Vero E6 cells 7 dpi with NP swab material from patient 1.  
220 The nearly complete genome sequence (and the virus isolate) were designated SARS-CoV-2/human/UF-  
221 19/2020, and this genome sequence has been deposited in GenBank (accession no. MT668716) and in  
222 GISAID (accession no. EPI\_ISL\_480349). Because the amount of virus RNA was below the threshold  
223 that could be easily sequenced by our NGS methods, Sanger sequencing was used to sequence SARS-  
224 CoV-2 RNA purified from the collection media of air samplers 1-1, 1-3, 2-1, and 2-3. One complete  
225 SARS-CoV-2 sequence was attained for RNA purified in the material collected by air sampling 1-1, and  
226 three nearly complete sequences for 1-3, 2-1, and 2-3, respectively. After alignment, comparisons of the  
227 three partial sequences with the complete sequence of SARS-CoV-2 in air sampling 1-1 indicated that the  
228 same consensus genome sequence were present in the virions that had been collected in all the air  
229 samplings. Moreover, they were an exact match with the corresponding sequences of the virus isolated  
230 from patient 1. This complete genome sequence of the virus collected by the air samplers (and the virus  
231 therein) were considered the same isolate and designated SARS-CoV-2/Environment/UF-20/2020, and

232 this genome sequence has been deposited in GenBank (accession no. MT670008) and in GISAID  
233 (accession no. EPI\_ISL\_477163). The virus' genomic sequence currently falls within GISAID clade  
234 B.1(GH), which is characterized by mutations C241T, C3037T, A23403G, G25563T, S-D614G, and  
235 NS3-Q57H relative to reference genome WIV04 (GenBank accession no. MN996528.1). As of 10 July  
236 2020, SARS-CoV-2 clade B.1(GH) was the predominant virus lineage in circulation in the USA.

237

## 238 **Discussion**

239 There are substantial epidemiologic data supporting the concept that SARS-CoV, which is highly related  
240 to SARS-CoV-2,<sup>3</sup> was transmitted via an aerosol route.<sup>31-33</sup> For SARS-CoV-2, there have also been two  
241 epidemiologic reports consistent with aerosol transmission.<sup>15,34</sup> However, despite these reports,  
242 uncertainties remain about the relative importance of aerosol transmission of SARS-CoV-2, given that so  
243 far, only one study has provided weak evidence of virus isolation from material collected by air  
244 samplers.<sup>21</sup> In other reports, attempts to isolate the virus were not successful. The current study takes  
245 advantage of a newer air sampling technology that operates using a water-vapor condensation mechanism,  
246 facilitating the likelihood of isolating the virus in tissue culture.

247 As reported in air sampling tests performed by others<sup>9-11,21</sup> and in our previous report,<sup>23</sup> airborne  
248 SARS-CoV-2 was present in a location with COVID-19 patients. The distance from the air-samplers to  
249 the patients ( $\geq 2$  m) suggests that the virus was present in aerosols. Unlike previous studies, we have  
250 demonstrated the virus in aerosols can be viable, and this suggests that there is an inhalation risk for  
251 acquiring COVID-19 within the vicinity of people who emit the virus through expirations including  
252 coughs, sneezes, and speaking.

253 The amount of airborne virus detected per liter of air was small, and future studies should address (a)  
254 whether this is typical for COVID-19, (b) if this represented virus production relative to the phase of  
255 infection in the patient, (c) if this was a consequence of active air flow related to air exchanges within the

256 room, (d) or if the low number of virus was due to technical difficulties in removing small airborne  
257 particles from the air.<sup>26</sup>

258 Our findings reveal that viable SARS-CoV-2 can be present in aerosols generated by a COVID-19  
259 patient in a hospital room in the absence of an aerosol-generating procedure, and can thus serve as a  
260 source for transmission of the virus in this setting. Moreover, the public health implications are broad,  
261 especially as current best practices for limiting the spread of COVID-19 center on social distancing,  
262 wearing of face-coverings while in proximity to others and hand-washing. For aerosol-based  
263 transmission, measures such as physical distancing by 6 feet would not be helpful in an indoor setting,  
264 provide a false-sense of security and lead to exposures and outbreaks. With the current surges of cases, to  
265 help stem the COVID-19 pandemic, clear guidance on control measures against SARS-CoV-2 aerosols  
266 are needed, as recently voiced by other scientists.<sup>35</sup>

267

268

## 269 **Contributors**

270 JAL, ML, ZHF, AJ, AEF, KC, JGM Jr, and C-YW conceived and designed the study. JAL, ML, KC, JG  
271 M Jr, and C-YW curated the data. JAL, ML, JGM Jr, and C-YW performed formal analyses of the data.  
272 JAL, ML, ZHF, AJ, JGM Jr. obtained funding for the work; JAL, TBT, MG, MU, SNS, KM, CJS, MMA,  
273 MAE, JCL, KS, and TBW performed experiments; JAL, M L, TBT, SNS, CJS, JCL, KS, TBW, JGM Jr,  
274 and C-YW established methods; JAL, ML, JCL, JGM Jr, and C-YW administered the project; JAL, ML,  
275 ZHF, AJ, JGM Jr, and C-YW provided resources; JAL, ML, JCL, JGM Jr, and C-YW supervised the  
276 project; JAL, JGM Jr., and C-Y Wu wrote the original manuscript draft; all authors revised the  
277 manuscript critically. All authors read and approved the final version of the manuscript.

278

## 279 **Declaration of interests**

280 The authors proclaim they have no conflicts of interest to report.

281 **Acknowledgements**

282 The authors thank Dr. Katherine Deliz (UF Environmental Engineering Sciences) for access to her  
283 laboratory for some essential engineering tasks, Drs. Christine Angelin and David Kaplan (UF  
284 Environmental Engineering Sciences) for providing critical supplies not readily available from vendors  
285 due to the COVID-19 pandemic, and Mark Dykes and Brad Pollitt of UF Health/Shands Hospital  
286 Facilities for providing room configuration and ventilation system info. Funding of work reported in this  
287 publication was partly supported by the National Science Foundation under Grant No. 2030844, partially  
288 by National Institute of Environmental Health Sciences of the National Institutes of Health award number  
289 1R43ES030649, and funds made available by the UF Emerging Pathogens Institute and the Office of the  
290 Dean, UF College of Medicine. The contents are solely the responsibility of the authors and do not  
291 necessarily represent the official views of the National Science Foundation and the National Institutes of  
292 Health.

293

294 **References**

- 295 1. Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2.  
296 *Nat. Med.* 2020; **26**: 450–52.
- 297 2. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus:  
298 Implications for virus origins and receptor binding. *The Lancet* 2020; **395**: 565–74.
- 299 3. Gorbalenya AE, Baker SC, Baric RS, et al. Coronaviridae Study Group of the International Committee  
300 on Taxonomy of Viruses. The species *Severe acute respiratory syndrome-related coronavirus*:  
301 Classifying 2019-nCoV and naming it SARS-CoV-2. *Nat. Microbiol.* 2020; **5**: 536–44.
- 302 4. Li Q, Guan X, Wu P, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-  
303 infected pneumonia. *N Engl J Med.* 2020; **382**: 1199–207.
- 304 5. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J*  
305 *Med.* 2020; **382**: 1708-20.

- 306 6. Jiang S, Du I, Shi Z. An emerging coronavirus causing pneumonia outbreak in Wuhan, China: Calling  
307 for developing therapeutic and prophylactic strategies. *Emerg. Microbes Infect.* 2020; **9**: 275–77.
- 308 7. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N.*  
309 *Engl. J. Med.* 2020; **382**: 727–33.
- 310 8. Lamers MM, Beumer J, van der Vaart J, et al. SARS-CoV-2 productively infects human gut  
311 enterocytes. *Science* 2020; **369**: 50 – 4.
- 312 9. Liu Y, Ning Z, Gio M, et al. Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals. *Nature*  
313 2020; **582**: 557–60.
- 314 10. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens.  
315 *JAMA* 2020; **323**: 1843-44.
- 316 11. Holshue ML, DeBolt C, Lindquist S, et al. First case of 2019 novel coronavirus in the United States.  
317 *N Engl J Med.* 2020; 382: 929-36.
- 318 12. Xiao F, Tang M, Zheng X, Liu Y, Li X, Shan H.. Evidence for gastrointestinal infection of SARS-  
319 CoV-2. *Gastroenterology* 2020; **158**: 1831–33.e3.
- 320 13. World Health Organization. Modes of transmission of virus causing COVID-19: implications for IPC  
321 precaution recommendations: scientific brief, 27 March 2020; (WHO/2019-  
322 nCoV/Sci\_Brief/Transmission\_modes/2020.2) (accessed 9 July 2020).
- 323 14. CDC. Coronavirus Disease 2019 (COVID-19). Frequently asked questions. 2020;  
324 <https://www.cdc.gov/coronavirus/2019-ncov/faq.html> (accessed 9 July 2020).
- 325 15. Hamner L, Dubbel P, Capron I, et al. High SARS-CoV-2 attack rate following exposure at a choir  
326 practice - Skagit County, Washington, March 2020. *MMWR Morb Mortal Wkly Rep.* 2020; **69**: 606–10.
- 327 16. Morawska I, Cao J. Airborne transmission of SARS-CoV-2: The world should face the reality.  
328 *Environ Int.* 2020; **139**: 105730.
- 329 17. Jayaweera M, Perera H, Gunawardana B, Manatunge J. Transmission of COVID-19 virus by droplets  
330 and aerosols: a critical review on the unresolved dichotomy. *Environ Res.* 2020; **188**: 109819.
- 331 18. Wang J, Du G. COVID-19 may transmit through aerosol. *Ir J Med Sci.* 2020; **24**: 1-2.

- 332 19. Fineberg HV. Rapid expert consultation on the possibility of bioaerosol spread of SARS-CoV-2 for  
333 the COVID-19 pandemic (April 1, 2020). In: The National Academies Press N.R.C., ed. Washington,  
334 DC: The National Academies Press, National Research Council. 2020.
- 335 20. Guo ZD, Wang ZY, Zhang SF, et al. Aerosol and surface distribution of severe acute respiratory  
336 syndrome coronavirus 2 in hospital wards, Wuhan, China, 2020. *Emerg. Infect. Dis.* 2020; **26**: 1583-91.
- 337 21. Santarpia JL, Rivera DN, Herrera V, et al. Aerosol and surface transmission potential of SARS-CoV-  
338 2. *medRxiv*; posted 23/06/2020, doi: <https://doi.org/10.1101/2020.03.23.20039446>.
- 339 22. Chia PY, Coleman KK, Tan YK, et al. Detection of air and surface contamination by SARS-CoV-2 in  
340 hospital rooms of infected patients. *Nat Commun.* 2020; **11**: 2800.
- 341 23. Lednicky JA, Shankar SN, Elbadry MA, et al. Collection of SARS-CoV-2 virus from the air of a  
342 clinic within a university student health care center and analyses of the viral genome. *Aerosol Air Qual.*  
343 *Res.* 2020; **20**: 1167–71.
- 344 24. van Doremalen N, Morris DH, Holbrook MG, et al. Aerosol and surface stability of SARS-CoV-2 as  
345 compared with SARS-CoV-1. *N. Engl. J. Med.* 2020; **382**:1564-67.
- 346 25. Fears AC, Klimstra WB, Duprex P, et al. Persistence of severe acute respiratory syndrome  
347 coronavirus 2 in aerosol suspensions. *Emerg. Infect. Dis.* **2020** (<https://doi.org/10.3201/eid2609.201806>).
- 348 26. Pan M, Lednicky JA, Wu CY. Collection, particle sizing and detection of airborne viruses. *J Appl*  
349 *Microbiol.* 2019; **127**: 1595- 611.
- 350 27. Lednicky J, Pan M, Loeb J, et al. Highly efficient collection of infectious pandemic influenza H1N1  
351 virus (2009) through laminar-flow water based condensation. *Aerosol Science and Technology* 2016;  
352 **50**:7, i-iv, DOI: 10.1080/02786826.2016.1179254.
- 353 28. Pan M, Bonny TS, Loeb J, et al. Collection of viable aerosolized influenza virus and other respiratory  
354 viruses in a student health care center through water-based condensation growth. *mSphere* 2017; **11**:  
355 2(5):e00251-17.
- 356 29. Wood DE, Lu J, Langmead B. Improved metagenomic analysis with Kraken 2. *Genome Biol.* 2019;  
357 **20**: 257.

- 358 30. Bankevich A, Nurk S, Antipov D, et al. SPAdes: a new genome assembly algorithm and its  
359 applications to single-cell sequencing. *J. Comput. Biol.* 2012; **19**: 455–77.
- 360 31. Yu IT, Li Y, Wong TW, et al. Evidence of airborne transmission of the severe acute respiratory  
361 syndrome virus. *N. Engl. J. Med.* 2004; **350**: 1731-39.
- 362 32. Li Y, Duan S, Yu IT, Wong TW. Multi-zone modeling of probable SARS virus transmission by  
363 airflow between flats in block E, Amoy Gardens. *Indoor Air* 2005; **15**: 96-111.
- 364 33. McKinney KR, Gong YY, Lewis TG. Environmental transmission of SARS at Amoy Gardens. *J.*  
365 *Environ. Health* 2006; **68**: 26-30.
- 366 34. Park SY, Kim YM, Yi S, et al. Coronavirus disease outbreak in call center, South Korea. *Emerg.*  
367 *Infect. Dis.* 2020; **26**:10.3201/eid2608.201274.
- 368 35. Morawska L, Milton DK. It is Time to Address Airborne Transmission of COVID-19 [published  
369 online ahead of print, 2020 Jul 6]. *Clin Infect Dis.* **2020**;ciaa939.

370

371

372

373

374

375

376

377

378

379

380

381

382

383



384 **Tables**

Table 1. SARS-CoV-2 N-gene rRT-PCR primers and probe.

<b>Primer/probe name</b>	<b>Description</b>	<b>Oligonucleotide sequence (5' to 3')</b>	<b>Label</b>
Led-N-F	SARS CoV-2 N Forward Primer	5'-GGGAGCAGAGGCGGCAGTCAAG-3'	None
Led-N-R	SARS CoV-2 N Reverse Primer	5'-CATCACCGCCATTGCCAGCCATTC-3'	None
Led-N-Probe <sup>a</sup>	SARS CoV-2 N Probe	5' FAM-CCTCATCACGTAGTCGCAACAGTTC- BHQ1-3'	FAM, BHQ1

385 <sup>a</sup>This TaqMan® probe is 5'-end labeled with the reporter molecule 6-carboxyfluorescein (FAM) and with  
386 quencher Black Hole Quencher 1 (BHQ-1) at the 3'- end.

387

388

389

390

391

392

393

394

395

396

397

398

399

400

401

402

403

404

405 Table 2. Results of rRT-qPCR tests of materials collected by air samplers.

Sample ID	Approx. distance (m) from head of patient 1 <sup>b</sup>	Approx. distance (m) from head of patient 2 <sup>b</sup>	rRT-qPCR test	Cq value	SARS-CoV-2 genome equivalents/25 µL rtRT-PCR test	SARS-CoV-2 genome equivalents/L of air
1-1 BioSpot	2	4.6	+	36.02	2.82E+03	94
1-2 BioSpot + HEPA	2	4.6	-	-	-	-
1-3 BioSpot	2	0 (PD <sup>b</sup> )	+	37.69	9.12E+02	30
2-1 VIVAS	4.8	3	+	37.42	1.15E+03	44
2-2 VIVAS+ HEPA	4.8	3	-	-	-	-
2-3 VIVAS	4.8	0 (PD <sup>d</sup> )	+	38.69	4.68E+02	16
SARS-CoV-2 vRNA	N/A <sup>c</sup>	N/A	+	29.53	2.20E+05	N/A
N-gene <sup>a</sup> DNA control - 1	N/A	N/A	+	26.56	1.00E+06	N/A
N-gene DNA control - 2	N/A	N/A	+	31.21	1.00E+05	N/A
N-gene DNA control - 3	N/A	N/A	+	34.71	1.00E+04	N/A
N-gene DNA control -4	N/A	N/A	+	37.74	1.00E+03	N/A
N-gene DNA control - 5	N/A	N/A	+	40.41	1.00E+02	N/A
N-gene DNA control - 6	N/A	N/A	+	-	1.00E+01	N/A
Known positive (NP swab <sup>e</sup> )	N/A	N/A	+	24.12	8.36E+06	N/A
Negative (no RNA) control	N/A	N/A	N/A	-	0	N/A

406 <sup>a</sup>N-gene, N-gene plasmid (positive control template).

407 <sup>b</sup>Distance from sampler inlet nozzle to patient's head.

408 <sup>c</sup>N/A, Not applicable.

409 <sup>d</sup>PD, patient discharged.

410 <sup>e</sup>NP, Nasal-pharyngeal swab from a person screened for SARS-CoV-2 at the UF EPI High-Throughput

411 COVID-19 Research Testing Facility.

412

413 Table 3. Estimate of viable virus counts based on TCID<sub>50</sub> tests.

Sample ID	Virus genome equivalents/L of air <sup>a</sup>	TCID <sub>50</sub> /100 µl	Viable virus count/L air
1-1 BioSpot	94	2.68E+04	74
1-2 BioSpot + HEPA	-	0	0
1-3 BioSpot	30	6.31E+03	18
2-1 VIVAS	44	1.00E+04	27
2-2 VIVA S+ HEPA	-	0	0
2-3 VIVAS	16	2.15E+03	6

414 <sup>a</sup>From Table 2.

415

416

417 **Figure legends**

418 Figure 1. Schematic diagram of room with depiction of patient bed and air-sampler locations.

419

420 Figure 2. Cytopathic effects in Vero E6 cells inoculated with material collected from the air during air  
421 sampling 1-1. [A] Mock-infected Vero E6 cells, 10 days post-inoculation with sterile collection medium.  
422 [B]. Large cytoplasmic vacuoles in Vero E6 cells inoculated with collection medium from BioSpot  
423 sample 1-1 at 4 dpi. [C] Early focus of infection 7 dpi. [D] Focus of infection 10 dpi. Rounded cells that  
424 are detaching, some in clumps, are present. Attached cells remaining in this focus of infection have dark  
425 cytoplasm, some have large cytoplasmic inclusion bodies, and some cells are elongated. Original  
426 magnifications at 400X.

427

428

429

430

431

432

433

434

435

436

437

438

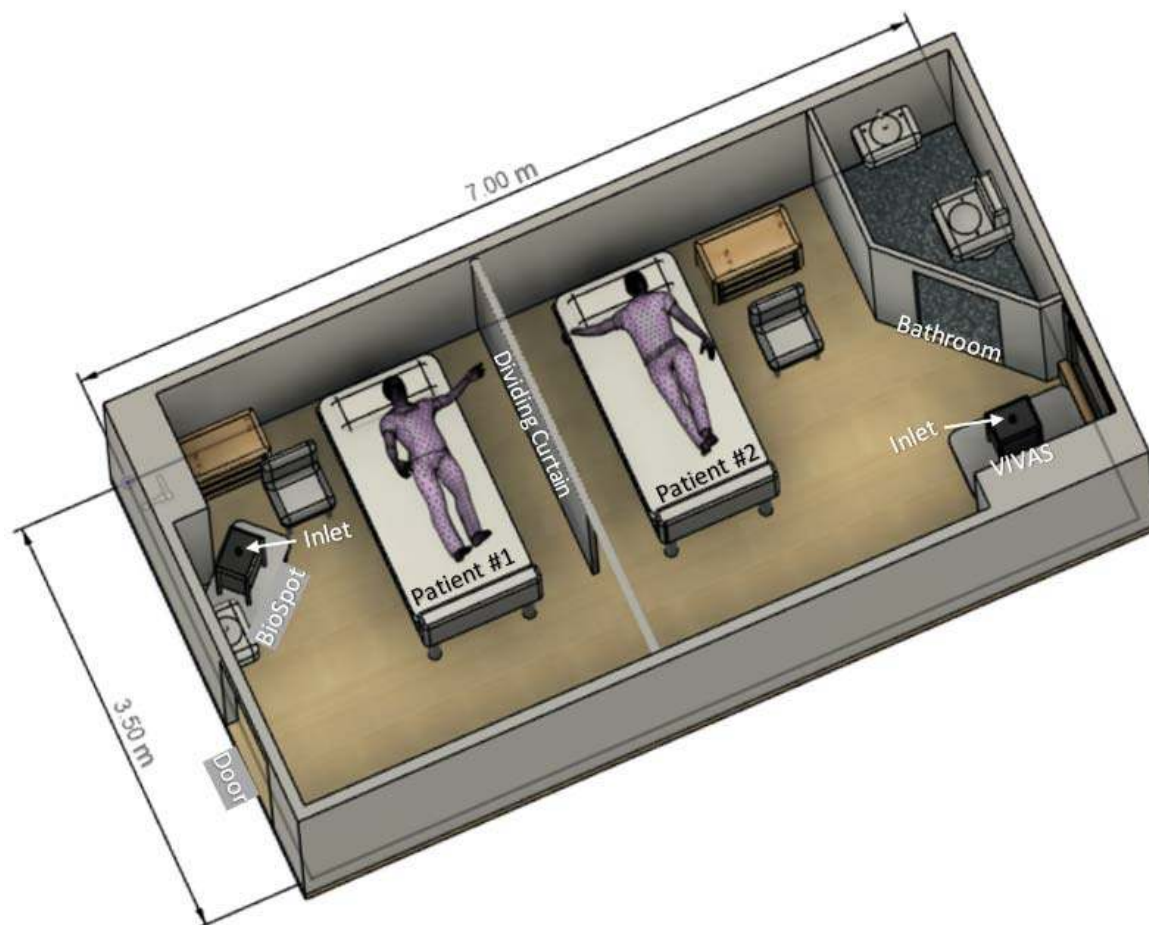
439

440

441

442

443 Figure 1.



444

445

446

447

448

449

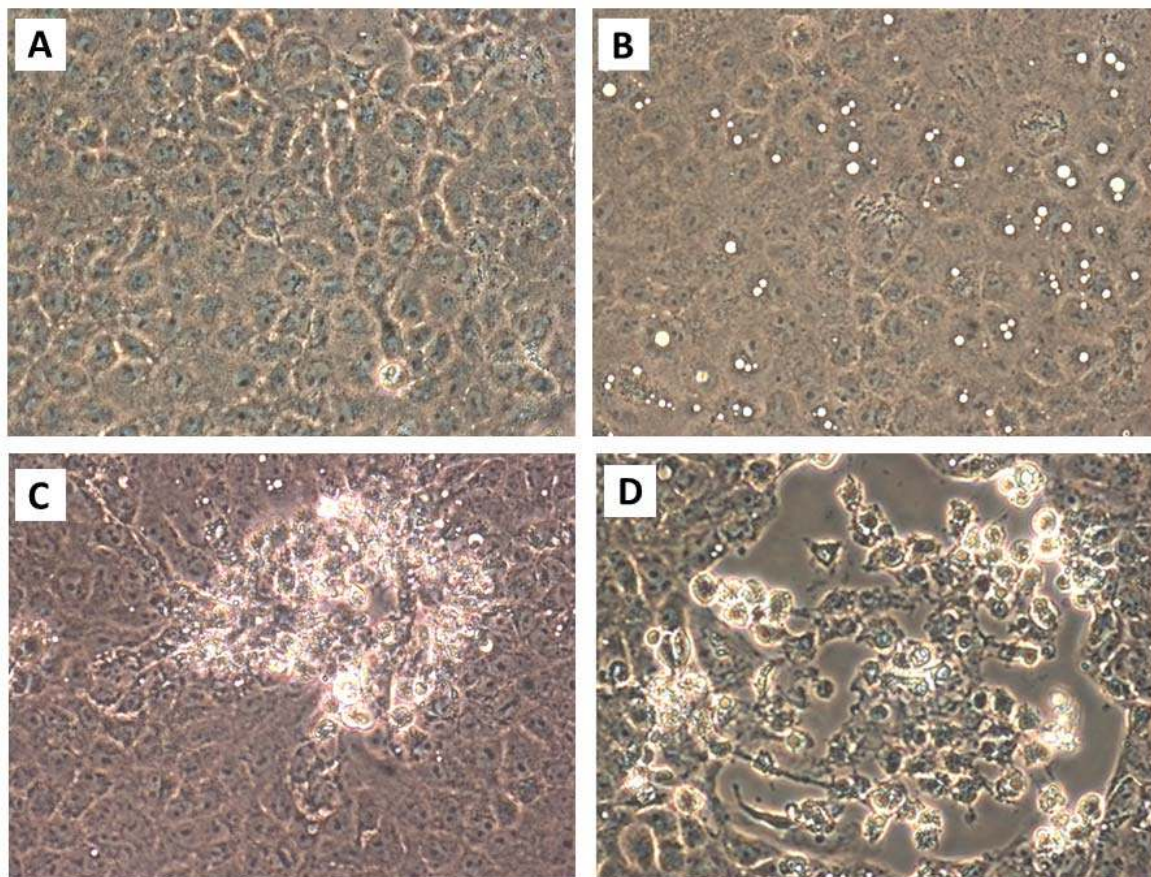
450

451

452

453

454 Figure 2.



455

456