

SARS-CoV-2 Environmental contamination in hospital rooms is uncommon using viral culture techniques

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## **Abstract**

We assessed environmental contamination of inpatient rooms housing COVID-19 patients in a dedicated COVID-19 unit. Contamination with SARS-CoV-2 was found on 5.5% (19/347) of surfaces via RT-PCR and 0.3% (1/347) of surfaces via cell culture. Environmental contamination is uncommon in hospitals rooms; RNA presence is not a specific indicator of infectious virus.

Keywords: COVID-19, Environmental Contamination, SARS-CoV-2, Pandemic.

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1 *Introduction*

2 Over 44 million confirmed cases of coronavirus disease 2019 (COVID-19) have occurred as of October 1<sup>st</sup>  
3 2021.<sup>1</sup> The primary route of SARS-CoV-2 transmission is exposure to respiratory droplets. However,  
4 SARS-CoV-2 can persist on environmental surfaces in the laboratory and healthcare setting.<sup>2,3</sup> Therefore,  
5 SARS-CoV-2 contaminated surfaces in the healthcare environment could potentially result in  
6 transmission of SARS-CoV-2. Previous studies have mainly assessed healthcare environmental SARS-CoV-  
7 2 contamination utilizing RT-PCR as a marker for contamination. However, few have utilized cell culture  
8 since this work is required to be completed in one of fourteen biosafety level 3 (BSL3) laboratories in the  
9 United States<sup>4</sup> Additionally, even fewer studies have used RT-PCR and cell culture in parallel, so the  
10 correlation of these methods has been inadequately assessed. The objective of this study was to assess  
11 SARS-CoV-2 hospital room contamination and compare the presence of SARS-CoV-2 RNA to infectious  
12 virus.

13  
14 *Methods*

15 We performed a prospective observational study of inpatient rooms housing patients with SARS-CoV-2  
16 infection in a dedicated COVID-19 unit at Duke University Hospital Durham, North Carolina. Patient  
17 rooms were screened and enrolled in the study between October 2020 and June 2021.

18  
19 *Inpatient Room Conditions*

20 Eligible patient rooms included rooms housing a COVID-19 positive patient with a first positive SARS-  
21 CoV-2 test within 24 hours of enrollment. Admission testing for COVID-19 was completed on all patients  
22 at the study hospital. All rooms were single-occupant rooms. A previous study demonstrated that the air

23 inside these patient rooms was exchanged roughly 14 times every hour, which is higher than the  
24 American Society of Heating, Refrigerating and Air-Conditioning Engineers–recommended ventilation  
25 (i.e.,  $\geq 6$  air exchanges per hour for recovery rooms or  $\geq 12$  air exchanges per hour for airborne infection  
26 isolation).<sup>5</sup> Routine disinfection was performed in rooms while COVID-19 patients were occupying them.  
27 Terminal disinfection in these rooms included bleach solutions for the floor and surfaces followed by  
28 ultraviolet C (UV-C) light treatment.

29

### 30 Environmental Sample Collection

31 Environmental samples were obtained on study day 1 (within 24 hours of the first positive SARS-CoV-2  
32 test) and again on days 3, 6, 10 and 14. Rooms were excluded if samples were not obtained on study  
33 days 1 and 3. Surface samples were obtained with nylon FLOQSwab® (Copan, Murrieta, California) pre-  
34 moistened with viral transport media (VTM) (Redoxica, Little Rock, Arkansas) from six locations with a  
35 pre-defined surface area of 20x20 cm inside the patient room (each bedrail, sink, medical prep area,  
36 room computer, exit door handle) and one outside the patient room (nursing station computer) and  
37 stored in 1.5mL of VTM during transport.

38

### 39 RNA Extractions, RT-PCR and Viral Culture

40 Sample swabs were vortexed for 10 seconds to remove viral particles from the swab. Swabs were  
41 discarded and RNA extractions were completed on the vortexed VTM using QIAamp® Viral RNA Mini Kits  
42 (Qiagen, Hilden, Germany). RT-PCR was completed on all samples using the US Centers for Disease  
43 Control and Prevention’s 2019-nCoV Real-Time RT-PCR (reverse-transcription polymerase chain  
44 reaction) assay protocol targeting the viral nucleocapsid (N) gene.<sup>6</sup> SARS-CoV-2 virus culture work was

45 performed in a BSL3 laboratory at the Duke Regional Biocontainment Laboratory. Samples positive for  
46 SARS-CoV-2 via RT-PCR were inoculated onto Vero E6 cells in 2 passages by transferring 250µL of  
47 supernatant at 7 days post inoculation for a total 14 days of incubation. Cells were monitored for  
48 cytopathic effect (CPE) every 48 hours. The cells and supernatant were harvested 14 days post-  
49 inoculation and were screened for SARS-CoV-2 by molecular assay. Infectious SARS-CoV-2 was  
50 confirmed when CPE was detected in inoculated wells and SARS-CoV-2 was detected in inoculated wells  
51 by real-time RT-PCR, at least 2 cycle thresholds (Cts) below the original sample. The SARS-CoV-2 isolate  
52 USA-WA1/2020 (BEI Resources, Manassas, Virginia) was used as the positive control.

53

#### 54 Analysis

55 Study data were collected and managed using REDCap electronic data capture hosted at Duke  
56 University. The demographic characteristics of the study population were summarized using descriptive  
57 statistics. This study was deemed exempt non-human research by the Duke University Institutional  
58 Review Board and institutional health, safety, environment protocols for culturing of SARS-CoV-2 virus  
59 was strictly adhered to, including BSL-3 requirements.

60

#### 61 *Results*

62 We enrolled 20 patients between October 2020 and June 2021. Patients in these rooms had a median  
63 age of 65 (Interquartile range [IQR], 50-73) and 12 (60%) were female. The median length of hospital  
64 stay was 6 days ([IQR], 3-11), the median length of stay in the study room was 5 days ([IQR], 3-12) and  
65 16 (80%) of study rooms were previously occupied by a COVID-19 positive patient. 11 (55%) of patients

66 were on supplemental oxygen, and 15 (75%) were actively symptomatic: 8 (40%) pyrexia, 6 (30%) cough,  
67 8 (40%) shortness of breath, and 5 (25%) diarrhea.

68

69 A total of 347 individual samples were obtained from 20 patient rooms and screened for SARS-CoV-2  
70 RNA;140 on day 1, 140 on day 3, 48 on day 6, and 14 on day 10. Overall, 19 (5.5%) samples were  
71 positive via RT-PCR;9 from bedrails (9.2%), 4 from sinks (8.0%), 4 from room computers (8.0%), 1 from  
72 the medical prep area (2.0%) and 1 from the exit door handle (2.0%). Notably, all nursing station  
73 computer samples were negative (Figure 1). Of the 19 positive samples, 6 were from day 1, 10 on day 3,  
74 2 on day 6 and 1 on day 10. All 19 SARS-CoV-2 RNA positive samples were screened for infectious virus  
75 via cell culture. Notably, only one (0.3%) sample, obtained on day 3 from the bedrails of a symptomatic  
76 patient with diarrhea and a fever, demonstrated CPE and the harvested inoculates were SARS-CoV-2 RT-  
77 PCR positive, indicating viral growth.

78

## 79 *Discussion*

80 The primary route of SARS-CoV-2 transmission is exposure to respiratory droplets. However, SARS-CoV-2  
81 contaminated surfaces in the healthcare environment could potentially lead to indirect transmission of  
82 SARS-CoV-2. In our study, the frequency of environmental contamination of SARS-CoV-2 in rooms  
83 housing COVID-19 infected patients was low (19/347,5.4%) via RT-PCR and lower (1/347,0.3%) via cell  
84 culture.

85

86 In general, our results are similar to previous studies.<sup>7</sup> For example, Colaneri et. al found SARS-CoV-2  
87 RNA in 7.7% of environmental samples.<sup>3,8</sup> Our RT-PCR results differed from some studies such as Zhou

88 et al. (52.3% positive surface samples), however, this was likely due to sample timing as these studies  
89 were completed early in the pandemic (April 2020) compared to ours.<sup>9</sup> Cheng et al. performed a similar  
90 study of fomites in inpatient hospital rooms housing COVID-19 positive patients and found similar RT-  
91 PCR results to our study: 5.0% of all samples were positive compared to our 5.5%, and, among shared  
92 study fomites, the bed rails were most likely to be contaminated at 5.4% compared to our 9.2%.<sup>10</sup> Our  
93 RT-PCR results and those just cited differed from some other studies such as Zhou et al. (52.3% of  
94 surface sample positive); likely because this study was conducted early in the pandemic prior to  
95 availability of therapeutic agents.

96 Few studies have examined environmental contamination using cell culture techniques.<sup>7</sup> Wang et al. did  
97 not find SARS-CoV-2 RNA or infectious virus in any environmental samples in a Chinese hospitals'  
98 isolation ward. Our cell culture results match prior studies that deployed RT-PCR and cell culture  
99 concurrently with all studies reporting higher contamination rates with RT-PCR than cell culture.  
100 However, unlike our study most studies did not demonstrate any positive cell culture samples including  
101 Colaneri et al. (26 environmental samples, healthcare emergency unit), Wang et al. (36 samples, of  
102 isolation wards), and Zhou et al. (218 samples, acute healthcare settings).<sup>3,8,9</sup> However, Santarpia et al.  
103 (163 samples, COVID-19 patient isolation rooms) found some evidence of intact SARS-CoV-2 virions in  
104 cell culture but did not observe CPE.<sup>11</sup> In summary, our data adds to published literature demonstrating  
105 that viable virus uncommonly contaminates room surfaces housing COVID-19 patients.

106  
107 Our study has several limitations. Patients were potentially later in their disease since timing of hospital  
108 presentation and admission does not necessarily reflect timing of infection. The CDC states that  
109 infectious virus is not typically shed after day 7 of symptom onset and it is possible our data included  
110 patients who were close to, or past, that day.<sup>12</sup> We attempted to control for this by enrolling patients

111 within 24 hours of their first positive SARS-CoV-2 test, however, patients could have been symptomatic  
112 days before. This study was also completed in an acute healthcare setting in a COVID-19 specific unit so  
113 these results are not generalizable to other healthcare environments such as emergency departments,  
114 non-COVID-19 units or outside of the healthcare setting. Lastly, this study has a relatively small sample  
115 size and patients were not selected randomly.

116

117 In conclusion, our results suggest that RT-PCR inflates the SARS-CoV-2 contamination rate of the  
118 healthcare environment and does not indicate the presence of live infectious virus. Importantly, even  
119 the detection of live infectious virus via cell culture does not indicate that an infectious does of SARS-  
120 CoV-2 is present. More studies including RT-PCR and viral cell culture assays are needed to determine  
121 the importance of discovering SARS-CoV-2 RNA versus infectious virus in the clinical environment.

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127

128 *Potential Conflicts of Interest*

129 All authors report no conflict of interest with this work. DA reports grant to institution during the  
130 conduct of the study from CDC Prevention Epicenter Program; grant to institution from NIH/NIAID and  
131 AHRQ outside of the conduct of the study and contract to institution from CDC outside of the conduct of  
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133 Major Sports, LLC.

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173 **Figure legend:**

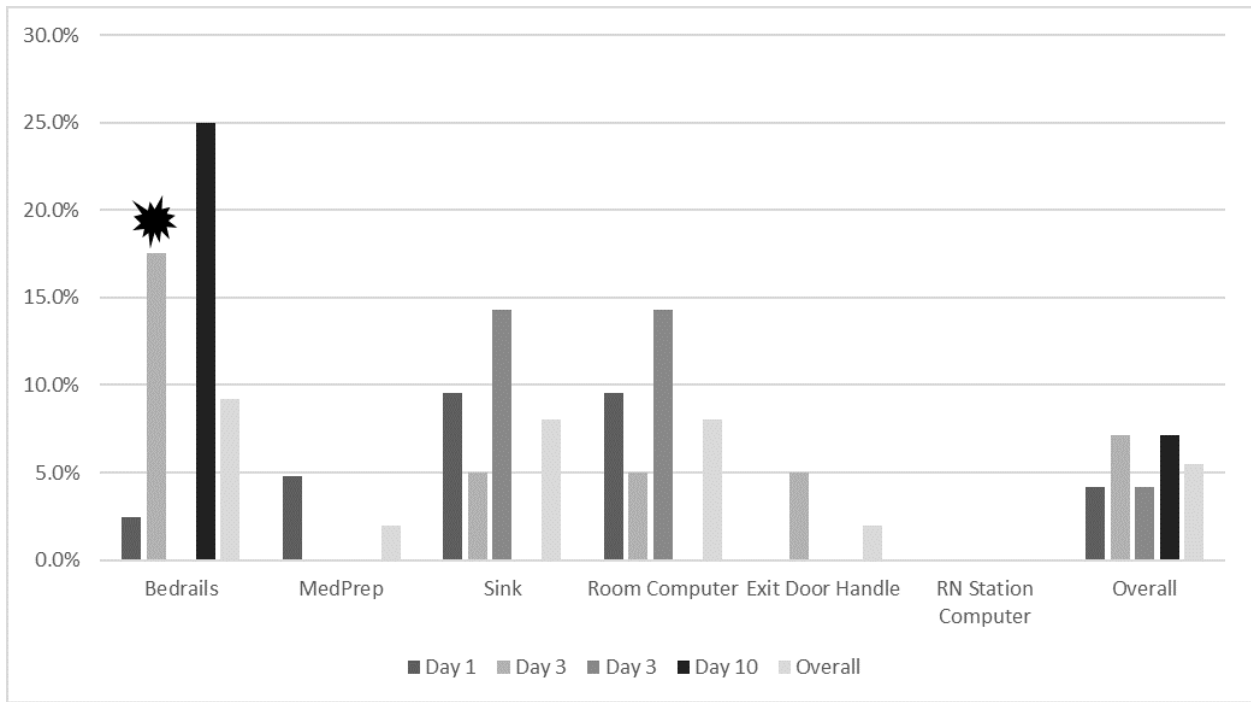
174 Figure 1. Proportion of SARS-CoV-2 Positive Environmental Samples by Sample Location and Day

175 Asterisk Indicates positive cell culture sample

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Figure 1



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