

# Transmission Potential of SARS-CoV-2 in Viral Shedding Observed at the University of Nebraska Medical Center

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**Abstract:** Lack of evidence on SARS-CoV-2 transmission dynamics has led to shifting isolation guidelines between airborne and droplet isolation precautions. During the initial isolation of 13 individuals confirmed positive with COVID-19 infection, air and surface samples were collected in eleven isolation rooms to examine viral shedding from isolated individuals. While all individuals were confirmed positive for SARS-CoV-2, symptoms and viral shedding to the environment varied considerably. Many commonly used items, toilet facilities, and air samples had evidence of viral contamination, indicating that SARS-CoV-2 is shed to the environment as expired particles, during toileting, and through contact with fomites. Disease spread through both direct (droplet and person-to-person) as well as indirect contact (contaminated objects and airborne transmission) are indicated, supporting the use of airborne isolation precautions.

**One Sentence Summary:** SARS-CoV-2 is shed during respiration, toileting, and fomite contact, indicating that infection may occur in both direct and indirect contact.

## Main Text:

Respiratory viral infections are among the leading causes of disease and mortality globally. In recent years, several highly transmissible respiratory viruses with epidemic potential have emerged. The most significant pandemics of the 20<sup>th</sup> Century, notably, were influenza viruses in 1918, 1957, and 1968. In 2003 a global alert was issued for an emerging yet unknown illness known as severe acute respiratory syndrome (SARS) caused by a novel coronavirus (SARS-CoV)(1). The SARS-CoV outbreak resulted in roughly 8000 cases and 800 deaths in over 30 countries with substantial economic impact. Since then several other viral respiratory pathogens have emerged including Middle East respiratory syndrome coronavirus (MERS-CoV), adenovirus-14, and virulent strains of influenza viruses. Soon after the discovery of SARS, new coronaviruses NL63 and HKU1 were identified (2, 3). The rapid emergence of these respiratory viruses underscores the epidemic potential and overall threat to global health security that these pathogens pose. In this context, experts recognize the severity of the current pandemic caused by COVID-19. The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) originated in Wuhan, China (4) in late 2019 and then spread globally by mid-March of 2020 has caused more than 180,000 confirmed cases and 7000 deaths in 96 countries. On March 11, 2020, the World Health Organization (WHO) declared the ongoing spread of SARS-CoV-2 a global

pandemic. The COVID-19 pandemic may represent the most significant public health emergency that the world has faced in a century.

Understanding the modes of transmission of emerging infectious disease is a key factor in protecting healthcare workers and implementing effective public health measures. The lack of evidence on SARS-CoV-2 transmission dynamics has led to shifting isolation guidelines between airborne and droplet isolation precautions by the WHO, U.S. CDC and other public health authorities. Other emerging coronaviruses (e.g. SARS and MERS) have been suggested to have airborne transmission potential (5, 6) in addition to more direct contact and droplet transmission. At least one study suggests that MERS-CoV has the possibility of transmission from mildly ill or asymptomatic individuals (7). Surface samples taken in patient care areas for MERS and SARS have shown positive PCR results (6); however, experts question the possibility of transmission through contact with surfaces that have been contaminated by an infected person, either by the direct contact of the infected person or the settling of virus-laden particles onto the surface(8). Nonetheless, coronaviruses have been implicated in nosocomial outbreaks with reports of transmission related to environmental contamination (9,10). Nosocomial transmission of SARS-CoV-2 has been reported, but the role of aerosol transmission and environmental contamination remains unclear (11).

The University of Nebraska Medical Center (UNMC), with its clinical partner Nebraska Medicine, monitored and cared for thirteen individuals with confirmed SARS-CoV-2 infection as of March 6<sup>th</sup>, 2020. The thirteen confirmed SARS-CoV-2 cases were managed in the Nebraska Biocontainment Unit (NBU) for individuals requiring hospital care and the National Quarantine Unit (NQU) for isolation of asymptomatic or mildly ill individuals not requiring hospital care. Key features of the NBU and NQU included: 1) individuals isolated in their rooms with private bathrooms; 2) infection prevention and control (IPC) protocols for each unit including wear of personal protective equipment by staff with hand hygiene and changing of gloves between rooms; and were not doffing until exiting the unit 3) entry and exit into rooms by staff was limited; 4) all NBU and NQU rooms were negative pressure equipped (12).

To improve the understanding of potential environmental transmission risk of SARS-CoV-2, refine IPC practices and protocols within the NBU and NQU specifically, and inform outbreak control strategies more broadly, we initiated an ongoing study to obtain surface and air samples in 2 NBU hospital and 9 NQU residential isolation rooms where individuals who tested positive for SARS-CoV-2 were being monitored. Samples were obtained in the NQU on days 5-9 of activation, i.e. when mildly ill or asymptomatic individuals infected with SARS-CoV-2 were housed in their rooms for five to nine days. Samples were obtained in the NBU on day 10, when Patients 1 and 2 had been admitted for ten days. Additional samples were obtained in the NBU on day 18, after Patient 3 had been admitted to the unit for four days. Three types of samples were taken during this survey: surface samples, high volume air samples, and low volume personal air samples. The surface samples fell into three general categories of location: common room surfaces, personal items, and toilets. Personal items were those items considered to be handled routinely by individuals in isolation and included: cellular phones, exercise equipment, television remotes, and medical equipment. Room surfaces were areas such as ventilation grates, tabletops, and window ledges. Toilet samples were taken to evaluate the potential for viral shedding during toileting. Air samples were collected both in isolation rooms and in the hallways of the NBU and NQU during sampling activities. Air samples were collected in the room while patients were present. Air

samples were taken in the hallways during sampling activities and samplers were placed on the floor adjacent to rooms where sampling activities were taking place. Personal air samplers were worn by sampling personnel on two occasions during sampling activities: once during sampling at the NQU when 6 individual rooms were sampled, and once in the hospital NBU when one room was sampled. It is important to note that all individuals in NQU isolation had mild illness such as low fever, cough, or body aches, had been advised to maintain a 6-foot distance from staff members who entered their room as well as wear a procedure mask when interacting with these personnel. Individuals in isolation were instructed that they could remove the mask during air sampling activities; however, many individuals were uncomfortable removing their mask and therefore the impact of infected individuals wearing masks can neither be assessed in this study nor can its impacts be removed.

Surface and aerosol samples were analyzed by RT-PCR targeting the E gene of SARS-CoV-2 (13). Of the 163 samples collected in this study, 126 (77.3%) had a positive PCR result for SARS-CoV-2. Due to the need to cause minimal disruption to individuals in isolation and undergoing hospital care, the precise surface area sampled in this study was not uniform, so results are presented as concentration of gene copies present in the recovered liquid sample (copies/ $\mu$ L). Viral gene copy concentrations recovered from each sample type were generally low and highly variable from sample to sample ranging from 0 to 1.75 copies/ $\mu$ L (Figure 1A and 2), with the highest concentration recovered from an air handling grate in the NBU. Since both the sampling time and flow rate were known for all aerosol samples collected in this study, the airborne concentration was calculated for all air samples (copies/L of air; Figure 2).

Overall, 76.5% of all personal items sampled were determined to be positive for SARS-CoV-2 by PCR (Figure 1B and 2A). Of these samples, 81.3% of the miscellaneous personal items, which included exercise equipment, medical equipment (spirometer, pulse oximeter, nasal cannula), personal computers, iPads and reading glasses, were positive by PCR, with a mean concentration of 0.217 copies/ $\mu$ L. Cellular phones were 83.3% positive for viral RNA (0.172 copies/ $\mu$ L mean concentration) and remote controls for in-room televisions were 64.7% percent positive (mean of 0.230 copies/ $\mu$ L). Samples of the toilets in the room were 81.0% positive, with a mean concentration of 0.252 copies/ $\mu$ L. Of all room surfaces sampled (Figure 1B and 2A), 80.4% were positive for SARS-CoV-2 RNA. This included 75.0% of the bedside tables and bed rails indicating the presence of viral RNA (mean concentration 0.263 copies/ $\mu$ L), as did 81.8% of the window ledges (mean concentration 0.219 copies/ $\mu$ L) sampled in each room. The floor beneath patients' beds and the ventilation grates in the NBU were also sampled. All five floor samples, as well as 4 of the 5 ventilation grate samples tested positive by RT-PCR, with mean concentrations of 0.447 and 0.819 copies/ $\mu$ L, respectively.

Air samples, both in the rooms and in the hallway spaces (Figure 1B and 2), provide information about airborne viral shedding in these facilities. In room air samples were 63.2% positive by RT-PCR (mean concentration 2.86 copies/L of air). In the NQU, samplers were placed either on the bedside table or a desk, wherever there was space. No attempt was made to ensure the sampler was placed a specific distance from the individual in the room, so, while distance between sampler and individual was neither defined nor consistent, individuals in the room did not directly interact with the sampler. In the NBU, for the first two sampling events performed on Day 10, the sampler was placed on the window ledge away from the patient (NBU Room A occupied by Patient 1) was

positive for viral RNA (Table 1; 3.76 copies/L of air). During the sampling event on Day 16 in NBU Room B occupied by Patient 3, one sampler was placed near the patient and one was placed near the door greater than 6ft from the patient's bed while the patient was receiving oxygen (1L) via nasal cannula. Both samples were positive by PCR, with the one closest to the patient indicating a higher airborne concentration of RNA (4.07 as compared to 2.48 copies/L of air). Samples taken outside the rooms in the hallways were 66.7% positive (Figure 1B and 2B), with a mean concentration of 2.59 copies/L of air. Both personal air samplers from sampling personnel in the NQU showed positive PCR results after 122 minutes of sampling activity (Figure 2B), and both air samplers from NBU sampling indicated the presence of viral RNA after only 20 minutes of sampling activity (Figure 2B). The highest airborne concentrations were recorded by personal samplers in NBU while a patient was receiving oxygen through a nasal cannula (19.17 and 48.21 copies/L). Neither individuals in the NQU or patients in the NBU were observed to cough while sampling personnel were in the room wearing samplers during these events.

Air samples that were positive for viral RNA by RT-PCR were examined for viral propagation in Vero E6 cells. Cytopathic effect was not observed in any sample, to date, and immunofluorescence and western blot analysis have not, so far, indicated the presence of viral antigens suggesting viral replication. However, the low concentrations of virus recovered from these samples makes finding infectious virus in these samples difficult. Further experiments are ongoing to determine viral activity in these samples.

During the sampling, individuals in isolation were recording symptoms and oral temperatures twice a day. Reports of symptoms for the 3 days preceding each sample collection were provided. The maximum temperature, during that period, was recorded, as was the presence of any symptoms. For the 3 days preceding each sampling event, 57.9% of patients recorded a temperature greater than 99.0 F, and 15.8% had a temperature greater than 100F. Independent of temperature, 57.9% of patients reported other symptoms, primarily cough. Because sampling personnel were allowed to use judgement and feedback from patients when collecting samples, between 5 and 16 samples were collected from each room, with a mean of 7.35 samples per room and a mode of 6 samples per room. Percentage of positive samples from each room ranged between 50% and 100% (Figure 1B and 2A). A Pearson product moment correlation of percent positive samples and total number of samples for each room had an  $R^2$  value of 0.01, indicating that there was no relationship between the number of samples taken and the percent of positive samples observed. When the percent of positive samples taken was compared to the maximum reported oral temperature of the patient for the previous three days, a Pearson's  $R^2$  of 0.01 indicated no relationship between elevated body temperature and shedding of virus in the environment. Further, recorded oral temperature was compared with the gene copy concentration for each in-room sample type (Figure 2A). These  $R^2$  values ranged from 0.00 (cell phones and remotes) to 0.22 (bedside tables), indicating small to no significant relationship between body temperature and environmental contamination, with bedside tables having the strongest correlation followed by toilets ( $R^2 = 0.20$ ) and windowsills ( $R^2 = 0.19$ ).

Taken together, these data indicate significant environmental contamination in rooms where patients infected with SARS-CoV-2 are housed and cared for, regardless of the degree of symptoms or acuity of illness. Contamination exists in all types of samples: high and low-volume air samples, as well as surface samples including personal items, room surfaces, and toilets. Samples of patient toilets that tested positive for viral RNA are consistent with other reports of

viral shedding in stool (14). The presence of contamination on personal items is also reasonably expected, particularly those items that are routinely handled by individuals in isolation, such as cell phones and remote controls, as well as medical equipment that is in near constant contact with the patient.

The variability in the degree of environmental contamination (as measured by the percentage of positive samples) room to room is of interest. On average, a higher percentage of positive samples were detected in the NBU where patients were hospitalized for inpatient care with both hospital NBU rooms sampled on Day 10 and 18 having the highest percentage of positive samples (85.6% over 3 rooms). However, samples of the residential NQU rooms from the first pass of sampling (Days 5 to 7) had a similar average (84.6% over 8 rooms). It is interesting to note that the average percentage of positive samples per room dropped to 64.9% (over the same 8 rooms and individuals and one additional room) during the second sampling period (Days 8 to 9). This may indicate a reduction in viral shedding by these individuals. The lack of any statistically defensible relationship between the evidence of environmental contamination and body temperature indicates that infected individuals may shed viral RNA to their environment without clearly identifiable symptoms, such as fever, at least in convalescence.

It is interesting to note the presence of viral RNA on the floor under the bed of the patients and on the window ledges (which were not obviously used by the patient) in the hospital NBU. Airflow in NBU suites enters from the top center of the room and exits at grates near the head of the patient's bed on either side of the room. Airflow modelling (15) has suggested that turbulent eddies may form under the patient's bed, which may cause the observed contamination under the bed, while the dominant airflow likely carries particles away from the patient's bed towards the edges of the room, likely passing by the windows resulting in some deposition there.

Although this study did not employ any size-fractionation techniques in order to determine the size range of SARS-CoV-2 droplets and particles, the data is suggestive that viral aerosol particles are produced by individuals that have the COVID-19 disease, even in the absence of cough. First, in the few instances where the distance between individuals in isolation and air sampling could be confidently maintained at greater than 6 ft, 2 of the 3 air samples were positive for viral RNA. Second, 66.7% of hallway air samples indicate that virus-containing particles were being transported from the rooms to the hallway during sampling activities. It is likely that the positive air samples in the hallway were caused by viral aerosol particles transported by personnel exiting the room (16,17). Finally, personal air samplers worn by sampling personnel were all positive for SARS-CoV-2, despite the absence of cough by most patients while sampling personnel were present.

Recent literature investigating human expired aerosol indicates that a significant fraction of human expired aerosol is less than 10  $\mu\text{m}$  in diameter across all types of activity (e.g. breathing, talking, and coughing; 18) and that upper respiratory illness increases production of aerosol particles (less than 10  $\mu\text{m}$ ; 19). Taken together these results suggest that virus expelled from infected individuals, including from those who are only mildly ill, may be transported by aerosol processes in their local environment, potentially even in the absence of cough or aerosol generating procedures. Further, a recent study of SARS-CoV-2 in aerosol and deposited on surfaces, indicates infectious aerosol may persist for several hours and on surfaces for as long as 2 days (20). Despite wide-spread environmental and limited SARS-CoV-2 aerosol contamination associated with hospitalized and mildly ill individuals, effective implementation of airborne isolation precautions including N95 filtering facepiece respirators and powered air purifying respirator use adequately protected health



care workers, in the NQU and NBU facilities, preventing health care worker infections. Health care workers were closely monitored and screened for COVID-19 suggesting the value in implementing IPC protocols that maintain airborne isolation standards including respiratory protection and include routine systematic environmental cleaning and disinfection of patient care areas and surrounding environments.

## References and Notes:

1. WHO issues a global alert about cases of atypical pneumonia. *Indian J Med Sci.* 2003;57(5):206-7.
2. van der Hoek L, Pyrc K, Jebbink MF, Vermeulen-Oost W, Berkhout RJ, Wolthers KC, et al. Identification of a new human coronavirus. *Nat Med.* 2004;10(4):368-73.
3. Woo PC, Lau SK, Chu CM, Chan KH, Tsoi HW, Huang Y, et al. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J Virol.* 2005;79(2):884-95.
4. Zhu, Na, et al. "A Novel Coronavirus from Patients with Pneumonia in China, 2019." *New England Journal of Medicine* (2020).
5. Tellier BMC article
6. Booth TF1, Kournikakis B, Bastien N, Ho J, Kobasa D, Stadnyk L, et al. Detection of airborne severe acute respiratory syndrome (SARS) coronavirus and environmental contamination in SARS outbreak units. *J Infect Dis.* 2005; 191:1472–1477.
7. Omrani AS, Matin MA, Haddad Q, Al-Nakhli D, Memish ZA, Albarrak AM. A family cluster of Middle East respiratory syndrome coronavirus infections related to a likely unrecognized asymptomatic or mild case. *Int J Infect Dis.* 2013;17:e668–72.
8. Morawska, L Droplet Fate in Indoor Environments, or can we Prevent the Spread of Infection. *Indoor Air.* 2006. 16(5):pp. 335-347.
9. Chowell G, Abdirizak F, Lee S, et al. Transmission characteristics of MERS and SARS in the healthcare setting: a comparative study. *BMC Med.* 2015;13:210. doi: [10.1186/s12916-015-0450-0](https://doi.org/10.1186/s12916-015-0450-0)
10. Bin SY, Heo JY, SongMS, et al. Environmental contamination and viral shedding in MERS patients during MERS-CoV outbreak in South Korea. *Clin Infect Dis.* 2016;62(6):755-760. doi:[10.1093/cid/civ1020](https://doi.org/10.1093/cid/civ1020)
11. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA.* Published online February 7, 2020. doi:[10.1001/jama.2020.1585](https://doi.org/10.1001/jama.2020.1585)
12. Beam, Elizabeth L. et al. Personal protective equipment processes and rationale for the Nebraska Biocontainment Unit during the 2014 activations for Ebola virus disease *American Journal of Infection Control*, Volume 44, Issue 3, 340 – 342
13. World Health Organization (2020, January 13). Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR. [https://www.who.int/docs/default-source/coronaviruse/wuhan-virus-assay-v1991527e5122341d99287a1b17c111902.pdf?sfvrsn=d381fc88\\_2](https://www.who.int/docs/default-source/coronaviruse/wuhan-virus-assay-v1991527e5122341d99287a1b17c111902.pdf?sfvrsn=d381fc88_2)
14. Ong SWX, Tan YK and Chia PY, Air, Surface Environmental, and Personal Protective Equipment Contamination by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) From a Symptomatic Patient, *JAMA.* Published online March 4, 2020. doi:[10.1001/jama.2020.3227](https://doi.org/10.1001/jama.2020.3227)

15. Hewlett AL, Whitney SE, Gibbs SG, et al., Mathematical Modeling of Pathogen Trajectory in a Patient Care Environment. *Infection Control and Hospital Epidemiology*. 2013;34(11): 1181-1188
16. S.A. Batterman, Characterization of particulate emissions from occupant activities in offices *Indoor Air*, 11 (1) (2001), pp. 35-48
17. Wang J, Chow TT. Numerical investigation of influence of human walking on dispersion and deposition of expiratory droplets in airborne infection isolation room. *Building and Environment*. 2011 Oct 1;46(10):1993-2002.
18. Johnson GR, Morawska L, Ristovski, ZD, et al. Modality of human expired aerosol size distributions. *Journal of Aerosol Science*. 2011; 42: 839–851. doi:10.1016/j.jaerosci.2011.07.009
19. Lee J, Yoo D, Ryu S et al., Quantity, size distribution, and characteristics of cough generated aerosol produced by patients with an upper respiratory tract infection. *Aerosol and Air Quality Research*. 2019; 19: 840–853.
20. van Doremalen N, Bushmaker T, Morris D, et al. Aerosol and surface stability of HCoV-19 (SARS-CoV-2) compared to SARS-CoV-1. *New England Journal of Medicine*. Published online March 17, 2020. DOI: 10.1056/NEJMc2004973
21. GenBank. Wuhan seafood market pneumonia virus isolate Wuhan-Hu-1, complete genome. <https://www.ncbi.nlm.nih.gov/nuccore/MN908947>

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## Supplementary Materials:

### Methods

Surface and personal items were collected using 3x3 sterile gauze pads prewetted with 3 mL of phosphate buffered saline (PBS). Large area surface samples were collected by wiping in an “S” pattern in 2 directions to cover as much of the available surface as possible. Smaller items (e.g. cellular phones, remote controls) were wiped in one direction on every available surface. Following collection, samples were packed in 50 mL conical tubes. Hand hygiene and glove changes were performed between the collection of every sample.

### Personal Items

Several categories of personal items were sampled consistently between all quarantine rooms and assayed for evidence of viral contamination: cellular phones and television remote controls. In addition, individuals were asked about what items they used or handled frequently and several additional samples were collected based on those responses: exercise equipment, pots used to heat water, a nasal cannula and a spirometer. In addition, both the rim and seat of the toilet were sampled together.

## Room Surfaces

Several surfaces were sampled in each room. For rooms in the National Quarantine Unit, both the windowsill and the bedside table were sampled. For rooms in the Nebraska Biocontainment Unit, samples were taken on the windowsill, the bed rail or bedside table, under the patient's bed and on the air conditioning return grate nearest the door.

## Air Samples

Several types of air samples were collected both in patient rooms, on personnel performing sampling and, in the hallways, outside of patient rooms. Stationary air samples, both inside and outside of patient rooms, were collected using a Sartorius Airport MD8 air sampler operating at 50 Lpm for 15 minutes. Samples are collected onto a 80mm gelatin filter. Additional air samples were collected using Personal Button Samplers (SKC, Inc.) using AirCheck pumps (SKC, Inc.) sampling at 4 Lpm. These samples were collected onto 25 mm gelatin filters.

## Sample Recovery, RNA Extraction and Reverse Transcriptase PCR

Surface samples were recovered by adding 15 mL of sterile PBS to the conical vial containing the gauze pad and manually shaking the conical for 1 minute. 25 mm gelatin filters were removed from filter housing and placed in a 50 mL conical tube and then dissolved by adding 10 mL of sterile PBS. 80 mm gelatin filters were removed from their filter housing, carefully folded and placed in a 50 mL conical tube and then dissolved by adding 15 mL of sterile PBS. RNA extractions were performed using a Qiagen DSP Virus Spin Kit (QIAGEN GmbH, Hilden, Germany) 200 to 400ul of initial sample was used for RNA extraction, and a negative extraction control was included with each set of extractions. Samples were eluted in 50ul of Qiagen Buffer AVE. RT-PCR was performed using Invitrogen Superscript III Platinum One-Step Quantitative RT-PCR System. Each PCR run included a positive synthetic DNA control and a negative, no template control. Reactions were set up and run with initial conditions of 10 minutes at 55°C and 4 minutes at 94°C then 45 cycles of 94°C for 15 sec and 58°C for 30 seconds, QuantStudio™ 3 (Applied Biosystems™, Inc) utilizing the following reagents:

6.1 µL	nuclease free water
12.5 µL	Invitrogen 2X Master Mix
0.4 µl	MgSO <sub>4</sub>
0.5 µl	Primer/Probe Mix (IDT)* ( <i>Primers 10uM, Probe 5uM</i> )
0.5 µl	SuperScript III Platinum Taq
5.0 µL	<u>extracted sample RNA, nuclease free water or positive control</u>
25.0 µL	Total

In order to quantify the number of viral gene copies present in each sample from the measured Ct values, a standard curve was developed using synthetic DNA. A 6- log standard curve was run in duplicate beginning at a concentration of  $1 \times 10^3$  copies/µL. The data was fit with the exponential function:



$$\text{copy concentration} \left( \frac{\text{copies}}{\mu\text{L}} \right) = 3 \times 10^6 e^{-0.421 \times Ct}$$

Where Ct is the cycle time where amplification is definitive. The equation was then used to convert all measured Ct values from all samples into gene copy concentrations. The average and standard deviation concentrations were calculated from the triplicate values for each sample.

The primers and probe used in this study (below) are based on a previously published assay (13) targeting the E gene of SARS-CoV-2, which produces the envelope small membrane protein.

The gene was used as a target based on its similarity to previously identified coronavirus, including SARS-CoV strain Frankfurt and two Bat SARS-related CoV (GenBank Acc. No. MG772933.1 and NC\_014470). The positive control consisted of ssDNA, targeting the E and N gene (below), in a 1:1 mixture at  $10^3$  copies/ $\mu\text{L}$ . ssDNA was based on the 2019-nCoV genome sequence published in Genbank (21). The minimum concentration detected by this assay was between  $1 \times 10^{-1}$  and  $1 \times 10^{-2}$  copies/ $\mu\text{L}$ .

\*E gene target primers and probe:

Probe: 5'/56-FAM/ACACTAAGCC/ZEN/ATCCTTACTGCGCTTCG/3AIBkFG/-3'

Primer 1: 5'-ATATTGCAGCAGTACGCACACA-3'

Primer 2: 5'-ACAGGTACGTTAATAGTTAATAGCGT-3'

ssDNA E Target Sequence:

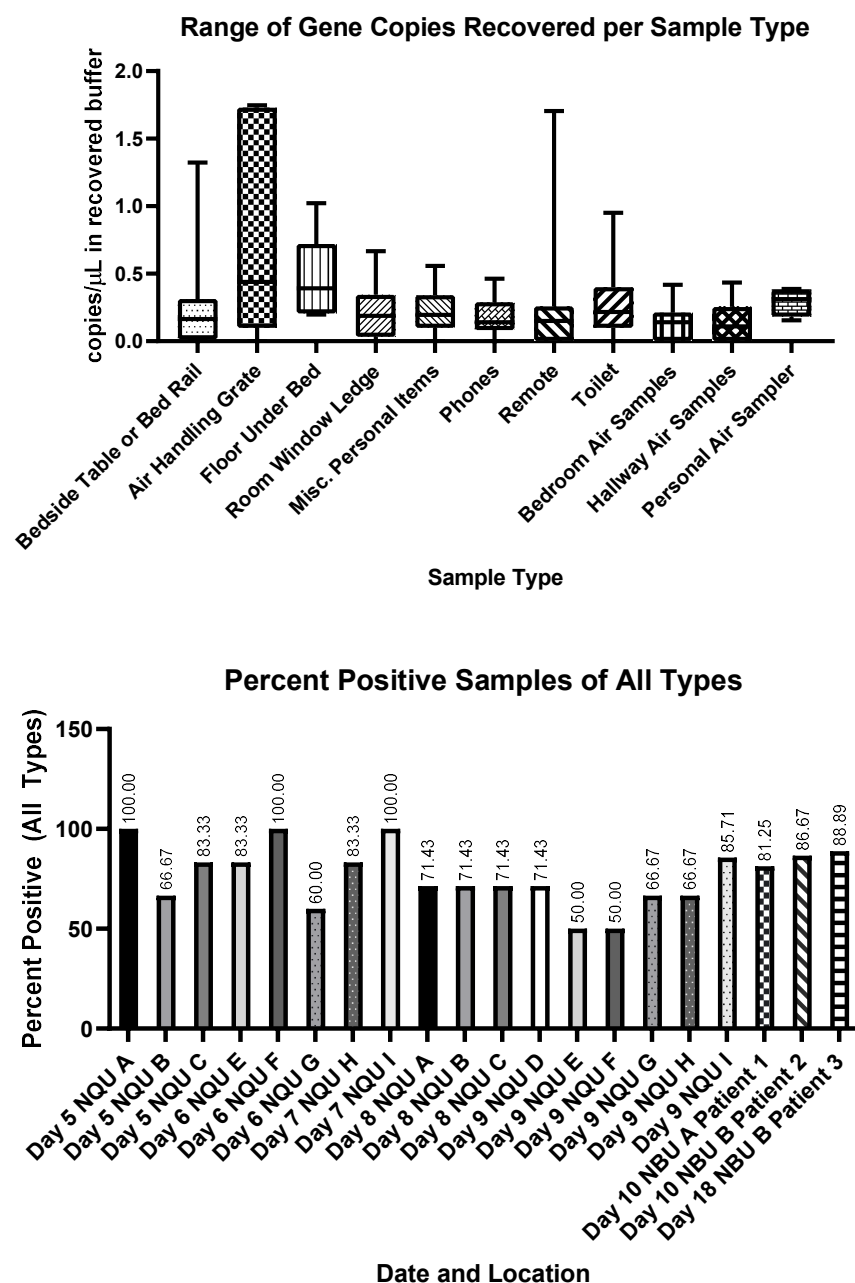
5'TTCGGAAGAGACAGGTACGTTAATAGTTAATAGCGTACTTCTTTTCTTGCTTTCGTG  
GTATTCTTGCTAGTTACACTAGCCATCCTTACTGCGCTTCGATTGTGTGCGTACTGCTGC  
AATATTGTAAACGTG-3'

ssDNA N Target Sequence:

5'ACCAAAAGATCACATTGGCACCCGCAATCCTGCTAACAATGCTGCAATCGTGCTACA  
ACTTCCTCAAGGAACAACATTGCCAAAAGGCTTCTACGCAGAAGGGAGCAGAGGCGG  
CAGTCAAGCCTCTTCTCGTTCCTCATCACGTAGT-3'

## Cell Culture Assays

Vero E6 cells were used to culture virus from environmental samples. The cells were cultured in Dulbeccos's minimal essential medium (DMEM) supplemented with heat inactivated fetal bovine serum(10%), Penicillin/Streptomycin (10,000 IU/mL & 10,000  $\mu\text{g/mL}$ ) and Amphotericin B (25  $\mu\text{g/mL}$ ). For propagation, 100  $\mu\text{L}$  of undiluted or samples diluted 1:1 in sterile water were added to T25 culture flasks. The cells were monitored daily to detect virus-induced CPE. After 5-7 days cell supernatants and lysates were collected. Samples were evaluated for cytopathic effect, immunofluorescence and western blot analysis using a mouse monoclonal SARS-CoV spike antibody (BEI NR-618) were used to determine the presence of viral antigens.



**Fig. 1. A.** Box and whisker plot demonstrating the max and min (whiskers), median (line) and 25<sup>th</sup> and 75<sup>th</sup> percentile gene copy concentrations (copies/μL) for all types of samples collected in this study. **B.** Percentage of positive samples recovered in each room sampling. Bar patterns from the same room and individual sampled on multiple dates are identical.

Room	Day	Bedside Table or Bed Rail (copies/μL)	Air Handling Grate (copies/μL)	Floor Under Bed (copies/μL)	Room Window Sledge (copies/μL)	Misc. Personal Items (copies/μL)	Phone (copies/μL)	Remote (copies/μL)	Toilet (copies/μL)	All Samples (copies/μL)	Samples Collected	% Positive	Oral Temp. (°F)	Other Symt
NQU A	5	1.315	0.114	0.140	0.290	Exercise Bike	0.140	0.242	0.122	0.211	6	100.00%	102.3	Y
NQU B	5	0.054	0.093	0.140	0.394	Hot Pot	0.352	0.325	UND	NC	6	66.67%	98.2	Y
NQU C	5	UND	NC	0.248	0.429	Hot Pot	0.146	0.252	0.224	0.198	6	83.33%	99.1	N
NQU E	6	0.118	0.005	0.209	0.362	IPad	UND	NC	0.415	0.122	6	83.33%	100.5	Y
NQU F	6	0.312	0.272	0.010	0.018		0.414	0.205	0.036	0.062	5	100.00%	98.6	Y
NQU G	6	UND	NC	UND	NC		0.319	0.301	0.043	0.075	5	60.00%	99.1	N
NQU H	7	0.157	0.272	0.200	0.346		UND	NC	0.281	0.252	5	83.33%	98.5	Y
NQU I	7	0.201	0.349	0.130	0.225		0.031	0.053	0.246	0.214	5	100.00%	99.1	N
NQU A	8	0.197	0.342	0.390	0.407	Exercise Bike	0.462	0.402	0.443	0.114	7	71.43%	100.4	Y
NQU B	8	0.305	0.328	0.250	0.228	Hot Pot	0.132	0.228	UND	NC	7	71.43%	97.9	N
NQU C	8	0.081	0.140	UND	NC	Hot Pot	0.100	0.174	UND	NC	7	71.43%	95.5	N
NQU D	9	0.191	0.310	0.174	0.302	IPad	0.146	0.252	UND	NC	7	71.43%	ND	ND
NQU E	9	UND	NC	UND	NC		0.278	0.317	0.248	0.222	6	50.00%	99	Y
NQU F	9	UND	NC	0.148	0.256		0.115	0.199	UND	NC	6	50.00%	98.8	Y
NQU G	9	0.508	0.308	UND	NC		0.189	0.327	0.177	0.306	6	66.67%	99.1	N
NQU H	9	0.575	0.237	0.667	0.319	Dumbbells	UND	NC	UND	NC	9	66.67%	98.7	N
NQU I	9	0.093	0.162	0.139	0.241	Treadmill	0.138	0.239	0.229	0.255	7	85.71%	98.5	N
NBU A	10	0.226	0.206	0.140	0.242	Yoga Mat	0.157	0.272	UND	NC	7	85.71%	98.5	N
Patient1		0.170	0.162	0.198	0.367	IPad	0.428	0.419	0.203	0.351	16	81.25%	98.3	Y
		0.333	0.309	0.438	0.070	Spirometer	UND	NC	0.126	0.219	3,760	3,435		
		0.144	0.250	0.445	0.363	O2 Cannula	0.152	0.263	0.215	0.372	self from patient	UND	NC	Y
NBU B	10	UND	NC	1.747	0.307	Laptop	0.133	0.230	0.215	0.372	15	86.67%	99.2	Y
Patient2				0.322	0.336	Glasses								
				1.021	0.307	Pulse Ox								
						Finger Monitor								
NBU B	18	1.322	0.238		0.440				0.226	0.195	9	88.89%	99.1	Y
Patient3				0.196	0.478	Lotion tube			UND	NC	9	88.89%	99.1	Y
Percent Positive		75.0%	80.0%	100.0%	81.8%	81.3%	83.3%	64.7%	81.0%	63.2%			57.9%	
R <sup>2</sup> to Fever		0.22			0.19	0.01	0.00	0.00	0.20	0.06		0.01		

**Figure 2. A. Results of all in-room samples collected in this study.** UND denotes undetected, NC denotes Not Calculated. ND denotes No Data. In the case where no data was obtained, the patient had been transferred from another facility less than 24 hours prior to sampling.

Location	Day	Hallway Air Samples (copies/L of air)		Personal Air Samples (copies/L of air)	
NQU	5	UND	NC		
	5	UND	NC		
	6	5.757	5.096		
	6	6.004	5.902		
	7	2.077	3.597		
	7	UND	NC		
	8	8.688	3.688		
	8	2.361	4.090		
	8	2.294	3.972		
	9			7.392	19.204
				5.366	7.150
NBU	10	UND	NC		
	10	2.994	5.186		
	10	0.979	1.695		
	18			19.174	49.817
				48.216	67.164
Percent Positive		66.7%		100.0%	

**Figure 2. B.-Results of hallway air samples and personal air samples.** UND denotes undetected, NC denotes Not Calculated. The dotted background indicates the sample found to contain culturable SARS-CoV-2 virus.

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