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SARS-CoV-2 detection and genomic sequencing from hospital surface samples collected at UC Davis

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Running title: SARS-CoV-2 detection and sequencing in a hospital

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Abstract

**Rationale:** There is little doubt that aerosols play a major role in the transmission of SARS-CoV-2. The significance of the presence and infectivity of this virus on environmental surfaces, especially in a hospital setting, remains less clear.

**Objectives:** We aimed to analyze surface swabs for SARS-CoV-2 RNA and infectivity, and to determine their suitability for sequence analysis.

**Methods:** Samples were collected during two waves of COVID-19 at the University of California, Davis Medical Center, in COVID-19 patient serving and staff congregation areas. qRT-PCR positive samples were investigated in Vero cell cultures for cytopathic effects and phylogenetically assessed by whole genome sequencing.

**Measurements and Main Results:** Improved cleaning and patient management practices between April and August 2020 were associated with a substantial reduction of SARS-CoV-2 qRT-PCR positivity (from 11% to 2%) in hospital surface samples. Even though we recovered near-complete genome sequences in some, none of the positive samples (11 of 224 total) caused cytopathic effects in cultured cells suggesting this nucleic acid was either not associated with intact virions, or they were present in insufficient numbers for infectivity. Phylogenetic analysis suggested that the SARS-CoV-2 genomes of the positive samples were derived from hospitalized patients. Genomic sequences isolated from qRT-PCR negative samples indicate a superior sensitivity of viral detection by sequencing.

**Conclusions:** This study confirms the low likelihood that SARS-CoV-2 contamination on hospital surfaces contains infectious virus, disputing the importance of fomites in COVID-19 transmission. Ours is the first report on recovering near-complete SARS-CoV-2 genome sequences directly from environmental surface swabs.

**Key words:** SARS-CoV-2, fomites, hospital surface contamination, viral genome sequencing, COVID-19
Introduction

There is a paucity of data regarding survival and infectivity of the SARS-CoV-2 virus on surfaces in closed environments, although some data are available for other coronaviruses (1, 2). Early in the pandemic, testing of artificially generated aerosols on copper, stainless steel, cardboard, and plastic surfaces found a rapid decay of viral viability within a few days (3). Another study examining survival on PPE showed that the virus decayed rapidly on cotton but survived for up to 21 days on some other surface material (4). More recent evaluation of a variety of surfaces showed that infectious virions could survive for up to 28 days in laboratory conditions including high titer virus and in the dark (5). However, it is unclear in all of these cases how this relates to virus survival and the potential for its transmission outside the laboratory. A study of high-touch surfaces in a community setting attempted to estimate transmission risk, but there are still too many unknowns to do this with any confidence (6). It is known that SARS-CoV-2 can survive on skin for about nine hours and may allow or extend viral survival on surfaces following contact (7).

A key complication in studies of SARS-CoV-2 environmental viability relates to how long the viral RNA can be detected on surfaces. A large number of studies have used qRT-PCR to detect SARS-CoV-2 viral RNA indoors (8–20) reviewed in (21) and found that the virus was detectable up to several weeks after it was presumably deposited (22). The amount of viral RNA detected seems to be inversely correlated with cleaning protocols (23). This probably explains otherwise surprising results such as the lack of viral RNA detected in an oncology ward housing patients with COVID-19 (24), or the very low probability of detection in an ICU (25). Several studies detected SARS-CoV-2 RNA in these environments but were unable to culture infectious SARS-CoV-2 virions (26–28). However, viable SARS-CoV-2 was successfully cultured and sequenced from the air of the hospital room with a COVID-19 patient using a water vapor condensation method (29).

In this study, we assessed environmental contamination with SARS-CoV-2 in a hospital setting by both qRT-PCR and a viral culture assay. We examined surfaces, and also sampled HVAC filters since these have been previously shown to contain SARS-CoV-2.
in healthcare settings (30, 31) and in homes (22). In addition, we sequenced partial and complete genomes from surfaces and compared them phylogenetically to identify the source of the virus.

**Materials and Methods**

**Swab sample collection at the UC Davis Medical Center (UCDMC)**

UCDMC is a 625-bed academic medical center in Northern California. While there are multiple ICUs and medical floors, during the first 6 months of the pandemic, most patients with active COVID-19 were hospitalized in 3 intensive care units (ICU) and 2 medical wards. Both the ICU and medical wards have the ability to place individual rooms as well as the entire ward under negative pressure, and that was the case during the study. Samples were collected using standard Puritan cotton-tipped swabs with plastic handles and placed into Trizol as described below. The first set of samples was collected in April 2020, and the second set between late July/early August 2020. Clinical staff swabbed an approximately 10cm x 10 cm area for several seconds, as if trying to clean it with a scrubbing motion and rotating the swab.

*Heating, ventilation, and air conditioning (HVAC) swab collection:* Swabs were moistened in saline, brushed across the air filters, and then placed into 500 ul of Trizol(R). For safety reasons, the air pressure in the HVAC system was temporarily reduced during sampling. Sampling took place on the filters which protect the evaporator coils from dust, meaning that the sampled dust was unfiltered directly from the hospital floor. Samples were collected both from the floor with a number of COVID-19 patients, as well as from another floor with no known COVID-19 patients. All samples were frozen at -80 °C until processing.

*Surface sampling:* During the first collection, swabs were pre-moistened in sterile saline and then placed into 500 uL Trizol(R); during the second round, swabs were either pre-moistened with Trizol(R) or viral transport media (VTM, Innovative Research™) and then
placed into their respective individual containers after sample collection. All samples were stored frozen at -80 °C until processing.

*Surface sampling (for viability testing):* For viability testing, a pair of swabs were held together for the swabbing. One was placed in Trizol for qRT-PCR (as described above) and the other into VTM. All samples were stored frozen at -80 °C until processing.

**qRT-PCR**

RNA extraction from swabs was performed using the Zymo Research Direct-zol-96RNA kit (#R2054). Briefly, 500 ul of pure ethanol was added to the 500 ul of Trizol+swab. The mixture was transferred to a 1-96 plate extraction performed according to the manufacturer instructions. RNA was eluted in 25 ul water and cDNA was made using the SuperScriptIII ThermoFisher kit (#18080051). SARS-CoV-2 screening was performed by qRT-PCR using Taqman Universal Master Mix II+UNG (ThermoFisher #4440038). Primers and probes and cycling conditions to detect segments of the N and RdRp genes were performed following the CDC

(https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html) and Corman et al. protocols (Corman et al. 2020). qRT-PCR was run for 45 cycles and any positive signal was reported.

**Vero cell culture and SARS-CoV-2 infection studies**

Vero E6 cells (ATCC #CRL-1586) were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 100 IU/ml of penicillin-streptomycin (Pen-Strep; Gibco). The mNeonGreen SARS-CoV-2 (icSARS-CoV-2-mNG) virus (Xie et al. 2020) was kindly provided by the UTMB World Reference Center for Emerging Viruses and Arboviruses and Dr. Scott Weaver, and was propagated and titered in Vero E6 cells. All swab samples and positive controls were diluted in D10-CoV medium consisting of DMEM supplemented with 10% FBS, 100 IU/ml Pen-Strep, 250 µg/ml Amphotericin B (Gibco) and 250 µg/ml Gentamicin (Quality Biologicals).

Six-well plates of Vero E6 cells (~60% confluent) were infected with either 300 uL of the viral transport medium from qRT-PCR positive environmental swab samples diluted 1:1
in D10-CoV medium, or 300 µL of mNeonGreen SARS-CoV-2 (icSARS-CoV-2-mNG) 10-fold serially diluted in D10-CoV medium to infect wells with $10^5$ PFU to $10^0$ PFU per well. Following 1h incubation at 37 °C, rocking plates every 15 minutes, the cells were replenished with fresh D10-CoV medium and incubated at 37 °C + 5% CO$_2$ for five days. A mock-treated control consisting of cells only maintained in D10-CoV medium was included in the assay and treated identically. All samples were tested in duplicate. Two and five days post-infection, the cells were assessed microscopically for any visible cytopathic effect. Five days post infection, 2 mL of cell culture supernatant was collected from each well and mixed with 6 mL of Trizol LS reagent (Ambion). Cell lysates were harvested by adding 1 mL of Trizol LS reagent to the cell monolayer. All Trizol-treated samples were used for RNA extraction and qRT-PCR.

**SARS-CoV-2 viral genome sequencing**

We prepared RNA extractions for Oxford Nanopore (ONT) MinION sequencing of SARS-CoV-2 viral genomes. We made modifications to the ARTIC Network Protocol (v2) (32), to optimize sequencing of environmental samples. Our complete protocol is available online [https://www.protocols.io/view/ncov-2019-environmental-sample-sequencing-protocol-brnbm5an](https://www.protocols.io/view/ncov-2019-environmental-sample-sequencing-protocol-brnbm5an). In brief: we conducted random hexamer primed reverse transcription and amplified cDNA using v3 primers, which tile the entire viral genome (save for non-coding regions at the genome ends) with overlapping 400 bp fragments. We concentrated PCR products using the Zymo Select-a-Size DNA Clean & Concentrator Kit (Zymo Research, Irvine CA), ligated barcodes using the Oxford Nanopore Native Barcoding kit, and ligated sequencing adaptors. Samples were run on ONT R9.4 or R10.3 flow cells. We followed the ARTIC Network bioinformatics SOP, which in brief involved high accuracy basecalling and demultiplexing using ONT Guppy, mapping reads to the Wuhan-Hu-1 (accession MN908947) reference, polishing with Nanopolish, and consensus generation (code for analysis available [https://github.com/sociovirology/sars_cov2_environmental_seq](https://github.com/sociovirology/sars_cov2_environmental_seq)).

**Results and Discussion**
**Improved cleaning protocol and patient management was associated with decreased recovery of SARS-CoV-2 RNA from hospital surface samples**

During the first wave of COVID-19 (March-April, 2020) the role of fomites in transmission was controversial and studies providing supporting evidence for it were lacking. Some of our hospital personnel also became ill with COVID-19 at that time. To investigate whether the infection clusters among health care workers were associated with SARS-CoV-2 contaminated areas, we collected 56 swabs in April 2020, from a variety of frequently used locations. Six of these samples (11%) tested positive for SARS-CoV-2 by qRT-PCR (Figure 1). While the positive locations were in the proximity of hospitalized COVID-19 patients, none of these areas were related to where the hospital personnel cluster infections were suspected to originate from.

During a three-month period between April and August 2020, important changes took place to improve cleaning protocols with a change in the frequency/duration/composition of cleaning material in the hospital. In addition to the cleaning protocol changes, improved patient management of respiratory secretions took place. This included earlier intubation, rapid sequence ventilation, and changes in the management of high O₂ flow nasal cannulas. To investigate whether changes in cleaning practices and patient management impacted the outcomes compared to our earlier findings, we performed a follow-up study by collecting an additional 168 swabs. Out of these, only five tested positive for SARS-CoV-2 by qRT-PCR (Supplementary Tables 1 & 2). None of the HVAC samples were positive by qRT-PCR.
Figure 1: Representative areas sampled by swabs for SARS-CoV-2 at the UCDMC. Positive samples are shown in red, negative samples in green. The top panel represents an ICU room, and the bottom panel represents a ward room. Each dot represents a single swab.
Thus, our results show a substantial decrease in positive samples from 11% to 2% between April and August. This trend is particularly significant in the light that in mid-August, 2020, a second surge of COVID-19 cases were admitted, substantially increasing the number of patients in the hospital (Figure 2).

![Figure 2: SARS-CoV-2 positive patients at UCDMC during the first and the second wave of COVID-19. Weekly totals of COVID-19 patients, and the cumulative total number from early March until mid-August, 2020. The blue arrows indicate the sampling dates.](image)

We propose that together, the improved cleaning protocols and patient management practices likely contributed to decreased presence of aerosolized (and deposited) virions in the rooms where COVID-19 patients were cared for. It was still unclear however, whether the recovered viral RNA from the samples collected from hospital surfaces could be a feasible source of infection.

**Hospital surface SARS-CoV-2 RNA did not exhibit infectious nature in a Vero cell culture model in vitro**

To investigate whether the SARS-CoV-2 qRT-PCR positivity in hospital surface samples was associated with potential infectivity, a total of five swabs (identified as positive by qRT-PCR) were tested. We used an *in vitro* infection assay to detect the presence of infectious virus particles. Each of the wells of Vero E6 cells incubated with individual swab samples appeared identical to the mock-infected cells and showed no signs of cytopathic
effect (CPE) by microscopy for up to five days post-infection (dpi) (Figure 3). This lack of CPE in swab-inoculated wells was consistent in two biologically independent infection assays in all tested samples. In contrast, positive control samples infected with 10-fold serial-dilutions from $10^5$ to 1 PFU of mNeonGreen SARS-CoV-2 showed notable CPE and mNeonGreen expression throughout the course of infection, even in wells infected with only 1 PFU (Figure 3). Therefore, the lack of CPE in the environmental swab samples indicated the absence of infectious virus particles or samples with a viral load below the detection limit for viral culture.

To confirm this result, supernatant and cell lysates from the swab and positive control inoculated Vero E6 cells were collected five dpi from each independent experiment. Total RNA from each sample was analyzed by qRT-PCR assay in duplicate, and while no signal was observed with the N1 primer set, a low signal (CT 28, 37) was detected in two of the samples with the N2 primer set. A repeat of this experiment in triplicate for each sample only yielded low signal in a single reaction (CT 37). In combination with the lack of viral infectivity in cell culture assays, our data suggest that the signal most likely represented relic RNA from the original swab and not due to the replication of viral particles in culture.
Figure 3. Micrographs of Vero E6 cells five days after inoculation. Cells were either mock-infected (upper left), inoculated with swab samples (representative of all five tested samples, upper right), or infected with one PFU of mNeonGreen SARS-CoV-2 (phase contrast, lower left; mNeonGreen lower right).

**Viral genome sequencing**

In order to determine the genome sequences from the isolated samples, we generated a total of 17,567,849 reads across five separate MinION sequencing runs (Supplementary Table 3), of which 6,670,616 were used for mapping after demultiplexing and quality control. The negative control in Run 4 yielded reads that mapped to the reference genome, therefore samples were re-sequenced in Run 5. Negative controls in Runs 1-3 and 5 had no reads mapping to the reference genome. At least one positive control (included in Runs 4 and 5), per run produced reads that mapped to the reference genome.
The genome coverage obtained from samples was assigned to three groups: >15% (n = 61), 20-40% (n = 5), >75% (n = 5). The percent of the genome covered at a 5X depth quickly declined as a function of increasing mean Ct values (Figure 4). There was a notable threshold of Ct ~ 38, above which no sample achieved >10% genome completeness.

Figure 4. Environmental swabs with Ct values below 38 yielded enough sequence reads to cover a substantial portion of the SARS-CoV-2 genome. The percent of the SARS-CoV-2 reference genome (isolate Wuhan-Hu-1) covered at ≥ 5X decreased steeply as a function of the mean Ct value (using CDC N1, N2, and Berlin RdRP primers). The colored points represent individual swab samples, some of which were re-run in independent sequencing runs.
Whole-genome PCR and sequencing yields more effective detection of SARS-CoV-2 than qRT-PCR

While there was a steep drop-off in achieving a full genome sequence with increasing Ct values, the sequencing protocol was able to detect SARS-CoV-2 in samples with undetermined Ct scores by PCR, with an average of 6.27% coverage (range: 2.19-14.78%). Using a sequencing cutoff of >2% genome coverage, sequences of SARS-CoV-2 were amplified in 15 samples that had no detectable Ct by PCR, whereas five samples that did not have a detectable Ct were not amplified by sequencing (at >2% coverage). This uncoupling of detection by qRT-PCR vs sequencing is likely due to the fact that qRT-PCR targets only a small portion of the genome and sequencing primers cover the entire genome (e.g. (33)). Furthermore, environmental samples in particular may have been degraded or diluted, affecting the genomic RNA available for reverse transcription, as observed in multiple studies of environmental samples ((34–36).

Generation of near-complete genomes from environmental samples

We recovered two near-complete genomes from two different patient rooms, D14 and T7 Blue. These samples were collected from two surfaces, the floor and a soiled linens basket lid. Genome coverage and Ct values for D14 were 99.26% (Mean Ct = 36.49) and T7 Blue 91.75% (Mean Ct = 36.89), both with a depth cutoff of 5X to call a base. The sample from room D14 had an average depth of 371.21 ± 171.30 reads (mean ± SD). The sample from room T7 had an average depth of 377.14 ± 185.03.

Effect of protocol modifications for environmental sample sequencing

The ARTIC protocol was modified in two major ways to accommodate the lower sample concentration in environmental samples compared to clinical samples: concentration and cleaning of PCR products and making duplicate barcoding reactions. Concentration of PCR products increased the genome coverage from 96.31% to 99.02% (sample from room D14) and from 76.08% to 91.75% (sample from room T7 Blue), compared to the standard ARTIC protocol. Duplicate barcoding reactions only marginally increased genome coverage in the sample from room D14 from 99.02% to 99.26%.
Recovered Genome Sequences are from clade 19B may have originated from a single patient, or from multiple patients infected with similar viruses

To compare the near-complete genome sequences generated, we conducted phylogenetic analyses. We first determined that the pairwise identity between these two genomes was 93.8%, with several polymorphisms present. We conducted a phylogenetic analysis using NextStrain (37) to compare the sequences with other viruses detected through local subsampling in California and Sacramento County specifically. Both sequences were placed in clade 19B (Figure 5a), which were the first sequenced variants that circulated (along with 19A) in Asia early in the epidemic (38). We included all publicly available samples sequenced from UCDMC in the phylogeny (Figure 5b). Both sequences clustered with UCDMC sample USA/CA-CZB-1145/2020, and notably these three samples clustered in an entirely different clade than the rest of the UCDMC samples, which were in clade 20C that arose in Europe. Thus, it appears likely these samples were derived from a single patient (or from multiple patients infected with similar viruses) from which USA/CA-CZB-1145/2020 originated.
Figure 5. Phylogenetic comparison of the SARS-CoV-2 sequences obtained from environmental swabs at UCDMC. A. Near-complete genomes obtained from environmental samples clustered in clade 19B. The phylogenetic tree was generated using the NextStrain protocol, and compares sequences to others amplified in
Sacramento County in California. B. Environmental genome sequences may have originated from a single patient, or from multiple patients infected with similar viruses. All publicly available patient samples originating from UC Davis are shown as blue points at the tips of the phylogeny. Note that most sequences from UC Davis in this time period are members of the 20C clade, as opposed to the environmental sequences that are members of clade 19B together with sample USA/CA-CZB-1145/2020.

Conclusions

Eleven percent of samples collected at the UC Davis Medical Center in April 2020 were positive for SARS-CoV-2 whereas a larger follow-up experiment in August found only 2% of swabs positive, which is likely due to improved cleaning protocols and improved management of patient respiratory secretions. No infectious virus was detectable from surfaces, in agreement with previous studies. However, near-complete genome sequences were amplified from two surfaces, suggesting that some viral genomes are present, but may not be infectious. Viral sequences were amplified from several samples which appeared negative by qRT-PCR, highlighting the potential to obtain viral sequences in some PCR negative samples. Genome sequences from the positive samples at the first sampling point suggest that the environmental contamination was linked to a single lineage of virus, most likely from a single patient.
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Supplementary Table 1: Locations of samples positive for SARS-CoV-2 by qRT-PCR. “U” is Undetermined (at 45 cycles of qRT-PCR). All patient rooms were occupied by known COVID-19 cases. The 1st wave was in the spring of 2020, and the second was in late summer 2020.

| Time         | Ct (N1) | Ct (N2) | Location                  | Surface          |
|--------------|---------|---------|---------------------------|------------------|
| 1st wave     | 36      | 39      | Patient room D14          | Floor            |
| 1st wave     | 32      | 34      | Patient room D14          | Floor            |
| 1st wave     | U       | 37      | Patient room T7 Blue      | Vent tubing arm  |
| 1st wave     | U       | 38      | Patient room T7 Blue      | Keyboard         |
| 1st wave     | 37      | 39      | Patient room T7 Blue      | Telemetry screen alarm button |
| 1st wave     | 36      | 37      | Patient room T7 Blue      | Soiled linen lid |
| 2nd wave     | 39      | 41      | Floor Samples             | Women’s bathroom|
| 2nd wave     | 35      | 37      | ICU Patient Room          | Linen Cart- lid  |
| 2nd wave     | U       | 42      | ICU Patient Room          | Room Door Handle |
| 2nd wave     | 38      | U       | Floor Nursing Workspace   | Floor            |
| 2nd wave     | 44      | U       | Floor Patient Room        | Floor            |
Supplementary Table 2: Locations and qRT-PCR results for all samples collected. Undetermined is at 45 cycles of qRT-PCR.

| Sample number | Location                | Object/Surface                          | CDC_N1      | CDC_N2      |
|---------------|-------------------------|-----------------------------------------|-------------|-------------|
| 1             | Hospitalist room P2     | food table                              | Undetermined| Undetermined|
| 2             | Hospitalist room P2     | Keyboard                                | Undetermined| Undetermined|
| 3             | Hospitalist room P2     | conference table                        | Undetermined| Undetermined|
| 4             | Hospitalist room P2     | armrest (chair at conference table)     | Undetermined| Undetermined|
| 5             | Residents' room T5      | Door knob/keypad, interior              | Undetermined| Undetermined|
| 6             | Residents' room T5      | Keyboard                                | ND          | ND          |
| 7             | Residents' room T5      | microwave panel and door                | Undetermined| Undetermined|
| 8             | Residents' room T5      | Telephone                               | Undetermined| Undetermined|
| 9             | Residents' room T5      | Keyboard                                | Undetermined| Undetermined|
| 10            | Residents' room D6      | Keyboard                                | Undetermined| Undetermined|
| 11            | Residents' room D6      | conference table                        | Undetermined| Undetermined|
| 12            | Residents' room D6      | Door knob/keypad, interior              | Undetermined| Undetermined|
| 13            | Residents' room D6      | Telephone                               | Undetermined| Undetermined|
| 14            | Residents' room D6      | armrest (chair at conference table)     | Undetermined| Undetermined|
|               |                         | AND "desktop"                           |             |             |
| 15            | Patient room D14        | bedrails (L)                            | ND          | ND          |
| 16            | Patient room D14        | bedrails (R)                            | Undetermined| Undetermined|
| 17            | Patient room D14        | IV pump console                         | Undetermined| Undetermined|
| 18            | Patient room D14        | 02 wall dial                            | Undetermined| Undetermined|
| 19            | Patient room D14        | marker/pen                              | Undetermined| Undetermined|
| 20            | Patient room D14        | Floor                                   | **35.92**   | **38.71**   |
| 21            | Patient room D14        | thermometer handle                      | Undetermined| Undetermined|
| 22            | Patient room D14        | propac                                   | Undetermined| Undetermined|
| 23            | Patient room D14        | mouse                                   | Undetermined| Undetermined|
| 24            | Patient room D14        | Keyboard                                | Undetermined| Undetermined|
| 25            | Patient room D14        | rover                                    | Undetermined| Undetermined|
| 26            | Patient room D14        | Ergotran                                | Undetermined| Undetermined|
|   | Patient room   | Item                                              | Floor | Position | Number |
|---|----------------|---------------------------------------------------|-------|----------|--------|
| 26| Patient room D14 | Floor                                             | 32.09 | Determined |        |
| 27| Patient room D14 | IV bag                                            | Undetermined | Undetermined |        |
| 28| Patient room D14 | Nightstand                                        | Undetermined | Undetermined |        |
| 29| Patient room D14 | hand sanitizer (in room)                          | Undetermined | Undetermined |        |
| 30| Patient room D14 | Window                                            | Undetermined | Undetermined |        |
| 31| Patient room D14 | light switch                                      | ND    | ND       |        |
| 32| Patient room D14 | light switch                                      | Undetermined | Undetermined |        |
| 33| Patient room D14 | Door knob/keypad, interior                        | Undetermined | Undetermined |        |
| 34| Patient room D14 | food table                                        | Undetermined | Undetermined |        |
| 35| Patient room D14 | isolation/PPE cart (room exterior)                | Undetermined | Undetermined |        |
| 36| Patient room D14 | Nightstand                                        | Undetermined | Undetermined |        |
| 37| Patient room T7 Blue | vent tubing arm                                 | 36.83 | Undetermined |        |
| 38| Patient room T7 Blue | Vent screen and knobs                           | Undetermined | Undetermined |        |
| 39| Patient room T7 Blue | Keyboard                                         | Undetermined | 37.62 |        |
| 40| Patient room T7 Blue | Ergotron                                         | Undetermined | Undetermined |        |
| 41| Patient room T7 Blue | Floor                                             | Undetermined | 43.06 |        |
| 42| Patient room T7 Blue | Door knob/keypad, interior                       | Undetermined | Undetermined |        |
| 43| Patient room T7 Blue | floor                                             | Undetermined | Undetermined |        |
| 44| Patient room T7 Blue | rover                                             | Undetermined | Undetermined |        |
| 45| Patient room T7 Blue | IV pump console                                   | Undetermined | Undetermined |        |
| 46| Patient room T7 Blue | Arterial line plunger                            | Undetermined | Undetermined |        |
| 47| Patient room T7 Blue | telescropy screen alarm button                   | 37.07 | 38.6     |        |
| 48| Patient room T7 Blue | CRRT console                                      | Undetermined | Undetermined |        |
| 49| Patient room T7 Blue | pluerevac box handle                              | Undetermined | Undetermined |        |
| 50| Patient room T7 Blue | marker/pen                                        | Undetermined | Undetermined |        |
| 51| Patient room T7 Blue | soiled linen lid                                  | 36.36 | 37.41    |        |
| 52| Patient room T7 Blue | mouse                                             | Undetermined | Undetermined |        |
| 53| Patient room T7 Blue | CRRT console                                      | Undetermined | Undetermined |        |
| 54| Patient room T7 Blue | endotracheal tube straps                          | Undetermined | Undetermined |        |
| 55| Patient room T7 Blue | bedrails (R)                                      | ND    | ND       |        |
| 56| Patient room T7 Blue | bedrails (L)                                      | Undetermined | Undetermined |        |
| 57| Patient room T7 Blue | marker/pen                                        | Undetermined | Undetermined |        |
| 58| Patient room T7 Blue | hand sanitizer (in room)                          | Undetermined | Undetermined |        |
| 59  | Patient room T7 Blue | doffing table (exterior, BD universal viral transport swab) | Undetermined | Undetermined |
|-----|---------------------|-------------------------------------------------------------|-------------|-------------|
| 60  | Patient room T7 Blue | sanitizer pump (exterior, BD Eswab for bacteria used)       | Undetermined | Undetermined |
| ENV-1| Floor Samples       | Hand sanitizer dispenser                                    | Undetermined | Undetermined |
| ENV-2| Floor Samples       | Sticker table screening area                                | Undetermined | Undetermined |
| ENV-3| Floor Samples       | Floor sample                                               | Undetermined | Undetermined |
| ENV-4| Floor Samples       | Information desk counters middle window                    | Undetermined | Undetermined |
| ENV-5| Floor Samples       | Circular table between gift shop and bathroom               | Undetermined | Undetermined |
| ENV-6| Floor Samples       | Water fountain in front of bathroom 1P154214                | Undetermined | Undetermined |
| ENV-7| Floor Samples       | Women's bathroom 1P154                                       | 39.3        | Undetermined |
| ENV-8| Floor Samples       | Women's bathroom 1P154                                      | Undetermined | Undetermined |
| ENV-9| Floor Samples       | Women's bathroom 1P154                                      | Undetermined | Undetermined |
| ENV-10| Floor Samples       | ATM in front of cardiovascular services                     | Undetermined | Undetermined |
| ENV-11| Elevator Button     | Elevator in front of cardiovascular services                | Undetermined | Undetermined |
| ENV-12| Elevator Button     | Davis Tower, exterior elevator button, level 1              | Undetermined | Undetermined |
| ENV-13| Elevator Button     | Davis Tower, interior elevator button, right side            | Undetermined | Undetermined |
| ENV-14| Floor Samples       | Patient transport wheelchairs                               | Undetermined | Undetermined |
| ENV-15| Floor Samples       | Patient transport wheelchairs                               | Undetermined | Undetermined |
| ENV-16| Floor Samples       | Information desk's mouse computer for self-serving PAVLNI060 | Undetermined | Undetermined |
| ENV-17| Floor Samples       | Floor Sample                                               | Undetermined | Undetermined |
| ENV-18| Floor Samples       | D14→ intercom button by the elevators                      | Undetermined | Undetermined |
| ENV-19| Offices of Ed, Pulm/ Crit Care Staff | Door handle                                  | Undetermined | Undetermined |
| ENV-20| Offices of Ed, Pulm/ Crit Care Staff | D10 intercom button for P1C4                          | Undetermined | Undetermined |
| ENV-21| Offices of Ed, Pulm/ Crit Care Staff | D10 Door handle for P1C4                                 | Undetermined | Undetermined |
| ENV-22| Offices of Ed, Pulm/ Crit Care Staff | Outdoor cafeteria courtyard dining table                    | Undetermined | Undetermined |
| ENV-23| Offices of Ed, Pulm/ Crit Care Staff | Outdoor cafeteria conference door chair arms                | Undetermined | Undetermined |
| ENV-24| Offices of Ed, Pulm/ Crit Care Staff | ER internal entrance door handle                          | Undetermined | Undetermined |
| ENV-25 | Offices of Ed, Pulm/Crit Care Staff | ER "first nursing/registration" counter | Undetermined | Undetermined |
| ENV-26 | Offices of Ed, Pulm/Crit Care Staff | West Entrance- hand sanitizer dispenser | Undetermined | Undetermined |
| ENV-27 | Offices of Ed, Pulm/Crit Care Staff | West Entrance Floor Sample | Undetermined | Undetermined |
| ENV-28 | Offices of Ed, Pulm/Crit Care Staff | South Elevator- External buttons ↑↓ buttons 1st floor | Undetermined | Undetermined |
| ENV-29 | Offices of Ed, Pulm/Crit Care Staff | Wellness Check counters at West Entrance | Undetermined | Undetermined |
| ENV-30 | Offices of Ed, Pulm/Crit Care Staff | Investigational drug services pharmacy door handle | Undetermined | Undetermined |
| ENV-31 | Hallways | Stair 1 Floor 1 "subbasement to 8th floor" West Entrance/East Wing | Undetermined | Undetermined |
| ENV-32 | Hallways | Door handle facing North Addition | Undetermined | Undetermined |
| ENV-33 | Hallways | Hallway to North Addition floor sample middle | Undetermined | Undetermined |
| ENV-34 | Hallways | Handicap button to exit hospital | Undetermined | Undetermined |
| ENV-35 | Hallways | Handicap button to exit hospital | Undetermined | Undetermined |
| ENV-36 | Hallways | D8 Reception counter (Transplant Unit) eastxxx | Undetermined | Undetermined |
| ENV-37 | Hallways | Door handle of UT8→ ICU | Undetermined | Undetermined |
| ENV-38 | Hallways | University Tower elevators internal buttons | Undetermined | Undetermined |
| ENV-39 | Hallways | University Tower elevator external buttons | Undetermined | Undetermined |
| ENV-40 | Lab Space | Beckman Centrifuge (Left) | Undetermined | Undetermined |
| ENV-41 | Lab Space | Thermo Centrifuge (Mid) | Undetermined | Undetermined |
| ENV-42 | Lab Space | Eppendorf Centrifuge (Right) | Undetermined | Undetermined |
| ENV-43 | Lab Space | Freezer Handle | Undetermined | Undetermined |
| ENV-44 | Lab Space | Refrigerator Handle | Undetermined | Undetermined |
| ENV-45 | Lab Space | Infectious waste lid and foot pedal | Undetermined | Undetermined |
| ENV-46 | Lab Space | iPad | Undetermined | Undetermined |
| ENV-47 | Lab Space | pipettors | Undetermined | Undetermined |
| ENV-48 | Lab Space | Lab Bench | Undetermined | Undetermined |
| ENV-49 | Lab Space | Hand soap Handle | Undetermined | Undetermined |
| ENV-50 | Lab Space | Floor beneath lab area | Undetermined | Undetermined |
| ENV-51 | Lab Space | Bleach bottle | Undetermined | Undetermined |
| ENV-52 | Lab Space | Entrance door handle | Undetermined | Undetermined |
| ENV-53 | Research pt. room | Vitals equipment | Undetermined | Undetermined |
| ENV  | Location                      | Item                            | Status   | Status |
|------|-------------------------------|---------------------------------|----------|--------|
| ENV 54 | Research pt. room               | Supplies door handle             | Undetermined | Undetermined |
| ENV 55 | Research pt. room               | Chair armrest (right)            | Undetermined | Undetermined |
| ENV 56 | Research pt. room               | Backrest                         | Undetermined | Undetermined |
| ENV 57 | Research pt. room               | Hand soap                        | Undetermined | Undetermined |
| ENV 58 | Research pt. room               | Infectious waste bin             | Undetermined | Undetermined |
| ENV 59 | Research pt. room               | Air vent?                        | Undetermined | Undetermined |
| ENV 60 | Research pt. room               | Floor                            | Undetermined | Undetermined |
| ENV 61 | Research pt. room               | DVD player buttons               | Undetermined | Undetermined |
| ENV 62 | Research pt. room               | Doorknob                         | Undetermined | Undetermined |
| ENV 63 | Research pt. room               | Ethanol Spray Bottle             | Undetermined | Undetermined |
| ENV 64 | Floor Sample                    | In front of South elevator       | Undetermined | Undetermined |
| ENV 65 | Floor Sample                    | E6 entrance (by east elevator entrance) | Undetermined | Undetermined |
| ENV 66 | Door Handle                     | E6 entrance                      | Undetermined | Undetermined |
| ENV 67 | Elevator Button                 | Middle Elevator/Buttons          | Undetermined | Undetermined |
| ENV 68 | Floor Sample                    | Side by 6004                     | Undetermined | Undetermined |
| ENV 69 | Door Handle                     | E5 double door                   | Undetermined | Undetermined |
| ENV 70 | Floor Sample                    | Double door                      | Undetermined | Undetermined |
| ENV 71 | XXX                            | Single door entry to patient rooms (E5) | Undetermined | Undetermined |
| ENV 72 | Door Handle                     | South Wing Level 3               | Undetermined | Undetermined |
| ENV 73 | Floor Sample                    | In front of room 3005             | Undetermined | Undetermined |
| ENV 74 | Floor Sample                    | In front of level 3              | Undetermined | Undetermined |
| ENV 75 | Greeter                        | Front Entry Badge                | Undetermined | Undetermined |
| ENV 76 | Greeter                        | Front Entry Badge                | Undetermined | Undetermined |
| ENV 77 | Greeter                        | Front Entry Badge                | Undetermined | Undetermined |
| ENV 78 | Divider                        | Pharmacy Divider/counter         | Undetermined | Undetermined |
| ENV 79 | Divider                        | Pharmacy Divider/counter ("other side") | Undetermined | Undetermined |
| ENV 80 | Divider                        | Security Main Entrance/ Divider  | Undetermined | Undetermined |
| ENV 81 | ER                             | ER wellness cheek                | Undetermined | Undetermined |
| ENV 82 | ER                             | Greeter RN badge/ ER entry       | Undetermined | Undetermined |
| ENV 83 | Divider                        | Divider in ER main entry         | Undetermined | Undetermined |
| ENV 84 | Divider                        | Divider in ER registration desk  | Undetermined | Undetermined |
| ENV 85 | Entrance Door                   | Hand Sanitizer dispenser         | Undetermined | Undetermined |
| ENV  | ER                        | Description                               | Value 1  | Value 2  |
|------|---------------------------|-------------------------------------------|----------|----------|
| ENV 86 | ER                        | PA registration Rep Badge                | Undetermined | Undetermined |
| ENV 87 | ER                        | ER wheelchair handle                     | Undetermined | Undetermined |
| ENV 88 | ER                        | ER wheelchair patient arm rest           | Undetermined | Undetermined |
| ENV 89 |                          | courtyard table                          | Undetermined | Undetermined |
| ENV 90 |                          | courtyard table divider                  | Undetermined | Undetermined |
| ENV 91 |                          | Staff Badge: Pharmacist Resident         | Undetermined | Undetermined |
| ENV 92 |                          | Vocera: Staff pharmacist                  | Undetermined | Undetermined |
| ENV 93 | ICU Patient- COVID+       | Floor- Patient Left                      | Undetermined | Undetermined |
| ENV 94 | ICU Patient- COVID+       | Floor- Patient Right                     | Undetermined | Undetermined |
| ENV 95 | ICU Patient- COVID+       | Floor- Near Door                         | Undetermined | Undetermined |
| ENV 96 | ICU Patient- COVID+       | Ventilator Tubing Intake                 | Undetermined | Undetermined |
| ENV 97 | ICU Patient- COVID+       | Ventilator Tubing Outflow                | Undetermined | Undetermined |
| ENV 98 | ICU Patient- COVID+       | Ventilator                               | Undetermined | Undetermined |
| ENV 99 | ICU Patient- COVID+       | Linen Cart- pedal                        | Undetermined | Undetermined |
| ENV 100 | ICU Patient- COVID+      | Linen Cart- lid                          | 35.16     | 37.27     |
| ENV 101 | ICU Patient- COVID+      | Bedside Table                            | Undetermined | Undetermined |
| ENV 102 | ICU Patient- COVID+      | Bedrail- Left Lower                      | Undetermined | Undetermined |
| ENV 103 | ICU Patient- COVID+      | Bedrail- Left Upper                      | Undetermined | Undetermined |
| ENV 104 | ICU Patient- COVID+      | Bedrail- Right Lower                     | Undetermined | Undetermined |
| ENV 105 | ICU Patient- COVID+      | Infusion Pump                            | Undetermined | Undetermined |
| ENV 106 | ICU Patient- COVID+      | Biohazard Bins                          | Undetermined | Undetermined |
| ENV 107 | ICU Patient- COVID+      | Hand sanitizer dispenser- near doorway   | Undetermined | Undetermined |
| ENV 108 | ICU Patient- COVID+      | Room Door Handle                        | Undetermined | 41.91      |
| ENV 109 | ICU Patient- COVID+      | Nurse Rover Device                       | Undetermined | Undetermined |
| ENV 110 | ICU Patient- COVID+      | Bedside Buttons- Left                    | Undetermined | Undetermined |
| ENV 111 | ICU Patient- COVID+      | Whiteboard markers                      | Undetermined | Undetermined |
| ENV 112 | ICU Patient- COVID+      | Light Switches                           | Undetermined | Undetermined |
| ENV 113 | Floor Neighboring COVID+ | Floor patient Foot of bed               | Undetermined | Undetermined |
| ENV 114 | Floor Neighboring COVID+ | Floor patient Right of bed              | Undetermined | Undetermined |
| ENV 115 | Floor Neighboring COVID+ | Floor patient Left of bed               | Undetermined | Undetermined |
| ENV 116 | Floor Neighboring COVID+ | Floor patient Bathroom door             | Undetermined | Undetermined |
| ENV 117 | Floor Neighboring COVID+ Floor patient | Floor- Main room door | Undetermined | Undetermined |
|---------|---------------------------------------|-----------------------|--------------|--------------|
| ENV 118 | Floor Neighboring COVID+ Floor patient | Handle- Bathroom door | Undetermined | Undetermined |
| ENV 119 | Floor Neighboring COVID+ Floor patient | Floor- Bathroom floor | Undetermined | Undetermined |
| ENV 120 | Floor Neighboring COVID+ Floor patient | Sink- Bathroom | Undetermined | Undetermined |
| ENV 121 | Floor Neighboring COVID+ Floor patient | Toilet | Undetermined | Undetermined |
| ENV 122 | Floor Neighboring COVID+ Floor patient | Workstation Keyboard (in room) | Undetermined | Undetermined |
| ENV 123 | Floor Neighboring COVID+ Floor patient | Computer monitor | Undetermined | Undetermined |
| ENV 124 | Floor Neighboring COVID+ Floor patient | Linen cart | Undetermined | Undetermined |
| ENV 125 | Floor Neighboring COVID+ Floor patient | Trash bin | Undetermined | Undetermined |
| ENV 126 | Floor Neighboring COVID+ Floor patient | Handle- Main room door | Undetermined | Undetermined |
| ENV 127 | Floor Neighboring COVID+ Floor patient | Hand sanitizer dispenser- near room sink | Undetermined | Undetermined |
| ENV 128 | Floor Neighboring COVID+ Floor patient | Sink- in room sink pedals | Undetermined | Undetermined |
| ENV 129 | Floor Neighboring COVID+ Floor patient | Whiteboard markers | Undetermined | Undetermined |
| ENV 130 | Floor Neighboring COVID+ Floor patient | Sharps lid and container | Undetermined | Undetermined |
| ENV 131 | Floor Neighboring COVID+ Floor patient | Bedrail- left side | Undetermined | Undetermined |
| ENV 132 | Floor Neighboring COVID+ Floor patient | Bedside Buttons- Left | Undetermined | Undetermined |
| ENV 133 | Floor Neighboring COVID+ Floor patient | Bedside Table | Undetermined | Undetermined |
| ENV 134 | Floor Neighboring COVID+ Floor patient | O2 Flow Regulator knob | Undetermined | Undetermined |
| ENV 135 | Floor Neighboring COVID+ Floor patient | Device Plug | Undetermined | Undetermined |
| ENV 136 | Floor Neighboring COVID+ Floor patient | Stethoscope (in room) | Undetermined | Undetermined |
| ENV 137 | Floor Nursing Workspace | Floor- Nursing Workspace | 37.97 | Undetermined |
| ENV 138 | Floor Nursing Workspace | Nurse Desk | Undetermined | Undetermined |
| ENV 139 | Floor Nursing Workspace | Nurse Sink | Undetermined | Undetermined |
| ENV 140 | Floor Nursing Workspace | Nurse Phone | Undetermined | Undetermined |
| ENV-141 | Floor Nursing Workspace | Rover in Nurse Space | Undetermined | Undetermined |
| --- | --- | --- | --- | --- |
| ENV-142 | Floor Nursing Workspace | Nurse's Vocera (who has been in COVID+ patient room) | Undetermined | Undetermined |
| ENV-143 | Floor Nursing Workspace | Nurse's Badge (who has been in COVID+ patient room) | Undetermined | Undetermined |
| ENV-144 | Floor Patient- COVID+ | Floor- Left of bed | 43.73 | Undetermined |
| ENV-145 | Floor Patient- COVID+ | Floor- Foot of bed | Undetermined | Undetermined |
| ENV-146 | Floor Patient- COVID+ | Floor- Right of bed | Undetermined | Undetermined |
| ENV-147 | Floor Patient- COVID+ | Workstation Keyboard (in room) | Undetermined | Undetermined |
| ENV-148 | Floor Patient- COVID+ | Workstation Desk Surface | Undetermined | Undetermined |
| ENV-149 | Floor Patient- COVID+ | Floor- Bathroom | Undetermined | Undetermined |
| ENV-150 | Floor Patient- COVID+ | Handle- Bathroom door | Undetermined | Undetermined |
| ENV-151 | Floor Patient- COVID+ | Sink- Bathroom | Undetermined | Undetermined |
| ENV-152 | Floor Patient- COVID+ | Toilet | Undetermined | Undetermined |
| ENV-153 | Floor Patient- COVID+ | Bedrail- Left | Undetermined | Undetermined |
| ENV-154 | Floor Patient- COVID+ | Bedrail- Right | Undetermined | Undetermined |
| ENV-155 | Floor Patient- COVID+ | Infusion Pump | Undetermined | Undetermined |
| ENV-156 | Floor Patient- COVID+ | Bedside Table | Undetermined | Undetermined |
| ENV-157 | Floor Patient- COVID+ | O2 Flow Regulator knob | Undetermined | Undetermined |
| ENV-158 | Floor Patient- COVID+ | Trash bin | Undetermined | Undetermined |
| ENV-159 | Floor Patient- COVID+ | Handle- Main room door | Undetermined | Undetermined |
| ENV-160 | Floor Patient- COVID+ | Bedside Buttons- Left | Undetermined | Undetermined |
| ENV-161 | Floor Patient- COVID+ | Hand sanitizer dispenser | Undetermined | Undetermined |
| ENV-162 | Floor Patient- COVID+ | Stethoscope (in room) | Undetermined | Undetermined |
| ENV-163 | Floor Patient- COVID+ | Linen Cart | Undetermined | Undetermined |
| ENV-164 | Floor Patient- COVID+ | Sharps lid and container | Undetermined | Undetermined |
| ENV-165 | Floor Patient- COVID+ | Patient Call Light | Undetermined | Undetermined |
| ENV-166 | Floor Patient- COVID+ | Device Plug | Undetermined | Undetermined |
| ENV-167 | Floor Patient- COVID+ | Thermometer | Undetermined | Undetermined |
| ENV-168 | Floor Patient- COVID+ | Sink- Antechamber Pedals | Undetermined | Undetermined |
Supplementary Table 3: Sequencing information for 5 MinION runs, detailing number of raw reads generated and the amount retained at each step of the bioinformatics pipeline.

| Run Number | Total Reads | Barcoded Reads | Unclassified Reads | Quality Controlled Reads | Percent Reads Passing QC |
|------------|-------------|----------------|--------------------|-------------------------|--------------------------|
| Run 1      | 405,968     | 142,204        | 263,765            | 63,048                  | 15.53                    |
| Run 2      | 14,804,576  | 8,694,698      | 6,109,879          | 6,404,977               | 43.26                    |
| Run 3      | 1,576,082   | 996,781        | 579,302            | 50,420                  | 3.2                      |
| Run 4      | 1,223       | 341            | 883                | 19                      | 1.55                     |
| Run 5      | 780,000     | 232,771        | 547,230            | 152,152                 | 19.51                    |