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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 I-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. 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).

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 COVD-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 O_2 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.

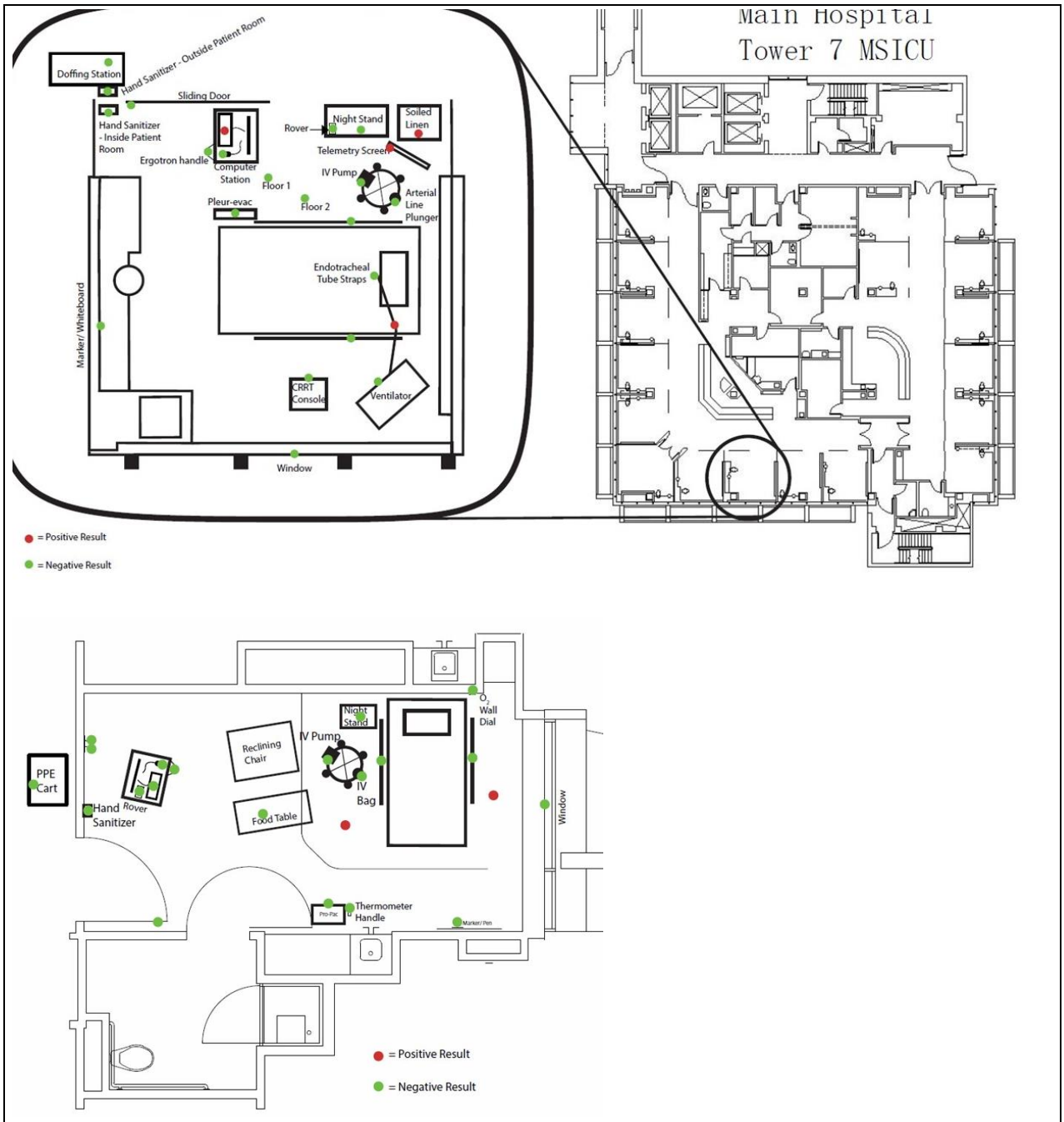

Figure 1: Representative areas sampled by swabs for SARS-CoV-2 at the UCDMC. Positive samples are shown in red, negative samples in green. The top panel represents an ICU room, and the bottom panel represents a ward room. Each dot represents a single swab.

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).

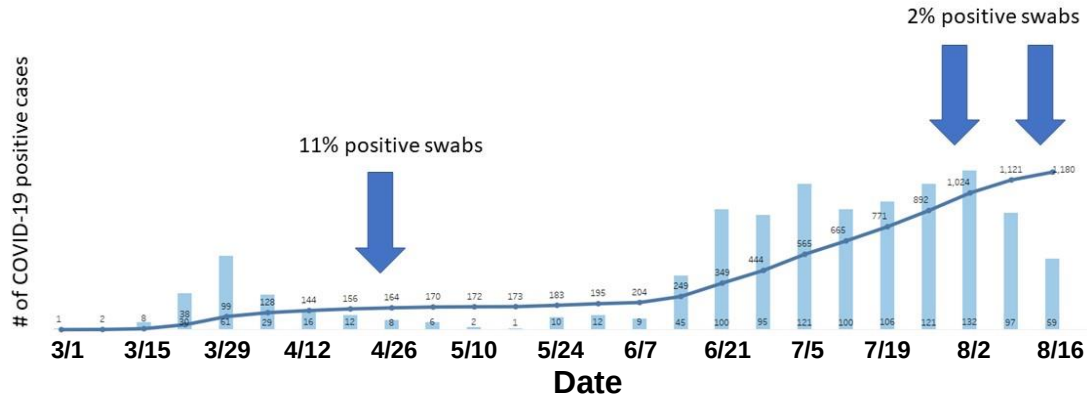

Figure 2: SARS-CoV-2 positive patients at UCDMC during the first and the second wave of COVID-19. Weekly totals of COVID-19 patients, and the cumulative total number from early March until mid-August, 2020. The blue arrows indicate the sampling dates.

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.

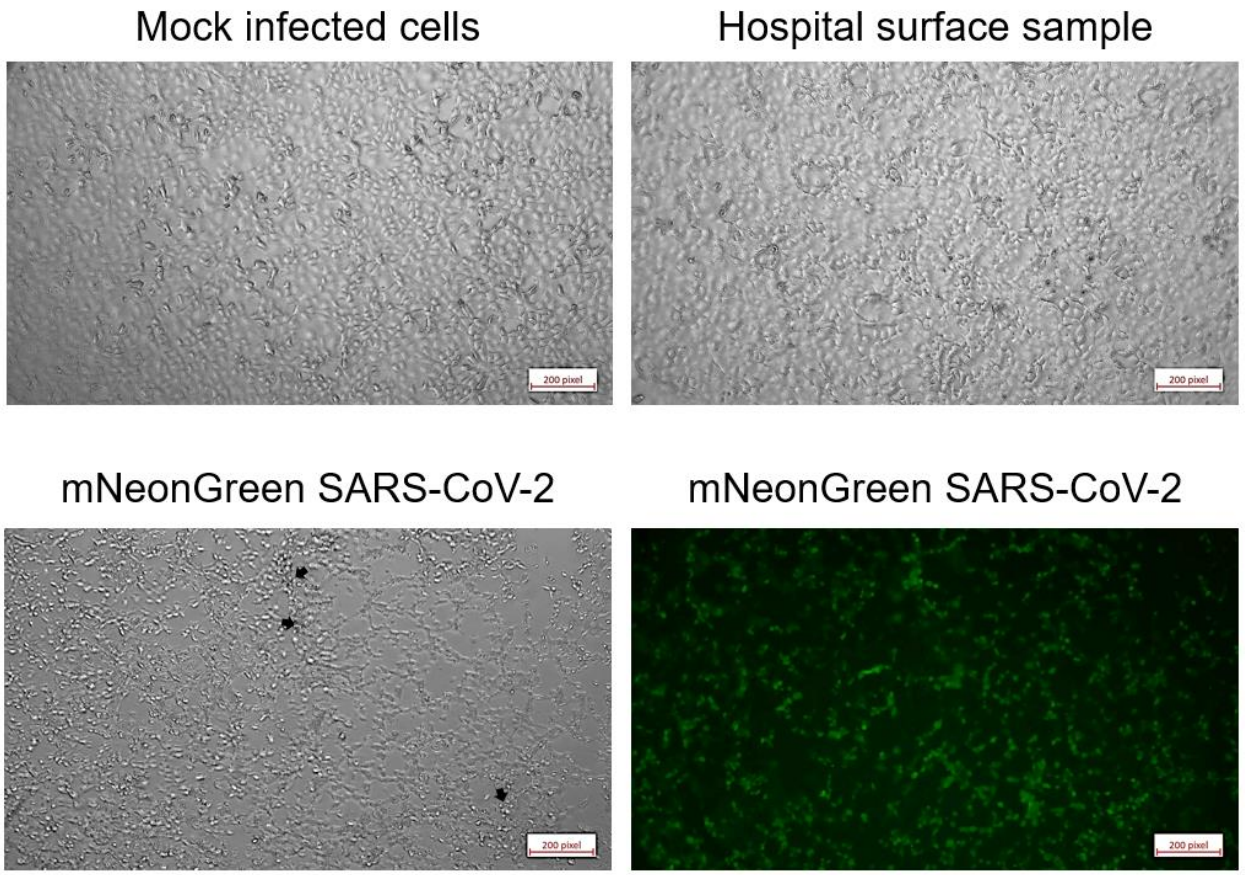

Figure 3. Micrographs of Vero E6 cells five days after inoculation. Cells were either mock-infected (upper left), inoculated with swab samples (representative of all five tested samples, upper right), or infected with one PFU of mNeonGreen SARS-CoV-2 (phase contrast, lower left; mNeonGreen lower right).

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

(details in GitHub repository https://github.com/sociovirology/sars_cov2_environmental_seq).

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.

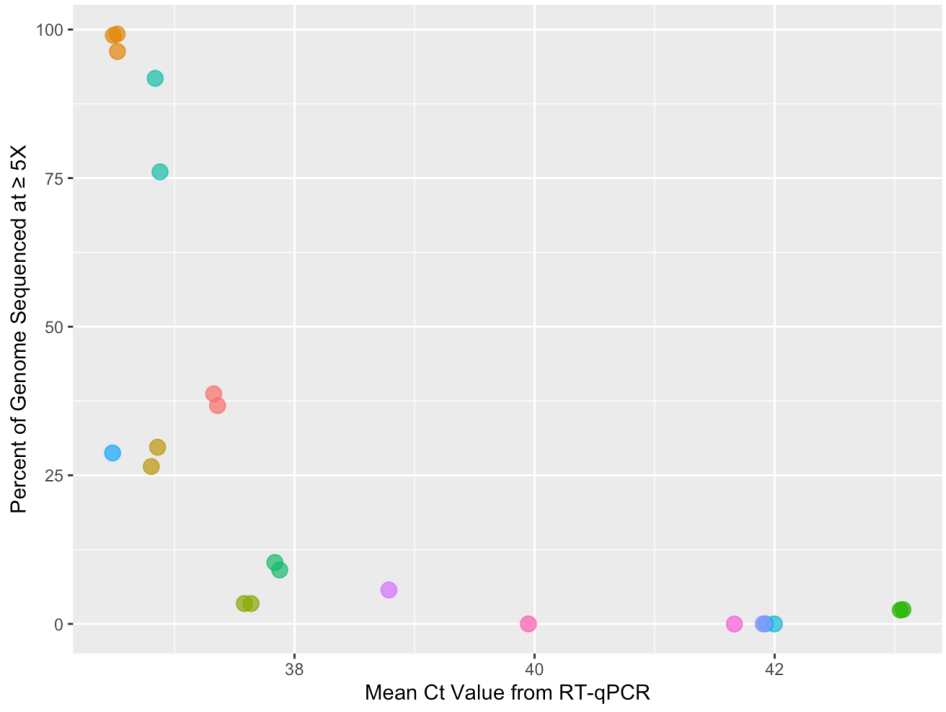

Figure 4. Environmental swabs with Ct values below 38 yielded enough sequence reads to cover a substantial portion of the SARS-CoV-2 genome. The percent of the SARS-CoV-2 reference genome (isolate Wuhan-Hu-1) covered at ≥ 5X decreased steeply as a function of the mean Ct value (using CDC N1, N2, and 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)). Furthemore, 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.

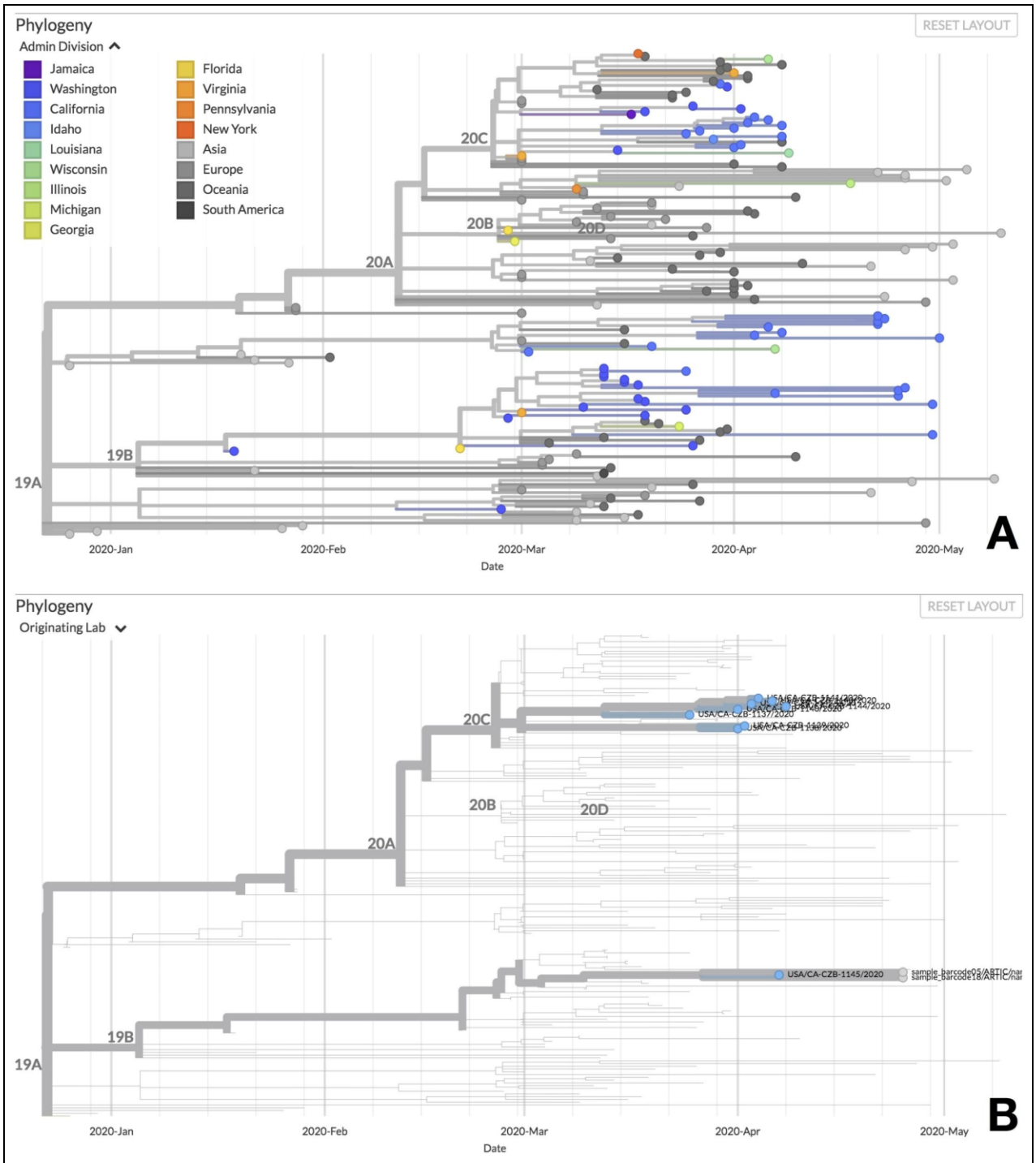

Figure 5. Phylogenetic comparison of the SARS-CoV-2 sequences obtained from environmental swabs at UCDMC. **A.** Near-complete genomes obtained from environmental samples clustered in clade 19B. The phylogenetic tree was generated using the NextStrain protocol, and compares sequences to others amplified 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.

Acknowledgements

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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	37	U	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) AND "desktop"	Undetermined	Undetermined
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	Ergotron	Undetermined	Undetermined

27	Patient room D14	Floor	**32.09**	**33.72**
28	Patient room D14	IV bag	Undetermined	Undetermined
29	Patient room D14	Nightstand	Undetermined	Undetermined
30	Patient room D14	hand sanitizer (in room)	Undetermined	Undetermined
31	Patient room D14	Window	Undetermined	Undetermined
32	Patient room D14	light switch	ND	ND
33	Patient room D14	light switch	Undetermined	Undetermined
34	Patient room D14	Door knob/keypad, interior	Undetermined	Undetermined
35	Patient room D14	food table	Undetermined	Undetermined
36	Patient room D14	isolation/PPE cart (room exterior)	Undetermined	Undetermined
37	Patient room T7 Blue	Window	Undetermined	Undetermined
38	Patient room T7 Blue	vent tubing arm	**36.83**	Undetermined
39	Patient room T7 Blue	Vent screen and knobs	Undetermined	Undetermined
40	Patient room T7 Blue	Keyboard	Undetermined	**37.62**
41	Patient room T7 Blue	Ergotron	Undetermined	Undetermined
42	Patient room T7 Blue	Floor	Undetermined	43.06
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	Door knob/keypad, interior	Undetermined	Undetermined
47	Patient room T7 Blue	Nightstand	Undetermined	Undetermined
48	Patient room T7 Blue	telemetry screen alarm button	**37.07**	**38.6**
49	Patient room T7 Blue	Arterial line plunger	Undetermined	Undetermined
50	Patient room T7 Blue	pluerevac box handle	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 PAVLNIO60	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-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-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	Floor- Foot of bed	Undetermined	Undetermined
ENV-114	Floor Neighboring COVID+ Floor patient	Floor- Right of bed	Undetermined	Undetermined
ENV-115	Floor Neighboring COVID+ Floor patient	Floor- Left of bed	Undetermined	Undetermined
ENV-116	Floor Neighboring COVID+ Floor patient	Floor- 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