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Determination of the Presence of SARS-CoV-2 in Environmental Surface and Air Samples from Public Areas in Las Vegas

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DETERMINATION OF THE PRESENCE OF SARS-COV-2 IN ENVIRONMENTAL SURFACE AND AIR SAMPLES FROM PUBLIC AREAS IN LAS VEGAS

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ABSTRACT

DETERMINATION OF THE PRESENCE OF SARS-COV-2 IN ENVIRONMENTAL SURFACE AND AIR SAMPLES FROM PUBLIC AREAS IN LAS VEGAS

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Contaminated surfaces and airborne spread are found to be among the main ways of coronavirus disease 2019 (COVID-19) transmission. Studies conducted in the hospital setting have shown that SARS-CoV-2 is found on frequently touched surfaces, personal protective equipment, and in air samples of patient isolation rooms. However, few environmental sampling studies have been done in public areas. Taking in consideration that COVID-19 cases may be symptomatic, presymptomatic, and asymptomatic, environmental monitoring may be essential for prompt detection of the virus. The objective of this study was to determine whether SARS-CoV-2 can be detected on environmental surfaces and from air samples in public areas in Las Vegas. In total, 300 surface samples were collected from high-touch surfaces from public areas and a public health facility (PHF) in Las Vegas. In addition, 18 air samples were collected from public areas, a PHF, and COVID-19 testing and vaccination sites. Environmental samples were analyzed with reverse-transcriptase polymerase chain reaction (RT-PCR) using SARS-CoV-2 specific primers and probes. Results showed that 58 out of 300 (19.3%) surface environmental

samples tested positive for SARS-CoV-2, 45 at the PHF and 13 in public areas. Concentrations ranged from 10^2 to 10^6 viral particles per sample. Materials that tested positive were plastic, stainless steel, rubber, metal, vinyl, ceramic, artificial leather, glass, wood, and paper. No air sample tested positive. Moreover, results showed that the N gene assay had greater sensitivity to detect SARS-CoV-2 compared to the S and ORF gene assays. Besides frequently touched surfaces, SARS-CoV-2 was detected from floors, shoes, mop water, surfaces in contact with the floor, and floor areas around toilets. Restroom surfaces were frequent SARS-CoV-2 contamination locations. These results indicate surfaces and areas where SARS-CoV-2 may be detected, and the extent and distribution of environmental contamination. Future research should focus on determining the infectivity of the virus in the environment and its potential to cause infection.

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CHAPTER 1

BACKGROUND

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped single-stranded ribonucleic acid (RNA) virus that emerged in 2019. Coronaviruses are the largest RNA viruses, and belong to the realm Riboviria, order Nidovirales, suborder Cornidovirineae, family Coronaviridae, and subfamily Orthocoronavirinae (Cavanagh, 1997). Moreover, coronaviruses are divided into Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus genera, and infect mammals and birds (Kirtipal et al., 2020). SARS-CoV-2 is a human coronavirus (HCoV) that belongs to the Betacoronavirus genera (Pal et al., 2020). Coronaviruses predominantly cause respiratory infections, as well as gastrointestinal and neurological diseases (Arbour et al., 2000).

Coronaviruses are named after the Latin word “corona” which means “crown”, due to a crown-like appearance of viral spike proteins observed under an electronic microscope. The first coronavirus was discovered in 1937, when it was isolated from chicken embryos (Ludwig & Zarbog, 2020). The first HCoVs were detected in the 1960s, and were named HCoV-229E and HCoV-OC43 (Hamre & Procknow, 1966). In 2002, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) emerged, and in 2004 and 2005 HCoV-NL63 and HCoV-HKU1 were discovered, respectively. HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1 are called endemic HCoVs, and are found to cause one third of all common cold infections in humans (Ludwig & Zarbog, 2020; Masters, 2006). In 2012, the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) was discovered. SARS-CoV-2, the most recent HCoV that emerged in 2019, has led to the COVID-19 pandemic (Kirtipal et al., 2020).

SARS-CoV-2 shares 96% of its genome with bat coronaviruses, and 75% with the SARS-CoV genome (Perlman, 2020). Moreover, coronaviruses are characterized by a high genomic recombination rate, due to errors in their genetic material duplication system. This characteristic contributes to the virus spillover from one species to another, resulting in transmission from animals to humans (Duffy et al., 2008).

According to Li et al. (2005), bats are found to be the primary hosts for SARS-like coronaviruses. In addition, the infection transmission to humans potentially happened via intermediate hosts. Based on the study of Malaiyan et al. (2021), potential hosts of SARS-CoV-2 may be *Rhinolophus affinis* (bat) and *Manis javanica* (Malayan pangolin).

Lauring & Malani (2021) report that, as SARS-CoV-2 spreads, its genetic material undergoes high rates of mutations, which contributes to the development of new variants of the virus. The Centers for Disease Control and Prevention (CDC) classifies SARS-CoV-2 variants as Variants Being Monitored, Variants of Concern, Variants of Interest or Variants of High Consequence, based on how easily the virus can spread from person to person, severity of symptoms, and treatment options (CDC, 2021a).

SARS-CoV-2 has four structural proteins: the spike (S) protein, membrane (M) protein, envelope (E), and nucleocapsid (N) protein (Wu et al., 2020a). The S, M, and E proteins form the viral coat, whereas the N protein helps packaging of RNA and genome protection. The S protein is a glycoprotein of the viral envelope that helps viral attachment and entry into host cells (Malaiyan et al., 2021). In addition, the N gene is highly conserved (Naqvi et al., 2020). Moreover, the SARS-CoV-2 genome contains ten open reading frames (ORF) genes which code for non-structural proteins that are not essential for RNA replication. All these gene targets are currently used to detect SARS-CoV-2 in clinical and environmental samples. As new SARS-

CoV-2 variants continue to emerge, it is important to determine which gene targets are the most sensitive in prompt detection of the virus.

A study conducted by Peeri et al. (2020), showed that COVID-19 has lower morbidity and mortality, but higher infectivity rates compared to SARS and MERS. However, per CDC data, after heart disease and cancer, COVID-19 was the third leading cause of death in the U.S. in 2020 (CDC, 2021b). Therefore, it is essential to understand all the routes of COVID-19 transmission and the significance of SARS-CoV-2 environmental contamination in public areas.

CHAPTER 2

INTRODUCTION

In December 2019, the world faced an outbreak of a new emerging pathogen called SARS-CoV-2. This new virus was detected in the city of Wuhan, China, and was found to cause coronavirus disease 2019 (COVID-19) (CDC, 2020a). In January 2020, the World Health Organization (WHO) characterized this outbreak as a public health emergency of international concern, resulting in the WHO's highest level of alarm (WHO, 2021). On March 11th 2020, the WHO declared COVID-19 as a pandemic (WHO, 2020).

The most common symptoms of COVID-19 are fever, cough, shortness of breath, muscle aches, fatigue, sore throat, headache, new loss of smell and taste, congestion, nausea, diarrhea, and vomiting (CDC, 2021c). COVID-19 symptoms may progress to severe pneumonia, respiratory failure, and death (Wu et al., 2020b). However, COVID-19 may also result in an asymptomatic and presymptomatic infection (CDC, 2021d)**.** Therefore, people with COVID-19 may be infectious and spread the virus without having any symptoms. This fact is potentially contributing to the increased spread of COVID-19, as asymptomatic and presymptomatic carriers may not be detected.

Since SARS-CoV-2 emerged, scientists have been trying to understand the method of transmission. Based on the CDC data, COVID-19 is transmitted from person to person via respiratory droplets, through airborne spread, from contact with animals, and contact with contaminated surfaces (CDC, 2021e). Furthermore, if a person touches their mouth, nose or eyes after touching a surface contaminated with SARS-CoV-2 respiratory droplets, they may become infected (CDC, 2021e).

The study of van Doremalen et al. (2020) found that SARS-CoV-2 can potentially be transmitted via air and fomites. In their experiment, this virus was viable in the air for three hours, and on fomites for days. In this study, scientists found that SARS-CoV-2 was viable for 72 hours on plastic and on stainless steel. Moreover, SARS-CoV-2 remained infective on cardboard for 24 hours, and on copper for four hours.

Chia et al. (2020) conducted research in a hospital setting, and detected SARS-CoV-2 on high-touch surfaces in patient isolation rooms during and after their first week of disease. In addition, their study showed that SARS-CoV-2 was detected in air samples of patient isolation rooms, although there were 12 air exchanges per hour. They discovered that viable SARS-CoV-2 can be detected on the outer layer of surgical masks up to seven days after viral application in the laboratory. There are data indicating the presence of SARS-CoV-2 in the hospital setting. Among the most contaminated surfaces are personal protective equipment (PPE), medical equipment, and sanitizer dispensers (Ye et al., 2020). However, few SARS-CoV-2 environmental sampling studies have been conducted in public areas.

An experimental study by Harbourt et al. (2020), revealed that SARS-CoV-2 can be found on skin for 96 hours and on bank notes for at least 4 hours. A study by Marshall et al. (2020) found that places where SARS-CoV-2 was detected with a significant prevalence had 10 times greater chances of having COVID-19 positive employees, compared to the places where there were no positive environmental samples. Moreover, this study indicated that the presence of asymptomatic COVID-19 cases may be detected by environmental monitoring of workplaces. Break room door handles, faucets, workbenches, and break room chairs were the surfaces where SARS-CoV-2 was mostly found (Marshall et al., 2020). This finding supports the observation that the virus may persist on certain surfaces for days.

Harvey et al. (2020) collected environmental surface samples from 33 different locations in Massachusetts. Researchers analyzed samples from a gas station, laundromat, grocery store, trash can, crosswalks, bank, liquor store, metro entrance, restaurant, post office box, and convenience store. In their study, 29 of 348 (8.3%) environmental surface samples were SARS-CoV-2 positive, indicating that multiple locations and more frequent environmental sampling should be conducted in order to obtain an indication of SARS-CoV-2 presence in environmental samples. Researchers in this study detected low level SARS-CoV-2 contamination on public surfaces, and estimated that COVID-19 infection from these surfaces is possible, but with low risk.

Riddell et al. (2020) found that SARS-CoV-2 stability on different surfaces depends of temperature and humidity. They found that this virus can remain viable on stainless steel at room temperature (20°C, 50% relative humidity) for more than 28 days. However, elevated temperatures decreased the stability of SARS-CoV-2 on stainless steel. The virus was detected on this surface after 7 days at 30°C, and less than 48h at 40°C. In addition, they found that SARS-CoV-2 can be detected on paper and plastic banknotes for 28 days at room temperature. Multiple studies have shown that other coronaviruses, such as the ones that cause Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS), and endemic HCoV can remain on glass, metal, and plastic for nine days (Kampf et al., 2020). Furthermore, surface transmission played a crucial role in the spread of other coronaviruses that cause MERS, porcine epidemic diarrhea virus, HCoV-229E, and HCoV-OC43 (Riddell et al., 2020).

Bin et al. (2016) conducted a MERS environmental sampling study at a hospital, and found that surfaces frequently touched by patients infected with MERS and medical staff were contaminated by MERS-CoV RNA. Virus was detected on surfaces up to five days since the last

positive patient's polymerase chain reaction (PCR) test. Moreover, they conducted cell culture and virus isolation, and discovered that the virus was viable, and that it could be spread from fully recovered patients. In addition, they concluded that stricter environmental surface disinfection should be conducted, and that the isolation period should be based on laboratory results, and not only clinical presentation. In their research, Wei et al. (2020a) found significant environmental SARS-CoV-2 contamination in COVID-19 asymptomatic patients' surroundings.

Cai et al. (2020) discovered that there was a correlation between COVID-19 spread in a shopping mall in China and contaminated surfaces. Cases reported no close contact to other COVID-19 positive cases from the mall; however, they all used the same areas at the shopping mall (i.e., restrooms, elevators). There was a correlation found between a cluster of female COVID-19 cases and a restroom custodian who tested positive, indicating that the frequently touched surfaces or the air may be a source of infection.

During a COVID-19 outbreak on a cruise ship, Yamagishi et al. (2020) conducted environmental sampling in the cabins of COVID-19 positive passengers, and detected SARS-CoV-2 RNA in 58 of 601 (close to 10%) environmental samples. Samples were collected between Day 1 and Day 17 after COVID-19 positive passengers left the cruise ship. Surfaces that tested positive 14 days after being contaminated were the floor area around the toilets, and pillows. They collected air samples from COVID-19 positive bathrooms and bedrooms, and did not detect SARS-CoV-2 RNA; however, one positive surface sample was obtained from a ceiling vent. An important finding of this study is that SARS-CoV-2 RNA was detected both in symptomatic and asymptomatic case cabins.

A SARS-CoV-2 air sampling study conducted in a hospital revealed that most positive samples were detected in patients' restrooms (Ding et al., 2021). A hospital environment study

done by Cheng et al. (2020), however, did not detect any positive air samples in the ICU. In their study, gelatin membranes were to sample one cubic meter of air.

Taking into consideration that different SARS-CoV-2 environmental sampling studies had varied results, there is a need for further research on the presence of this virus on surfaces and in the air. Surface and air environmental sampling for SARS-CoV-2 is important as it may indicate if the public health COVID-19 mitigation measures need to be modified. In addition, conducting environmental surveillance of work places may enable businesses to detect asymptomatic carriers early, conduct employee testing, and perform additional workplace sanitation. Furthermore, environmental surveillance of public areas is of benefit to the general population, as locations testing positive can be properly cleaned and disinfected. Through environmental surveillance, new surfaces that potentially present a great risk for SARS-CoV-2 contamination may be detected, and more attention can be focused on their proper disinfection.

This study addressed the presence of SARS-CoV-2 in environmental samples of public areas in Las Vegas. SARS-CoV-2 is a new pathogen that is currently causing a devastating pandemic. COVID-19 treatment, vaccination and mitigation measures are still being researched and evaluated. In addition, new COVID-19 variants are emerging. Therefore, it is essential to understand if SARS-CoV-2 can be detected on different surfaces and in air samples.

This study may help reveal the gaps in knowledge in terms of the COVID-19 cases that did not have close contact with other COVID-19 positive cases. This will help us understand potential COVID-19 modes of transmission via contaminated surfaces. In addition, environmental sampling can be conducted after COVID-19 positive cases report being at certain locations to detect whether surfaces that the case touched are SARS-CoV-2 positive.

Currently, in the U.S. there are more than 47 million confirmed COVID-19 cases, and more than 770,000 people have died due to COVID-19 (CDC, 2021f). Nevada has more than 452,000 COVID- 19 cases, whereas Clark County counts more than 348,000 cumulative cases (NV Health Response, 2021a). To our knowledge, no studies of SARS-CoV-2 surface contamination in Las Vegas have been conducted. SARS-CoV-2 environmental contamination in Las Vegas may potentially lead to increased COVID-19 spread both nationally and internationally. As Las Vegas is one of the most visited cities in the world, COVID-19 mitigation measures are essential for maintaining public health.

Objective

The objective of this study was to determine whether SARS-CoV-2 RNA could be detected in environmental surface and air samples in selected public areas in Las Vegas. Based on previous studies that showed approximately a 10% positivity rate, we sampled 300 surface samples from selected locations (e.g., doorknobs, light switches, gas station buttons, faucets, toilet flush buttons). A total of 150 environmental surface samples were collected from public areas, and 150 environmental surface samples were collected from a public health facility (PHF). In addition, 18 air samples were collected from public areas, PHF, and COVID-19 testing and vaccination sites. Environmental samples were analyzed with reverse transcription polymerase chain reaction (RT-PCR), using SARS-CoV-2 specific primers and probes.

Research Questions

1) Can SARS-CoV-2 RNA be detected in environmental surface samples in public areas in Las Vegas?

2) Can SARS-CoV-2 RNA be detected in air samples in public areas in Las Vegas?

CHAPTER 3

MATERIALS AND METHODS

Study Design

This study was conducted in high traffic public areas and in a PHF in Las Vegas. A literature review was conducted to compile all the relevant research that has been done pertaining to this topic. An Institutional Biosafety Committee (IBC) application was submitted and received approval for sampling and analysis of SARS-CoV-2. No Institutional Review Board (IRB) approval was needed, as human subjects were not included in this study. Public areas in Las Vegas where the sampling was conducted were selected indoor and outdoor frequently visited places that were open and accessible to people. The identity of areas sampled in this study is confidential. All samples were collected and analyzed with the proper use of PPE. Appropriate safety training courses were taken and approval was received before sampling at the PHF was conducted.

Environmental Surface Sampling

Environmental surface sample collection was conducted from December 2020 until April 2021 due to the increased number of COVID-19 cases and COVID-19 circulation in the community. Environmental surface sampling was conducted in high traffic public areas and in a PHF in Las Vegas. In total, 300 environmental surface samples were collected; 150 environmental surface samples were collected from public areas, and 150 environmental surface samples were collected from a PHF. Surfaces sampled were frequently touched surfaces in high traffic places, such as gas stations, traffic lights, post office, car washes, restrooms in grocery stores, and shopping malls (Appendix A). These locations were selected based on the high

number of people that visited them. Information about types of surface materials sampled was recorded.

Surface environmental sampling was conducted with sterile foam tipped applicators (Puritan, Guilford, ME) that were placed in 3 ml viral transport medium (VTM) (Hardy Diagnostics, Santa Maria, CA). Sample collection kits were stored at 4°C until ready to be used. Prelabeled kits were transported on ice in a designated, biohazard labeled cooler to the sampling location. The sampling was conducted with moistened swabs in an overlapping pattern, according to CDC sampling protocols (CDC, 2012). Exposed swabs were placed in VTM. All sampled surfaces were disinfected with an isopropyl alcohol wipe after sample collection. After environmental surface sampling, the samples were placed in the cooler, and were taken to the Emerging Diseases Laboratory (EDL) located at the University of Nevada Las Vegas (UNLV) within 24 h. The samples were stored at -20°C until extraction and PCR analysis.

Environmental Air Sampling

Environmental air sample collection was conducted from April 2021 until July 2021. Air sampling was conducted in high traffic public areas, at the COVID-19 testing and vaccination sites, and in a PHF in Las Vegas. Air samples were collected independently from environmental surface samples. A total of 18 air samples were collected with the MD8 Airport Portable air sampler with gelatin membranes (Sartorius, Goettingen, Germany). Of the 18 samples, 3 samples were collected at the PHF (Site 1), 7 samples were collected at high traffic public areas, and 8 samples were collected from COVID-19 testing and vaccination sites. The total air sample volume was 250 L, with an air flow rate of 50 L/min, for 5 minutes per sample. The gelatin membrane used for air sampling was stored at 4°C and transported on ice in a portable cooler to the sampling location. Upon sample collection, the membrane was placed in the 4°C cooler, and

taken to the EDL within 24 h. The samples were processed by dissolving the gelatin membranes in sterile water and processing samples according to the air sampling analysis protocol developed in our laboratory (Appendix B). After sample processing, samples were stored at -20°C until RNA extraction and PCR analysis.

SARS-CoV-2 RNA Extraction

To isolate SARS-CoV-2 RNA from both surface and air samples, RNA extraction was performed in the biosafety cabinet (BSC) at the EDL, using the QIAamp DSP Viral RNA extraction kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol (Appendix C). The volume of each sample that was extracted was 420 µL. The final extract volume of 60 µl was stored at -70°C until PCR analysis.

Reverse Transcription Polymerase Chain Reaction Analysis

Reverse Transcription Polymerase Chain Reaction (RT-PCR) is used to detect viral pathogens that contain RNA. This PCR employs a step to convert RNA to DNA, and the target DNA is amplified to detect the pathogen. This method was authorized for COVID-19 testing (FDA, 2021a).

PCR analysis of samples was conducted with the QuantStudio™ 6 Pro Real-Time PCR instrument (ThermoFisher Scientific, Waltham, MA), using a SARS-CoV-2 PCR assay kit (ThermoFisher). A one- step RT- PCR Master Mix (ThermoFisher) was used. Each PCR reaction had 25µl of total volume that consisted of 5 µl sample extract, 9.5 µl ultrapure water, 1.25 µl N gene assay (i.e., primers and probes), 6.25 µl 4X TaqMan® PCR Master Mix, 2.5 µl 10X IPC Mix, and 0.5 µl 50X IPC DNA. Amplification was conducted in standard mode, with the following conditions: 25°C for 2 min, 50°C for 15 min, 95°C for 2 min, and 40 cycles of

95°C for 3 sec followed by 60°C for 30 sec. All samples were amplified in duplicate. A non-template control (NTC) that contained nuclease free water, and a positive control (SARS-CoV-2 RNA, ThermoFisher) were included in each PCR analysis.

In this study, the SARS-CoV-2 N gene assay was used as some studies indicated that it is more conserved and stable, compared to the S and ORF genes (FDA, 2021b; CDC, 2020b; Naqvi et al, 2020). A TaqMan® internal positive control (IPC) (ThermoFisher), using VIC™ /TAMRA probe was used to determine if there was PCR inhibition in the environmental samples. The reaction mix threshold was set to 0.100, and the baseline was set between 3-15. Serial dilutions of standards with known RNA concentration were analyzed in duplicate, along with environmental samples. Positive samples, as determined by a fluorescent signal, confirmed the presence of SARS-CoV-2 RNA in the environmental samples. Upon amplification, the instrument software created a standard curve of cycle threshold (Ct) values, which was used to confirm the presence and concentration of SARS-CoV-2 RNA in the environmental samples. Cycle threshold represents the cycle at which fluorescence (i.e., amplification) is first detected. Ct values are inversely proportionate to the number of target copies present in the sample. Standard serial dilutions used to conduct quantitative analysis contained 50 SARS-CoV-2 RNA genome copies, 500 RNA copies, 5,000 RNA copies, and 50,000 viral RNA copies. PCR Ct values of these standards were used to calculate the number of viral particles per PCR reaction, and the number of viral particles per 3 ml sample.

Mean Ct values of duplicate samples were used to analyze the amplification results. PCR data were entered into a spreadsheet for further analysis to determine the number and location of positive samples, as well as the number of SARS-CoV-2 RNA copies per sample. Positive samples were verified by additional PCR analyses using the S and ORF gene markers

(ThermoFisher). Negative samples were analyzed using an IPC PCR to rule out false negative results due to environmental inhibition.

CHAPTER 4

RESULTS

Environmental Surface Sample Analysis

A total of 300 surface environmental samples and 18 air samples were collected from public areas in Las Vegas. Out of 150 samples collected at the PHF, 45 tested positive (30%). In addition, out of 150 samples collected in public areas, 13 tested positive (8.7%). Overall, results showed that 58 out of 300 surface environmental samples (19.3%) tested positive for SARS-CoV-2 RNA.

Moreover, 31 samples tested positive for both PCR replicates (+/+) on the first PCR analysis. In addition, 27 environmental samples tested positive in one PCR replicate and negative in the other replicate (+/-). All samples that tested +/- were re-analyzed. After conducting additional N gene PCR analyses of all the +/- samples, results showed that 9 samples tested +/- again, 4 samples tested +/+, and 14 samples tested -/- (Table 1).

Inhibition was found in only one surface environmental sample collected from the PHF restroom light switch. After retesting that sample, no inhibition was found, and the sample tested negative.

Environmental Air Sample Analysis

A total of 18 air environmental samples were collected from public areas, PHF (Site 1), and two COVID-19 testing and vaccination sites in Las Vegas. No air sample tested positive. Of the 18 air samples collected, 7 samples were collected in outdoor areas, and 11 samples were collected indoors. No inhibition was found in any of the air samples. It is possible that SARS-CoV-2 may have been present at concentrations below the limit of detection of the assay.

Surfaces and Materials where SARS-CoV-2 RNA was found

Surfaces where SARS-CoV-2 RNA was detected were public restroom door locks, escalator rubber handrails, a bus pass machine, elevator buttons, gas station pumps, credit card pin pads and gas selection buttons, a condominium pin pad, traffic light buttons, a floor area around the toilet at a retail store, a trash can in a grocery store restroom, university library toilet seats and sinks, university facility restroom doorknobs, and a university library door handle (Table 2).

Surfaces that tested positive at the PHF were the entrance door, the decontamination area desk, handrails, a clothes locker, restroom faucets and a flush button, the trash can next to the toilet, sinks, door handles, a desk shelf, the front desk, coffee tables, a keyboard and mouse, a couch, dining tables, a toilet seat, the area around toilet seats, a surface inside a toilet seat and a staff restroom urinal, a shower head, a pen, a copy machine, chairs, the security team radio, staff's shoes, books, doorknobs, a phone, a linen cart, staff chairs and oxygen tank wheels, and cleaning stations wheels.

Materials on which SARS-CoV-2 was found were plastic, stainless steel, rubber, glass, metal, vinyl, ceramic, artificial leather, wood, and paper. SARS-CoV-2 was detected most often on plastic and stainless-steel surfaces (Figure 1); however, these surfaces were sampled the most frequently. A total of 18 positive surface samples were found on plastic (out of 122 samples), and 16 positive samples were from stainless steel (out of 123 samples).

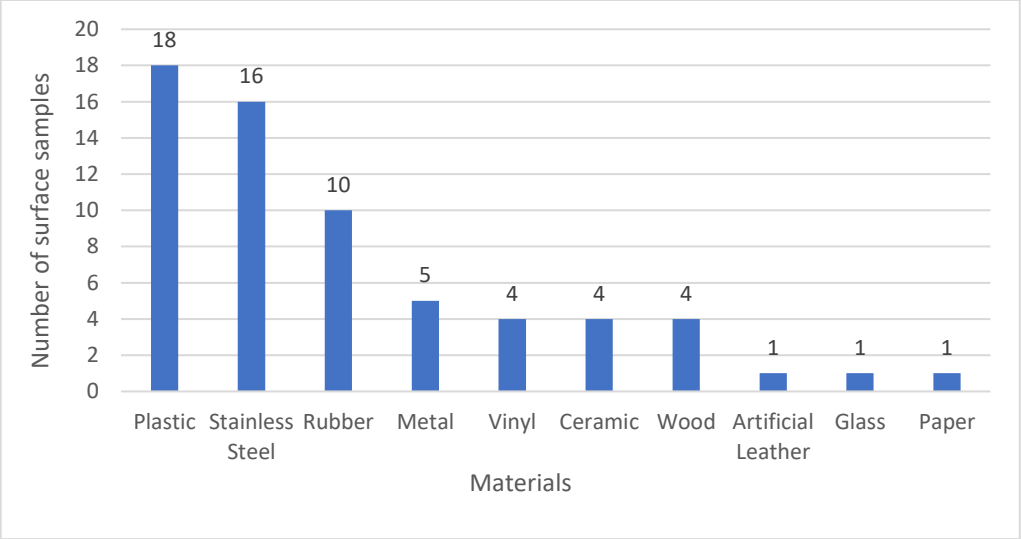

Figure 1. Materials that tested positive for SARS-CoV-2

An interesting finding was that all the floor samples, as well as samples of the objects that were in contact with the floor (e.g., chair wheels, cart wheels) from the PHF tested positive, including staff's shoes.

A positive mop water sample was collected from a mopping bucket that was used for floor cleaning. This finding indicated that the viral RNA can be found in the floor-cleaning water that was in contact with the SARS-CoV-2 contaminated floor. However, no PHF tap water control sample was collected to exclude tap water as a source of SARS-CoV-2 RNA in the mop water sample.

Locations

Out of 58 positive samples, 45 tested positive at the PHF, whereas 13 tested positive in other public areas in Las Vegas. Samples from public areas that tested positive were the university library door handle, the door locks in a male restroom, a Las Vegas Boulevard

escalator rubber handrail, a Las Vegas Boulevard bus pass machine, Las Vegas elevator buttons, gas station pump buttons, traffic light buttons, a keypad of the condominium, the floor around a toilet at a retail store on Las Vegas Boulevard, a grocery store metal trash can in a female restroom, university library female restroom toilet seats, university library female restroom sinks, and university facility male and female restrooms door knobs (Table 1).

ZIP Code Sample Distribution

ZIP codes of the sampling locations were recorded (Table 3). ZIP codes where positive samples were collected were 89107, 89109, 89113, 89117, 89119, and 89145. ZIP codes were not pre-selected; sampling locations were selected based on convenience, due to changes in COVID-19 mitigation measures implemented during the sampling timeframe. The largest number of positive samples was detected at the 89107 ZIP code, as most of the samples tested positive were from the PHF, which is located in that ZIP code. Based on the Southern Nevada Health District's data that are available on their website, the range and number of cases on the collection date for that ZIP code were recorded to provide information on the COVID-19 circulation in that area. In addition, the highest number of cases was noted at the 89119 ZIP code. Samples collected from this ZIP code were samples from the University and may be related to the high circulation of people in this part of the city.

Comparing Positive Samples based on N, S, and ORF gene

After testing samples with the N gene PCR assay, we selected 10 samples that tested +/- on both PCRs and 10 +/+ samples that had the strongest signal (lowest mean Ct values), to test with the S and ORF gene assays for comparison. Results showed that all 10 +/+ samples tested positive for the S gene. However, only three out of ten +/- samples tested +/- again, whereas seven samples tested -/-. No +/- sample tested +/+ for the S gene.

For the ORF gene assay, the results showed that all +/+ samples tested positive, and that two +/- samples tested +/- again. The remaining eight +/- samples all tested negative.

In summary, both S and ORF gene assays showed all double positive (+/+) results for those that were double positive with the N gene assay. The lowest mean Ct value for detecting +/+ samples was observed with the N gene assay, followed by the S gene assay, whereas the highest Ct value (the weakest signal) was observed with the ORF gene assay. Moreover, the S and ORF gene assays were less sensitive for obtaining positive results for samples that tested +/- for the N gene assay. Results showed that the N gene assay had greater sensitivity compared to the S and ORF gene assays.

Quantification

Upon amplification, the instrument software created a standard curve of Ct values, which were used to confirm the presence and concentration of SARS-CoV-2 RNA in the environmental samples. Ct values of positive samples ranged from 25.8 to 38.4. A Ct of 40 is considered negative. Quantification was conducted based on the N gene target only, as this target showed the highest detection rate. Based on the Ct values of SARS-CoV-2 standard dilutions, the PCR software calculated the standard curve plot ($r^2 = 0.984$) with the Y-intercept and slope values (Figure 2). Based on the standard curve equation and mean Ct values of environmental samples, the concentrations of SARS-CoV-2 RNA copies per PCR reaction and per 3 ml sample were calculated (Table 4).

The lowest number of SARS-CoV-2 RNA copies detected was 697 per sample, and was obtained from the PHF Clothes Locker sample. The highest number of SARS-CoV-2 RNA copies was 7,783,891 per sample, and was found in the PHF Restroom Sink sample. This sample had the lowest mean Ct value of 25.8. A sample that also had a high number of RNA

copies was PHF Site 2 Restroom 2- Floor Area Around Toilet with 3,931,680 RNA copies, and a Ct value of 26.7.

Sensitivity of the Assays

The limits of SARS-CoV-2 detection in environmental surface and air samples were calculated. Assuming the detection of one to ten RNA copies in the PCR reaction as determined by the standard curve, sensitivities of the assays were calculated based on the sample volume, the fraction of the sample processed for RNA extraction, and the amount of RNA extract used in the PCR assay. For environmental surface samples, the limits of detection were between 86 and 860 RNA copies per sample. The limits of detection for the air samples were between 571 and 5710 viral particles per m^3.

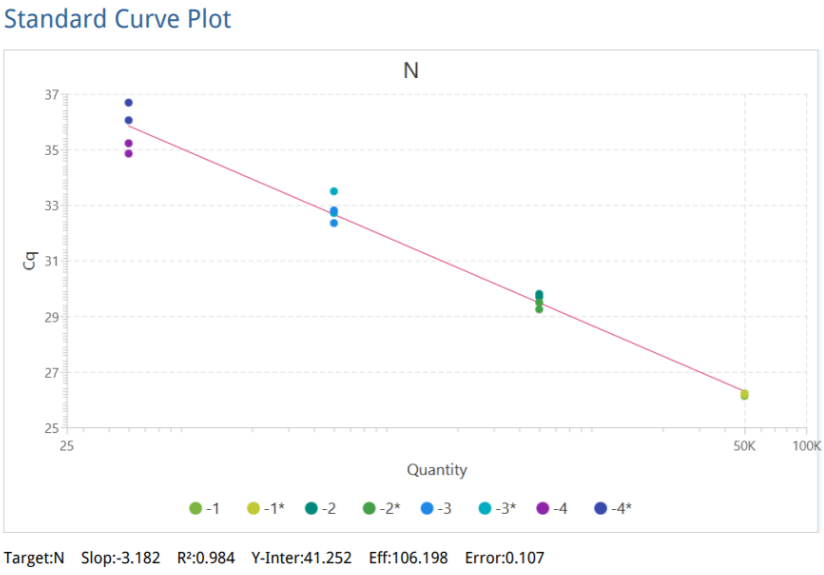

Figure 2. Standard curve plot with Y intercept, R^2, and slope values for serial dilutions of SARS-CoV-2 RNA with the N gene RT-PCR assay (Cq = Ct; Quantity = RNA copies per reaction).

CHAPTER 5
DISCUSSION

Materials

An interesting finding in this study was that SARS-CoV-2 was detected on numerous frequently touched environmental surfaces in public areas in Las Vegas. SARS-CoV-2 was detected on plastic, stainless steel, rubber, metal, glass, vinyl, ceramic, artificial leather, wood, and paper. In this study, plastic and stainless steel were two surfaces on which SARS-CoV-2 was detected most frequently. This finding is supported by van Doremalen et al. (2020) who detected SARS-CoV-2 on stainless steel and plastic, and found that this virus remains the longest on these two surfaces. Furthermore, Gidari et al. (2021) found that plastic and stainless steel needed higher doses of UV-C light to reach viral particle reduction. Liu et al. (2021) found that SARS-CoV-2 can be detected under experimental conditions on stainless steel, plastic, ceramic, glass, and wood, and remain viable for seven days. In this study, SARS-CoV-2 was found on all of these surfaces.

Abrahão et al. (2021) conducted a study in Brazil and found SARS-CoV-2 on metal, plastic, glass, wood, as well as on the floor of a bus station. In addition, most of their positive samples were collected at a health facility, which supports the results of our study. Furthermore, a study by Lui et al. (2020) revealed that SARS-CoV-2 can be found on wooden chopsticks used by symptomatic and postsymptomatic patients. Based on an experiment conducted by the Institute of Museum and Library Services, SARS-CoV can be detected on artificial leather eight days after viral application (IMLS, 2020). Another study found that under laboratory conditions, SARS-CoV-2 is detectable on glass, stainless steel, vinyl, and paper for 28 days after inoculation

(Ridel et al., 2020). However, to our knowledge, no environmental surface sampling study has been conducted that detected SARS-CoV-2 on rubber.

Surface Transmission

According to CDC data, the risk of SARS-CoV-2 transmission from surfaces is less than 1 in 10,000 (CDC, 2021g). This risk depends on prevalence rates in a community, number of viral particles that the infected person excretes, air flow and ventilation in indoor places, environmental factors such as heat and evaporation, time between surface contamination and human contact with that surface, transfer of viral particles from surface to mucosa, and minimum infective dose (MID) of the virus. MID is the minimal number of viral particles needed to cause an infection in humans (CDC, 2021g). However, the contribution that SARS-CoV-2 surface contamination has on COVID-19 transmission is still being researched.

SARS-CoV-2 on Floor Surfaces

Results showed that all the floor samples collected at the PHF, as well as samples of the objects that were in contact with the floor (i.e., chair wheels, cleaning stations wheels, linen cart wheels, oxygen tank wheels) tested positive for SARS-CoV-2, including staff's shoes. In addition, all the samples from staff shoes (3/3, 100%) tested positive for SARS-CoV-2. This finding is supported by the results of Guo et al. (2020) who detected SARS-CoV-2 on half of the samples from medical staff shoes. They found that 7 out of 10 intensive care unit (ICU) floor samples tested positive. Moreover, they concluded that gravity and air flow may contribute to the increased presence of SARS-CoV-2 viral particles on the floors.

Another interesting finding of this study was that SARS-CoV-2 was detected in the mop water used for cleaning the floor of the PHF. To our knowledge, no environmental sampling

study has shown a similar finding. However, studies have confirmed the presence of SARS-CoV-2 in wastewater (Ahmed et al., 2020; La Rosa et al., 2021).

SARS-CoV-2 in Restrooms

Restroom floors are important surfaces where SARS-CoV-2 was detected in this study. Another study conducted in a clinical setting showed that samples collected from patients' restrooms, such as samples from toilets and sinks, tested positive for SARS-CoV-2 (Ong et al., 2020). Similarly, the results of this study showed that samples taken from restroom surfaces such as toilet seats, flush buttons, sinks, faucets, trash cans, door locks, door knobs, door handles, and areas around toilets tested positive. A total of 4 out of 9 samples (44.4%) from areas around toilets tested positive. This finding is in agreement with the study of Yamagishi et al. (2020) who discovered that SARS-CoV-2 was mostly detected on the floor area around toilets.

The detection of SARS-CoV-2 RNA on toilet seats supports the study of Peng et al. (2020) who detected viral RNA in human urine. Liu et al. (2021) found that SARS-CoV-2 can remain viable for a few hours in human feces, and for three to four days in urine samples. They concluded that urine and feces are a significant source of SARS-CoV-2 transmission, as these excreta can be aerosolized. A study by Dancer et al. (2021) found that public restrooms present important locations where SARS-CoV-2 can be transmitted. Moreover, they found that SARS-CoV-2 was detected on numerous surfaces, including sinks. In this study, the highest number of SARS-CoV-2 RNA copies was detected in PHF patient restroom sinks. This may indicate that respiratory and/or oral excreta from COVID-19 patients contain a high number of viral particles that may remain on sink surfaces. This finding is important, as SARS-CoV-2 was detected in both PHF and public restrooms surfaces in this study (Table 1).

Frequently touched surfaces

The PHF in which the samples were collected was a facility for COVID-19 patients. Results indicate that surfaces frequently touched by patients tested positive for SARS-CoV-2. This finding is supported by the study of Ben-Shmuel et al. (2020) who found that surfaces frequently touched by patients and medical staff in two hospitals tested positive. A surface sample from an N-95 respirator used at the PHF was negative. This finding is supported by the study of Wei et al. (2020b) who similarly did not find SARS-CoV-2 on PPE samples in non-ICUs.

A hospital environment study conducted by Ye et al. (2020) found that objects that were most often contaminated with SARS-CoV-2 in a medical center in Wuhan, China were printers, desktop/keyboard, and door knobs frequently touched by patients and medical professionals. This finding is in agreement with the results of this study, which found that a copy machine, keyboard/mouse, and doorknobs at the PHF tested positive, as they were frequently touched surfaces. Cheng et al. (2020) sampled 377 environmental surfaces in a hospital, and found a 5% positivity rate. Harvey et al. (2020) found an 8.3% positivity rate among their 348 environmental surface samples. In this study, of 300 environmental surface samples the positivity rate was 19.3%, which exceeded the expected 10% positivity rate.

Air Samples

The research of Yamagishi et al. (2020) and Cheng et al. (2020) did not detect SARS-CoV-2 in any air samples, which is in agreement with the results of this study. However, a study of Razzini et al. (2020) showed that all air samples collected at the ICU and patients' corridors in a hospital in Milan, Italy tested positive for SARS-CoV-2, while no positive air samples were obtained in a non-ICU area. In their study, they used the MD8 Airport Portable Air Sampler and

gelatin membranes, similar to our study. However, the air volume sampled was 2,000 L, which is greater than the 250 L sampled in this study. In this study, it is possible that air samples tested negative for SARS-CoV-2 because viral particles were present at concentrations below the detection limit of the assay. In order to improve air sampling test sensitivity, future studies may increase the air sampling volume, as well as the number of air samples collected.

Sampling Timeframe and Test Positivity Rate

The environmental surface sampling timeframe in this research project was from December 2020 until April 2021. Kahn & McIntosh (2005) found that coronavirus infections occur mostly during winter and spring. However, a SARS-CoV- 2 seasonal pattern has yet to be determined. The sampling in this study coincided with the largest surge of COVID to date in Southern Nevada. Moreover, according to Nevada Health Response data, the highest test positivity rate (22.3%) was recorded on 12/8/20 (NV Health Response, 2021b). Therefore, the environmental sampling for this project was begun on that date. After the State of Nevada adjusted the data calculation to a 14-day moving average with a 7-day lag, the data currently available at the website shows an 18.6% test positivity rate for 12/8/20 (Table 3). The test positivity rate was 5.5% on 4/22/21 when the environmental surface sampling was completed. In addition to 4/22/21, air samples were collected on 7/20/21 and 7/21/21. Test positivity rates for those two days were 11.8% and 12.1%, respectively.

PCR Assay Sensitivity

This study demonstrated that the N gene PCR assay was more sensitive compared to the S and ORF assays, respectively. However, as genomic sequencing of viral RNA was not conducted in samples, it is unknown whether SARS-CoV-2 variants were also detected. According to Naqvi et al. (2020), the N gene is a highly conserved genetic region. However,

Wang et al. (2020), found that the N gene is one of the least conserved genes. Tahan et al. (2021), discovered that the S gene and E gene assays may not detect SARS-CoV-2 variants, due to mutations in those gene regions. Therefore, multiple gene target PCR tests may be used to improve detection of variants.

Sample Quantification

Quantification of SARS-CoV-2 RNA was conducted to understand the number of viral particles present in each sample. According to Karimzadeh et al. (2021), the MID of SARS-CoV-2 is approximately 100 viral particles. Van Damme et al. (2021) concluded that a lower dose of viral inoculum will lead to milder disease, whereas a higher dose will lead to severe clinical presentation. However, most of the published studies have been referring to person-to-person respiratory droplets transmission. In this study, viability of SARS-CoV-2 viruses collected from environmental samples was not determined, therefore, the potential for infection from surfaces is unknown.

ZIP Codes

A study by Harvey et al. (2020), found that peaks in surface sample positivity rates were detected 7 days before peaks in COVID-19 cases in the same ZIP code. However, as continuous environmental monitoring was not conducted, the findings of this study may not reflect the circulation of COVID-19 in ZIP codes where samples were collected.

Study Limitations

This study was limited by a relatively long sample collection time frame that encompassed variable environmental conditions and infection rates in the community. A positive PCR analysis could not confirm whether the virus detected was viable and had potential to cause an infection. Moreover, samples were collected from only six ZIP code areas in Las

Vegas. Furthermore, data on time between surface contamination and next human contact with that surface were not collected. Relative humidity and temperature data, and data on the cleanliness status of surfaces sampled were not recorded. In addition, some swab samples were used to collect samples from multiple surfaces (i.e., gas station buttons and pin pads), and some surfaces sampled were made of more than one material. Moreover, as the virus mutates, it cannot be stated with certainty whether SARS-CoV-2 variants were detected. In addition, surface and air sampling were not conducted at the same times and locations, so no conclusions can be made about potential relationships between air and surface contamination.

CHAPTER 6

CONCLUSION

The results of this study provided information on the extent and distribution of environmental SARS-CoV-2 contamination in public areas in Las Vegas. They helped in identification of types of materials where SARS-CoV-2 can be detected, and advanced the knowledge and understanding of SARS-CoV-2 presence in the environment.

Moreover, in this study a protocol was developed for SARS-CoV-2 environmental surface and air sampling in public areas that could be used for future studies. SARS-CoV-2 RNA was detected in environmental surface samples in public areas in Las Vegas, with a 19.3% positivity rate, which was greater than the 10% positivity rate expected based on previous studies.

In this study, SARS-CoV-2 was detected on plastic, stainless steel, rubber, metal, glass, vinyl, ceramic, artificial leather, wood, and paper. Plastic and stainless steel were identified as surfaces on which SARS-CoV-2 was detected the most frequently. This study contributed to the knowledge about SARS-CoV-2 presence on floors and on objects that are in contact with floors. Moreover, this research revealed the mop water used for floor cleaning, as a potential source of SARS-CoV-2 RNA. In addition, objects that are in close contact with floors, such as cart wheels and shoes may be significant virus carriers. Frequent changing of floor cleaning water and proper use of disinfectants should be adopted, as floor cleaning water may be a source of SARS-CoV-2 RNA spread. In addition, future studies may include testing for the presence of SARS-CoV-2 on rubber, as this material is widely used and was shown in this study to be a source of contamination.

Future work should be focused on collecting more surface and air samples from non-healthcare indoor and outdoor areas (e.g., concerts, sporting events, airports, grocery stores, pharmacies) in order to better understand the presence of SARS-CoV-2 in the environment. Continuous monitoring for SARS-CoV-2 in areas with high circulation of people is essential for pandemic control, especially as the virus continues to mutate.

Although PCR assay sensitivity is still being researched, our results indicate that in time-sensitive conditions, the SARS-CoV-2 N gene assay may be used for environmental sample PCR analysis. However, the best practice would be to conduct environmental sample analysis with multiple gene PCR tests. In addition, future work should be focused on understanding the sensitivity of different gene assays. Genomic sequencing of environmental samples should be conducted in order to understand if variants could be detected using N, S, and ORF gene primers and probes.

Future work should focus on cell culture and virus isolation to understand SARS-CoV-2 viability. Moreover, understanding how long the virus can maintain viability on public area surfaces would be beneficial for future environmental sampling studies. To better understand the relationship between COVID-19 prevalence rates per ZIP code and positive environmental samples, ZIP codes should be pre-selected and more ZIP codes should be included in future studies, and environmental sampling should be conducted routinely. In addition, future studies may include concomitant air and surface sampling to provide more information on potential sources of environmental contamination. Moreover, an increase in air volume sampled may increase the probability of SARS-CoV-2 detection of low concentrations of SARS-CoV-2 in air samples.

In this study a new method was developed for SARS-CoV-2 environmental surveillance in public areas. Environmental monitoring is significant as an early detection tool that may indicate SARS-CoV-2 circulation in the community. This study revealed important findings that may be beneficial for infection control practices in public areas and public health facilities, universities, as well as businesses.

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APPENDIX A

Sampling Locations

Sampling Locations (number of samples)
Condominiums (4)
Bus Station (1)
Hardware Stores Restrooms (16)
Grocery Stores Restrooms (28)
Las Vegas Boulevard Escalators (4)
Las Vegas Boulevard Elevators (3)
Traffic Lights (walk buttons) (3)
Retail Store Restrooms (12)
Retail Store Carts (2)
Post Office (1)
Water Mill (4)
Carwash (2)
Department Store Restroom (1)
Casino Parking (3)
Bus Pass Machine (1)
University Library (20)
University Restrooms (9)
University Locations (6)
Shopping Mall Restrooms (20)
Public Health Facility Site 1 (10)
Public Health Facility Site 2 (140)

APPENDIX B

Flow diagram illustrating MD8 Air sampling and processing protocol

Sampling

Power on the MD8 AirPort Sartorius sampler

Press ↑↓ button to scroll through menu options, press ↑ or ↓ to adjust

Change sampling parameters to:

- Sampling volume default: 250 L
- Air flow rate L/min: 50

Scroll through menu to the 2nd option (after the AirPort MD8 screen)

Gently place a disposable filter holder on the adapter, reserve bag

Press power button to start sampling

Confirm the sampling parameters from the display

After sampling, return the exposed filter holder to the labeled bag

↓

Processing

Pre-warm a 15 ml centrifuge tube with 5 ml sterile ultrapure water for each sample in a 40°C water bath × 15 min

Pre-warm incubator shaker to 40°C

Obtain a Petri dish for each sample, label, and place in the shaker

Transfer warm water to Petri dish, gently swirl by hand; reserve and label centrifuge tube

Using forceps place exposed membrane on top of the water in Petri dish

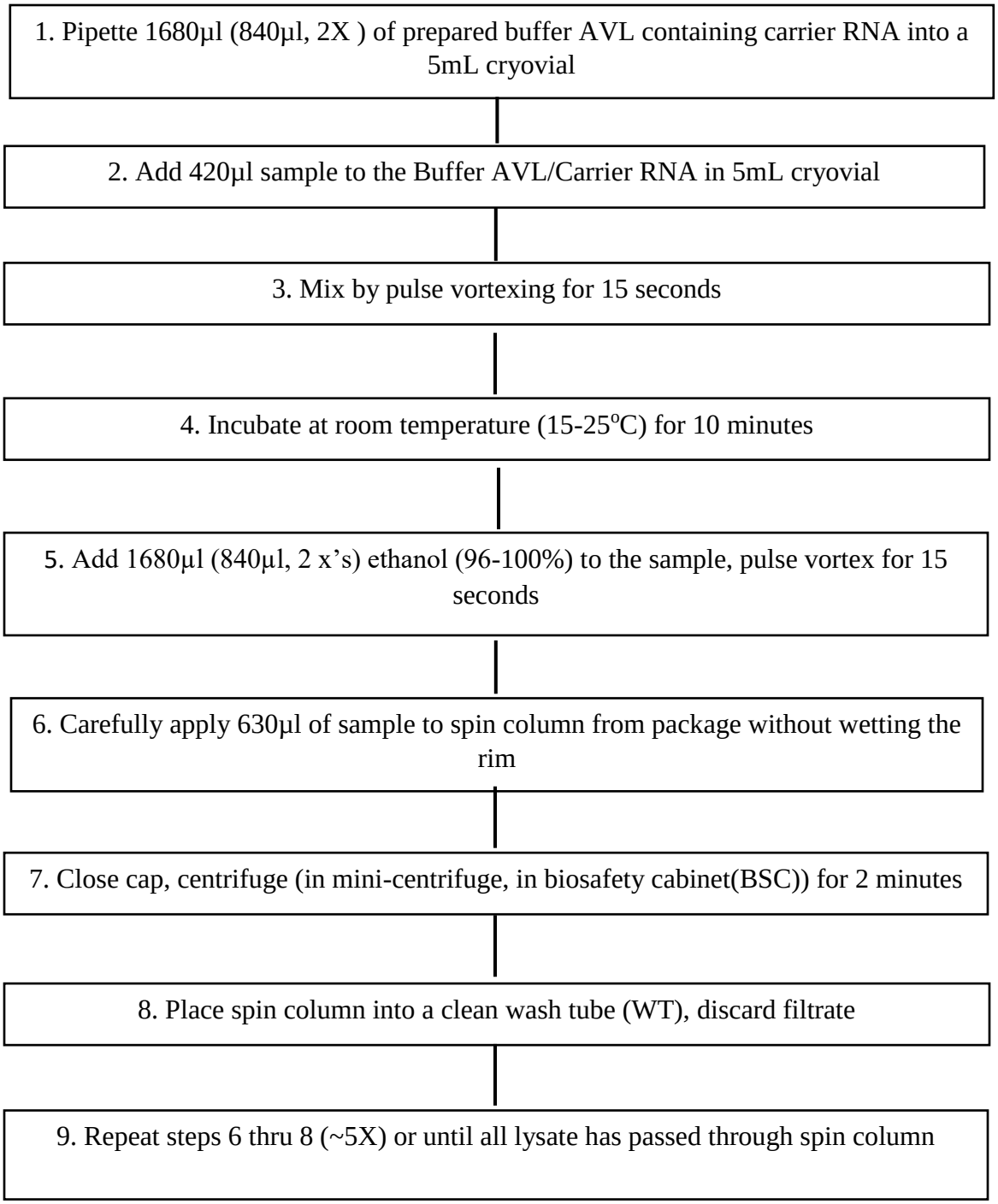
Incubate @ 40°C, 100 rpm × 4 min

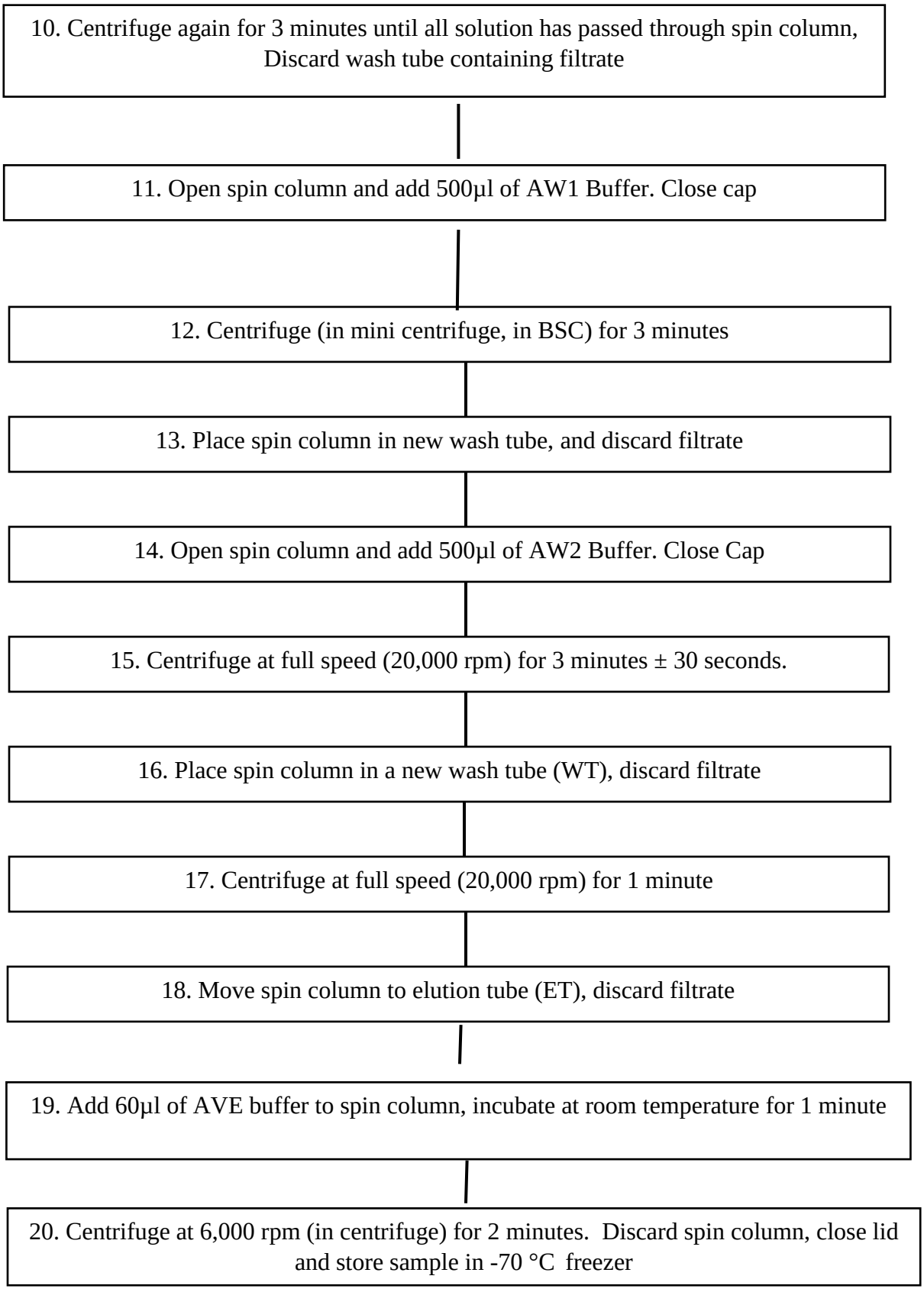
Transfer sample (i.e., dissolved membrane) to the labeled 15 ml centrifuge tube that contained the warm water (measure and record volume)

Store/aliquot sample for RNA extraction as done with surface samples

APPENDIX C

RNA Extraction Protocol for Environmental Sampling of SARS-CoV-2




10. Centrifuge again for 3 minutes until all solution has passed through spin column, Discard wash tube containing filtrate
11. Open spin column and add 500µl of AW1 Buffer. Close cap
12. Centrifuge (in mini centrifuge, in BSC) for 3 minutes
13. Place spin column in new wash tube, and discard filtrate
14. Open spin column and add 500µl of AW2 Buffer. Close Cap
15. Centrifuge at full speed (20,000 rpm) for 3 minutes ± 30 seconds.
16. Place spin column in a new wash tube (WT), discard filtrate
17. Centrifuge at full speed (20,000 rpm) for 1 minute
18. Move spin column to elution tube (ET), discard filtrate
19. Add 60µl of AVE buffer to spin column, incubate at room temperature for 1 minute
20. Centrifuge at 6,000 rpm (in centrifuge) for 2 minutes. Discard spin column, close lid and store sample in -70 °C freezer

APPENDIX D

Tables

Table 1: List of positive (+/+) samples and partial positive (+/-) PCR results (N/A = Not Applicable).

Sample Name	First PCR	Second PCR
University Library Door Handle	(+/-)	(+/-)
Hardware Store Door Locks in Male Restroom	(+/+)	N/A
Las Vegas Boulevard Escalator #4	(+/-)	(-/-)
Las Vegas Boulevard RTC Bus Pass Machine	(+/-)	(-/-)
Las Vegas Elevator 2 Buttons	(+/+)	N/A
Gas Station- Gas Pump Buttons, Gas Selection Buttons, Credit Card Pin Pad Buttons	(+/-)	(-/-)
Traffic Light Buttons	(+/+)	N/A
Gas Station- Gas Pump Buttons, Credit Card Pin Pad Buttons, Gas Selection Buttons AND Keypad of Condominium	(+/+)	N/A
Retail Store Restroom on Las Vegas Boulevard- Floor Around Toilet	(+/-)	(+/+)
Grocery Store Metal Trash Can in Female Restroom	(+/+)	N/A
PHF Site 1 Entrance Door	(+/-)	(-/-)
PHF Site 2 Outdoor Decontamination Area Desk	(+/-)	(-/-)
PHF Site 2 Handrail 1	(+/-)	(+/-)
PHF Site 2 Clothes Locker	(+/+)	N/A
PHF Site 2 Handrail 2	(+/-)	(+/+)
PHF Site 2 Restrooms-Faucets and Flush Buttons	(+/+)	N/A
PHF Site 2 Restrooms- Trash Cans Next to Toilet Seat	(+/-)	(-/-)
PHF Site 2 Restrooms- Sinks	(+/+)	N/A
PHF Site 2 Restrooms- Door Handles	(+/-)	(+/-)
PHF Site 2 Desk Shelf in Front of Restrooms	(+/-)	(-/-)
PHF Site 2 Front Desk	(+/-)	(-/-)
PHF Site 2 Coffee Table 1	(+/+)	N/A
PHF Site 2 Coffee Table 2	(+/+)	N/A
PHF Site 2 Shower Room Door Handles	(+/-)	(-/-)
PHF Site 2 Keyboard and Mouse	(+/-)	(+/-)
PHF Site 2 Front Desk	(+/-)	(-/-)
PHF Site 2 Couch 2	(+/+)	N/A
PHF Site 2 Table 1	(+/+)	N/A

Sample Name	First PCR	Second PCR
PHF Site 2 Table 2	(+/-)	(+/-)
PHF Site 2 Restroom 2- Toilet Seat	(+/+)	N/A
PHF Site 2 Restroom 1- Area Around Toilet	(+/+)	N/A
PHF Site 2 Toilet 1 Surface Inside Toilet Seat	(+/-)	(+/+)
PHF Site 2 Dining Table 3	(+/+)	N/A
PHF Site 2 Shower Head	(+/-)	(+/-)
PHF Site 2 Front Desk and Pen	(+/+)	N/A
PHF Site 2 Copy Machine	(+/+)	N/A
PHF Site 2 Restroom 2- Area Around Toilet	(+/+)	N/A
PHF Site 2 Chairs 1	(+/-)	(+/-)
PHF Site 2 Chairs 3	(+/+)	N/A
PHF Site 2 Security Team Radio	(+/+)	N/A
PHF Site 2 Medical Staff Shoes	(+/+)	N/A
PHF Site 2 Security Staff Shoes	(+/+)	N/A
PHF Site 2 Books	(+/-)	(-/-)
PHF Site 2- Researcher's Shoes	(+/+)	N/A
PHF Site 2 Mop Water	(+/+)	N/A
PHF Site 2 Restroom 1 Door Knob	(+/-)	(+/+)
PHF Site 2 Shower Room Door Knobs	(+/-)	(-/-)
PHF Site 2 Patient's Phone	(+/-)	(+/-)
PHF Site 2 Linen Carts Wheels	(+/+)	N/A
PHF Site 2 Restroom 1 Area Around Toilet	(+/-)	(-/-)
PHF Site 2 Cleaning Station 1 Wheels	(+/+)	N/A
PHF Site 2 Cleaning Station 2 Wheels	(+/+)	N/A
PHF Site 2 Medical Staff Chair Wheels	(+/+)	N/A
PHF Site 2 Oxygen Tank Wheels	(+/+)	N/A
PHF Site 2 Medical Staff Restroom Urinals	(+/-)	(-/-)
University Library Female Restroom Toilet Seats	(+/-)	(+/-)
University Library Female Restroom Sinks	(+/+)	N/A
University Facility Male and Female Restroom Door Knob	(+/+)	N/A

Table 2. List of positive sample surface types and materials

Sample Name	Surface Type (Object)	Sampling Surface Material
University Library Door Handle	Door Handle	Stainless Steel
Hardware Store Door Locks in Male Restroom	Door Lock	Stainless Steel
Las Vegas Boulevard Escalator #4	Escalator Rubber	Rubber
Las Vegas Boulevard RTC Bus Pass Machine	Buss Pass Machine Button	Metal, plastics
Las Vegas Elevator 2 Buttons	Elevator Button	Stainless Steel
Gas Station- Gas Pump Buttons, Gas Selection Buttons, Credit Card Pin Pad Buttons	Gas Station Buttons	Stainless Steel, Plastic
Traffic Light Buttons	Traffic Light Button	Stainless Steel
Gas Station- Gas Pump Buttons, Credit Card Pin Pad Buttons, Gas Selection Buttons AND Keypad of Condominium	Gas Station Buttons	Stainless Steel, Plastic
Retail Store Restroom on Las Vegas Boulevard- Floor Around Toilet	Floor Around Toilet	Vinyl floor
Grocery Store Metal Trash Can in Female Restroom	Trash Can	Metal
PHF Site 1 Entrance Door	Metal Door	Metal
PHF Site 2 Outdoor Decontamination Area Desk	Table	Plastic
PHF Site 2 Handrail 1	Handrail	Stainless Steel
PHF Site 2 Clothes Locker	Lock	Stainless Steel
PHF Site 2 Handrail 2	Handrail	Stainless Steel
PHF Site 2 Restrooms-Faucets and Flush Buttons	Faucet and Flush button	Stainless Steel
PHF Site 2 Restrooms- Trash Cans Next to Toilet Seat	Trash Can	Metal, Plastic
PHF Site 2 Restrooms- Sinks	Sink	Ceramic
PHF Site 2 Restrooms- Door Handles	Door Handle	Stainless Steel
PHF Site 2 Desk Shelf in Front of Restrooms	Cabinet	Metal
PHF Site 2 Front Desk	Front Desk	Wood
PHF Site 2 Coffee Table 1	Table	Wood
PHF Site 2 Coffee Table 2	Table	Plastic
PHF Site 2 Shower Room Door Handles	Door Handle	Stainless Steel
PHF Site 2 Keyboard and Mouse	Keyboard and Mouse	Plastic

Sample Name	Surface Type (Object)	Sampling Surface Material
PHF Site 2 Front Desk	Front Desk	Wood
PHF Site 2 Couch 2	Couch	Artificial Leather (synthetic leather)
PHF Site 2 Table 1	Table	Plastic
PHF Site 2 Table 2	Table	Plastic
PHF Site 2 Restroom 2- Toilet Seat	Toilet Seat	Plastic
PHF Site 2 Restroom 1- Area Around Toilet	Floor Around Toilet	Vinyl floor
PHF Site 2 Toilet 1 Surface Inside Toilet	Toilet	Ceramic
PHF Site 2 Dining Table 3	Table	Plastic
PHF Site 2 Shower Head	Shower Head	Stainless Steel
PHF Site 2 Front Desk and Pen	Front Desk and Pen	Wood and plastic
PHF Site 2 Copy Machine	Copy Machine	Plastic
PHF Site 2 Restroom 2- Area Around Toilet	Floor Around Toilet	Vinyl floor
PHF Site 2 Chairs 1	Chairs	Plastic
PHF Site 2 Chairs 3	Chairs	Plastic
PHF Site 2 Security Team Radio	Radio	Plastic, rubber
PHF Site 2 Medical Staff Shoes	Staff Shoes	Rubber
PHF Site 2 Security Staff Shoes	Staff Shoes	Rubber
PHF Site 2 Books	Books	Paper
PHF Site 2- Researcher's Shoes	Researcher's Shoes	Rubber
PHF Site 2 Mop Water	Water	Water
PHF Site 2 Restroom 1 Door Knob	Door Knob	Stainless Steel
PHF Site 2 Shower Room Door Knobs	Door Knob	Stainless Steel
PHF Site 2 Patient's Phone	Phone	Plastic, glass
PHF Site 2 Linen Carts Wheels	Cart Wheels	Rubber
PHF Site 2 Restroom 1 Area Around Toilet Seat	Floor Around Toilet	Vinyl floor
PHF Site 2 Cleaning Station 1 Wheels	Cart Wheels	Rubber
PHF Site 2 Cleaning Station 2 Wheels	Cart Wheels	Rubber
PHF Site 2 Medical Staff Chair Wheels	Chair Wheels	Rubber
PHF Site 2 Oxygen Tank Wheels	Tank Wheels	Rubber
PHF Site 2 Medical Staff Restroom Urinals	Urinals	Ceramic

Sample Name	Surface Type (Object)	Sampling Surface Material
University Library Female Restroom Toilet Seats	Toilet seat	Plastic
University Library Female Restroom Sinks	Sink	Ceramic
University Facility Male and Female Restroom Door Knob	Door Knob	Stainless Steel

Table 3. Sampling Date, ZIP codes and Number of cases on sampling date

Sample Name	Date Collected	ZIP Codes	Range of COVID-19 cases in the ZIP code on the sampling date (per SNHD color coding data)	Total number of COVID-19 cases on the sampling date per ZIP code	Test Positivity Rate in Nevada on the sampling date
University Library Door Handle	12/8/2020	89119	2338-3726	2971	18.60%
Hardware Store Door Locks in Male Restroom	12/14/2020	89113	1556-2685	1958	20.20%
Las Vegas Boulevard Escalator #4	12/14/2020	89109	5-1555	538	20.20%
Las Vegas Boulevard RTC Bus Pass Machine	12/14/2020	89109	5-1555	538	20.20%
Las Vegas Elevator 2 Buttons	12/14/2020	89109	5-1555	538	20.20%
Gas Station- Gas Pump Buttons, Gas Selection Buttons, Credit Card Pin Pad Buttons	12/14/2020	89117	2686-3993	2940	20.20%
Traffic Light Buttons	12/14/2020	89145	5-1555	1410	20.20%
Gas Station- Gas Pump Buttons, Credit Card Pin Pad Buttons, Gas Selection Buttons AND Keypad of Condominium	12/21/2020	89117	2884-4258	3183	19.30%
Retail Store Restroom on Las Vegas Boulevard- Floor Around Toilet Seat	12/21/2020	89109	5-1686	571	19.30%
Grocery Store Metal Trash Can in Female Restroom	12/30/2020	89145	5-1838	1672	18.50%
PHF Site 1 Entrance Door	2/5/2021	89107	4014-5791	4701	14.70%
PHF Site 2 Outdoor Decontamination Area Desk	2/5/2021	89107	4014-5791	4701	14.70%
PHF Site 2 Handrail 1	2/5/2021	89107	4014-5791	4701	14.70%
PHF Site 2 Clothes Locker	2/5/2021	89107	4014-5791	4701	14.70%
PHF Site 2 Handrail 2	2/5/2021	89107	4014-5791	4701	14.70%
PHF Site 2 Restrooms- Faucets and Flush Buttons	2/16/2021	89107	4142-5908	4774	10.08%

Sample Name	Date Collected	ZIP Codes	Range of COVID-19 cases in the ZIP code on the sampling date (per SNHD color coding data)	Total number of COVID-19 cases on the sampling date per ZIP code	Test Positivity Rate in Nevada on the sampling date
PHF Site 2 Restrooms-Trash Cans Next to Toilet Seat	2/16/2021	89107	4142-5908	4774	10.08%
PHF Site 2 Restrooms-Sinks	2/16/2021	89107	4142-5908	4774	10.08%
PHF Site 2 Restrooms-Door Handles	2/16/2021	89107	4142-5908	4774	10.08%
PHF Site 2 Desk Shelf in Front of Restrooms	2/16/2021	89107	4142-5908	4774	10.08%
PHF Site 2 Front Desk	2/16/2021	89107	4142-5908	4774	10.08%
PHF Site 2 Coffee Table 1	2/16/2021	89107	4142-5908	4774	10.08%
PHF Site 2 Coffee Table 2	2/16/2021	89107	4142-5908	4774	10.08%
PHF Site 2 Shower Room Door Handles	2/16/2021	89107	4142-5908	4774	10.08%
PHF Site 2 Keyboard and Mouse	2/16/2021	89107	4142-5908	4774	10.08%
PHF Site 2 Front Desk	2/16/2021	89107	4142-5908	4774	10.08%
PHF Site 2 Couch 2	2/18/2021	89107	4163-5926	4789	9.60%
PHF Site 2 Table 1	2/18/2021	89107	4163-5926	4789	9.60%
PHF Site 2 Table 2	2/18/2021	89107	4163-5926	4789	9.60%
PHF Site 2 Restroom 2-Toilet Seat	2/18/2021	89107	4163-5926	4789	9.60%
PHF Site 2 Restroom 1-Area Around Toilet Seat	2/18/2021	89107	4163-5926	4789	9.60%
PHF Site 2 Toilet 1 Surface Inside Toilet Seat	2/18/2021	89107	4163-5926	4789	9.60%
PHF Site 2 Dining Table 3	2/18/2021	89107	4163-5926	4789	9.60%
PHF Site 2 Shower Head	2/18/2021	89107	4163-5926	4789	9.60%
PHF Site 2 Front Desk and Pen	2/18/2021	89107	4163-5926	4789	9.60%
PHF Site 2 Copy Machine	2/18/2021	89107	4163-5926	4789	9.60%
PHF Site 2 Restroom 2-Area Around Toilet Seat	2/18/2021	89107	4163-5926	4789	9.60%
PHF Site 2 Chairs 1	2/18/2021	89107	4163-5926	4789	9.60%
PHF Site 2 Chairs 3	2/18/2021	89107	4163-5926	4789	9.60%
PHF Site 2 Security Team Radio	2/18/2021	89107	4163-5926	4789	9.60%

Sample Name	Date Collected	ZIP Codes	Range of COVID-19 cases in the ZIP code on the sampling date (per SNHD color coding data)	Total number of COVID-19 cases on the sampling date per ZIP code	Test Positivity Rate in Nevada on the sampling date
PHF Site 2 Medical Staff Shoes	2/25/2021	89107	4234-5979	4834	7.30%
PHF Site 2 Security Staff Shoes	2/25/2021	89107	4234-5979	4834	7.30%
PHF Site 2 Books	2/25/2021	89107	4234-5979	4834	7.30%
PHF Site 2- Researcher's Shoes	2/25/2021	89107	4234-5979	4834	7.30%
PHF Site 2 Mop Water	2/25/2021	89107	4234-5979	4834	7.30%
PHF Site 2 Restroom 1 Door Knob	2/25/2021	89107	4234-5979	4834	7.30%
PHF Site 2 Shower Room Door Knobs	2/25/2021	89107	4234-5979	4834	7.30%
PHF Site 2 Patient's Phone	2/25/2021	89107	4234-5979	4834	7.30%
PHF Site 2 Linen Carts Wheels	3/4/2021	89107	4124-6040	4850	6.20%
PHF Site 2 Restroom 1 Area Around Toilet Seat	3/4/2021	89107	4124-6040	4850	6.20%
PHF Site 2 Cleaning Station 1 Wheels	3/4/2021	89107	4124-6040	4850	6.20%
PHF Site 2 Cleaning Station 2 Wheels	3/4/2021	89107	4124-6040	4850	6.20%
PHF Site 2 Medical Staff Chair Wheels	3/4/2021	89107	4124-6040	4850	6.20%
PHF Site 2 Oxygen Tank Wheels	3/4/2021	89107	4124-6040	4850	6.20%
PHF Site 2 Medical Staff Restroom Urinals	3/4/2021	89107	4124-6040	4850	6.20%
University Library Female Restroom Toilet Seats	4/22/2021	89119	4381-6312	5252	5.50%
University Library Female Restroom Sinks	4/22/2021	89119	4381-6312	5252	5.50%
University Facility Male and Female Restroom Door Knob	4/22/2021	89119	4381-6312	5252	5.50%

Table 4. SARS-CoV-2 RNA copies per reaction and per sample in +/+ samples

Sample Name	Number of viral RNA copies per reaction	Number of viral RNA copies per sample
Door Locks in Male Restroom	26	2259
Las Vegas Elevator 2 Buttons	33	2785
PHF Site 2 Clothes Locker	8	697
PHF Site 2 Restrooms- Faucets and Flush Buttons	704	60300
PHF Site 2 Restrooms- Sinks	90848	7783891
PHF Site 2 Coffee Table 1	3200	274162
PHF Site 2 Coffee Table 2	944	80887
PHF Site 2 Couch 2	123	10570
PHF Site 2 Table 1	2117	181418
PHF Site 2 Restroom 2- Toilet Seat	63	5372
PHF Site 2 Restroom 1- Area Around Toilet	303	25997
PHF Site 2 Dining Table 3	3945	337966
PHF Site 2 Front Desk and Pen	25	2169
PHF Site 2 Copy Machine	40	3460
PHF Site 2 Restroom 2- Area Around Toilet	45888	3931680
PHF Site 2 Chairs 3	51	4331
PHF Site 2 Security Team Radio	81	6899
PHF Site 2 Medical Staff Shoes	120	10300
PHF Site 2 Security Staff Shoes	62	5329
PHF Site 2- Researchers' Shoes	2878	246612
PHF Site 2 Mop Water	590	50512
PHF Site 2 Linen Carts Wheels	138	11820
PHF Site 2 Cleaning Station 1 Wheels	1799	154173
PHF Site 2 Cleaning Station 2 Wheels	344	29494
PHF Site 2 Medical Staff Chair Wheels	317	27124
PHF Site 2 Oxygen Tank Wheels	253	21697
University Library Female Restroom Sinks	207	17699

University Facility Male and Female Restroom Door Knob	24	2076
Traffic Light Buttons	90	7707
Gas Station- Gas Pump Buttons, Credit Card Pin Pad Buttons, Gas Selection Buttons AND Keypad of Condominium	92	7853
Grocery Store Metal Trash Can in Female Restroom	83	7151

CURRICULUM VITAE

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Thesis Title: Determination of the Presence of SARS-CoV-2 in Environmental Surface and Air Samples from Public Areas in Las Vegas

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