Technical Whitepaper

Validation of Disinfection for SARS-CoV-2 in a Senior Living Facility Using RT-qPCR

This paper was developed in collaboration between LuminUltra, IWC Environmental, and Source Molecular

Abstract

The emergence of SARS-CoV-2 and its associated COVID-19 pandemic rapidly spread throughout the world testing modern technology in an unprecedented manner. Due to the high rate of asymptomatic carriers, the disinfection of public spaces is increasingly important as global restriction begin to lift. This study evaluated the use of an environmental RT-qPCR assay for SARS-CoV-2 to validate disinfection protocols in a senior living facility known to be contaminated with the virus. Initial an environmental-friendly IPMP-based disinfectant was used in the contaminated rooms. However, after analysis it was determined that SARS-CoV-2 was still present. An additional round of disinfection was completed with sodium hydroxide which eliminated the virus in 8 of 9 samples tested. The protocol described allowed the biohazard cleaning team to efficiently confirm whether disinfection was successful or whether further actions would be required. Overall, the study points to the need for monitoring in addition to disinfection practices to reduce the risk of community transmission.

Introduction

During the COVID-19 pandemic the demand for clinical diagnostic has generally exceeded demand globally. SARS-CoV-2, which causes COVID-19, is highly contagious and has been shown to remain viable on surfaces for several days after exposure depending on the surface material indicating surfaces as a potential transmission pathway [1]. This is particularly alarming as the asymptomatic infection rate has been reported to range from 18-78% [2] and these carriers could be vectors of community transmission.

Ensuring effective disinfection strategies will be critical to ensuring public safety as countries start to phase out quarantines and other restrictions. Airlines, public transportation, healthcare facilities, hotels, businesses, and the like are promoting their cleaning regimens as effective in disinfecting SARS-CoV-2 thereby assuring the public they will be safe to return. However, by and large, they have no means to rapidly validate their cleaning and disinfection practices thereby confirm they have been effective.

Current Centers for Disease Control and Prevention (CDC) guidance for disinfection of surfaces to prevent COVID-19 recommends using a disinfectant approved by the US EPA for use against SARS-CoV-2 [3,4]. In absence of an EPA approved disinfectant, alternative disinfectants including 0.1% bleach or 70% alcohol can be used. Research on other known coronaviruses, including SARS, MERS and HCoV, showed that not all biocidal agents provide effective disinfection. Ethanol (62-71%), hydrogen peroxide (0.5%) and sodium hypochlorite (0.1%) were found to be effective, while

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benzalkonium chloride (0.05-0.2%) and chlorhexidine digluconate (0.02%) were less effective [5]. Validating disinfectants as well as validateing cleaning and disinfecting protocols will be vital to reducing transmission of SARS-CoV-2.

Senior living facilities have been shown to be particularly vulnerable to COVID-19. The objective of this study was to validate cleaning and disinfection strategies used in a senior living facility in the midwestern USA where nine residents had recently confirmed positive for COVID-19. Validation was done using reverse transcription quantitative polymerase chain reaction (RT-qPCR). Effective validation testing strategies will aid in the disinfection of known contaminated surfaces as well as allow potential contamination to be tracked over time, providing an increased margin of safety for employees, guests, patients, and ultimately the general public.

Methodology

A senior living facility in midwestern USA had nine residents test positive for COVID-19 in April 2020. After moving the COVID-19 patients to a hospital equipped to treat them, their individual resident rooms remained vacant and uncleaned for approximately four days until a biohazardous cleaning team was able to clean and disinfect.

Prior to cleaning and disinfecting, three samples were taken from “high touch” areas in four of the COVID-19 case room. The samples are designated as follows:

1. Bathroom: sink faucets, toilet flusher, countertop
2. Bedroom: remotes, phone, wall switch plates
3. Handles: Doorknobs, safety rails

Samples were taken using a swab wetted with sterile phosphate-buffered saline. Once the sample was taken the swab was placed in tubes with RNA preservation solution. Necessary sample data, including sample ID, sample location, data and time of collection, area swabbed and other comments were entered into a proprietary mobile application (*IWC Smart Test*) for sample tracking.

After sampling, the rooms were then disinfected using an environmental-friendly commercial disinfectant with 2-isopropyl-5-methylphenol (IPMP) as the active ingredient. Approximately 1.5 hours after disinfection, another set of swab samples were collected from the same locations and stored in RNA preservation solution.

All samples were transported to an ISO/IEC 17025:2005 accredited commercial laboratory for analysis by RT-qPCR. The analysis targeted both the N1 and N2 gene fragments. Following the guidance document for clinical diagnostic from the CDC [6], the results we’re interpreted as follows:

- Positive for SARS-CoV-2: Detection of N1 and N2
- Inconclusive for SARS-CoV-2: Disagreement between N1 and N2
- Not Detected: No detection of either N1 or N2

Various quality control and assurance steps were also conducted including positive and negative control for N1 and N2, and an extraction blank using mouse ACTB.

Upon analysis of the results, it was determined that an additional round of disinfection and validation should be completed with a 5% Sodium Hypochlorite solution. During the second round, one room was

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treated with the same IPMP-based disinfectant as a control. This occurred 4 days after the initial use of the IPMP-based disinfectant. Again, pre and post disinfection samples were collected and analyzed.

In all cases PPE associated with biohazardous cleaning was utilized.

Results and Discussion

Disinfection with IPMP-based Commercial Disinfectant

The results from the initial disinfection protocol using the IPMP-based environmental-friendly commercial disinfectant are shown in Table 1.

Table 1: SARS-CoV-2 results prior to and after disinfection with a IPMP-based disinfectant

Room	Location	Pre-Disinfection Result	Post-Disinfection Result
1	Bathroom	Positive	Positive
	Bedroom	Positive	Positive
	Handles	Positive	Positive
2	Bathroom	Inconclusive	Positive
	Bedroom	Inconclusive	Inconclusive
	Handles	Inconclusive	Positive
3	Bathroom	Positive	Positive
	Bedroom	Positive	Positive
	Handles	Positive	Positive
4	Bathroom	Positive	Positive
	Bedroom	Positive	Positive
	Handles	Positive	Positive

All of the pre- and post-disinfection samples tested positive or inconclusive for SARS-CoV-2. In practice an inconclusive result would yield the same action as a positive result – disinfect and test again. Interestingly, the IPMP-based disinfectant was not observed to be effective at removing or disinfecting SARS-CoV-2. This could be due to several factors including insufficient IPMP concentration, insufficient contact time or the antiviral action of IPMP may be incompatible with RT-qPCR validation testing as it does not distinguish between active and inactive virus. There are several IPMP-based disinfectants on the USEPA's List N of disinfectant for use against SARS-CoV-2. It is important to note that the list was generated based on results from other viruses, including human coronavirus similar to SARS-CoV-2. Further disinfection validation may be prudent.

The results also show that even after 4 days unoccupied, SARS-CoV-2 is well distributed and detectable in all the rooms tested.

Disinfection with Sodium Hypochlorite

The positive test results post-disinfection was a significant concern to all parties. Thus, another round of disinfection and validation testing was conducted 4 days later, during which the rooms remained quarantined. Instead of the IPMP-based disinfectant, Rooms 1-3 were disinfected with a 5% Sodium Hypochlorite solution. As a control, Room 4 was disinfected with the IPMP-based disinfectant used initially. The results are shown in Table 2.

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Table 2: SARS-CoV-2 results prior to and after disinfection with 5% NaOCl (Room 1-3) or a IPMP-based commercial disinfectant(Room4)

Room	Location	Pre-Disinfection Result	Post- Disinfection Result
1	Bathroom	Positive	Not Detected
	Bedroom	Positive	Not Detected
	Handles	Positive	Not Detected
2	Bathroom	Positive	Not Detected
	Bedroom	Positive	Not Detected
	Handles	Positive	Not Detected
3	Bathroom	Not Detected	Not Detected
	Bedroom	Positive	Inconclusive
	Handles	Positive	Not Detected
4 Control	Bathroom	Positive	Positive
	Bedroom	Positive	Inconclusive
	Handles	Positive	Not Detected

After disinfection with 5% Sodium Hypochlorite, 8 of 9 samples had no detectable SARS-CoV-2. The remaining sample was inconclusive, potentially pointing to ineffective disinfection procedure. Room 4 which was treated with the IPMP-based disinfectant and 2 of the 3 samples were still positive or inconclusive following disinfection. This is critical information as the current CDC guidance document [3] states that if an area has been unoccupied for 7 days it will only need routine cleaning and not disinfection. In this case after approximately 8 days, SARS-CoV-2 was still present in 11 of 12 samples potentially indicating disinfection should be conducted in cases with known SARS-CoV-2 contamination. Continuing to gather data as countries lift restrictions will be critical to refining and strengthening guidance.

The second round of testing confirmed several things: sodium hypochlorite is effective at killing SARS-CoV-2, disinfection efficacy can be determined using RT-qPCR and that maintaining an effective disinfection procedure is important. Again, the IPMP-based disinfectant was not observed to perform well. As described above this could be due to other factors including user error.

Conclusions and Next Steps

The purpose of the field trial was to ascertain the efficacy of an environmental SARS-CoV-2 RT-qPCR test under real world conditions. The opportunity to use the test in conjunction with the biohazard cleaning team at a facility that had confirmed cases was an ideal platform. The test exceeded expectations and provided actionable data for the cleaning teams. Using the test to validate cleaning protocols along with other CDC recommended guidelines will further increase the surveillance and traceability.

The results further highlighted that results need to be obtained as quickly as possible. Waiting several days for results is not conducive to reducing the risk of community transmission. Therefore, the next step of this research is to deploy a testing solution for localized field laboratories – this will allow potentially contaminated surfaces to be checked and allow contaminated surfaces to be clean and validated within hours ensuring our most vulnerable population is adequately protected.

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