On-Site Field Study

New Environmental RT-qPCR Test for SARS-CoV-2

to Validate Cleaning and Disinfection Practices

Proguardeum™ Disinfectant Solution Dry Mist Application

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Prepared by

Brian Waymire, CEO; Bill Pearson, Chief Scientific Officer, Certified Water Technologist
IWC Environmental Solutions | 704 S. State Road 135, Ste D348, Greenwood, IN 46143

ON SITE FIELD STUDY

RT-qPCR testing to validate disinfection of the SARS-CoV-2

A continuation field study using an on-site, new environmental RT-qPCR test protocol for SARS-CoV-2 to validate cleaning and disinfection practices.

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Brian Waymire, CEO; Bill Pearson, CSO, CWT / IWC Environmental Solutions

ABSTRACT

The emergence of the COVID-19 and its rapid pandemic spread throughout the world has tested modern technology in an unprecedented manner. It has led to a litany of approaches to both mitigate and test for the disease. Countries and businesses are now searching for answers on how and when to reopen their doors and return to some semblance of normalcy after extensive, government mandated, shelter-in-place shutdowns have rocked their economies. An important concern now, is how to quickly assure the public that the 2019 novel coronavirus has been mitigated and cleared from their spaces of business (office buildings, hotels, restaurants, manufacturing facilities, institutions, transportation services, airlines, cruise ships and the like) by their cleaning and disinfection efforts. This paper will describe a second field study using the latest, on-site, qPCR environmental surface testing protocol for SARS-CoV-2 to validate cleaning and disinfection practices. This test is currently available for use.

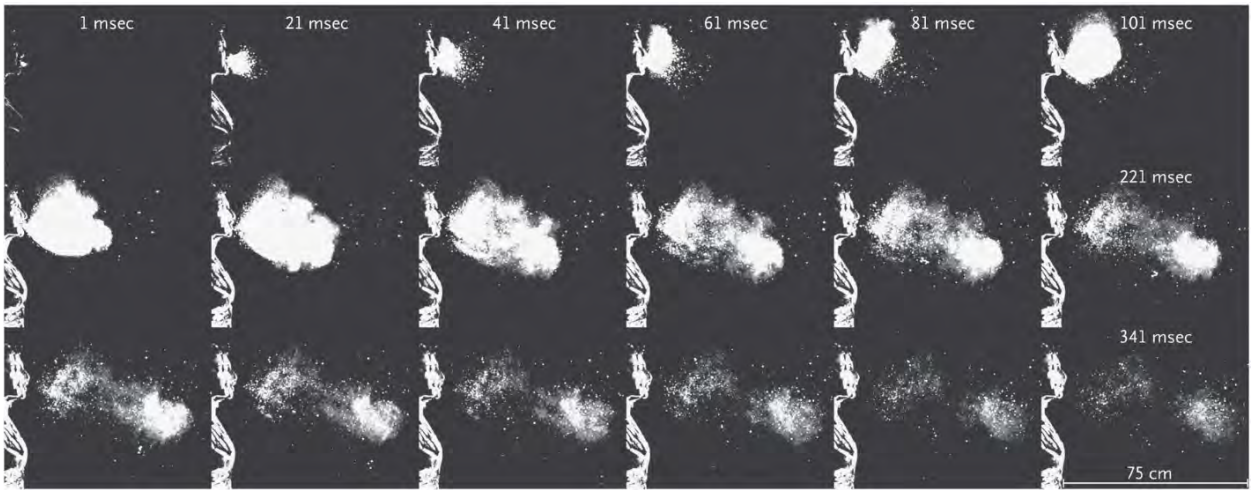


Figure 1. 20-millisecond sequences of a human sneeze ejecting droplets of fluid. (Courtesy NEJM)

BACKGROUND

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 and 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 and spread of disease.

The current guidance from the Centers for Disease Control and Prevention (CDC) for disinfection of surfaces to prevent COVID-19 recommends using a disinfectant that meets the criteria required for the US EPA List N: Disinfectants for Use Against SARS-CoV-2. [3,4]

EPA approved disinfectants include hypochlorous (200ppm), alternative disinfectants including bleach (0.1%), and alcohol (70%). 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 benzalkonium chloride (0.05-0.2%) and chlorhexidine digluconate (0.02%) were less effective. [5] Validating disinfectants as well as verifying cleaning and disinfecting protocols will be vital to reducing transmission of SARS-CoV-2.

Airlines, public transportation, healthcare facilities, hotels, businesses, and the like are promoting cleaning regimens as effective in killing (removing) the virus from their particular facility surfaces (spaces) to assure the public they will be safe. However, they are not 'validating' or demonstrating their cleaning and disinfection practices onsite are effective. A new RT-qPCR, on-site, rapid test for the 'severe acute respiratory syndrome coronavirus 2' (SARS-CoV-2) has been developed and can be utilized to 'validate' the cleaning and disinfection practices used to mitigate the virus and spread of disease. Baseline testing of buildings before reopening and tracking trends within the building over time can provide a margin of safety for employees, guests, patients, and ultimately the general public.

METHODOLOGY

RT-PCR, or reverse transcriptase polymerase chain reaction, is a close cousin of PCR, or polymerase chain reaction. PCR heats DNA samples to make them separate, then uses primers to fill in the empty halves to create double the amount of DNA. Once 20-40 cycles of duplication occur, there is enough DNA to analyze. RT-PCR copies RNA instead of DNA, making it useful for detecting viruses (which are RNA-based). It works through transcribing RNA into complementary DNA (cDNA), then the PCR process is performed on the cDNA. RT- PCR can detect RNA from the smallest sample, even down to a single cell. It should be noted that real-time PCR and RT-PCR are two different processes. Real-time PCR, which can be used for both PCR and RT-PCR, is a faster process than standard PCR or RT-PCR, as it collects data during the duplication process and not afterward, in order to automatically collect data and perform data analysis. [7]

Quantitative PCR (qPCR) and RT-qPCR allows the detection of DNA or RNA as well as the type. RT-qPCR uses fluorescent labeling, which allows quantification of cDNA by calculating the amount of fluorescence.

The newly developed test for SARS-CoV-2 includes an enhanced method of RNA purification to achieve maximum stability and mitigation of potential environmental interferences. The RNA extraction procedure that can be used with either laboratory or available mobile PCR instrumentation is based on a simplified version of patented DNA extraction procedures. Obtaining the highest purity RNA greatly reduces the occurrence of false negatives along with the removal of impurities for the highest accuracy testing.

Gene expression, or mRNA synthesis, is a critical part of protein synthesis. Gene expression isan area of active inquiry for molecular biologists and aids in understanding numerous biological pathways and diseases. Using reverse transcriptase, a researcher can create cDNA copies of the RNA in a sample, and then perform qPCR on the cDNA. The resulting readings provide information about the amount of otherwise difficult-to-measure mRNA present in the original, un-reverse-transcribed sample. The kind of data generated by a qPCR run depends heavily on the choices of primers and dyes utilized in the process.

Typically, a single qPCR sample collects data on a single gene when standard dye is used. The dyes often used in qPCR are nonspecific and will merge multiple genes into a single reading if multiple primers are used in the same mix. However, using qPCR instrumentation with fluorescent DNA probes rather than dyes, allows them to "multiplex" qPCR and measure multiple DNA targets, with each target corresponding probe fluorescing at a different frequency. [8]

Summary: SARS-CoV-2 Environmental Real-Time-PCR Panel

- Biomarker detection by reverse transcriptase, qPCR (RT-qPCR)
- Results reported herein apply only to the sample matrices as received.
- Results reported herein relate to the genetic material extracted from the sample matrix process and included in the analysis.
- Results reported based Guidance of CDC-006-00019, Revision: 02, Instructions for Use
  - **Positive SARS-CoV-2** = Detection of N1 & N2 (regions of nucleocapsid 'N' gene)
  - **Not Detected** = No detection of either N1 or N2
  - **Inconclusive** = Disagreement between N1 and N2 / **should be reported as Positive, if not resampled and tested, to rule out a False Negative**
  - **Invalid** = No detection of ACTB
- Method – RNA purification to achieve maximum stability and mitigation of potential interferences from the environment.
- RT-qPCR – Reverse transcription quantitative polymerase chain reaction. With reporting as detection/non-detection of viral RNA SARS-Cov-2.
- Approved Preservation method for destruction of active virus.
- Ability to remove false negatives with internal control: every sample has synthetic RNA amplified and detected on a different fluorescent channel. If SARS-CoV-2 viral RNA is not detected and the internal control does not amplify, then sample is rerun as some inhibition or issue has occurred. If the internal control is detected, but not SARS-CoV-2 RNA, then the sample is truly virus-free. The combination of a negative, positive, and internal control gives the highest confidence in test results. Inhibitor examples include: hemoglobin, Ca^{2+}, polysaccharides, cleaners, dyes, and the like.
- The on-site instruments use 4 channel qPCR (vs. 1 channel) – having 4 fluorescent channels.
- **Sensitivity: Limit of detection is 10 GU (genomic units) per reaction and 1 GU = 1 cell.**

SECOND FIELD STUDY

SARS-CoV-2 DISINFECTION VALIDATION TESTING USING RT-qPCR

IWC Environmental Solutions provided proprietary RT-qPCR Validation Testing services to a Midwestern Skilled Nursing Facility where there were fifty-two (52) residents sick (confirmed positive) with COVID-19. This study was designed to further research and show the efficacy of IWC's SARS-CoV-2 environmental validation testing in conjunction with Proguardeum Inc's patented and certified electrostatic dry misting process for application of Proguardeum™ Optimum, a medical-grade hypochlorous (HOCl) disinfectant solution.

IWC Environmental Solutions was tasked to provide their COVID-19 RT-qPCR environmental test for validation of cleaning and disinfection services. IWC Environmental Solutions used their proprietary (smartphone) ***Smart Test*** APP for tracking all aspects of the validation testing. IWC Environmental Solutions Staff performed the testing sampling for this study.

Four (4) rooms were selected on the second floor, where all 52 residents with confirmed COVID-19 were quarantined-housed. The IWC team collected 3 swab samples for each room from predesigned "high touch" areas provided and described for recording in the ***Smart Test*** APP. They included: (1) bathroom sink faucets, toilet flusher, countertop; (2) bedroom remotes, phone, wall switch plates; and (3) room doorknobs, handles, footboards, wheelchairs, and rails. The collected samples were appropriately logged as **Pre-Clean samples** then sealed and prepared for transport and testing.

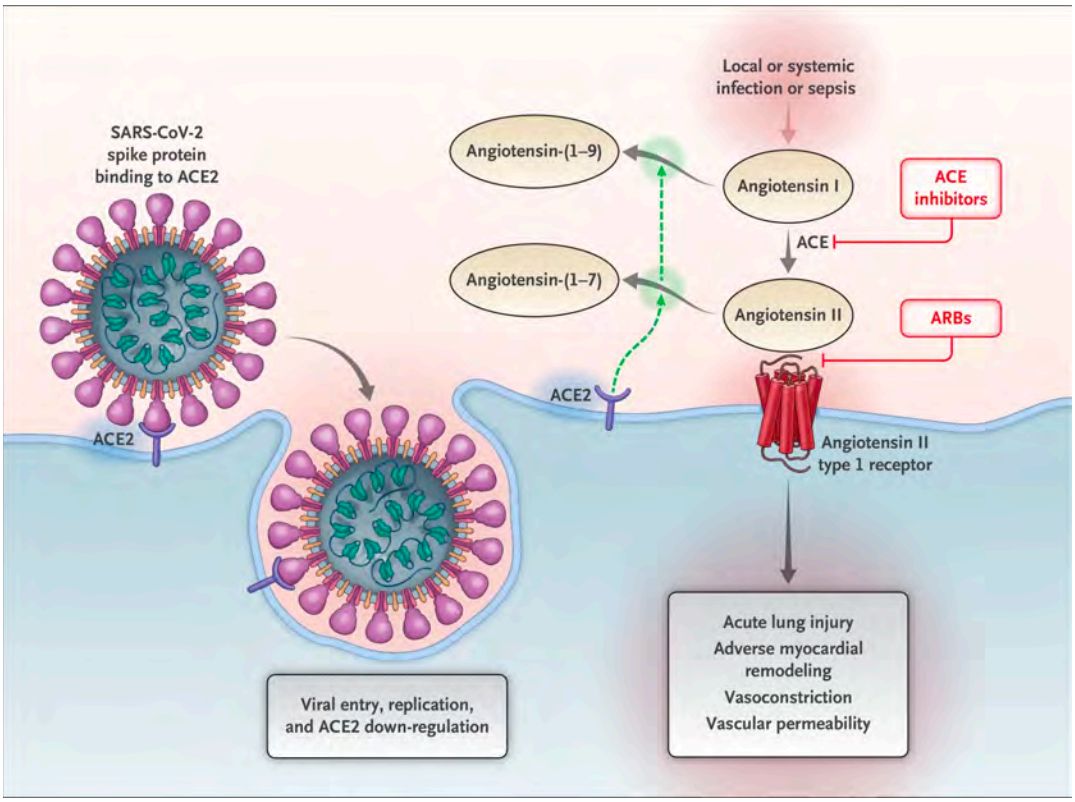
Note: ***Available on-site testing with mobile qPCR units was not provided for this study.***
Swab samples were sent to the lab for next-day processing and reporting of independent test results.

Approximately 2 hours after sampling the 4 rooms for pre-cleaning testing, the environmental cleaning team proceeded to sanitize each room using Proguardeum™ dry- misting application of hypochlorous (HOCl).

Approximately one and a half hours after the Proguardeum™ sanitization process was completed, another set of swab samples were collected from the same locations and in a similar manner as the first (pre-cleaning) sample set. They were subsequently logged as **Post-Clean samples** then sealed and prepared for transport to laboratory for testing. The swab sample collection procedure is described below.

Swab Sample Collection Recap: (Samples may be tested on-site or sent to laboratory.)

1. Wash hands per CDC guidelines and put on clean disposable gloves.
2. Scan Bar Code on the sample tube into the *Smart Test* APP.
3. Record the unique sample identification, date collected, time collected, area swabbed (cm^2), and other comments provided in *Smart Test* APP.
4. Select a swab and dip the swab tip into the PBS (blue cap) tube to moisten the tip.
5. Swab the desired sampling surface/s.
6. Carefully cut or break the swab stick into the tube at the location noted.
7. Place swab, sampling tip first, into pre-labeled Swab Storage Tube. Replace cap and secure.
8. Swirl the tube to ensure transport media contacts the entire swab tip.
9. Place closed Swab Storage Tube in the provided plastic zip bag and seal the plastic zip bag opening. (Up to five samples may be placed in each bag.)
10. Place closed plastic zip bag in the envelope provided for return to the lab per packing and shipping instructions.
11. Repeat previous steps for remaining samples.
12. Provide tracking number via email as soon as available.



Shown is the initial entry of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) into cells.
REF: https://www.nejm.org/doi/full/10.1056/NEJMsr2005760

RESULTS and DISCUSSION

TABLE 1: SARS-CoV-2 Results Pre & Post Disinfection with Proguardeum™ Disinfection Solution (HOCL)

Room	Location	Pre-Disinfection Result	Post-Disinfection Result
1	Bathroom	Positive	Not Detected
	Bedroom	Inconclusive / Pos	Not Detected
	Handles	Positive	Positive
2	Bathroom	Inconclusive / Pos	Not Detected
	Bedroom	Positive	Positive
	Handles	Positive	Positive
3 (214)	Bathroom	Inconclusive / Pos	Not Detected
	Bedroom	Positive	Not Detected
	Handles	Positive	Not Detected
4	Bathroom	Positive	Positive
	Bedroom	Positive	Positive
	Handles	Positive	Positive

Notes: SARS-CoV-2 Environmental Real-Time-PCR Panel

- Detection of biomarkers by reverse transcriptase qPCR (RT-qPCR)
- Results reported apply only to the sample matrices as received
- Results reported relate to the genetic material extracted from the sample matrix process and included in the analysis
- Results reported based Guidance of CDC-006-00019, Revision: 02, Instructions for Use
- **Positive SARS-CoV-2** = Detection of N1 and N2 (regions of nucleocapsid 'N' gene)
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- **Invalid** = No detection of ACTB

OBSERVATIONS

The data observed in **Table 1**, showing **9 of 12 pre-cleaning samples tested positive** (3 were Inconclusive Positive) using the new SARS-CoV-2 RT-qPCR environmental test protocol was consistent with a previous study for rooms having COVID-19 confirmed occupants. The post-disinfection samples resulted in 6 of 12 samples testing Not Detected while 6 (remained) testing Positive.

The study was conducted with COVID-19 occupied acute care rooms. One of the four rooms sampled for testing (room #214) was an empty room that had a COVID-19 patient prior to pre- and post-disinfection testing. It was used to conduct the same validity as a previous study. IWC was particularly interested in the results of the COVID-19 patient occupied rooms post cleaning. It was anticipated that test results from the occupied rooms would yield (remain) Positive for SARS-CoV-2 because of continued viral spread from the COVID-19 positive occupants. Thus, a validation of the Proguardeum™ disinfection process would likely only present for the unoccupied room and yield Non-Detects in concurrence with a previous study.

As described above, 1 of the 4 rooms sampled was vacant, having had a COVID-19 patient prior, and remained vacant during the field study. With confirmed COVID-19 patients remaining in the 3 other rooms, as well as the positive RT-qPCR environmental test results from these rooms, we suspected that the novel coronavirus was present on the high touch surfaces sampled for testing.

The unoccupied Room 214 tested positive for SARS-CoV-2 on 2 of the 3 pre-clean samples with the 3rd sample testing Inconclusive/Positive. The post-disinfection samples all yielded Non Detectable. This indicates validation of the Proguardeum™ process in disinfecting SARS-CoV-2 from the surfaces in this room.

It was suspected the 3 rooms occupied with COVID-19 patients would still yield predominately, if not all, positives on the post-disinfection sanitization. Viral spread (shedding) through the environment was most likely occurring even after the post cleaning (approximately 2 hours of time elapsed prior to sample collection). However, some non-detects were reported for surfaces after two hours post cleaning.

This field study further suggests that not only does the new environmental test for SAR-CoV-2 work, but that results are consistent with an earlier study of confirmed cases and testing of occupied and unoccupied spaces.

CONCLUSION

The purpose of the field trial was to ascertain the efficacy of a new Novel Coronavirus-19 Environmental Validation RT-qPCR Test under real world conditions. The opportunity to use the test on a facility that had confirmed COVID-19 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 tractability.

This validation testing protocol can be utilized as a powerful tool for businesses, schools, and governments to know what surfaces to clean and whether the selected sanitation methodology was successful as validated by data. With data analytics today and trend monitoring capabilities this test can potentially help to reasonably mitigate the risk of opening the country, provide actionable data to businesses, as well as further our understanding of SARS-CoV-2 and COVID-19 and maybe future pandemic viruses.

This study validates the efficacy of the Proguardeum™ process in disinfecting SARS-CoV-2 from the surfaces that remained untouched by patients both in occupied rooms and unoccupied rooms for an extended period of approximately two hours.

The study further highlighted that results need to be obtained as quickly as possible. Waiting several days or weeks 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 on-site services as well as for localized field laboratories – this will allow potentially contaminated surfaces to be checked and allow contaminated surfaces to be disinfected and validated within hours ensuring our most vulnerable population is adequately protected.

APPENDICES: 5/9/2020 SARS-CoV-2 Environmental Test Results

Client:	Midwest Skilled Nursing Facility
Sampled By:	IWC
Test Requested:	SARS-CoV-2 Real-Time RT-PCR Panel
Sample Type:	Swab
Date Received:	5-08-2020
Date Final:	5-09-2020

Test Site:	Barcode:	Location:	Location Comments:	Date Collected:	Time Collected:	Result:
R202 Pre	81	Room	Bed Rail Upper	5/07/2020	8:32 PM	Inconclusive
R202Pre	80	Room	Wheelchair / Bed Rail Lower	5/07/2020	8:33 PM	**Positive**
R202Pre	77	Room	Footboard and Chair Rail	5/07/2020	8:35 PM	**Positive**
R213Pre	85	Bathroom	Doorknob and Flusher	5/07/2020	8:37 PM	Inconclusive
R213Pre	84	Room	Chair and Footboard	5/07/2020	8:40 PM	**Positive**
R213Pre	78	Room	Table and Remote	5/07/2020	8:42 PM	**Positive**
R214Pre	79	Room	Table and Footboard	5/07/2020	8:46 PM	Inconclusive
R214Pre	76	Room	Door Handle and Chair	5/07/2020	8:48 PM	**Positive**
R214Pre	82	Room	Bathroom Doorknob and Guard Rail	5/07/2020	8:49 PM	**Positive**
R219Pre	83	Room	Table and Door Handle	5/07/2020	8:52 PM	**Positive**
R219Pre	75	Room	Electric Fan Buttons and Wheelchair	5/07/2020	8:53 PM	**Positive**
R219Pre	86	Bathroom	Bathroom Door and Toilet Flusher	5/07/2020	8:56 PM	**Positive**
R202Post	97	Room	Bed Rail Upper	5/07/2020	11:48 PM	Not Detected
R202Post	93	Room	Wheelchair / Bed Rail Lower	5/07/2020	11:49 PM	Not Detected
R202Post	98	Room	Footboard and Chair Rail	5/07/2020	11:51 PM	**Positive**
R213Post	88	Bathroom	Doorknob and Flusher	5/07/2020	11:54 PM	Not Detected
R213Post	87	Room	Chair and Footboard	5/07/2020	11:59 PM	**Positive**
R213Post	96	Room	Table and Remote	5/07/2020	12:00 PM	**Positive**
R214Post	95	Room	Table and Footboard	5/07/2020	12:05 PM	Not Detected
R214Post	91	Room	Door Handle and Chair	5/08/2020	12:07 AM	Not Detected
R214Post	90	Room	Bathroom Doorknob and Guard Rail	5/08/2020	12:09 AM	Not Detected
R219Post	89	Room	Table and Door Handle	5/08/2020	12:12 AM	**Positive**
R219Post	92	Room	Electric Fan Buttons / Wheelchair	5/08/2020	12:13 AM	**Positive**
R219Post	94	Bathroom	Bathroom Door / Toilet Flusher	5/08/2020	12:15 AM	**Positive**

NOTES: The data above is believed to be accurate and represents the best information currently available to IWC Environmental Solutions. Any interpretation or recommendation provided by IWC Environmental Solutions shall be left to the discretion of the client. IWC shall have no liability to the client with respect to decisions or recommendations made, actions taken, or courses of conduct implemented by the client as a result of or based upon the Test Results. In no event shall IWC be liable to the client with respect to the Test Results except for IWC Environmental Solutions' own willful misconduct or gross negligence nor shall IWC Environmental Solutions be liable for incidental or consequential damages or lost profits or revenues to the fullest extent such liability may be disclaimed by law, even if IWC Environmental Solutions has been advised of the possibility of such damages, lost profits or lost revenues. In no event shall IWC Environmental Solutions' liability with respect to the Test Results exceed the amount paid to IWC Environmental Solutions by the client therefor. The data and information on this, and other accompanying documents, represent only the sample(s) analyzed. This report is not to be reproduced in whole or in part without expressed consent of Source Molecular. Results apply to the sample as received.

REFERENCES

1. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, Tamin A, Harcourt JL, Thornburg NJ, Gerber SI, Lloyd-Smith JO. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. New England Journal of Medicine. 2020 Apr 16;382(16):1564-7.
2. He W, Yi GY, Zhu Y. Estimation of the basic reproduction number, average incubation time, asymptomatic infection rate, and case fatality rate for COVID-19: Meta-analysis and sensitivity analysis. medRxiv [Preprint]. [Posted May 5, 2020, Cited May 6, 2020]. Available from: https://doi.org/10.1101/2020.04.28.20083758
3. Centers for Disease Control and Prevention. Guidance for Cleaning and Disinfecting Public Spaces, Workplaces, Businesses, Schools, and Homes. [Cited on May 6, 2002]. Available from: www.cdc.gov/coronavirus/2019-ncov/community/pdf/Reopening_America_Guidance.pdf
4. List N: Disinfectants for Use Against SARS-CoV-2 [Internet]. [Cited on May 6, 2020]. Available from: www.epa.gov/pesticide-registration/list-n-disinfectants-use-against-sars-cov-2
5. Kampf G, Todt D, Pfaender S, Steinmann E. Persistence of coronaviruses on inanimate surfaces and its inactivation with biocidal agents. Journal of Hospital Infection. 2020 Feb 6.
6. Centers for Disease Control and Prevention. CDC 2019-Novel Coronavirus (2019-nCoV) Real- Time RT-PCR Diagnostic Panel (CDC-006-00019, Revision: 03). [Cited on May 6, 2020]. Available from: www.fda.gov/media/134922/download
7. www.thomasnet.com/articles/top-suppliers/rt-pcr-instrument-manufacturers- suppliers/#_What_is_RT-PCR?
8. www.epa.gov/pesticide-registration/list-n-disinfectants-use-against-sars-cov-2