International Journal of Biomedical Science and Engineering
2021; 9(4): 78-82
http://www.sciencepublishinggroup.com/j/ijbse
doi: 10.11648/j.ijbse.20210904.11
ISSN: 2376-7227 (Print); ISSN: 2376-7235 (Online)

Disinfectant Effectiveness Against SARS-CoV-2 and Surrogates Using Cell Culture and RT-PCR

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To cite this article:
Sudha Bhavanam, Mathew Diggle, Jiaao Yu, Xiaoli Lilly Pang. Disinfectant Effectiveness Against SARS-CoV-2 and Surrogates Using Cell Culture and RT-PCR. *International Journal of Biomedical Science and Engineering.* Vol. 9, No. 4, 2021, pp. 78-82.
doi: 10.11648/j.ijbse.20210904.11

Received: August 27, 2021; **Accepted**: September 24, 2021; **Published**: October 21, 2021

Abstract: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) causes the global COVID-19 pandemic. Limited studies have been performed on various types of disinfectants utilized to control the spread of this highly contagious virus. This study aimed to investigate the inactivation of SARS-CoV-2 surrogate virus, hCoV-229E using an *in vitro* to test the anti-infectivity activity of the humidifier buffers (A and B, Lumichem^{TM}). A real-time reverse transcriptase quantitative PCR (RT-qPCR) assay was used to evaluate the effectiveness of these disinfectants on the degradation of viral RNA in a time dependent manner. The effects of disinfectants on viral infectivity were determined using a tissue culture infectious dose (TCID50) assay of a surrogate virus, hCoV-229E, in MRC-5 cell culture. The results demonstrated that the Lumichem^{TM} buffers A and B had a 2 to 3-$\log_{10}$ reduction inactivation using cell culture after a short exposure compared to the control, indicating the disinfection efficacy of the tested anti-infectivity compounds. The Lumichem^{TM} buffers A and B in addition did not affect the viral genomic RNA of a surrogate virus, hCoV-229E, thus representing an additional benefit with a negligible impact to operators and those in close contact when providing in-situ operational cleaning.

Keywords: SARS-CoV-2, Coronavirus, COVID-19, Virus Inactivation, Buffers, RT-PCR

1. Introduction

The recent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing coronavirus disease 2019 epidemic affected all over the globe [1]. Previous studies have reported that due to the long-term survival of SARS-CoV-2 on the human skin surface [2-5] appropriate hand hygiene and wearing a face mask is the most important infection control tool. It has been recommended the use of disinfectants that have complete virucidal activity as a good approach in controlling the infection. Previous studies have suggested that alcohol-based disinfectants such as ethanol and isopropanol are effective against SARS-CoV-2 [6-8]. On the other hand, effectiveness of the disinfection, such as chlorhexidine gluconate and benzalkonium chloride, is unclear [4]. However, in these studies the evaluations were performed only *in vitro* and the evaluation of disinfection under conditions close to actual use is needed to evaluate these disinfectants. Hence, the evaluation of effectiveness of disinfection against SARS-CoV-2 in the environment is very important for preventing transmission. It has also been demonstrated that SARS-CoV-2 can be infectious on inanimate surfaces at room temperature for up to 9 days (reviewed in [4], contamination of frequently touched surfaces are therefore a potential source of viral transmission, studies are currently being carried out, which require appropriate inactivation methods to restrict the spread. Hence, we evaluated the disinfection effectiveness of compounds against clinical hCoV-229E samples in an *in vitro* and their ability to inactivate surrogate virus, hCoV-229E *in vitro*. We aim to measure the stability of viral RNA after varying time of exposure. In addition, viral infectivity was examined using viral culture and tissue culture infectious dose assay (TCID50) of a laboratory hCoV-229E strain. Since hCoV-229E has 79% genomic similarity with SARS-CoV-2 [9], it

was used as the surrogate virus in this study to understand the inactivation by sanitizer and the buffers.

2. Material and Methods

2.1. Human Coronavirus (hCoV)

A laboratory strain of hCoV-229E was purchased from ATCC (ATCC® VR-740™) and cultured in our laboratory.

2.2. Anti-infective Compounds Lumichem™ Buffers A and B

Two buffers of differing concentrations were tested *in vitro* model (Figure 1), and were supplied from an anti-infective company (Lumichem ™ LumiBio Ltd, UK) to establish any effect on presence of potential pathogens. Buffer A was a higher concentration than would be routinely used as an anti-infective agent and equated to 2.5 times the concentration of Buffer B. Buffer B was at the standard level of concentration used as an anti-infective. The buffer mode of action targets specific physiological activities and disrupts critical pathways of the viral cycle preventing transcription/replication without lysing the genomic material of the virus.

2.3. Recovery of hCoV RNA After Exposure to Lumichem™ Buffers A and B

The effect of humidifier buffers A and B on hCoVs RNA was analyzed at 0, 10, 30 and 60 seconds of exposure time at room temperature. Briefly, for each of the buffer tested, two parts of buffer (30 µl) were mixed with one part (15 µl) of virus suspension (hCoV-229E laboratory strain). A RT-qPCR assay was used to evaluate the effectiveness of buffers on the disintegration of viral RNA in a time dependent manner.

2.4. Nucleic Acid Extraction

Viruses on the swabs were eluted with 350 µl of PBS, and then viral RNA was extracted and eluted in 50 µL nuclease free water using MagaZorb® total RNA Mini-Prep Kits (Promega, Madison, WI) as recommended by the manufacturer. Viral RNA extracts were immediately stored at -80°C until testing.

Salmon DNA, as an internal control, was spiked to each sample or RNA extract and quantified by RT-qPCR as indicated in each experiment to monitor amplification inhibition. Five µl of Salomon DNA was added as background DNA to each nucleic acid extraction at a final concentration of 0.5 ng/µl. In addition, 1 µl of Salmon DNA was added to the final reaction mixture after the nucleic acid extraction, such that its efficacy as an inhibition control alone could be assessed.

2.5. Real-time Quantitative RT-PCR

Real-time qPCR was performed by using an ABI 7500 Sequence detection system apparatus with 7000 system software version 1.2.3 (Applied Biosystems, USA). Amplification and detection were carried out in 96-well plates in a 10 µl reaction mixture with 5 µl extracted RNA, 2.5 µl of Taqman fast virus One-Step Reverse Transcriptase qPCR master mix containing ROX as a passive reference dye, 20 uM (SARS-CoV-2); 10 µM (h229E) 10 µM forward and reverse primers, and 10 uM TaqMan probe. Amplification and detection were performed under the following conditions: an initial reverse transcription at 50°C for 30 min followed by PCR activation at 95°C for 20 S, followed by 45 cycles of amplification (denaturation at 95°C for 3 s, annealing at 60°C for 30 s. The cycle threshold (*Ct*) represented the refraction cycle number at which a positive amplification observed was measured. The negative RT-qPCR result was set as Ct value equal to 40. The primes (Integrated DNA Technologies Coralville, IA) and probe (Applied Biosystems, USA) used for RT-qPCR reactions are listed in Table 1.

Table 1. *Primer and Probe for RT-qPCR.*

Primer/Probe	Target	Sequence (5' – 3')	Reference
229E_Forward	Membrane protein gene (M)	TTCCGACGTGCTCGAACTTT	[14]
229E_Reverse		CCAACACGGTTGTGACAGTGA	
229E_Probe		TCCTGAGGTCAATGCA (NFQ-MGB)	

2.6. Viral Culture and Determination of Inactive Effectiveness

2.6.1. Cell Culture

Medical Research Council cell strain 5 (MRC-5) were purchased from ATCC. Cells were cultured using Modified Eagle Medium (MEM) supplemented (Sigma-Aldrich) with 10% fetal bovine serum (FBS), 2 µM/mL of L-Glutamine and 100 µg/ml of Gentamicin (Gibco; ThermoFisher). The cells were incubated in a 37°C in a 5% CO_2 incubator. When the cells reached 80% confluency, they were used for the inactivation test of the viruses on the different material surfaces.

2.6.2. In Vitro Model for Inactivation of hCoV-229E by Lumichem™ Buffers A and B

Briefly, two parts of buffer A and B (30 µl) were mixed with one part (15 µl) of virus suspension and the inactivation of hCoV-229E were tested in MRC-5 cells at time points 0, 5 minutes and 1, 2, 4, 6 and 24 hours. Samples were collected and analysed for the inactivation of hCOV-229E using cell culture.

2.6.3. Cell Culture and TCID50

Viral titers were determined using the TCID50 method in MRC-5 cells. Cells were inoculated with 10-fold serial dilutions of samples collected in 100 µl MEM (Sigma-Aldrich) supplemented with 10% FBS, 2 µM/mL of L-glutamine and 100

µg/ml of gentamicin in 96 well pates and incubated for 1 h at 37°C and 5% CO_2 atmosphere with 95% humidity. For each dilution step, four wells of replicates were inoculated. After one hour of incubation the inoculum was removed and 300 µl of maintenance MEM supplemented with 1% FBS, 2 µM/ml of L-glutamine and 100 µg/ml of gentamicin was added to each well. Cells were incubated for seven days and scored for cytopathic effect (CPE) on a daily basis. hCoV 229E induced CPE of infected cells was determined by observing rounded, detached cells in close association to each other. Evidence of inactivation was determined by absence of CPE in MRC-5 cells, indicating loss of infectivity. The TCID50 was calculated via the Reed-Muench formula [10]. The log-value was a measure of the effectiveness of the disinfectant in inactivating the virus. This value was calculated by the following formula: Inactivation log-value=log N_0−log N_x where N_0 is the $TCID_{50}$ of the control group, and N_x is the $TCID_{50}$ of the test group.

2.7. Statistical Analysis

All the graphs and statistical analyses were performed using GraphPad Prism software version 8.0 for windows (GraphPad Software, San Diego, CA, USA). The comparison of means between different groups of numerical variables was performed using non-parametric two-way ANOVA followed by the Friedman test, and a *p* value less than 0.05 was considered as statistically significant.

3. Results and Data Analysis

3.1. Time Dependent Effect of Lumichem™ Buffers A and B on Viral RNA Using RT-qPCR

Two parts of buffer (30µl) were mixed with one part of virus (15µl) suspension and the reduction in the viral RNA was measured at concentrations ($1x10^7$, $1x10^6$ and$1x10^5$ viral particles) and at varying time points (0, 10, 30 and 60 seconds) The results from the RT-qPCR demonstrate that both buffers A and B have similar Ct values in the test groups as control groups indicating that the buffers have no inhibitory effect on laboratory strain of hCoV-229E viral RNA and no obvious time-dependent reduction of viral RNA was observed even at longer exposure time (24 hours) (Table 2).

3.2. Inactivation of hCoV-229E by Lumichem™ Buffers A and B Using Cell Culture and TCID50

To examine the viral inactivation effect of buffer A and B virus stocks were exposed to buffers for varying amounts of time and the recovered suspension were placed in 96-well tissue culture plates, as indicated in Figure 2. Exposure of hCoV-229E to buffer A resulted in 2 log reduction at 5 minutes compared to control group (no exposure) with time-dependent inactivation up to 24 hours (Figure 1). The virus was almost completely inactivated and close to the detection limit (≤1.58 $TCID_{50}$ (log_{10}) per ml) within 24 hours.

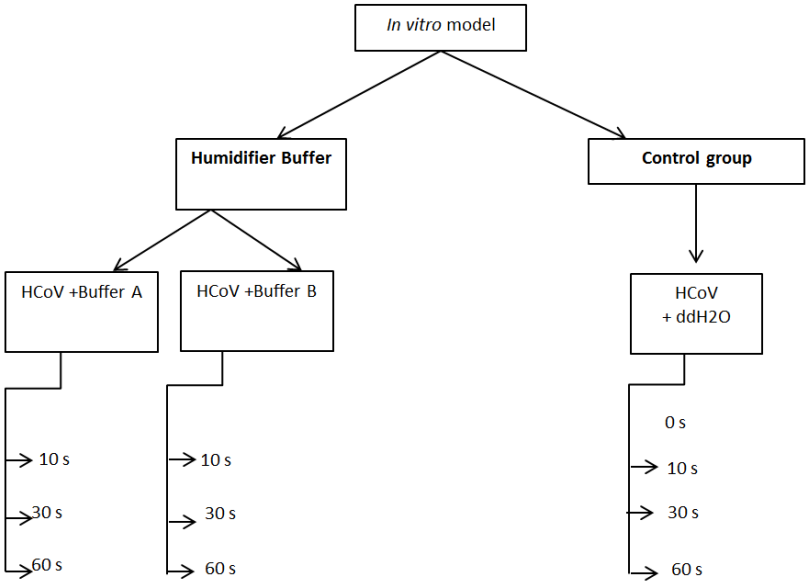

Figure 1. *Schematic diagram of the in vitro model to test the effectiveness of the humidifier buffers; s-seconds.*

Table 2. *Effectiveness of Lumichem™ buffers A and B against SARS-CoV-2 by RT-qPCR.*

Time (s)	hCoV-229E (1X10^6)			hCoV-229E (1X10^5)			hCoV-229E (1X10^4)		
	dH_20	Buffer A	Buffer B	dH_20	Buffer A	Buffer B	ddH_20	Buffer A	Buffer B
0	19.74	N/A	N/A	21.95	N/A	N/A	24.92	N/A	N/A
10	19.68	20.13±0.31	19.65±0.40	21.99	21.73±0.05	22.49±0.33	25.33	25.33±0.24	25.1±0.07
30	19.60	20.67±0.13	20.14±0.37	21.94	21.78±0.10	21.97±0.28	25.34	25.65±0.02	24.89±0.02
60	19.37	19.74±0.13	19.56±0.21	22.30	21.62±0.07	22.32±0.12	25.37	22.53±0.05	25.60±0.11

Results are represented as mean ± SD

* Negative RT-PCR results was set as Ct value at 40

N/A not available for RT-qPCR analysis.

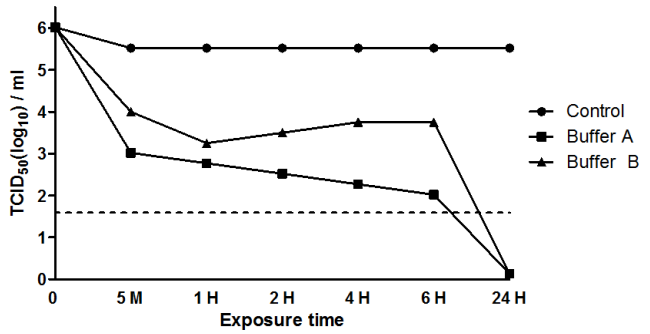

Figure 2. *Effect of Lumichem™ buffers A and B on the infectivity of hCoV 229E. Two parts of buffer A or B were mixed with one part of virus suspension and the samples were collected at each time point and titrated in MRC-5 cells. The results shown are representative of duplicates. Control group (without buffer treatment) were treated identically at each time point. Samples were titrated in MRC-5 cells using four replicates. The dotted line denotes the limit of detection of the assay.*

4. Discussion

Currently, there is a scarcity of evidence-based information on the effectiveness of various disinfectants to inactivate SARS-CoV-2. Although some work has been published on disinfection of other coronaviruses, whether this could be applied to SARS-CoV-2 had not been well-known [4]. In this study we intended to show whether commonly used disinfectants and disinfection procedures are effective to inactivate SARS-CoV-2.

The results from our study support the recommendations that these compounds may effectively control SARS-CoV-2 transmission and the pigskin model can be used as an *in vitro* screening method to compare the immediate and persistent antimicrobial efficacies of various products and to select an effective product for the elaborate *in vivo* study. There are several *in vivo* methods of testing the antimicrobial efficacy of a product against pathogenic bacteria and viruses [11]. However, these tests are not easy to perform, and because these tests involve human subjects, they cannot be used to test a product's disinfectant efficacy against many of the pathogenic microorganisms. Testing antimicrobial preparations with an inanimate surface does not reflect their true activity, which has generally been found to be much lower when applied to the skin [12].

In this study the efficacy of two buffers of differing concentrations Buffer A (higher concentration) and Buffer B (lower concentration) was tested *in vitro* for its effectiveness against of hCoV-229E. The results from this study indicate that Buffer A and B did not have any effect on the viral genome of SARS-CoV-2. However, the predictable virucidal efficacy usually is expressed as minimal $\log_{10}$ reduction in viral titer. The U.S. Environmental Protection Agency detailed in its 2018 disinfectant product guidance [13] that if the cytotoxicity is present in the cell-based infectivity assays the virus control titer should establish a$\geq$3 $\log_{10}$ reduction in viral titer beyond the cytotoxic level and the controls (cells not infected with virus) be negative of infectivity In this study we observed that there was no cytotoxic effect of the products studied in MRC5 cell lines indicating that they do not have the inhibitory effect on MRC5 cells. A 3 $\log_{10}$ reduction values to below detection limit was observed for humidifier buffer B and 2 $\log_{10}$ to below detection limit reduction was observed by humidifier buffer A in relatively short contact times (i.e., 5 min to 24 hours). The contact times required for humidifier buffers (A and B) were 5 min. We speculate that the possible mode of actions of the agents evaluated in this study have unique mechanisms of action. The mode of action for the humidifier buffers tested in this study may target specific physiological activities and disrupts critical pathways of the viral cycle preventing transcription/replication without lysing the genomic material of the virus, As the disinfectant effect of humidifier buffers can last after the application, The above results suggest the potential effectiveness of the humidifier buffers used in this study.

5. Conclusion

This initial evaluation is part of a specifically developed and highly sensitive collection of assays for the recovery and detection of a range of coronaviruses including SARS CoV-2 on a range of surrogate surface models. The environmental conditions were generated to represent abnormally high concentrations of virus which would not normally be detected on a routine basis. The results suggest that these novel methods of detection of pathogens were successful and highlighted the significant value such protocols have in the absence of a current gold standard. Moreover, the humidifier buffers used displayed a rapid and significant virustatic effect on the viability of coronaviruses with a unique mode of action which does not affect RNA and has no cytotoxic effect on cells, demonstrating a preventative measure on viral replication and proliferation compared to alternative modes of treatment considered virucidal used by traditional disinfectants.

Competing Interests

The author (s) declare no competing interests.

Funding

This research is supported by the industry collaboration funds (RES0050867).

Ethics

This study was approved by the Research Ethics Boards of the University of Alberta.

Acknowledgements

Authors would like to thank Dr. Yuanyuan Qiu for providing the technical guidance for the study, LumiBio UK

and Dr Andrew Metcalfe for the support and provision of compounds for testing.

References

[1] Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. *Int. J. Antimicrob. Agents* 55 (3), 105924 (2020).

[2] Hirose R, Ikegaya H, Naito Y *et al.*: Survival of SARS-CoV-2 and influenza virus on the human skin: Importance of hand hygiene in COVID-19. *Clin. Infect. Dis.* (2020).

[3] Chan KH, Sridhar S, Zhang RR *et al.*: Factors affecting stability and infectivity of SARS-CoV-2. *J. Hosp. Infect.* 106 (2), 226-231 (2020).

[4] Kampf G, Todt D, Pfaender S, Steinmann E: Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. *J. Hosp. Infect.* 104 (3), 246-251 (2020).

[5] Hirose R, Ikegaya H, Naito Y *et al.*: Reply to Gracely. *Clin. Infect. Dis.* (2021).

[6] Mukherjee S, Vincent CK, Jayasekera HW, Yekhe AS: Antiviral efficacy of personal care formulations against Severe Acute Respiratory Syndrome Coronavirus 2. *Infect. Dis. Health.* 26 (1), 63-66 (2021).

[7] Leslie RA, Zhou SS, Macinga DR: Inactivation of SARS-CoV-2 by commercially available alcohol-based hand sanitizers. *Am. J. Infect. Control* 49 (3), 401-402 (2021).

[8] Kratzel A, Todt D, V'kovski P *et al.*: Inactivation of Severe Acute Respiratory Syndrome Coronavirus 2 by WHO-Recommended Hand Rub Formulations and Alcohols. *Emerg. Infect. Dis.* 26 (7), 1592-1595 (2020).

[9] Chen Z, Boon SS, Wang MH, Chan RWY, Chan PKS: Genomic and evolutionary comparison between SARS-CoV-2 and other human coronaviruses. *J. Virol. Methods* 289, 114032 (2021).

[10] Ramakrishnan MA: Determination of 50% endpoint titer using a simple formula. *World J. Virol.* 5 (2), 85-86 (2016).

[11] Ijaz MK, Whitehead K, Srinivasan V *et al.*: Microbicidal actives with virucidal efficacy against SARS-CoV-2. *Am. J. Infect. Control* 48 (8), 972-973 (2020).

[12] Maillard JY, Messager S, Veillon R: Antimicrobial efficacy of biocides tested on skin using an ex-vivo test. *J. Hosp. Infect.* 40 (4), 313-323 (1998).

[13] U.S. Environmental Protection Agency. *Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing*. [EPA 712-C-17–004] (2018), https://www.regulations.gov/document?D=EPA-HQ-OPPT-2009-0150-0036.

[14] Vijgen L, Keyaerts E, Moes E, Maes P, Duson G, Van Ranst M: Development of one-step, real-time, quantitative reverse transcriptase PCR assays for absolute quantitation of human coronaviruses OC43 and 229E. *J. Clin. Microbiol.* 43 (11), 5452-5456 (2005).