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Short Report

Alcohol-free hand sanitizer and other quaternary ammonium disinfectants quickly and effectively inactivate SARS-CoV-2

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S U M M A R Y

Background: SARS-CoV-2 is the virus responsible for the current global pandemic, COVID-19. Because this virus is novel, little is known about its sensitivity to disinfection.
Methods: We performed suspension tests against SARS-CoV-2 using three commercially available quaternary ammonium compound (Quat) disinfectants and one laboratory-made 0.2% benzalkonium chloride solution.
Findings: Three of the four formulations completely inactivated the virus within 15 s of contact, even in the presence of a soil load or when diluted in hard water.
Conclusion: Quats rapidly inactivate SARS-CoV-2, making them potentially useful for controlling SARS-CoV-2 spread in hospitals and the community.

Introduction

Coronaviruses are enveloped viruses that commonly cause upper respiratory tract infections in humans and animals. Four known coronaviruses cause the common cold in humans, while another three have caused deadly outbreaks in the past 20 years, including SARS-CoV-2, the agent of coronavirus disease 2019 (COVID-19). COVID-19 has been particularly devastating, thus enhanced disinfection and other preventative measures against SARS-CoV-2 have been adopted worldwide to limit its spread.

Because SARS-CoV-2 is both a novel virus and a biosafety level-3 (BSL-3) agent, disinfection data for this specific virus are scarce. Consequently, studies using other coronaviruses have been used to draw conclusions about which disinfectants are most effective against it. While this approach is useful, it is also inherently speculative, because even viruses within the same family can respond differently to a given disinfectant [1]. In addition, experts disagree about which disinfectants should work best against SARS-CoV-2, especially when it comes to quaternary ammonium compounds (Quats). For instance, one prominent review article reported that benzalkonium chloride (a Quat) was probably "less effective" against SARS-CoV-2,

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Table I
Disinfectant properties

Product (Manufacturer Name)	Intended use for product	Active ingredient (concentration)	Suggested contact time (EPA List N)
Benzalkonium chloride (Beantown Chemical, Hudson, NH, USA)	Laboratory chemical	Benzalkonium chloride (0.2%)	N/A
Qimei® Hand Sanitizing Wipes (Zhejiang Qimei Commodity Company, Haining City, Zhejiang, China)	Sanitizing hands	Benzalkonium chloride (0.13%)	N/A
Cavicide (Metrex Research LLC, Orange, CA, USA)	Disinfecting medical devices, non-porous surfaces in healthcare facilities	Diisobutylphenoxyethoxyethyl dimethyl benzyl ammonium chloride (0.28%), isopropanol (17.20%)	2 min
Clean Quick (Procter & Gamble Company, Cincinnati, OH, USA)	Disinfecting food contact surfaces, dishes in third compartment sinks	Alkyl dimethyl benzyl ammonium chlorides (0.15%), alkyl dimethyl ethylbenzyl ammonium chlorides (0.15%)	10 min

N/A, not applicable.

which was cited by the Centers for Disease Control (CDC) of the United States as a reason to avoid using benzalkonium chloride-based hand sanitizer products [2,3]. At the same time, the Environmental Protection Agency (EPA) of the United States and Health Canada both list a benzalkonium chloride product on their official list of disinfectants recommended for use against SARS-CoV-2 [4]. Many nosocomial outbreaks of COVID-19 have been documented to date, and hospitals need laboratory-documented data for effective decontamination. Clearly, more research is needed in this area to help stem the current pandemic.

In this study, we tested 0.2% benzalkonium chloride and three commercial Quat disinfectants against SARS-CoV-2. The three commercial disinfectants we tested were Cavicide®, a widely used Quat hospital disinfectant; Clean Quick® Broad Range Quaternary Sanitizer, a multi-use Quat disinfectant safe for food contact surfaces; and fluid extracted from Qimei® Hand Sanitizing Wipes, a benzalkonium chloride household product. Specific details, including the compound used, the concentration tested, and the source for each disinfectant are included in Table I. A suspension test method was used, and surviving virus was assayed using a plaque assay with Vero E6 (ATCC CRL-1586) cells. We found that all four compounds, except for Clean Quick, effectively inactivated SARS-CoV-2 within 15 s of contact and in the presence of organic soil loads. In addition, dilution of the disinfectants in hard water had no significant effect on virus inactivation.

Methods

SARS-CoV-2 virus stocks (isolate USA-WA1/2020, NR-52281, obtained from BEI Resources, Manassas, VA, USA) with a concentration of 1×10^7 plaque forming units (pfu)/mL were used to prepare test suspensions containing 5% bovine serum albumin (BSA) or 0.5% bovine mucin. These suspensions were then exposed to the disinfectants for various contact times and held at room temperature. The ratio of virus inoculum to total solution was 1:10 by volume: one part virus per nine parts disinfectant. After the specified contact time, the disinfectant–virus mixtures were then diluted 1:10 into a neutralizer solution except for the Qimei wipe liquid, which was diluted 1:20 into neutralizer to address cytotoxicity concerns. Serial 1:10 dilutions of the neutralized mixtures were performed in sterile 1× PBS, and 200-μL aliquots of the resulting solutions were added to Vero E6 cells after removal of cell culture media. Plating was done in duplicate, and cells were then incubated at 37°C for 1 h. After incubation, 1.5 mL of overlay medium containing complete DMEM and 1.5% SeaPlaque™ GTG™ agarose (Lonza, Rockland, ME, USA) was added to each well, and cells were incubated for 72 hours at 37°C to allow plaque formation.

Once plaques were established, a 10% formaldehyde solution was added for 1 h to inactivate infectious virus, and top agar was removed. Cells were stained with 1% crystal violet, and plaques were counted manually using microscopy. All experiments were performed in a BSL-3 laboratory, with approval from the Institutional Biosafety Committee at Brigham Young University (protocol 2020-0069). Each test was then repeated on at least two different dates. More detailed information about our methods is included in the Supplementary Material.

Table II
Test results

Product	Concentration	Exposure time	Reduction in virus titre (pfu/mL, log 10)			
			No soil load	0.5% Mucin	5% BSA	Product diluted in hard water
Benzalkonium chloride	0.2% w/w in water	15 s	>3.19*	>2.97*	2.09	>3.19*
		30 s	>3.02*	>2.93*	>2.68*	>2.72*
Qimei Wipes	Undiluted	15 s	>2.97*	>2.64*	>2.64*	N/A
		30 s	>2.77*	>2.64*	>2.64*	N/A
Cavicide	Undiluted	15 s	>3.19*	>2.97*	>2.97*	N/A
		30 s	>2.88*	>2.97*	>2.97*	N/A
Clean Quick	200 parts per million in water	15 s	1.49	0.31	0.00	0.44
		30 s	>2.88*	0.57	0.42	1.86

N/A: these products were not tested with hard water because they are sold ready to use; they did not specify dilution prior to use.
* For these tests, the amount of inactivation detected was the maximum possible inactivation level the assay was able to detect. Variation in log reduction value for these data points is due to variation of the titre on different test dates, not variation in the inactivation activity of the disinfectant.

In addition to the test method, two simultaneous controls were run: a titre and a neutralizer control. For the titre control, serial 1:10 dilutions of the virus were performed in 1x PBS, and the virus was plated as described above. For the neutralizer control, each disinfectant was diluted 1:10 in neutralizer, and immediately after mixing, approximately 10^5 pfu of virus was added. After incubation at room temperature for 10 min, serial 1:10 dilutions were then performed in 1x PBS, and plating was performed as described above. The purpose of this control was to ensure that neither the neutralizer nor the neutralized disinfectants were cytotoxic or virucidal, and to ensure that the neutralizer effectively stopped the activity of the disinfectant.

Results and discussion

Full results of suspension tests are listed in Table II. Each of the compounds except Clean Quick was highly effective at inactivating SARS-CoV-2 within 15 s of contact and in the presence of a mucin or BSA soil load. Clean Quick was effective after 30 s of contact time in the absence of a soil load, which matches its intended use. The manufacturer's website states that Clean Quick is supposed to be used as either a "non-rinse sanitizer for third-sink sanitizing of dishes" or a sanitizer for pre-cleaned surfaces, either way with a contact time of at least 1 min. Under those conditions, it is likely to be effective against SARS-CoV-2 [5].

These results show that Quats are effective at inactivating SARS-CoV-2. Quats are already the most widely represented class of disinfectants on EPA's List N, the agency's official list of disinfectants recommended for use against SARS-CoV-2 based on prior studies with other viruses [4]. In addition, in July 2020, the EPA officially approved SARS-CoV-2 efficacy claims for 13 disinfectants based on laboratory testing with SARS-CoV-2 itself, and all of those disinfectants list Quats as their only active ingredients [4,6]. Clearly, Quats can be an effective tool for helping food establishments, hospitals, and the general public to control SARS-CoV-2.

Perhaps the most significant finding of this study is that benzalkonium chloride hand sanitizer is effective at inactivating SARS-CoV-2, which is useful for healthcare professionals to know. Since alcohol-based hand sanitizer is the only type recommended by the CDC and also the only type given expedited manufacturing approval by the U.S. Food and Drug administration, alcohol hand sanitizer has overwhelmingly been the dominant choice for SARS-CoV-2 control in the U.S., leading to acute supply shortages in the U.S. and elsewhere [3,7]. However, benzalkonium chloride has several advantages over alcohol for hand disinfection: it is non-toxic, less irritating to skin, and non-flammable [8]. In fact, switching from alcohol to benzalkonium chloride hand sanitizer can lead to better hand hygiene compliance from healthcare workers, possibly decreasing overall viral contamination of their hands [8]. Since both alcohols and Quats inactivate this virus, healthcare facilities and providers should choose a hand sanitizer product for COVID-19 control based on comfort, cost, and availability. In light of these results, the FDA should consider giving expedited approval to manufacturers of benzalkonium chloride hand sanitizers, thereby making both types of hand sanitizers more available.

To our knowledge, only two studies have been conducted on Quats and SARS-CoV-2 disinfection to date. Chin *et al.* tested 0.1% benzalkonium chloride against SARS-CoV-2 with no soil load, which was effective after a 5-min contact time [9]. Ijaz *et al.* went further, testing a 0.19% alkyl dimethyl benzyl ammonium chloride disinfectant with a 5% FBS soil load, which was effective after a contact time of 2 minutes [10]. Both studies used $TCID_{50}$ assays and suspension tests, although shorter contact times were not reported.

This study explores shorter, more commonly used contact times than previous studies, adding meaningful information to our understanding of SARS-CoV-2 disinfection. Unique features of these experiments include the relatively short contact times tested (15 s and 30 s) and the organic and inorganic matter added during testing, which included mucin, BSA, and hard water. These variables were designed to simulate real-world conditions: in practice, surfaces contaminated with SARS-CoV-2 probably contain mucus or other organic materials, and water used to dilute disinfectants may contain hard water ions. Another unique aspect of this study was the use of plaque assays to quantify viable SARS-CoV-2 virus. As a result, the number of infectious virus particles was determined, allowing precise log reductions to be calculated. By comparison, $TCID_{50}$ assays are less precise because any cytopathic effects at a given dilution are scored equally; similarly, genome copy number assays may detect genomes from non-infectious particles. In addition, most users of surface disinfectants do not leave them on surfaces for very long. Quat compounds, in particular, are known to vary in activity with changes in disinfectant formulation or soil load, thus accounting for these factors in laboratory tests is especially important [1].

We conclude that Quats are effective disinfectants for the inactivation of SARS-CoV-2. Beyond merely inactivating the virus, Quats act quickly, making them practical for use in healthcare settings where prompt disinfection is important. In particular, benzalkonium chloride hand sanitizer products could be used as effective alternatives to alcohol-based products, which may help reduce supply shortages and contribute to the containment of COVID-19.

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Conflict of interest statement
The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2020.11.023.

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