Department of Homeland Security Science and Technology Directorate Emerging Results: Evaluation of Disinfectant Efficacy Against SARS-CoV-2

Background

In order to control the spread of SARS-CoV-2, effective disinfectants are required, among other complementary measures such as social distancing and use of personal protective equipment (PPE). The Department of Homeland Security (DHS) Science and Technology Directorate (S&T) is executing laboratory studies to evaluate a panel of disinfectants (Table 1) for use against SARS-CoV-2. This document summarizes current methods and results using 70% isopropyl alcohol (IPA) and 0.26% sodium hypochlorite (bleach) as of the end of April 2020.

Table 1. Disinfectants Being Tested Against SARS-CoV-2 at NBACC

Active Ingredient(s)	Commerical Product Tested[†]	Comments
70% isopropyl alcohol	CiDehol® 70 (Decon Labs, Inc.)	Commonly found in alcohol wipe pads for medical procedures and electronics
0.26% sodium hypochlorite	1:32 Clorox® Germicidal Bleach (8.25%)	
70% ethanol	N.A., prepared using laboratory grade ethanol	Active ingredient in common hand sanitizer gels (e.g., Purell®)
Quaternary ammonium 1.1856% Alkyl (50% C14, 40% C12, 10% C16) dimethyl benzyl ammonium chlorides	Lysol®	
3% hydrogen peroxide	N.A., prepared using over the counter hydrogen peroxide solution	
Dual quaternary ammonium with surfactant 1-5% 4-Nonylphenol, branched, ethoxylated 1-3% Alkyl (68% C12, 32% C14) dimethyl ethylbenzyl ammonium 1-3% Alkyl Dimethyl Benzyl Ammonium Chloride (C12-C18)	Micro-Chem Plus™ Detergent Disinfectant (National Chemical Laboratories, Inc.)	Used in biocontainment laboratories and hospitals
Peracetic acid	Peraspray (Enviro Tech Chemical Services, Inc.)	
Acidified bleach (0.5% sodium hypochlorite/ 1% acetic acid)	Prepared using Clorox® Germicidal Bleach and Heinz® Distilled White Vinegar	Effective against Ebolavirus in previous study; short shelf life (hours)

[†]Evaluation of commercial products is not intended to be a DHS endorsement. These products were chosen based on product availability and their representation of a given disinfectant class.

Materials and Methods

Cell Culture

Vero (ATCC® CCL-81™) cells were used for propagation and microtitration of SARS-CoV-2. Cells were cultured at 37°C and 5% CO_2 in complete growth medium (gMEM) as previously described.[1] A VIAFILL reagent dispenser (INTEGRA Biosciences Corp.) was utilized to seed cells into 96-well, clear-bottom plates for virus microtitration assays. Cells were seeded at a density to achieve 100% confluency on the day of infection.

Virus Stock Production

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate USA-WA1/2020, NR-52281, was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH. This virus (passage 4) was propagated twice in Vero cells to yield working stocks (passage 6) that were used for this work. The viral stocks were sequenced and found to match the consensus sequence previously described (MN985325.1). All work with SARS-CoV-2 was performed at biosafety level-3 containment.

Test Matrix

SARS-CoV-2 was diluted 1:10 in simulated saliva to characterize the virus in a relevant bodily fluid. Simulated saliva was prepared according to previous recipes (Sup. Tbl. 1)[2,3] with the exceptions of KH_2PO_4 and K_2HPO_4, which were present at 15.4 mM and 24.6 mM, respectively. The simulated saliva was characterized for its pH, surface tension, viscosity, percent solids, and protein content and was found to be similar to previous reports.[2, 3] Simulated saliva was stored at 4°C for up to two weeks.

Viral Microtitration Assay

Virus-containing samples were serially-diluted (10^{-1} through 10^{-4}) in 96-well, clear bottom plates containing confluent monolayers of Vero cells. For each dilution, a total of ten replicate wells were infected. The infected plates were incubated at 37°C and 5% CO_2 for 4 days, and then individual wells were visually inspected using a Nikon TS100 microscope for the presence of virus-induced cytopathic effects (CPE) at each dilution as compared to a negative, media-only control. The median tissue culture infectious dose ($TCID_{50}$) for each sample was estimated using the Spearman-Karber method.[4, 5]

Disinfectants

Initial studies were conducted using 70% IPA and bleach, as these disinfectants were completely neutralized by dilution with gMEM and did not result in cytotoxic effects when applied to Vero cells (data not shown). CiDehol 70 (Decon Labs, Inc.), a ready-to-use 70% IPA spray, was used without preparation. Clorox Germicidal bleach (8.25% sodium hypochlorite) was diluted 1:32 in distilled water prior to use to yield a 0.26% sodium hypochlorite solution.

Disinfectant Efficacy

Disinfection of SARS-CoV-2 using 70% IPA and bleach was assessed using a method based upon ASTM International standard E2197. In brief, 10 µL of SARS-CoV-2 stock or gMEM (for disinfectant neutralization

controls) was diluted 1:10 in simulated saliva and then deposited onto 19 mm circular 304 stainless steel coupons. One set of coupons was immediately processed when the virus was in a wet droplet, while a second set of coupons was incubated under ambient conditions (room temperature and humidity) to allow the virus to dry to a film on the coupon. For disinfectant efficacy tests (n = 3), 50 µL of disinfectant was added to each coupon and permitted to incubate for a specified contact time. Several controls were performed in parallel and included virus recovery controls (n = 3) in which 50 µL of gMEM was added to each coupon in lieu of the disinfect and disinfectant neutralization controls (n = 3) in which virus was added post-neutralization, as described below, to ensure there was no remaining active disinfectant. All coupons were incubated for the appropriate disinfectant contact time, and then transferred to conical tubes containing 4 mL gMEM. The tubes were vortexed for 30 seconds at 2400 RPM to resuspend virus from the coupon surface. In addition, for disinfectant neutralization controls, 10 µL of SARS-CoV-2 virus was added. Infectivity in all samples was quantified via viral microtitration assay as described above. The infectivity reduction factor (RF) and % neutralization were determined as follows:

$$RF = \text{mean } \log_{10}TCID_{50}/mL \text{ (recovery ctl.)} - \text{mean } \log_{10}TCID_{50}/mL \text{ (disinfectant efficacy)}$$

$$\% \text{ neutralization} = 100*[1-1/10^{RF}]$$

For samples where disinfection resulted in no residual infectivity, the RF was calculated using the lower limit of quantitation for our virus microtitration assays, which was 0.2 log $TCID_{50}$/mL.

Emerging Results

For these disinfection tests, SARS-CoV-2 was either used directly as a virus stock in cell culture medium, or first diluted 1:10 in simulated saliva to represent a relevant contamination source from COVID-19 patients. These studies were based on the ASTM 2197 standard, in which droplets are applied to stainless steel coupons and then treated with disinfectant without agitation. This standard essentially models a worst-case cleaning scenario where disinfectant is simply sprayed on surface without any follow up wiping or scrubbing.

The contact time for the 70% IPA solution tested was not defined by the manufacturer, and so we chose to evaluate a short contact time of 30 seconds, as this would be useful in an operational context for DHS components. For virus suspended in culture medium, 70% IPA resulted in a > 99.4% (RF = 2.20 $\log_{10}$) reduction of SARS-CoV-2 infectivity for dried virus and a > 99.9% (RF = 3.03 $\log_{10}$) reduction for wet droplets, respectively. For virus in simulated saliva, 70% IPA resulted in a > 96.8% (RF = 1.50 $\log_{10}$) reduction of infectious virus for dried droplets and a > 99.2% (2.10 $\log_{10}$) reduction for wet droplets.

The contact time for the dilute bleach solution recommended by the manufacturer was 5 minutes. For virus suspended in culture medium, bleach resulted in a > 99.9% (RF = 3.07 $\log_{10}$) reduction of SARS-CoV-2 infectivity for dried virus and a > 99.9% (RF = 3.13 $\log_{10}$) reduction for wet droplets, respectively. For virus in simulated saliva, bleach resulted in a > 96.8% (RF = 1.50 $\log_{10}$) reduction of infectious virus for dried droplets and a > 99.0% (2.00 $\log_{10}$) reduction for wet droplets.

The lower percent reduction for dried virus compared to wet virus is a result of virus inactivation that occurs during the drying process, which results in a lower amount of viable virus present on the coupon prior to the addition of the disinfectant.

In summary, these results provide evidence that 70% IPA (30 seconds) and 0.26% sodium hypochlorite (5 minutes) are effective means to reduce SARS-CoV-2 contamination in saliva on a hard, nonporous surface.

Ongoing and Future Studies

Current efforts are focused on developing methods to neutralize cytotoxicity of the remaining disinfecting agents in the panel to enable testing. Unlike simple dilution into culture medium, which was effective for 70% IPA and dilute bleach, these agents must be chemically neutralized or removed from test sample to enable determination of residual viral infectivity. Current efforts are focused on using centrifugal concentrating filters that can retain virus while allowing disinfectant chemicals to pass through the filter. Upon development of these methods, further results will be made available on the efficacy of these disinfectants.