

ORIGINAL ARTICLE

Residual antimicrobial coating efficacy against SARS-CoV-2

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Funding information

United States Environmental Protection Agency, Grant/Award Number: 68HERC20F0231

Abstract

Aims: This study evaluated the residual efficacy of commercially available antimicrobial coatings or films against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on non-porous surfaces.

Methods and Results: Products were applied to stainless steel or ABS plastic coupons and dried overnight. Coupons were inoculated with SARS-CoV-2 in the presence of 5% soil load. Recovered infectious SARS-CoV-2 was quantified by TCID₅₀ assay. Tested product efficacies ranged from <1.0 to >3.0 log₁₀ reduction at a 2-h contact time. The log₁₀ reduction in recovered infectious SARS-CoV-2 ranged from 0.44 to 3 log₁₀ reduction on stainless steel and 0.25 to >1.67 log₁₀ on ABS plastic. The most effective products tested contained varying concentrations (0.5%–1.3%) of the same active ingredient: 3-(trihydroxysilyl) propyldimethyloctadecyl ammonium chloride. Products formulated with other quaternary ammonium compounds were less effective against SARS-CoV-2 in this test.

Conclusions: The residual antimicrobial products tested showed varied effectiveness against SARS-CoV-2 as a function of product tested. Several products were identified as efficacious against SARS-CoV-2 on both stainless steel and ABS plastic surfaces under the conditions evaluated. Differences in observed efficacy may be due to variation in active ingredient formulation; efficacy is, therefore, difficult to predict based upon listed active ingredient and its concentration.

Significance and Impact: This study highlights the formulation-specific efficacy of several products against SARS-CoV-2 and may inform future development of residual antiviral products for use on non-porous surfaces. The identification of antimicrobial coatings or films showing promise to inactivate SARS-CoV-2 suggests that these products may be worth future testing and consideration.

KEYWORDS

antimicrobial coating, COVID-19, disinfection, SARS-CoV-2, virucide

INTRODUCTION

The global outbreak of coronavirus disease 2019 (COVID-19), caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), sheds an unprecedented spotlight on infection control procedures in the public sector. Although the primary route of infection and transmission of SARS-CoV-2 is via respiratory droplet and aerosols (Meyerowitz et al., 2021), fomites and touch-surface transmission may represent a secondary risk (van Doremalen et al., 2020; Hulkower et al., 2011; Kampf et al., 2020; Marques & Domingo, 2021). Contamination of surfaces and environments by infected individuals is of special concern for public transportation, hotels and motels, airport lounges and other public-facing locations as communities re-open and human travel resumes. Studies with epidemiologically relevant pathogens (human parainfluenza virus, norovirus) show that pathogen persistence on inert surfaces in high-touch areas (e.g. schools, offices, cruise ships and others) can contribute to viral transmission and community spread (Boone & Gerba, 2007; Bright et al., 2010; Stobnicka et al., 2018). Disinfection of high-touch surfaces is of critical importance when reducing the spread of environmentally transmitted pathogens, including SARS-CoV-2. SARS-CoV-2 has been reported to survive for 2–3 days on plastic and stainless steel surfaces (van Doremalen et al., 2020), presenting a challenge for infection control.

The COVID-19 pandemic has accelerated the need for proven effective surface decontaminants, particularly those which offer longer term residual activity or prolonged protection. The US Centers of Disease Control and Prevention (CDC) recommendation for disinfection of SARS-CoV-2 is to use an Environmental Protection Agency (EPA)-registered product on List N (Disinfectants for Use Against SARS-CoV-2), or if a List N product is not available, a dilute bleach solution can be used (CDC, 2021; EPA, 2021). Traditional disinfectants have relatively rapid (e.g. on the order of minutes) antimicrobial activity when used in accordance with the product label, and are not required to show prolonged (e.g. hours to weeks) residual activity against viral or bacterial contamination. Thus, disinfectants require routine re-application in many settings that quickly become re-contaminated.

In October 2020, in response to the global COVID-19 pandemic, the EPA published guidance specifying testing requirements to register residual antimicrobial products with activity against SARS-CoV-2 (van Doremalen et al., 2020). These residual antimicrobial products are long-acting antimicrobial coatings, films, paints or surfaces which show antimicrobial activity within 2 h of exposure to microbes (i.e. contact time) and may have long-lasting activity over the weeks to months' timeframe. For a coating

or film to claim supplemental residual antimicrobial activity, and meet EPA registration requirements (EPA, 2020), the product must meet the EPA performance standard (3-log reduction) against bacteria (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) and any other public health pathogens to be added to a label claim (e.g. viruses such as SARS-CoV-2) on hard, non-porous surfaces (i.e. stainless steel) within a 2-h contact time when compared to untreated controls. The testing also requires demonstration of durability of the coating as demonstrated against wet and dry abrasion protocols.

There are many products registered by EPA as microbiostatic agents that inhibit the growth of odour and stain causing bacteria, fungi and algae. Many of these products claim effectiveness as residual coatings, providing protection on surfaces for days to weeks to months. However, products registered as microbiostats do not protect against food-borne or disease-causing pathogens. In theory, products which provide residual efficacy against disease-causing pathogens could be a highly useful supplement to current disinfection strategies. This study assessed a panel of commercially available antimicrobial products (coatings or films) for efficacy against SARS-CoV-2, with the intent of determining whether this category of antimicrobial products has potential for residual antiviral activity. In addition to antimicrobial films and coatings, copper alloy surfaces were evaluated for residual antimicrobial efficacy against SARS-CoV-2 in this study. Several studies have demonstrated the potent activity of copper for inactivating microbial and viral pathogens, with alloys containing 60% or higher concentrations of copper showing the highest biocidal activity (Mostaghimi et al., 2021; Rai et al., 2012). Therefore, the copper alloys C11000 (99% copper) and C26000 (70% copper) were chosen for evaluation against SARS-CoV-2. Testing was not intended to generate data supporting product or surface registration by the EPA, but rather to inform future development and testing of residual antivirals similar to the products and surfaces tested.

Chosen products were evaluated for efficacy against SARS-CoV-2 on hard surfaces (stainless steel and Acrylonitrile Butadiene Styrene [ABS] plastic), which are representative of surfaces commonly found in high-touch areas. A cyclohexane polymer antimicrobial film coating was chosen, along with liquid products containing <5.00% quaternary ammonium compounds (QACs) as the active ingredient in each formulation. QACs are cationic detergents that kill viruses and bacteria by disrupting the lipid membrane of the microbe and have gained widespread attention over the last 20 years as effective antimicrobial compounds. QAC derivatives (e.g. silicon quaternary ammonium salt) have been investigated for use as residual antimicrobial coatings and have shown efficacy against

several pathogens (Li et al., 2019; Xue et al., 2015). While QACs have shown some ability to inactivate enveloped viruses, including Influenza A (Tuladhar et al., 2012), results to date have been varied with respect to effectiveness against coronaviruses, including SARS-CoV-2. Recent work has reported low efficacy against human coronavirus by QACs in suspension and when applied to stainless steel (Kampf et al., 2020); however, one compound was effective against SARS-CoV-2 in suspension within 15 s of contact (Ogilvie et al., 2021). The wide variation in these results supports the need to further evaluate QACs and their derivatives for use against SARS-CoV-2 (Schrank et al., 2020). We report efficacy results for seven products tested as supplemental residual antimicrobials targeting SARS-CoV-2, with a discussion that follows on the applicability and future testing needs.

MATERIALS AND METHODS

Cells and virus

SARS-CoV-2 (isolate USA-WA1/2020) was obtained from the American Type Culture Collection (ATCC). All work done with SARS-CoV-2 was done in a Biosafety Level 3 (BSL-3) laboratory. SARS-CoV-2 virus was concentrated by membrane centrifugal concentration on each test day. For each test, 6 ml of SARS-CoV-2 was thawed, pooled and mixed prior to concentration via one hundred thousand (100 K) molecular weight cut-off (MWCO) centrifugal concentration (Pierce, Protein Concentrator PES, 100K MWCO, Cat# 88532; ThermoFisher Scientific). Sample was centrifuged for 10 min in the concentrator at 3000× g at room temperature (RT), with sample retentate (~0.2 ml) increased to a final volume of 2.5 ml with Dulbecco's Modified Eagle medium (DMEM) supplemented with 5% foetal bovine serum (FBS; Omega Scientific). Viral inoculum was transferred to a clean conical tube and kept on wet-ice until use (within 2 h of initial thaw). Concentrated SARS-CoV-2 virus used in this study had an average titre of 3.10×10^6 TCID₅₀ ml⁻¹.

Vero (African Green Monkey kidney) cells (ATCC CCL-81) were used for SARS-CoV-2 virus infectivity assays. Vero cells were incubated at 37°C, 5% CO₂ in DMEM (Gibco, ThermoFisher Scientific), supplemented with 10% FBS and 1% Penicillin–Streptomycin (Gibco, ThermoFisher Scientific), referred to as Complete Growth Medium (CGM). For cytotoxicity or virus infectivity assays, 1 day prior to the start of the assay, Vero cells were seeded in 96-well or 48-well tissue culture plates (CellTreat) at a density of 2.25×10^4 cells per well in 0.1 ml CGM (96-well plates) or 4.5×10^4 cells per well in 0.3 ml (48-well plates) to achieve 80%–85% confluency on the next day.

While 96-well plates were used for some initial cytotoxicity testing, all virus infectivity assays were conducted in 48-well plates.

Material coupons and chemical coatings

Fatigue-Resistant 301 stainless steel (0.01" thick; hardness rating of C40 on Rockwell Scale; meeting ASTM A666 specifications) and Impact-Resistant ABS Plastic (3/8" thick; hardness rating of R101–R109; meeting UL 94 HB specifications) were purchased from McMaster-Carr and cut into 3" × 0.75" coupons. Hand tin snips (301 stainless steel) or a miter saw (ABS Plastic) was used to cut coupons to size. Once cut, coupons were cleaned by soaking in a 1:100 diluted Liqui-Nox solution at pH 8.5 (Alconox), followed by rinsing in distilled water. Coupons were air-dried. Coupons were packaged in heat-sealed polyethylene in packs of 9 and were sterilized by Electron Beam (E-BEAM Services, Inc.) with a dose of 40 kGy.

The antimicrobial coatings and products, active ingredients, and method(s) of application tested in this study are listed in Table 1. Due to proprietary constraints, products were renamed using lettered identification. Products were initially evaluated for efficacy against Phi6, an enveloped viral bacteriophage that is a potential surrogate for SARS-CoV-2 (Calfee et al., 2021) and products which showed efficacy against Phi6 were selected for testing against SARS-CoV-2. To ensure consistency between the two studies, product identification nomenclature was maintained, as such product lettering is non-sequential (Table 1). Products were prepared at the recommended concentration according to the manufacturer's directions. The manufacturer-directed method of application for several of the products tested is by electrostatic sprayer (ESS). To ensure repeatability and consistency of product application at a smaller scale, a gravity-fed airbrush was used for the application of five out of six liquid chemicals. For all products except Product H, chemicals were spray-applied to either 301 stainless steel or ABS Plastic coupons using a gravity-fed airbrush one day prior to testing. During airbrush application, coupons were laid onto a flat surface and the product was applied evenly and completely to one side of the coupon. To apply products by airbrush, a 0.3-mm needle was used on a gravity-fed Master Airbrush (TCP Global) calibrated to dispense 1 ml per minute at 25 pounds per square inch (psi). The airbrush reservoir was loaded with 0.2 ml product per coupon to be sprayed. This ensured that approximately 0.1 ml of product is applied to each coupon. Application of product to each set of six coupons was performed as follows: at a height of 4 inches above coupons, the airbrush was sprayed left to

TABLE 1 List of Antimicrobial Products and Active Ingredients

Product ID	Product type	Active ingredient(s) ^a	Method of application
C	Microbiostat	3-(Trimethoxysilyl) propyldimethyloctadecyl ammonium chloride (<5.00%)	Gravity-fed airbrush
D	Disinfectant (non-residual), Microbiostat	3-(trihydroxysilyl) propyldimethyloctadecyl ammonium chloride (0.5%)	Gravity-fed airbrush
E	Microbiostat	3-(trihydroxysilyl) propyldimethyloctadecyl ammonium chloride (<1%)	Gravity-fed airbrush
F	Microbiostat	3-(trihydroxysilyl) propyldimethyloctadecyl ammonium chloride (1.3%)	Gravity-fed airbrush
H	Unregistered	1-Octadecanaminium,N,N-dimethyl-N-[3(trihydroxysilyl)propyl],chloride (<1%)	Electrostatic sprayer
Q	Antimicrobial polymer film	Cyclohexane (40%–60%) Polymer Solution	Peel & stick
V	Unregistered	3-(trimethoxysilyl) propyldimethyloctadecyl ammonium chloride (1–<3%)	Gravity-fed airbrush
Copper 11000	Surface	Copper ion	N/A
Copper 26000	Surface	Copper ion	N/A

^aPercentages of active ingredient is listed as a range if the formulation is proprietary.

right and back and forth across the coupon area until the entire area was covered, and the product was exhausted from the reservoir. Consistency was ensured using a template and standard application setup. Coupons were dried uncovered in a fume hood overnight at ambient laboratory conditions (average of 21.5°C and range of 30%–40% relative humidity) and stored in a closed gas-keted box until use.

For Product H, product was applied to coupons by the manufacturer using an ESS; coupons were dried overnight (20.8°C and 30% relative humidity). Coated coupons were stored in closed 50 ml conical tubes and shipped to Battelle Memorial Institute for efficacy testing.

Chemical testing

All products (except for Product H) were evaluated for efficacy against SARS-CoV-2 20–24 h following product application to coupons. Product H was evaluated for efficacy between 4 and 7 days after product application by the manufacturer. Coated and uncoated coupons were placed in individual sterile 10 cm petri dishes with the treated side facing up. Triplicate coupons (product treated and untreated, $n = 3$ each) were inoculated with 0.2 ml concentrated SARS-CoV-2 in DMEM supplemented with 5% FBS (5% soil loading) at an average titre of 6.61×10^5 TCID₅₀ per coupon. Viral inoculum was inoculated in droplets evenly across the entire surface of the coupon in a straight line. A gel-loading pipette tip was used to evenly spread the

inoculum across the entire coupon surface. Coupons were dried in the biosafety cabinet, uncovered, at ambient laboratory temperature and relative humidity for $2 \text{ h} \pm 5 \text{ min}$ (to allow a 2-h contact time between virus and chemical).

After the target contact time (2 h) between coated coupon surface and viral inoculum, residual infectious virus was extracted by adding each coupon to 5.0 ml low-FBS cell culture media (DMEM, 2% FBS, 1% PS) in conical tubes, followed by vortexing for 2 min (Vortex-Genie 2, Scientific Industries, Inc.) on the maximum setting (~3200 rpm) using a 50 ml conical adapter to extract up to 5 samples at a time. For copper coupons, virus was extracted in 5.0 ml low-FBS cell culture media (2% FBS) supplemented with 10 mM EDTA to chelate residual copper ions. Post-vortexing, samples were inverted three times and sample extract was passed through Sephadex (Sephadex G25 packed in PD-10 disposable columns, GE Healthcare) via centrifugation (2 min, 1000× g). Flow-through was retained and analysed immediately for infectivity via TCID₅₀ assay.

Each test matrix included controls for infectivity loss due to extraction (extraction control) as well as material and chemical negative controls (coupons inoculated with DMEM +5% FBS [no virus]). Extraction controls were inoculated with 0.2 ml concentrated SARS-CoV-2 and immediately extracted ($T = 0$) and assessed for infectivity as described for test samples. Material controls were generated from mock-inoculated coupons (coupons inoculated with 5% FBS in cell culture media in the absence of virus) without chemical treatment. Chemical inhibition

controls were included to evaluate inhibitory effects or viral loss due to un-neutralized chemical remaining in the sample post-extraction. Chemical inhibition controls consisted of coupons treated with antimicrobial coating, inoculated with 0.2 ml DMEM +5% FBS (no virus) and immediately extracted ($T = 0$) as described for test samples. The flow-through was then spiked with 0.2 ml concentrated SARS-CoV-2 inoculum and then infectivity was assessed via TCID₅₀ to determine whether residual chemical impacted viral recovery from the sample. When compared to virus recovered from untreated extraction controls, no viral loss was observed for all products in post-extraction spike tests with stainless steel coupons. For post-extraction spike tests with ABS plastic coupons, viral loss due to residual chemical was observed to be minimal (less than $0.03 \log_{10}$) for most products (Table S1). Product H showed variability in viral loss due to residual chemical, with no loss observed from stainless steel extracts and $0.32 \log_{10}$ loss observed from ABS plastic extracts (Table S1).

Cytotoxicity assays

All products and coupon materials were evaluated for cytotoxicity on Vero cells prior to testing. Products were applied to stainless steel or ABS plastic coupons as described. Material controls (using untreated coupons) were inoculated with 0.1 ml of cell culture media (DMEM supplemented with 5% FBS) and coupons were extracted in low-FBS containing media (DMEM supplemented with 2% FBS). Samples were extracted as described. Samples were analysed for cytotoxicity on Vero cells in 48-well tissue culture plates to mimic the TCID₅₀ assay method used. 0.2 ml of each sample was inoculated undiluted or diluted (1:10) into replicate rows of either 96-well or 48-well plates of Veros at 80–85% confluency. Cells were incubated (37°C, 5% CO₂) and assessed for cytotoxicity visually at various times across period of 5 days. Cells were analysed for cytotoxicity at day 5 via the CyQuant lactate dehydrogenase (LDH) Release Kit (Invitrogen, Cat# C20301; ThermoFisher Scientific). Cytotoxicity was evaluated using the following equation:

$$\% \text{Cytotoxicity} = \frac{\text{Treated LDH activity} - \text{untreated LDH activity}}{\text{Maximum LDH activity} - \text{untreated LDH activity}} \times 100. \quad (1)$$

Results are reported as percent cytotoxicity relative to the average maximum possible cell death. Maximum LDH activity is determined by quantifying the LDH released after chemical lysis of triplicate control wells included on the same plate of Vero cells.

TCID₅₀ assay for SARS-CoV-2

Quantification of infectious SARS-CoV-2 recovered from each sample was by TCID₅₀ assay. Vero cell monolayers were infected at 80%–85% confluency in 48-well plates; the additional capacity of 48-well plates provided more dilution of toxic test chemicals, necessary to reduce cytotoxic effects on host cells. Briefly, sample extracts were serially diluted (10-fold) in low-FBS containing media (DMEM supplemented with 2% FBS); 0.2 ml of each neat or diluted sample was plated onto replicate wells ($n = 12$) across two paired 48-well plates. Plates were rocked (30–35 min; 37°C, 5% CO₂) and 0.3 ml low-FBS containing media was added to each well. Plates were incubated for 5 days (37°C, 5% CO₂). At 5 days, wells were scored for cytopathic effect (CPE). Quantification of the titre was determined via the Reed–Muench method (Reed & Muench, 1938). The limit of detection (LOD) for this assay was calculated as $5 \text{ TCID}_{50} \text{ ml}^{-1}$ ($0.7 \log_{10} \text{ TCID}_{50} \text{ ml}^{-1}$) if undiluted samples are readable; and $50 \text{ TCID}_{50} \text{ ml}^{-1}$ ($1.7 \log_{10} \text{ TCID}_{50} \text{ ml}^{-1}$) if the lowest readable dilution is 1:10.

Calculations and statistical analysis

Calculation of percent reduction, log reduction and pooled error for log reduction, where appropriate, were determined using the equations below. Efficacy (Percent Reduction and Log Reduction) calculations utilized recovery data for time-matched controls (i.e. $T = 2$ control recoveries were compared to $T = 2$ test recoveries to determine efficacy at $T = 2$). For each chemical, a dynamic range (the widest possible window of infectivity able to be observed) was calculated for each test using Equation (5) below.

$$\% \text{Reduction} = \frac{(\text{Untreated titer}) - (\text{Treated sample titer})}{(\text{Untreated titer})} \times 100\%, \quad (2)$$

$$\log \text{Reduction} = \log_{10}(\text{Untreated titer}) - \log_{10}(\text{Treated sample titer}), \quad (3)$$

$$\text{Pooled error} = \left[\left(\frac{\text{StDev} \left(\log_{10} \frac{\text{TCID}_{50}}{\text{ml}} \text{untreated coupons} \right)^2}{3} \right) + \left(\frac{\text{StDev} \left(\log_{10} \frac{\text{TCID}_{50}}{\text{ml}} \text{treated coupons} \right)^2}{3} \right) \right]^{0.5}, \quad (4)$$

Dynamic range for chemical = $\log_{10}(\text{Untreated titer}) - \log_{10}\text{LOD}$. (5)

Pursuant to the EPA's 'Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing' (EPA 712-C-17-004) a product was considered efficacious against SARS-CoV-2 if a 99.9%, 3-log reduction, was demonstrated for the product at ≤ 2 h compared to untreated samples.

All test samples were performed in triplicate. Statistical significance was determined by ANOVA where applicable and *p*-value lower than 0.05 was considered statistically significant.

RESULTS

Neutralization of chemicals to mitigate cytotoxicity on vero cells

Each product and coupon material were evaluated for cytotoxicity on Vero cells. All products required

neutralization procedures to reduce cytotoxicity to levels that allowed CPE to be observed. However, products varied in the severity of cytotoxicity (Table 2). The average cytotoxicity of each product and coupon material before and after neutralization was evaluated via LDH-release assay (Table 2). Neither inert coupon material (301 stainless steel, ABS plastic) resulted in appreciable cytotoxicity in Vero cells (Table 2). Both copper materials C11000 and C26000 resulted in cytotoxicity in Vero cells observed during efficacy testing. C11000 required a 1:1000 dilution, resulting in an LOD of 4.39 $\log_{10}$ (Table S2). C26000 required a 1:100 dilution, resulting in an LOD of 3.39 $\log_{10}$ (Table S2).

Chemical products were evaluated for cytotoxic effect on Vero cells to evaluate the chemical effect on a standard TCID₅₀ assay format. While Products C, D and H were non-cytotoxic to Vero cells when undiluted in 96-well plates (Table 2), Products E and V showed high cytotoxicity. Thus, a 48-well plate TCID₅₀ assay was developed to reduce cytotoxic effects and generate comparable data when testing all products. The 48-well format, due to

TABLE 2 Cytotoxicity of antimicrobial coatings and films

Product	Volume (ml)	Plate format	Dilution	Percent cytotoxicity	Stdev	Lowest readable dilution	Assay LOD
C	0.10	96	Neat	0.07	1.24	Neat	1.4 log
			1:10	−0.84	0.69		
D	0.10	96	Neat	3.88	2.07	Neat	1.4 log
			1:10	0.22	0.75		
E	0.15	96	Neat	30.95	2.01	N/A	N/A
			1:10	0.96	1.56		
		48	Neat	18.73	4.55	1:20	2.7 log
			1:10	4.80	3.79		
F	0.15	48	Neat	5.92	2.03	1:10	2.4 log
			1:10	−1.68	2.58		
H	0.10	96	Neat	1.28	1.90	Neat	1.4 log
			1:10	−0.26	0.92		
Q	N/A	48	Neat	0.44	1.20	Neat	1.4 log
			1:10	0.17	1.01		
V	0.15	48	Neat	21.8	3.60	1:20	2.7 log
			1:10	29.29	7.33		
SS	N/A	48	Neat	−0.93	1.32	Neat	1.4 log
			1:10	0.70	2.26		
ABS	N/A	48	Neat	0.47	2.40	Neat	1.4 log
			1:10	−0.32	1.90		

Note: Cytotoxicity determined in 96- or 48-well format assay as noted. Results shown detail the average (and standard deviation) cytotoxicity of each product relative to the average maximum possible cell death (LDH release) per well. The lowest readable dilution and corresponding limit of detection are reported for each product. Assay LOD reflects the lowest attainable LOD (TCID₅₀ per coupon) when the product is used in a 48-well plate TCID₅₀ assay. Coupons were extracted in 5.0 ml extraction media.

Abbreviations: ABS, ABS plastic; N/A, not applicable; SS, stainless steel.

post-adsorption dilution, allowed testing with products which otherwise caused high cytotoxicity. The 48-well plate format also provided a wider dynamic range as a result of higher inoculum volume (0.1 ml for 96 wells vs. 0.2 ml for 48 wells), reducing assay LOD (50 TCID₅₀ per coupon, 96 wells vs. 25 TCID₅₀ per coupon, 48 wells). Although the 48-well plate format effectively reduced cytotoxicity levels induced by most products tested, Products E and F were only reduced to testable levels (<10%) when an additional dilution (1:10 or 1:2, respectively) was used in conjunction with the assay (Table 2). All infectivity assays were performed using the 48-well plate method. While the assay was performed on each chemical, the lowest readable dilution varied and directly influenced the LOD per chemical (Table 2).

Product E resulted in an average of $18.73 \pm 4.55\%$ cytotoxicity when measured undiluted on Vero cells in 48-well plates (Table 2), and an average of $4.80 \pm 3.79\%$ cytotoxicity after a 1:10 dilution. The anticipated LOD for Product E was 2.4 log TCID₅₀ per coupon. During testing with SARS-CoV-2, neat samples were diluted 1:2 prior to serial dilution to reduce cytotoxicity. The minimum readable dilution during testing was 1:20, resulting in a LOD of 2.7 log TCID₅₀ per coupon for Product E.

Product V resulted in high levels of cytotoxicity on Vero cells even after a 1:10 dilution (average of $29.29 \pm 7.33\%$ cytotoxicity). It was therefore anticipated that additional dilution(s) would be required to fully mitigate cytotoxicity. Thus, Product V samples were diluted 1:2 prior to serial dilution to mitigate cytotoxicity. The minimum readable dilution during testing with Product V was 1:20, resulting in a LOD of 2.7 log TCID₅₀ per coupon.

Extraction and recovery of SARS-CoV-2 from non-porous surfaces

Infectious SARS-CoV-2 has been recovered from stainless steel for up to 4 days depending on environmental conditions (Chin et al., 2020; van Doremalen et al., 2020), and another study determined the SARS-CoV-2 half-life ranges from 6.3 to 18.6 h (RT) on stainless steel or ABS plastic (Biryukov et al., 2020). Results from these studies show that SARS-CoV-2 stability is impacted by environmental and other conditions, including surface material. EPA registration of supplemental residual antimicrobial products (e.g. coatings or films) requires a minimum of 3-log reduction of test organism reached within a 2-h contact time (van Doremalen et al., 2020). This study used a 2-h contact time ($T = 2$) at ambient environmental conditions. During each test, a control coupon was extracted at $T = 0$ as a control to ensure efficient extraction of virus present on the coupon surface. The efficiency of the

extraction process using stainless steel or ABS Plastic coupons was evaluated at both $T = 0$ and $T = 2$ h. Recovery of infectious SARS-CoV-2 from coupons at $T = 0$ was $5.06 \pm 0.23 \log_{10}$ TCID₅₀ ($33.5\% \pm 17.67\%$ of the inoculum; Figure 1; Table S3) from stainless steel coupons and $4.98 \pm 0.26 \log_{10}$ TCID₅₀ ($52.47\% \pm 24.34\%$ of the inoculum; Figure 1; Table S3) from ABS Plastic. Extraction losses were $0.55 \pm 0.33 \log_{10}$ per stainless steel coupon and $0.32 \pm 0.22 \log_{10}$ per ABS Plastic coupon. There were no statistically significant differences in recovery from either coupon material.

Recovery of infectious SARS-CoV-2 at $T = 2$ h was an average of $4.80 \pm 0.37 \log_{10}$ TCID₅₀ ($16.41\% \pm 8.7\%$ of the inoculum) per stainless steel coupon and $4.43 \pm 0.34 \log_{10}$ TCID₅₀ ($15.01\% \pm 6.84\%$ of the inoculum) per ABS Plastic coupon (Figure 1; Table S3). While there was no significant difference between SARS-CoV-2 recovery from stainless steel at $T = 0$ versus $T = 2$ h (Figure 1), viral recovery from ABS plastic was reduced at $T = 2$ h compared to $T = 0$ h ($p = 0.058$). At $T = 2$ h, viral loss due to drying was an average of 0.26 log₁₀ (stainless steel; Table S3) and 0.55 log₁₀ (ABS Plastic; Table S3). Van Doremalen et al showed slightly increased recovery of SARS-CoV-2 on polypropylene plastic (mean half-life of 6.83 h) compared to stainless steel (mean half-life of 5.63 h; van Doremalen et al., 2020). The variability between our results and theirs may be due to the plastic type used for testing (polypropylene; van Doremalen et al., 2020 vs. ABS plastic).

Efficacy evaluation of chemicals against SARS-CoV-2 on non-porous surfaces

Efficacy testing was performed on seven commercially available products, and results showed variability in the ability of each to inactivate SARS-CoV-2 using a 2-h contact time (Table S4). All products were tested as described in the methods section. Briefly, all products were evaluated for residual efficacy against SARS-CoV-2 ($6.61\text{E}+05$ TCID₅₀ [$5.82 \log$ TCID₅₀] per coupon) in the presence of a 5% soil load (FBS). The dynamic range for each test is shown in Table S4, with dynamic range defined as the maximum quantifiable titre between the assay LOD and the concentration of SARS-CoV-2 recovered from chemically untreated control coupons inoculated with SARS-CoV-2 on each test day. LODs for each chemical are listed in Table 2. The current testing was not conducted for the purposes of registration, rather with the intended purpose of gathering performance data against SARS-CoV-2 under conditions that more closely represent actual use conditions to inform stakeholders' risk reduction measures. Therefore, our test methods differed from EPA registration methodologies. For example, this study

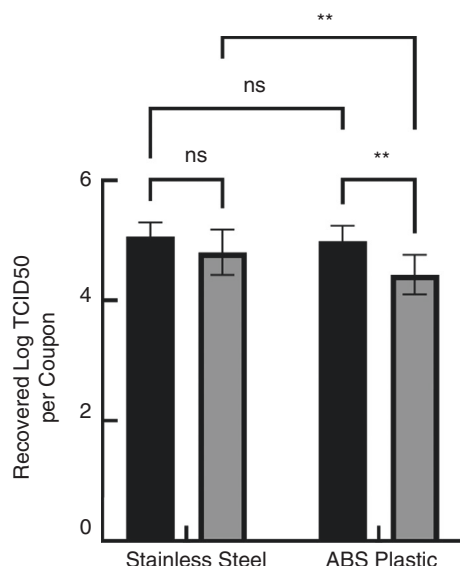


FIGURE 1 Recovery of severe acute respiratory syndrome coronavirus 2 from Stainless Steel and Acrylonitrile Butadiene Styrene (ABS) Plastic Coupons. Infectious virus (TCID₅₀) recovered at $T = 0$ (black bars) or $T = 2$ h (grey bars) from stainless steel coupons ($n = 7$, $T = 0$; $n = 21$, $T = 2$ h) and ABS plastic ($n = 6$, $T = 0$; $n = 18$, $T = 2$ h). Displayed is the mean and standard deviation. Statistical significance was determined by ordinary two-way ANOVA. ** $p \leq 0.01$; ns, $p > 0.05$

utilized larger test coupons, spray application of test products, and material types common to indoor environments. Nonetheless, a 3-log dynamic range was targeted for each test to reach the 3-log reduction benchmark set in the EPA guidance [11]. This dynamic range was not achieved for all tests, due to (a) SARS-CoV-2 titres recovered from untreated coupons and (b) increases in LODs due to product induced cytotoxicity. Therefore, results are presented with respect to their dynamic range, and complete kill of infectious virus is reported when observed.

Products D, E and F all contained the same active ingredient (Table 1) and were the most effective against SARS-CoV-2 in this test. Similar efficacy was observed for all three products; however, product cytotoxicity increased with higher concentrations of the QAC. Product D, with 0.5% QAC, did not cause appreciable cytotoxicity and was able to be evaluated under a <3.0 log dynamic range; in comparison, Products E and F ($<1\%$ and 1.3% QAC, respectively) required additional dilution which reduced the dynamic range for efficacy evaluation.

Product D was effective against SARS-CoV-2, reaching a 3.00 ± 0.40 log₁₀ reduction ($99.80\% \pm 0.23\%$ reduction) on stainless steel when compared to untreated coupons (Figure 2a,b; Table S4). However, the same product was not as effective against SARS-CoV-2 on ABS Plastic (log₁₀ reduction of 1.34 ± 0.35 ; Figure 2c,d; Table S4).

Products E and F resulted in a complete inactivation of SARS-CoV-2 (on stainless steel and ABS Plastic); no virus was recovered from coupons treated with Product E or F (Table S4). Although both products E and F resulted in complete inactivation of SARS-CoV-2, product-induced cytotoxicity limited the dynamic range to less than 3-log. The log₁₀ reduction able to be observed during testing was 2.10 log₁₀ (Product E) and 2.87 log₁₀ (Product F) on stainless steel and 1.60 log₁₀ reduction for both Products E and F on ABS Plastic (Table S4). Despite the lower dynamic range, both products resulted in high levels of inactivation. Product E resulted in $>99.4\%$ reduction on stainless steel (Figure 2b) and $>97.6\%$ reduction on ABS Plastic (Figure 2d). Product F resulted in $>99.9\%$ reduction on stainless steel (Figure 2b) and $>98\%$ reduction on ABS plastic coupons (Figure 2d).

Products C and H were the least effective at inactivating SARS-CoV-2 (log₁₀ reduction of <1.0 log). While other products were applied at the testing laboratory, Product H was applied to coupons by the manufacturer and shipped to the laboratory for efficacy testing. Product H testing commenced between 4 and 7 days post-application of the product versus next day for all other products. A limitation of our testing on Product H was the inability to control environmental conditions during shipping and handling, which may have affected product stability and efficacy as tested. However, all products tested, including Product H, are claimed to be stable and remain effective for months after product application under typical ambient conditions.

An antimicrobial polymer film (Product Q) was also tested. Product Q (a peel-and-stick product) was applied directly to stainless steel coupons one day prior to testing. Because the polymer film can be applied to any surface material, stainless steel was included as the sole test material for this product. Despite a dynamic range of 3.17 log for this test, Product Q (containing 40–60% cyclohexane polymer, Table 2) resulted in an average of 1.06 ± 0.71 log₁₀ reduction (a $70.67\% \pm 31.42\%$ reduction) after a 2-h contact time (Figure 2a,b; Table S4). Note that Product Q results were highly variable, ranging from 37.30% to 99.70% reduction across triplicate coupons.

In addition to antimicrobial coatings and films, there is growing interest in the virucidal activity of hard, copper-containing surface products. Copper alloy surfaces have been reported to inactivate several viruses in addition to displaying antibacterial activity (Cortes & Zuniga, 2020; Michels et al., 2015; Recker & Li, 2020). Therefore, the efficacy of Copper C11000 (99.9% Cu) and C26000 (70% Cu, 30% Zn) coupons were evaluated at the 2-h contact time (Table S2). The C26000 material resulted in a 0.21 log₁₀ reduction (out of a total 0.56 log dynamic range for this test) when compared with recovery from untreated stainless

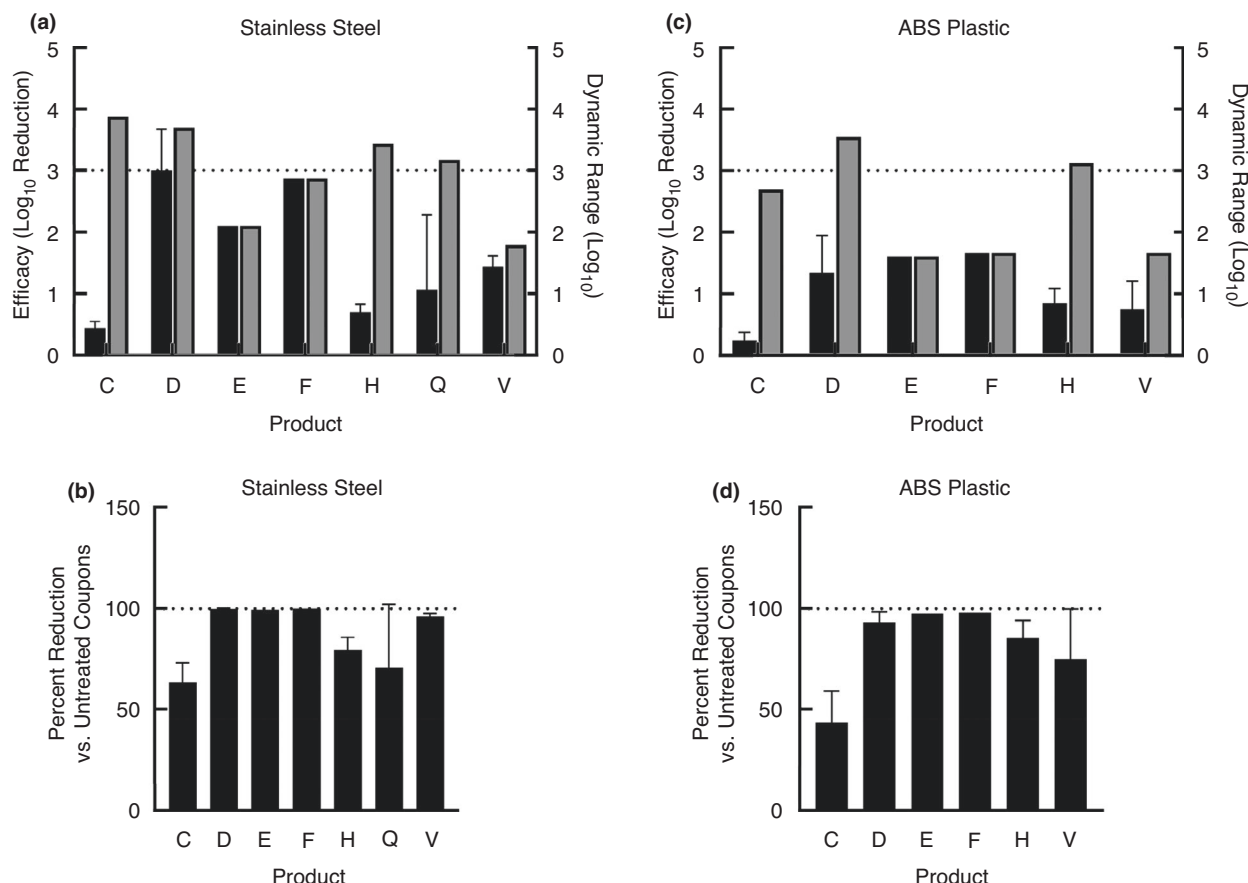


FIGURE 2 Efficacy of residual products against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on stainless steel or Acrylonitrile Butadiene Styrene (ABS) plastic. (a, b) Product efficacy (Log₁₀ reduction; black bars; left y-axis) and dynamic range (Log₁₀; grey bars; right y-axis) on stainless steel (a) or ABS (c) coupons (mean and standard deviation). The target 3-log reduction is indicated by a dashed line on the y-axis. (c, d) Percent reduction in infectious SARS-CoV-2 from treated stainless steel (c) or ABS (d) coupons compared to untreated coupons. Recovery of infectious virus from untreated coupons was set to 100% (dashed line on y-axis)

steel coupons. SARS-CoV-2 reduction from C11000 was not determined due to the high levels of cytotoxicity in the test (Table S2).

DISCUSSION

In the current study, residual antimicrobial products formulated with QACs were evaluated for efficacy against SARS-CoV-2 application on two common, high-touch materials (stainless steel and ABS plastic). The results from this testing suggest that efficacy of residual antimicrobial products containing QACs may be formulation specific. Efficacy results from the seven different products were highly variable, with substantial differences in efficacy across the panel of products tested, even between products with the same active ingredients. Tested product efficacies ranged from <1.0 to >3.0 log reduction at a 2-h contact time, despite several sharing active ingredients. The three most effective products tested, Products D, E and F, were all formulated with 3-(trihydroxysilyl)

propyldimethyloctadecyl ammonium chloride (CAS 199111-50-7), but each formulation contained varying concentrations of active ingredient (Product D: 0.5%, Product E: <1% and Product F: 1.3%; Table 2). Product H lists 1-Octadecanaminium,N,N-dimethyl-N-[3(trihydroxysilyl)propyl],chloride (<1%) which has the same CAS number as listed in Products D, E and F. Product C lists a different QAC (e.g. 3-(Trimethoxysilyl) propyldimethyloctadecyl ammonium chloride [CAS 27668-52-6]) at <5.00%. However, these two organosilanes are structurally very similar and when exposed to water, the trimethoxysilyl QACs undergo a chemical reaction which leads to the formation of trihydroxysilyl QACs (EPA, 2007). Differences in observed efficacy may be due to variation in active ingredient formulation; efficacy is, therefore, difficult to predict based upon listed active ingredient and its concentration.

In addition to liquid coatings, this study evaluated two self-disinfecting materials: copper alloys C11000 and C26000, and a peel-and-stick sulfonated polymer coating. Both copper alloys produced high levels of cytotoxicity,

reducing the ability of this test method to evaluate the antiviral activity of these copper materials. Antiviral activity of copper alloys has been demonstrated for RNA viruses using electron microscopy and real-time PCR amplification methods (Warnes et al., 2015) and suggest that additional studies would be useful to evaluate copper alloys for inactivation of SARS-CoV-2. The polymer coating (Product Q, a sulfonated cyclohexane polymer) was not consistently effective against SARS-CoV-2 under the conditions tested in this study (an average of 1.06 log₁₀ or 70.67% reduction in virus compared to stainless steel). However, the results of this test were highly variable, ranging from 37.30% to 99.7% reduction in virus across triplicate test samples. As a sulfonated polymer, the antiviral activity depends on the number of sulphononic acid moieties present on each polymer molecule and the degree of sulphonation has been shown to affect the kinetics of viral inactivation (Peddinti et al., 2019). The variability in antiviral efficacy observed for the polymer film in the present study may be due to variability in the degree of sulphonation or the application of the film itself onto stainless steel. The wide range of antiviral efficacy observed for this film in the present study, coupled with recent studies providing evidence for the use of polymer coatings to inactivate SARS-CoV-2 (Peddinti et al., 2021), suggests that further investigation into the antimicrobial stability of polymer coatings may be warranted.

For decontamination studies, SARS-CoV-2 presents a challenge due to the lower titres produced (in the absence of concentration methods). While not a hurdle for persistence testing, testing that includes chemicals, such as disinfectants, sanitizers or antimicrobial coatings, couples the lower viral concentrations with cellular cytotoxic effects which reduces the testable dynamic range. The EPA performance standard for registration (3-log reduction in infectious agent post-contact) is dependent on viral titre, assay LOD and infectious virus recovery from untreated coupons. This study was not intended to generate data supporting antimicrobial product registration by EPA for use of these products against SARS-CoV-2. Testing to support antimicrobial coating product registration would require data collection under GLP conditions, demonstration of product activity against *P. aeruginosa*, *S. aureus* and public health pathogens of interest (e.g. SARS-CoV-2) in addition to demonstration of product durability. This study was intended to demonstrate whether antimicrobial products were efficacious against SARS-CoV-2, and while demonstration of a 3-log reduction using study methods indicates potential residual efficacy of the product against SARS-CoV-2, results do not meet the requirements for product registration as listed above. However, this study identified several issues which impeded the ability to determine a <3-log range of

reduction; SARS-CoV-2 stock used for this study was not propagated above 6 log₁₀ ml⁻¹ and although ultrafiltration via centrifugation was used to increase starting titre, only an average of 29% titre increase was able to be realized (unconcentrated SARS-CoV-2: $2.41 \times 10^6 \pm 1.28 \times 10^6$ TCID₅₀ ml⁻¹; concentrated SARS-CoV-2: $3.10 \times 10^6 \pm 5.09 \times 10^6$ TCID₅₀ ml⁻¹).

Viral decay on coupon materials at 2-h post-inoculum was 0.26 log₁₀ on stainless steel and 0.55 log₁₀ on ABS plastic (Table S3). Losses during virus recovery procedures resulted in an additional 0.55 log₁₀ reduction from stainless steel and 0.32 log₁₀ reduction from ABS plastic (Table S3). Collectively, these losses totalled >0.80 log₁₀ for both materials (Table S3). This loss, when coupled with necessary dilutions to mitigate cytotoxicity (Table 2), resulted in conditions which were unable to preserve at least a 3-log dynamic range. For example, Products E and F were able to completely inactivate SARS-CoV-2 to below the assay LOD, however were unable to reach a 3-log reduction due to cytotoxic effects which reduced the assay LOD, thus reducing the dynamic range for testing those chemicals (Figure 2; Table S4).

It should be noted that viral aggregates may be present in the inoculum used for testing in this study. Viral aggregation occurs because of environmental or surface changes (Celik et al., 2020; Gerba & Betancourt, 2017b), presence of matrix (e.g. simulated saliva) components (Fedorenko et al., 2020) or simply by spontaneous formation (Gerba & Betancourt, 2017b). It is also possible that the method used in this study to concentrate the virus prior to inoculation (centrifugation) may promote aggregation of SARS-CoV-2. Aggregates of virus may display increased resistance to disinfectants or antimicrobials (Gerba & Betancourt, 2017a) and therefore may have implications for results of efficacy testing such as those reported in this study. If aggregation of SARS-CoV-2 is occurring either in the inoculum or upon contact with the surface materials (SS or ABS plastic) in this study, it is likely that the efficacy results reported herein represent a conservative estimate of the efficacy of each antimicrobial tested against SARS-CoV-2.

In conclusion, efficacy of residual antimicrobial chemicals against SARS-CoV-2 was variable, with inactivation ranging between 0.44 and 3 log reduction on stainless steel and between 0.25 and >1.67 log on ABS plastic. While some commercial products were effective against SARS-CoV-2, product-to-product variability and cytotoxic effects on cell culture hampered the ability of this study to observe full 3-log reduction (other than Product D). However, several products (Products E and F) did show complete inactivation within the dynamic range achieved. Products D, E and F share an active ingredient (Table 1); however, other products

with similar QAC active ingredients (Products C and H) did not show efficacy against SARS-CoV-2, despite having dynamic ranges able to observe much higher reduction levels (dynamic ranges: 3.43 [Product H] and 3.88 [Product C] on stainless steel and 3.12 [Product H] and 2.69 [Product C] on ABS plastic; Figure 2a,b). These data suggest that formulation or QAC-specific effects on SARS-CoV-2 may play a role in inactivation of SARS-CoV-2. These effects merit further investigation. In addition, while this study evaluated efficacy at 24-h post-chemical application, several products tested claim residual efficacy over longer time periods (e.g. several days to weeks). Follow-on weathering studies are merited to determine the length of antiviral effectiveness of products against SARS-CoV-2 in real-world settings.

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ACKNOWLEDGEMENTS

The United States Environmental Protection Agency through its Office of Research and Development funded and managed the research under contract number EP-C-16-014/Task Order 68HERC20F0231. We thank members of the Environmental Protection Agency (EPA) Office of Research and Development for helpful discussions and guidance on study objectives. We also thank Clint Fleshman, Demond Church, Veronica Fulwider, Cody Dudik, Joel Marx, Nola Bliss, Genevieve Mobilia, Erin Schwarzbach, Kirsten English and David Glasbrenner at Battelle Memorial Institute for assistance with experimental set up, cell culture support and method development.

CONFLICT OF INTEREST

Neither Battelle nor the EPA manufacture or provide the products or materials described herein, nor plan to manufacture or provide these products in the future.

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How to cite this article: Hardison, R.L., Ryan, S.P., Limmer, R.A., Crouse, M., Nelson, S.W., Barriga, D., et al. (2022) Residual antimicrobial coating efficacy against SARS-CoV-2. *Journal of Applied Microbiology*, 132, 3375–3386. <https://doi.org/10.1111/jam.15437>