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**Continuously Active Disinfectant Inactivates SARS-CoV-2 and Human Coronavirus 229E Two Days After the Disinfectant Was Applied and Following Wear Exposures**

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Running Title: Continuously Active Disinfectant SARS-CoV-2
Abstract

The surface environment in COVID-19 patient’s rooms may be persistently contaminated despite disinfection. A continuously active disinfectant demonstrated excellent sustained antiviral activity following a 48-hour period of wear and abrasion exposures with reinoculations. Reductions of $>4\log_{10}$ were achieved within a 1-minute contact time for SARS-CoV-2 and the human coronavirus, 229E.

Keywords: Persistent; germicides; disinfectants; sustained; coronavirus; SARS
Subject Category: Disinfection
Background

Hospital room environmental surfaces and noncritical medical devices are frequently contaminated and serve as a source of healthcare pathogens including the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although indirect transmission via fomites is not thought to be the primary way the virus spreads, the role of the contaminated healthcare environment in the transmission of SARS-CoV-2 among patients and/or healthcare personnel remains unclear. In laboratory testing, SARS-CoV-2 has been shown to remain viable on surfaces for hours to days.¹ The surface environment in COVID-19 patient’s rooms may be persistently contaminated with SARS CoV-2 RNA despite routine room cleaning and disinfection.²

We recently reported on a novel continuously active disinfectant against pathogens causing healthcare-associated infections such as methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus spp. (VRE), Candida auris and multi-drug-resistant organisms (MDROs) such as carbapenem-resistant Klebsiella pneumoniae.³⁴ Application of this continuously active disinfectant on portable equipment resulted in significant reductions in aerobic colony counts over 7 days, and in lower recoveries of S. aureus and enterococci.⁴ The aim of this study was to evaluate the residual efficacy of a continuously active disinfectant that is registered by the Environmental Protection Agency (EPA) to kill SARS-CoV-2 and human coronavirus 229E (HCoV-229E) 229E on surfaces for at least 24 hours.

Methods

We investigated the continuously active disinfectant against HCoV-229E and SARS-CoV-2 using the EPA Protocol #01-1A “Protocol for Residual Self-Sanitizing Activity of Dried Chemical Residuals on Hard, Non-Porous Surfaces”, with modifications for viruses.⁵ The method simulates dry and wet wiping by incorporating “wear” of the test surface as well as reinoculations of the test and control surfaces occurring over a period of at least 24 hours following product application. Glass surfaces (1”x1”) were initially inoculated with ≥5-log₁₀ of virus per carrier, treated with the novel disinfectant (3 sprays, 6 to 8 inches in distance), and allowed to dry overnight. The carriers were abraded using a standardized abrasion machine (Gardco Model D10V, Paul N. Gardner Co., Inc., Pompano Beach, FL) under multiple alternating dry and wet wiping conditions (6 dry cycles, 6 wet cycles, total 12 cycles [2 passes
per cycle=24 passes] interspersed with 6 reinoculations with \( \geq 3 \times \log_{10} \) of the test pathogen. The mean \( \log_{10} \) viral titer of the initial viral inoculum volume per carrier (0.010ml) for SARS-CoV-2 was 5.67±0.17, and the reinoculations titer was 3.67±0.17 and 3.92±0.09 on day 1 and day 2 (n=2), respectively. The mean \( \log_{10} \) viral titer of the initial viral inoculum volume per carrier (0.010ml) for HCoV-229E was 5.63±0.18, and the reinoculations titer was 4.00±0.35 and 3.75±0.00 on day 1 and day 2 (n=2), respectively. After the 48-hour period of wear exposures, the surfaces were reinoculated with \( \geq 5 \times \log_{10} \) of virus to assess sanitizing efficacy of the continuously active disinfectant to kill >99.9% following a 1-minute contact time. All viral preparation were amended with fetal bovine serum to achieve a 5% organic soil load. Carriers were neutralized using 1 ml of Letheen Broth Base (Neogen. Lansing, MI) followed by immediate passage through a Sephadex G-10 gel filter column via centrifugation (3,500 x g, 5 minutes).

Human coronavirus 229E (ATCC VR-740), an enveloped respiratory virus, was procured from the American Type Culture Collection (ATCC, Manassas, VA). Propagation and assay of HCoV-229E was performed using the human lung fibroblast MRC-5 cell line (ATCC CCL-171). SARS-CoV-2 Isolate USA-WA1/2020 was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, the National Institute of Allergy and Infectious Diseases, and the National Institutes of Health. SARS-CoV-2 (BEI NR-52281) was propagated and assayed using the Vero E6 cell line (ATCC CRL-1586). HCoV-229E and SARS-CoV-2 viral stocks were enumerated on their respective host cell lines seeded into 96-well cell culture trays using the TCID\textsubscript{50} technique.

The continuously active disinfectant is EPA-registered as Firebird F130 (Microban Products, Huntersville, NC) and marketed as Sani-24 by Professional Disposable International (Woodcliff Lake, NJ). The product has a disinfectant claim against 32 microorganisms and a residual claim against 5 bacteria.

Results

The continuously active disinfectant studied demonstrated excellent sustained antiviral activity (>4.0-\( \log_{10} \) reduction) within 1 minute against human coronavirus, 229E and for SARS-CoV-2 amended with 5% organic soil following a 48-hours period of wear and abrasion exposure.(Table 1) There was no reduction in viral titer for the control.
Conclusion

Environmental contamination plays an important role in the transmission of several key healthcare-associated pathogens, including MRSA, VRE and MDROs. Evidence in the literature supporting the role of the contaminated surface environment in the transmission of healthcare pathogens has been published.\(^6\) Many of the studies demonstrated that rooms are not adequately cleaned/disinfected and patients admitted to a room previously occupied by a patient colonized or infected with a pathogen (e.g., MRSA, VRE, *Clostridioides difficile*) have an increased likelihood of developing colonization or infection with that pathogen.\(^7\) To minimize this risk, improved terminal room decontamination (e.g., supplemental UV following cleaning and disinfection) of Contact Precaution patient rooms has led to a decreased rate of infection in patients subsequently admitted to the room where the prior occupant was colonized or infected.\(^8\) However, the limitation of these “no touch” technologies is that currently they can only be used for terminal room disinfection (i.e., not daily cleaning and disinfection) because they require removal of the patients, visitors and healthcare personnel from the room.

Since routine cleaning and disinfection of room surfaces by environmental services is frequently inadequate\(^7\) and surfaces rapidly become recontaminated\(^9\) by patients, visitors and staff, continuous room decontamination methods are being evaluated.\(^10\) This highlights the potential to interrupt transmission from contaminated surfaces via healthcare provider’s hands by suboptimal compliance with hand hygiene or inappropriate glove use.

A continuously active disinfectant is a continuous room decontamination method.\(^10\) That is, if an antimicrobial residue was left on a disinfected surface and it persists on the surface for \(\geq 24\) hours, it could reduce or eliminate the problem of continuous recontamination and minimize the role of environmental surfaces as reservoirs of pathogens by eliminating them on the treated surface. The intent of this technology is to make surfaces hygienically clean (not sterile), or free of pathogens in sufficient numbers to prevent human disease. This study subjected a continuously active disinfectant to wear and abrasion exposure over a 48-hour to assess residual antiviral efficacy against SARS-CoV-2 and HCoV-229E on surfaces for \(\geq 24\) hours.

Previous studies demonstrated persistent antimicrobial activity (i.e., 3-5 \(\log_{10}\) reduction) 24 hours post-application for many healthcare pathogens within a contact time of 5 minutes.\(^3,4\) This study demonstrated residual efficacy of the continuously active disinfectant to inactivate SARS-CoV-2 and HCoV-229E within 1 minute following 12 cycles of alternating dry and wet...
abrasions (6 dry and 6 wet) performed with reinoculations during the 48 hours after the product application. Based on our data using SARS-CoV-2 as well as studies with several common healthcare pathogens (e.g., MRSA, VRE), continuously active disinfectants can significantly reduce bacterial, viral and yeast populations that contact treated surfaces within a minute over ≥24 hours.\(^3\)\(^4\) If the microbial load on surfaces is pathogen-free or pathogens are significantly reduced, the treated environmental surface will not act as a reservoir/source for pathogens (including SARS-CoV-2) and be linked to disease transmission.

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**Disclosure**
Drs. Rutala and Weber are consultants to Professional Disposables International (PDI). Dr. Donskey received research funding from Clorox and PDI.
References


Table 1. Inactivation of SARS-CoV-2 and the human coronavirus 229E by a continuously active disinfectant following a 48-hour period of wear and abrasion exposure

<table>
<thead>
<tr>
<th>Carrier Treatment with Wears and Reinoculations</th>
<th>Contact Time</th>
<th>Mean Viral Recovery Titer per Carrier (Log&lt;sub&gt;10&lt;/sub&gt;)</th>
<th>HCoV 229E Log&lt;sub&gt;10&lt;/sub&gt; Reduction</th>
<th>SARS-CoV-2 Log&lt;sub&gt;10&lt;/sub&gt; Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Sterile NP-Water, n=3)</td>
<td>1 min</td>
<td>≥5.63 ± 0.18</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Continuously Active Disinfectant, n=3</td>
<td>1 min</td>
<td>≤ 1.50 ± 0.00</td>
<td>&gt;4.50</td>
<td>&gt;4.22</td>
</tr>
</tbody>
</table>