

Letter to the Editor

Why is there a discrepancy between laboratory test results and real-world efficacy of continuously active quaternary ammonium disinfectants?

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To the Editor—Continuously active quaternary ammonium disinfectants containing polymer coatings that bind to surfaces have been developed to provide persistent antimicrobial activity between episodes of cleaning.^{1,2} Environmental protection agency (EPA) registration as a disinfectant with 24-hour residual antimicrobial activity requires demonstration of a 5-log reduction in bacteria and/or a 3-log reduction in viruses within 10 minutes after 12 cycles of alternating wet and dry abrasions intended to simulate routine contacts that might occur between cleaning episodes.^{1,3,4} A product registered with the EPA as Firebird F130 (Microban Products, Huntersville, NC) and previously marketed by Professional Disposables International as Sani-24 has demonstrated residual activity against several bacterial pathogens and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).^{1,5} However, real-world assessments of these products have yielded mixed results.¹ In a recent randomized trial, a continuously active disinfectant significantly reduced total bioburden and recovery of clinically important pathogens,⁶ whereas no significant reductions occurred in another randomized trial.⁷

Why might there be a discrepancy between laboratory results and real-world efficacy of continuously active quaternary ammonium disinfectants? It is possible that the coatings may sometimes be removed in real-world settings as the products are easily removed by disinfectant or nondisinfectant wipes.^{1,8} The artificial methods used for laboratory testing may also exaggerate the potential for efficacy in real-world settings (ie, organisms deposited in a liquid inoculum during laboratory testing may be reduced more than organisms deposited without moisture in clinical settings).^{1,9}

Another factor that could affect real-world efficacy is variation in the amount of continuously active quaternary ammonium disinfectant applied to surfaces. For Firebird F130/Sani-24, the EPA registration (no. 42182-9) for residual disinfection indicates that sufficient product must be applied to ensure thorough wetness with 1 minute of wet contact time. It is plausible that insufficient product might be applied in real-world settings. The product may dry quickly on surfaces because it contains 68.6% ethanol and

might require reapplication to achieve 1 minute of wet contact time. Therefore, we compared the amount of product applied using Sani-24 Germicidal Spray and presaturated Sani-24 Germicidal Wipes with different wiping methods and tested for activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Sani-24 was applied to overbed tables using 5 methods: (1) spraying with Sani-24 Germicidal Spray following the manufacturer's recommendation (ie, 3 sprays at 15 cm) providing ~120 seconds of wet contact time, (2) wiping with 1 Sani-24 Germicidal Wipe with 2 passes over the surface providing ~60 seconds of contact time, (3) wiping with 1 Sani-24 Germicidal Wipe with 1 pass over the surface providing ~30 seconds contact time, (4) wiping with 2 Sani-24 Germicidal Wipes to thoroughly wet the surface providing ~120-seconds contact time, and (5) and wiping with 1 Sani-24 Germicidal Wipe that had first been applied to a 1.2 m² surface area resulting in reduced product application providing ~10 seconds contact time. For each method, the product was allowed to dry overnight before testing. The spray and 2-wipe applications left a palpable sticky residue on the surface.

A bromophenol blue colorimetric assay was used to assess the presence of quaternary ammonium on the surfaces ~24 hours after application.^{1,10} Bromophenol blue solutions turn from purple to blue when complexed with quaternary ammonium compounds. A color change has been correlated with a >99.9% reduction in *Staphylococcus aureus* and *Klebsiella aerogenes*.¹⁰ The supplemental material shows a bromophenol blue standard curve.

To assess antimicrobial activity on the surfaces, 6 log₁₀ of MRSA in 10 μL phosphate-buffered saline was inoculated onto treated surfaces. After 1 and 10 minutes of contact time, the surfaces were sampled with cotton swabs premoistened in Dey-Engley neutralizer and plated onto selective media for enumeration. Reductions were calculated in comparison to untreated control surfaces.

As shown in Figure 1.A, bromophenol blue solution turned from purple to blue with the spray and 2-wipe application, and the swab tip turned blue for the 1 wipe with 60 seconds contact time application. No blue color was detected for the 1 wipe with 30 second contact time and the used wipe applications. MRSA was reduced by ≥5.9 log₁₀ with the spray and 2-wipe applications, but only by ~1–2 log₁₀ when applied with a single wipe with 1–2 passes over the surface (Fig. 1B). No substantial reduction in MRSA occurred when a single wipe was applied after first wiping a 1.2 m² surface.

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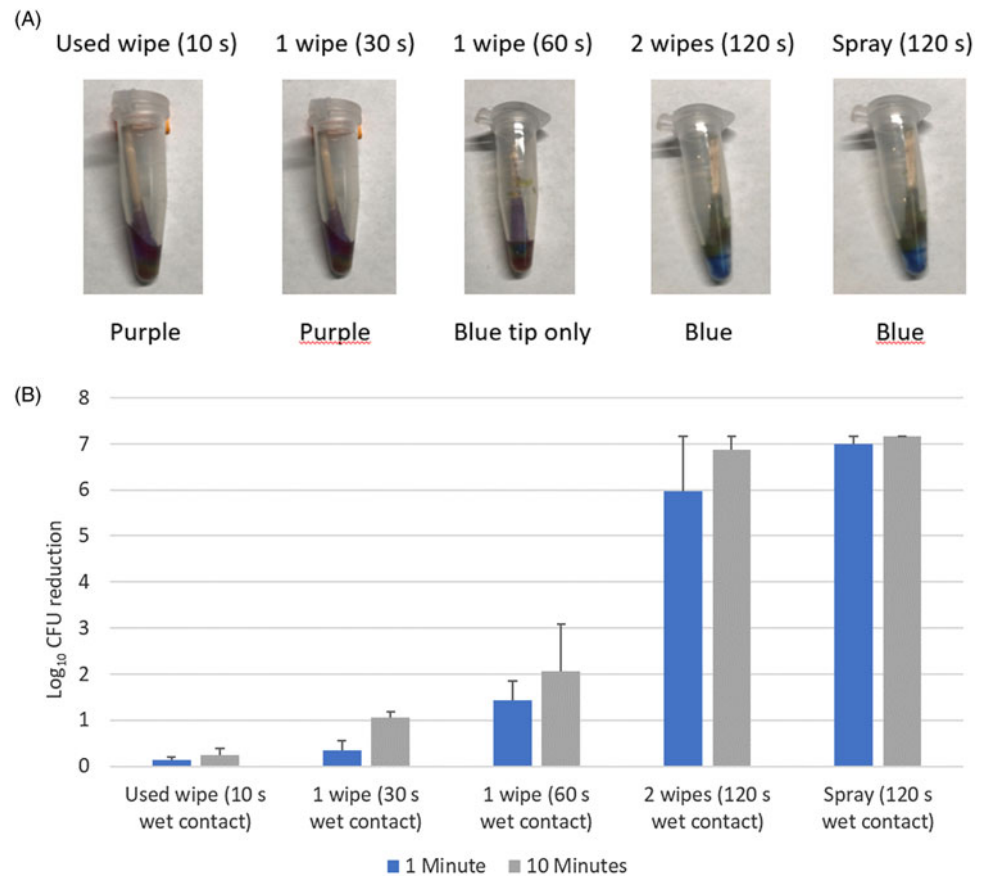


Figure 1. Detection of residual quaternary ammonium disinfectant on surfaces based on a bromophenol blue colorimetric assay (A) and reduction in methicillin-resistant *Staphylococcus aureus* (MRSA) inoculated onto the surface with 1 or 10 minutes of contact time (B). Seconds in parentheses indicate wet contact time of the disinfectants prior to drying. Change from purple to blue indicates detection of quaternary ammonium compound. s, second; CFU, colony-forming unit.

Our findings demonstrate that the amount of continuously active quaternary ammonium disinfectant detected on surfaces can vary considerably with different methods of application. Application as a spray or wipe with sufficient product to provide ~120 seconds of wet contact time provided optimal activity but may not be practical in some settings if a residue is left on surfaces. Quaternary ammonium disinfectant was only detected on surfaces with 60 seconds or longer contact time. A single wipe passed over the surface twice to provide ~60 seconds wet contact time resulted in a 2 log₁₀ reduction in MRSA, but ≤1 log₁₀ reductions occurred on surfaces with wet contact time of ≤30 seconds.

In summary, the method of application of continuously active quaternary ammonium disinfectants could substantially impact results in real-world settings. Our findings reinforce the manufacturer's recommendation that sufficient product must be applied to provide at least 60 seconds of wet contact time. Bromophenol blue testing could be a useful tool to assess the adequacy of product application.

Supplementary material. To view supplementary material for this article, please visit <https://doi.org/10.1017/ice.2024.15>

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