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Research Report

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Cleaning and Disinfection Investigation: Clorox Healthcare® Hydrogen Peroxide Cleaner Disinfectant Wipes

Purpose - This investigation was undertaken to:

1. Demonstrate the ability of ***Clorox Healthcare® Hydrogen Peroxide Cleaner Disinfectant Wipes*** to clean environmental surfaces coated with dried organic debris;
2. Demonstrate the ability of ***Clorox Healthcare® Hydrogen Peroxide Cleaner Disinfectant Wipes*** to kill vegetative bacteria using a single-step application procedure; and
3. Compare the cleaning and disinfecting capabilities of the ***Clorox Healthcare® Hydrogen Peroxide Cleaner Disinfectant Wipes*** with other commonly used disinfectant products.

Abstract

A new hydrogen peroxide disinfectant wipe was tested against high alcohol disinfectants on two types of environmental surfaces coated with dilutions of blood and bacteria. After treatment all demonstrable blood was removed and no detectable test bacteria remained following use with the hydrogen peroxide disinfectant. In contrast, treatment with two high alcohol containing disinfectants resulted in visible organic debris and quantifiable concentrations of challenge bacteria.

Materials and Methods

Test Specimen Preparation

Bacterial suspensions of stock *Methicillin-resistant Staphylococcus aureus (MRSA) ATCC #3359* and *Escherichia coli ATCC #25922* were prepared by aerobically culturing bacteria in 10 mL of trypticase soy broth at 37C for 24 hours. Bacterial concentrations were subsequently determined using a *Spectronic* 200 spectrophotometer. Suspensions for experiments were prepared by adding 1.5 x 10^4 cfu/mL bacteria to 5%, 25%, 50%, and 100% whole human blood. These mixtures were used to coat experimental environmental surfaces by adding 0.2 mL of each bacteria/blood suspension onto 6 environmental surface tiles. Two types of 2"x2" environmental surfaces were used: 1. hard formica tile squares; and 2. soft synthetic leather fabric commonly used on dental chairs attached onto tile squares. These are among the most common environmental surfaces found in a dental office. Suspensions were spread over the entire surface using a sterile cotton swab and allowed 1 hour to dry at room temperature. Five experimental replicates and one control were used for each assayed dilution.

Cleaning, Disinfection, and Replica Plating Procedures

Hard and soft surface tiles coated with bacteria/blood were then treated with one of 3 test disinfectants (Table 1).

Table 1. Test Disinfectants

Disinfectant	Company	Active Ingredients	EPA Registration #	Experimental Contact Times
Clorox Healthcare® Hydrogen Peroxide Cleaner Disinfectant Wipes	Clorox Healthcare®/Healthlink®	1.4% hydrogen peroxide	67619-25	5 min - TB 1 min – *MRSA* 30 sec – *E.coli*
PDI® Super Sani-Cloth®	Professional Disposables International	55% isopropanol + 0.5% quaternary ammonium	9480-4	2 min TB
Lysol® Brand III I.C.™ Disinfectant Spray	Reckitt Benckiser	58% ethanol + 0.1% quaternary ammonium	777-99-675	10 min TB

Materials and Methods (cont.)

Environmental surface disinfectants were evaluated using a single-step procedure to clean and disinfect. The manufacturers' directions were followed using specified tuberculocidal times designated to accomplish intermediate-level disinfection. All disinfectant wipes were applied onto tiles by the same individual and wiped 3-5 times with normal mechanical force. *Lysol® Brand III I.C.™ Disinfectant Spray* was sprayed 2-3 times onto experimental tiles before wiping 3-5 times with sterile 4"x4" dry wipes. Exploratory testing of ***Clorox Healthcare® Hydrogen Peroxide Cleaner Disinfectant Wipes*** was also conducted using designated contact times for challenge *MRSA* (1 minute) and *E.coli* (30 seconds) bacteria. Removal of organic debris from disinfectant-treated surfaces was assessed using *Red Detection Application*, a computerized process that quantifies the percentage of blood remaining on test surfaces. Detection of remaining viable bacteria on the surfaces was determined by replica plating tile squares on trypticase soy agar plates containing 5% sheep blood. Positive, untreated control tiles coated with bacteria/blood suspensions were replica plated for comparison. After aerobic incubation at 37°C, bacterial growth was noted, colonies counted and recorded as colony-forming units (cfu).

Results

Removal of organic debris from disinfectant-treated surfaces

Photographs were taken of each soft and hard surface tile before and after cleaning with the various products. Images were subsequently analyzed for residual blood on the surfaces using the color-specific, computerized *Red Detection Application* program.

The results of cleaning assays are shown in Figures 1-4 along with Tables 2-3. ***Clorox Healthcare® Hydrogen Peroxide Cleaner Disinfectant Wipes*** were able to remove all detectable blood with a single-step wipe procedure (Figures 1a-b and 3a-b). In contrast, visible bioburden was evident after application and wiping procedures using the two alcohol-based disinfectants, *Lysol® Brand III I.C.™ Disinfectant Spray* and *Super Sani-Cloth® Wipes*. Failure to remove all dried material was shown to be progressively worse as the concentrations of dried blood on the surfaces increased (Figures 2a-b and 4a-b). More than 50% of the organic material remained when these two disinfectants were challenged with surfaces containing undiluted whole blood (Tables 2-3).

Figure 1. Representative contaminated formica tiles (100%, 50%, 25%, 5% whole blood dilutions) (a) before and (b) after treatment with ***Clorox Healthcare® Hydrogen Peroxide Cleaner Disinfectant Wipes***.

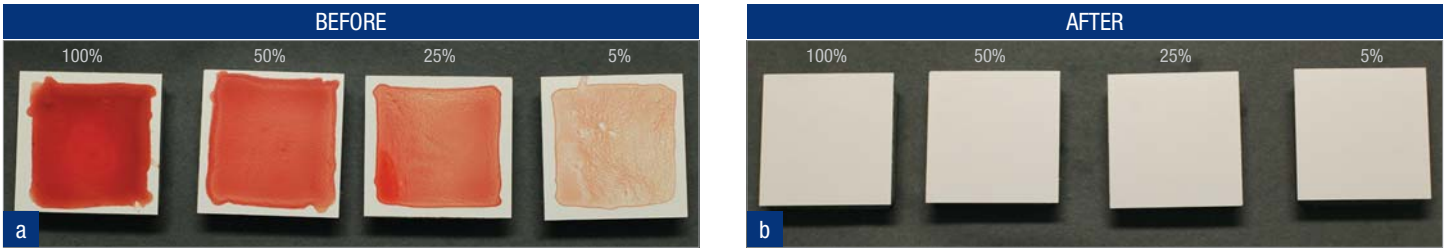

Figure 2. Representative contaminated formica tiles (100%, 50%, 25%, 5% whole blood dilutions) (a) before and (b) after treatment with a high alcohol-based surface disinfectant.

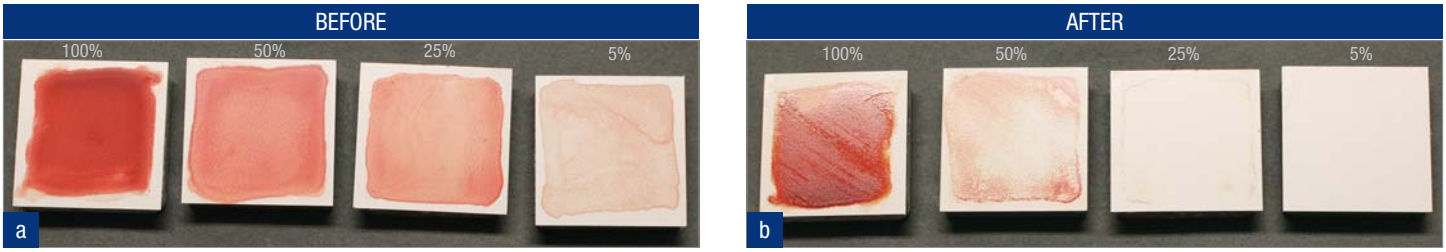

Table 2. Percent (%) of blood remaining on formica tile surfaces after treatment.*

Blood Dilution, %	Clorox HP (TB contact time**)	Clorox HP (bacterial contact time***)	Lysol IC	Super Sani-Cloth
100	0	0	55.0	61.5
50	0	0	12.7	42.8
25	0	0	0.6	9.0
5	0	0	0	0.6

**Percent of Blood determined using the Red Detection application.*

***TB contact time is 5 minutes.*

****Bacterial contact time for E.coli is 30 seconds and 1 minute for MRSA.*

Results (cont.)

Figure 3. Representative contaminated synthetic leather tiles (100%, 50%, 25%, 5% whole blood dilutions) (a) before and (b) after treatment with ***Clorox Healthcare® Hydrogen Peroxide Cleaner Disinfectant Wipes***.

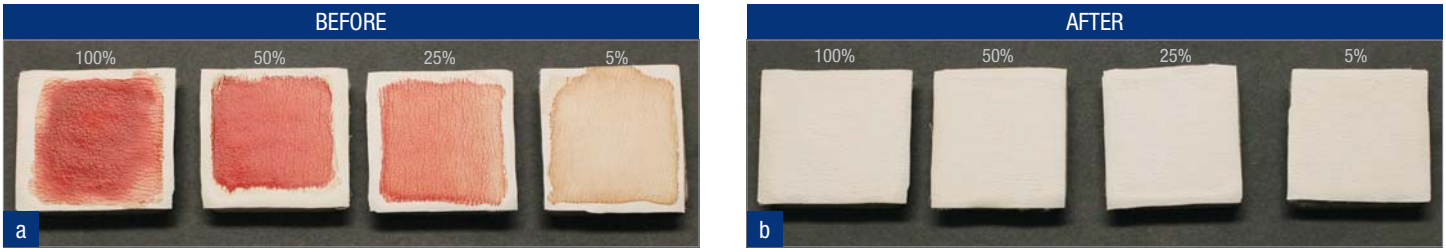

Figure 4. Representative contaminated synthetic leather tiles (100%, 50%, 25%, 5% whole blood dilutions) (a) before and (b) after treatment with a high alcohol-based surface disinfectant.

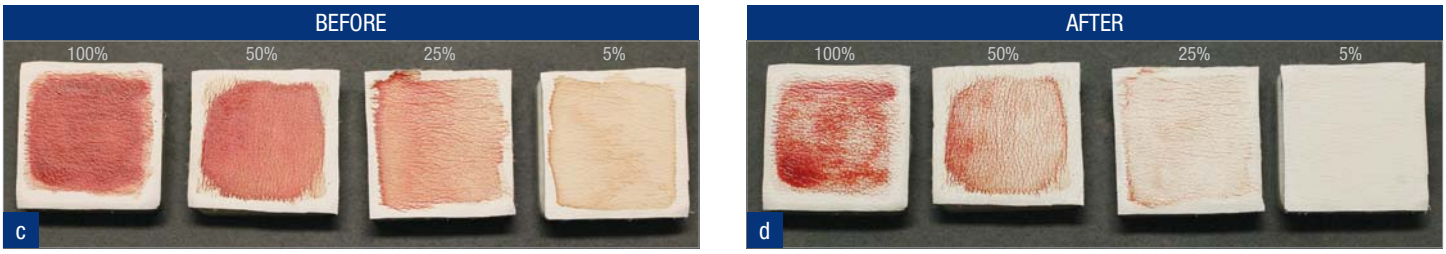

Table 3. Percent (%) of blood remaining on synthetic leather environmental surfaces after treatment.*

Blood Dilution, %	Clorox HP (TB contact time**)	Clorox HP (bacterial contact time***)	Lysol IC	Super Sani-Cloth
100	0	0	53.6	78.7
50	0	0	23.0	60.2
25	0	0	5.2	18.4
5	0	0	0	1.3

**Percent of Blood determined using the Red Detection application.*

***TB contact time is 5 minutes.*

****Bacterial contact time for E.coli is 30 seconds and 1 minute for MRSA.*

Cleaning and disinfection of bacteria in blood

Microbial growth from untreated bacteria/blood-coated tiles for each of the two test bacteria visually demonstrated that sufficiently high concentrations of test bacteria were applied onto contaminated surfaces (Figure 5). When the cleaning and disinfecting capabilities of the commercial preparations were evaluated using the replica plate technique, resultant microbial growth patterns were similar to observations noted for blood removal. In addition to removal of all visible blood from the tiles, no detectable *MRSA* or *E. coli* were found on surfaces treated with ***Clorox Healthcare® Hydrogen Peroxide Cleaner Disinfectant Wipes*** (Figure 6). This positive finding was noted even when undiluted whole blood was used to apply bacteria onto surfaces. In contrast, replica plate cultures from tiles treated with high alcohol *Lysol® Brand III I.C.™ Disinfectant Spray* and *Super Sani-Cloth® Wipes* yielded insufficient removal and/or destruction of applied bacteria (Tables 4-5; Figure 8).

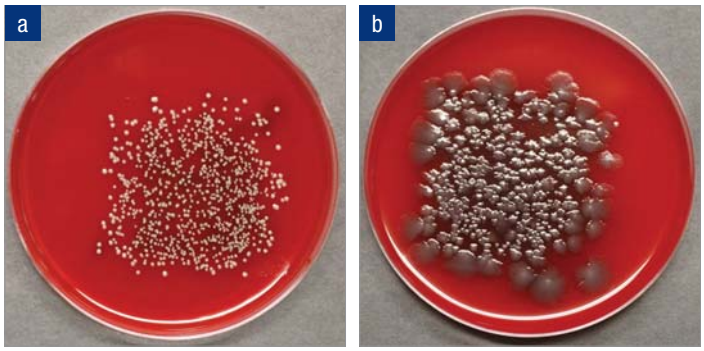

Figure 5. *MRSA* (a) and *E.coli* (b) colonies from untreated tiles (control) after being replica plated onto tryptic soy agar with 5% sheep blood.

Figure 6. Replica plate culture of remaining bacteria after tile surface treatment with ***Clorox Healthcare® Hydrogen Peroxide Cleaner Disinfectant Wipes.***

Results (cont.)

Experiments were also conducted with ***Clorox Healthcare® Hydrogen Peroxide Cleaner Disinfectant Wipes*** using product label contact times for *MRSA* (1 min) and *E.coli* (30 sec). The test methods for these studies were as described above with blood/bacteria suspensions serving as the cleaning and disinfectant challenges (Figure 7). Bacterial culture results showed that all of the *MRSA* and *E.coli* were removed and destroyed following a single application with the hydrogen peroxide disinfectant. (Tables 4-5; Figure 9)

Table 4. Percent (%) of bacteria remaining on synthetic leather tiles after single-step treatment with disinfectants.

Blood Dilution (%)	Clorox HP (TB contact time*)		Clorox HP (bacterial contact time**)		Lysol IC		Super Sani-Cloth	
	E.coli	*MRSA*	*E.coli*	*MRSA*	*E.coli*	*MRSA*	*E.coli*	*MRSA*
100	0	0	0	0	3.10	2.55	7.20	2.00
50	0	0	0	0	0	3.06	1.44	0.95
25	0	0	0	0	0	0.16	0	0
5	0	0	0	0	0	0.17	0	0

**TB contact time is 5 minutes.*
***Bacterial contact time for E.coli is 30 seconds and 1 minute for MRSA.*

Table 5. Percent (%) of bacteria remaining on formica tiles after single-step treatment with disinfectants.

Blood Dilution (%)	Clorox HP (TB contact time*)		Clorox HP (bacterial contact time**)		Lysol IC		Super Sani-Cloth	
	E.coli	*MRSA*	*E.coli*	*MRSA*	*E.coli*	*MRSA*	*E.coli*	*MRSA*
100	0	0	0	0	0	3.61	0	0.17
50	0	0	0	0	0	1.60	0	0.33
25	0	0	0	0	0	0.97	0	0
5	0	0	0	0	0	0.18	0	0

**TB contact time is 5 minutes.*
***Bacterial contact time for E.coli is 30 seconds and 1 minute for MRSA.*

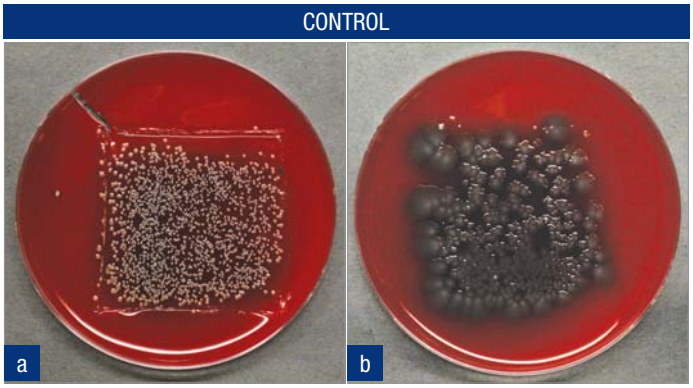

Figure 7. *MRSA* (a) and *E.coli* (b) colonies from untreated tiles (control) after being replica plated onto tryptic soy agar with 5% sheep blood.

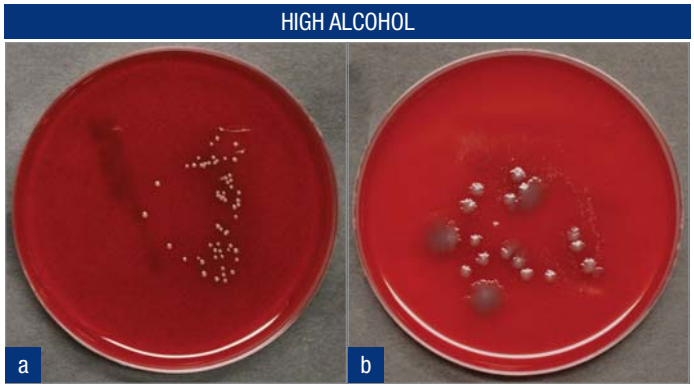

Figure 8. *MRSA* (a) and *E.coli* (b) colonies from tiles treated with an alcohol-based surface disinfectant.

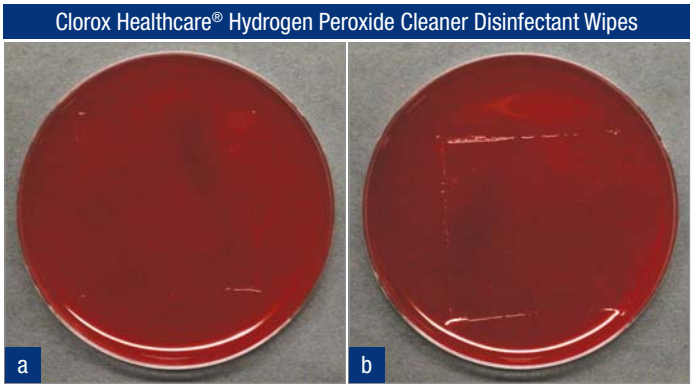

Figure 9. Replica plate culture of remaining bacteria after tile surface treatment using ***Clorox Healthcare® Hydrogen Peroxide Cleaner Disinfectant Wipes*** with the designated contact times for *MRSA* (a) and *E.coli* (b).

Discussion

The initial approach to environmental surface disinfection is the application of a basic principle: *clean first*. Cleaning is the physical removal of debris that can interfere with disinfection and also results in a reduction of the number of microorganisms present on the environmental surface. The chemical formulation of a disinfectant product can greatly affect the ability of the active agent to accomplish cleaning. Water-based cleaner-disinfectants have the greatest capacity to initially remove soil and organic debris, thereby allowing for a more effective disinfection procedure. While there are a variety of effective water-based disinfectant chemical classes, hydrogen peroxide, represented in this study by ***Clorox Healthcare® Hydrogen Peroxide Cleaner Disinfectant Wipes***, is both a broad-spectrum antimicrobial agent with excellent cleaning properties. These characteristics provide ideal features for its use. While this product is newer to the dental world, ***Clorox Healthcare® Hydrogen Peroxide Cleaner Disinfectant Wipes*** are already used extensively in other healthcare settings. Multiple scientific studies support its use as a fast and effective one-step cleaner-disinfectant in hospital settings.[1, 2, 3] These were reinforced by our studies which showed that ***Clorox Healthcare® Hydrogen Peroxide Cleaner Disinfectant Wipes*** were able to quickly and effectively clean blood-coated tiles (even at 30 seconds).

In contrast, infection control disinfectants that contain high concentrations of alcohols may not readily remove bioburden. The chemical action of alcohol explains this effect, as exposure of organic debris to alcohol denatures and dehydrates proteins. This chemical modification renders them more insoluble and adherent onto most surfaces. The denatured material also may actually protect contaminant micoorganisms from the agent's antimicrobial action for extended intervals.

Hydrogen peroxide technology, like bleach, has been shown to act faster than alcohol or quaternary ammonium based disinfectants. With regard to bacteria of clinical importance, such as *MRSA* and *E.coli*, the ***Clorox Healthcare® Hydrogen Peroxide Cleaner Disinfectant Wipes*** were shown in this study to effectively clean surfaces and kill all test organisms within the prescribed 1-minute *(MRSA)* and 30-second *(E.coli)* contact times.

Conclusion

A single-step procedure using ***Clorox Healthcare® Hydrogen Peroxide Cleaner Disinfectant Wipes*** was highly effective in removal of all detectable organic debris from contaminated soft and hard surface tiles. These disinfectants were also able to accomplish effective antimicrobial activity against the test strains of *MRSA* and *E. coli*. In contrast, treatment of test surfaces with products containing greater than 55% alcohol demonstrated visible organic debris and higher concentrations of challenge bacteria.

References:

1. *Boyce J, Havill NL. Evaluation of a new hydrogen peroxide wipe disinfectant. Infect Control Hosp Epidemiol 2013; 34:521-3.*

2. *Rutala W, Gergen MF, Sickbert-Bennett, EE, Williams DA. Effectiveness of improved hydrogen peroxide in decontaminating privacy curtains contaminated with multidrug-resistant pathogens. Am J Infect Control 2014; 42:426-8.*

3. *Rutala W, Gergen MF, Weber DJ. Efficacy of improved hydrogen peroxide against important healthcare-associated pathogens. Infect Control Hosp Epidemiol 2012; 33:1159-61.*