EPA
www.epa.gov/research
technical BRIEF
INNOVATIVE RESEARCH FOR A SUSTAINABLE FUTURE

Effectiveness of Disinfecting and Sporicidal Wipes against *Bacillus atrophaeus*, a *Bacillus anthracis* Surrogate

INTRODUCTION

Disposable wipe materials can, when saturated with a sporicidal liquid, facilitate the inactivation of biological agents such as *Bacillus anthracis* (*B. anthracis*) spores, the causative agent of anthrax. Disinfecting and sporicidal wipes are frequently used in healthcare settings; they are appealing because they are readily available and easy to use. Sporicidal wipes are registered as antimicrobial products effective against *C. difficile* spores under the U.S. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). Sporicidal wipes have not been developed specifically for use against *B. anthracis*. When using such wipes to decontaminate surfaces contaminated with *B. anthracis* spores, efficacy predictions of currently registered sporicidal wipes may be inaccurate, as wipes are generally tested and registered against *C. difficile* spores. FIFRA registration requires efficacy against the actual organism or an approved surrogate. *B. atrophaeus* has been shown to be a suitable surrogate for spray-based decontamination methods using hypochlorite based solutions, when tested side-by-side against *B. anthracis Ames* [1,2]. Therefore, this work was conducted using *B. atrophaeus* as a surrogate for *B. anthracis*.

RESEARCH

Various bench scale studies were conducted to evaluate the effectiveness of commercially available sporicidal wipes against *B. atrophaeus*, under conditions reflective of their realistic field decontamination use. A first study [3] identified wipes that were most effective in inactivation of *B. atrophaeus* as present on various materials with a (contaminated) surface area of 12” x 12” (1.0 ft^2). A second study [4] was conducted to investigate the applicability and limitations in use of the sporicidal wipes when making an effort to inactivate *B. atrophaeus* spores from larger, 42” x 42” (12.25 ft^2), glass and painted drywall materials. This research effort was followed by a third study [Addendum to Reference 4] that extended the efficacy measurements across multiple materials (laminate, acrylic, Viton™ fluoroelastomer (DuPont Performance Elastomers LLC, Wilmington, DE), painted metal, stainless steel, and glass) with an intermediate 28” x28” size. Sporicidal wiping of a surface started in

U.S. Environmental Protection Agency
Office of Research and Development

August 2017
EPA/600/S-17/182

the top left corner with horizontal strokes working downwards. After folding the wipe inwards, vertical wiping occurred starting in the top left corner working to the right. After a second folding of the wipe, the surface was wiped diagonally from the top left corner to the bottom right area. Lastly, the circumference was wiped after the final folding of the wipe.

INITIAL EVALUATION OF DISINFECTING AND SPORICIDAL WIPES

The potential for sporicidal wipes to decontaminate small, contained areas of biological contamination with *B. atrophaeus* was first reported in [3]. This study identified wipes that completely inactivated *B.* spores on a glass Petri dish (following *ASTM E2896-12 Standard test method for quantitative petri plate method for determining the effectiveness of antimicrobial towelettes* [5]). Tests were then conducted with larger indoor material coupons (12" x 12" surface area) to determine the wipes' efficacy against *B. atrophaeus* on the larger surface area. Table 1 identifies the selected sporicidal and disinfecting wipes, manufacturer, and active ingredient. Table 2 summarizes the efficacies, measured as the log reduction in viable spores, using the manufacturer-prescribed contact times for *C. difficile* as to achieve a 6 $\log_{10}$ reduction of *B. atrophaeus* spores. From the results shown in Table 2 it is clear that not all commercially available wipes are able to kill *B. atrophaeus* spores as evaluated. Registration as an antimicrobial product effective against *C. difficile* has a high correlation with observed high efficacy against *B. atrophaeus* within the limitations of this study. The observed low log reduction for three of the seven wipes compared to the other four can be associated with the physical removal process by the wipe only as a significant number of viable spores were recovered from these three wipes [3].

Table 1. Product Information and Application Notes

Product	EPA Registered for *C. difficile* [1] (EPA Reg. No.)	Manufacturer [2]	Active Ingredients
Clorox® Healthcare™ bleach germicidal wipe	YES (67619-12)	Clorox Professional Products Co.	Sodium hypochlorite (0.1–1.0 %)
Sani-Cloth® bleach germicidal disposable wipe	YES (9480-8)	Professional Disposables Internationals, Inc.	Sodium hypochlorite (<1.0 %)
Dispatch® hospital cleaner disinfectant towel with bleach	YES (56392-8)	Clorox Professional Products Co.	Sodium hypochlorite (<1.0 %)
Hype-Wipe® disinfecting towel with bleach	YES (70590-1)	Current Technologies, Inc.	Sodium hypochlorite (0.525 %)
Steriplex® SD wipe	NO	SteriScience, Inc.	Hydrogen peroxide (0.02%), peracetic acid (0.15%)
Lysol® disinfecting wipe	NO	Reckitt Benckiser North America	Alkyl dimethyl benzyl ammonium chloride (0.1-1.0%)
Clorox® disinfecting wipe	NO	Clorox Professional Products Co.	Alkyl dimethyl benzyl ammonium chloride (0.145%)

[1]: Source: LIST K: EPA's Registered Antimicrobial Products Effective against Clostridium difficile Spores. Available from https://www.epa.gov/pesticide-registration/selected-epa-registered-disinfectants. Last accessed May 16, 2017

[2]: See Reference 1 for complete manufacturer/distributor information

Table 2. Efficacy Results for Sporicidal Wipes Against *B. atrophaeus* [3], 12" x 12" Surface Size

Product	Contact time (min)	Mean log reduction (LR) per material (12" x 12" surface)					
		Petri dish	Stainless steel	Glass	Composite epoxy	Painted drywall	LDPE
Clorox® Healthcare™ bleach germicidal wipe	3	≥ 6.1	≥ 7.3	≥ 7.5	7.4	7.2	≥ 7.3
Sani-Cloth® bleach germicidal disposable wipe	4	≥ 6.1	≥ 7.4	≥ 7.5	≥ 7.3	≥ 7.3	≥ 7.5
Dispatch® hospital cleaner disinfectant towel with bleach	5	≥ 6.1	≥ 7.4	7.4	7.4	5.7	≥ 7.4
Hype-Wipe® disinfecting towel with bleach	4	≥ 6.2	≥ 7.3	≥ 7.3	≥ 7.5	≥ 7.3	≥ 7.5
Steriplex® SD wipe	5	2.6					
Lysol® disinfecting wipe	10	**1.5**					
Clorox® disinfecting wipe	4	**1.4**					

Color coding: Green: LR ≥ 6; Yellow: 4 ≤ LR ≤ 6; Orange: 2 ≤ LR ≤ 4; Red and bold font: LR ≤ 2. Disinfecting wipes were not tested beyond initial Petri dish test. LR values indicated as "≥" indicate no viable spores detected following wiping of the surface. LDPE = low density polyethylene. Diagonal lines identify product/material combinations that were not included in the test matrix. Source: Meyer, K.M. et al., J Appl. Microbiol., (117): 1634-1644, (2014).

EXPANSION OF WIPED SURFACE AREA

As a general rule, the area requiring decontamination should be saturated with enough sporicidal liquid to remain wet for the required contact time (Table 2). Two feasibility studies [4] were performed to determine whether sporicidal wipes can be used to efficaciously decontaminate a larger surface area. Two of the four sporicidal wipes that were efficacious in the 12" x 12" tests (i.e., a better than 6-$\log_{10}$ reduction in viable spores across the majority of materials) were considered for decontamination of the most challenging material (painted drywall) with glass as a reference material. This effort started with tests for the decontamination of a 42" x 42" area followed by tests with a reduced area of 28" x 28" based on the outcomes of the 42" x 42" area study. A 42" x 42" surface is an area that is within arm's reach of an adult person and equates to the experimental setup that allowed for inoculation of *B. atrophaeus* spores onto nine 14" x 14" areas in a 3 x 3 distribution grid. Similarly, the 28" x 28" area consisted of four 14" x 14" areas in a 2 x 2 distribution grid. Decontamination efficacy was determined for test areas that were completely contaminated (all 14" x 14" areas were contaminated) and for test conditions where only one 14" x 14" area (1/9th of the 3 x 3 or 1/4th of the 2 x 2 grid) of the surface was contaminated ("hot spot decontamination").

Results for an even spore distribution on 42" x 42" size surface

As summarized in Table 3, neither of the two sporicidal wipes included in this study achieved a mean 6 $\log_{10}$ reduction in viable *B. atrophaeus* spores on the two tested materials when the 42" x 42" surface was evenly contaminated.

Table 3. Efficacy Results for Sporicidal Wipes Against *B. atrophaeus* [4], 42”x 42” Surface

Product	Minimum contact time (min)	Mean log reduction (LR) ± SE per material (n=3)	
		Glass	Painted drywall
Clorox® Healthcare™ bleach germicidal wipe	30	2.9 ± 0.2	2.2 ± 0.2
Hype-Wipe® disinfecting towel with bleach	30	4.5 ± 0.4	3.1 ± 0.2

Color coding: Green: LR ≥ 6; Yellow: 4 ≤ LR ≤ 6; Orange: 2 ≤ LR ≤ 4; Red: LR ≤ 2. SE: Standard Error. Diagonal line identifies product/material combination that was not included in the test matrix. Source: U.S. EPA. 2015. EPA/600/R15/188.

Results for hot spot spore distribution on 42” x 42” surface

Figure 1 shows the log reduction in spores at various 14” x 14” hot spot locations (inoculated prior to decon with 10^7 spores/ft^2) when wiping the whole 42” x 42” surface. The observed efficacy for the top left hot spot location tends to be higher than for hot spots in the center or bottom right. Wiping of the whole surface distributes the available sporicidal liquid from the wipe across the surface with a preference for more liquid left at the top than at the middle or bottom sections. This explains the higher efficacy for the top left hot spot. In the case of the presence of a hot spot in the bottom right, much of the sporicidal liquid from the wipe has been left on the not-contaminated sections before the wipe reaches the contaminated bottom right hot spot. The amount of liquid on both sporicidal wipes is insufficient to reach full decontamination for any hot spot on this large surface. Note that further interpretation is complicated since the wipe motion was horizontal; then vertical; then diagonal, and finally the circumference of the area. All mean $\log_{10}$ reductions were less than 6.0 except for one condition (center “hot spot” on glass and decontaminated using the Hype-Wipe®).

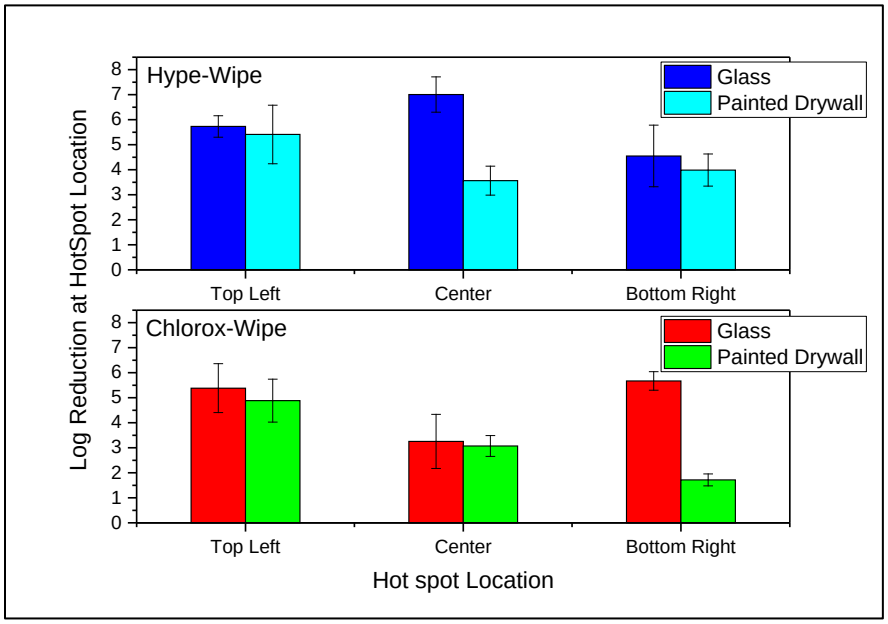
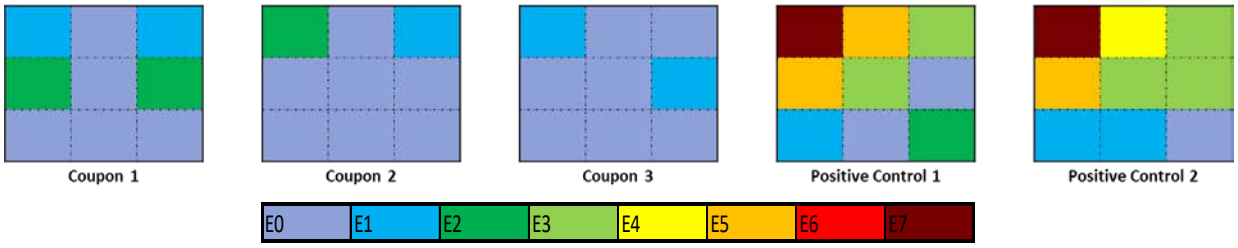


Figure 1. Decontamination efficacy (LR) as function of 14”x14” hot spot location on the 42”x 42” surface.

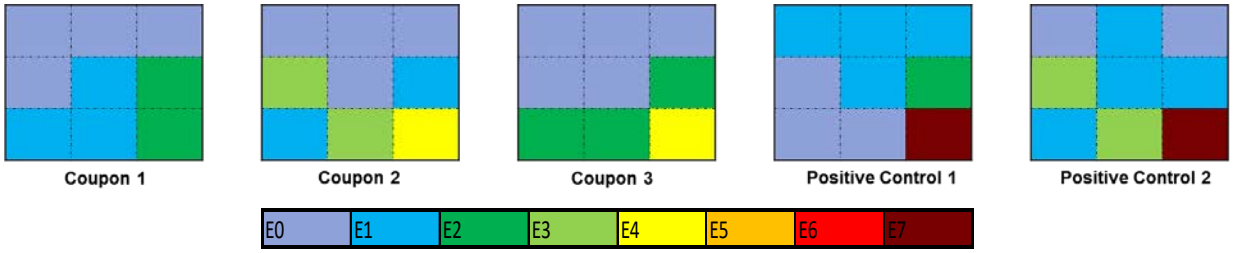
For the 42”x 42” surface sizes, the transfer of spores from a 14” x 14” hot spot to adjacent areas on the same surface was measured. As shown in Figure 2 for a glass surface and using the Hype-Wipe® disinfecting towel, a redistribution of spores occurs due to the wiping process. Whereas the redistribution shown here does not exceed 10^2 spores per 14”x 14” subsection, this example was the best outcome, i.e., the lowest number of redistributed spores (mean $1.6x10^2$ redistributed spores; n=3) observed across all 12 redistribution tests.



Spore concentration per 14” x 14” section color coded scheme. E0 indicates zero spores recovered.

Figure 2. Redistribution of spores from a hot spot on glass as observed following wiping with a Hype-Wipe® sporicidal wipe.

Figure 3 illustrates a noticeable higher redistribution of spores (mean $2.4x10^3$ spores; n=3) due to the wiping process (same wipe material) of the painted drywall surface in the presence of a hot spot in the bottom right corner. The observed lower efficacy for decontaminating the bottom right corner hot spot (Figure 1) is directly associated with the larger redistribution of spores.



Spore concentration per 14” x 14” section color coded scheme. E0 indicates zero spores recovered.

Figure 3. Redistribution of spores from a hot spot on drywall as observed following wiping with a Hype-Wipe® sporicidal wipe.

The combination of observed low efficacy and significant redistribution of spores on a 42” x 42” surface following wiping of such surface size indicates that a single sporicidal wipe cannot be used to achieve a 6 $\log_{10}$ reduction in viable spores. The research did not address whether a second sporicidal wipe would have inactivated the residual spores.

Results for hot spot spore distribution on 28” x 28”

Results from the 42” x 42” hot spot tests indicated that the most challenging condition to decontaminate is when the hot spot is in the bottom right corner (assuming wiping starts in the diagonally opposed top left corner). The hot spot spore distribution tests on the intermediate 28” x 28” surface was limited to this test condition and included six building materials. As can be seen in Figure 4,

the mean $\log_{10}$ reduction at the hot spot location was larger than six for five out of the six materials tested. The noted exception was for wiping of the painted metal, similarly to the lower log reduction for the 12” x 12” painted drywall material.

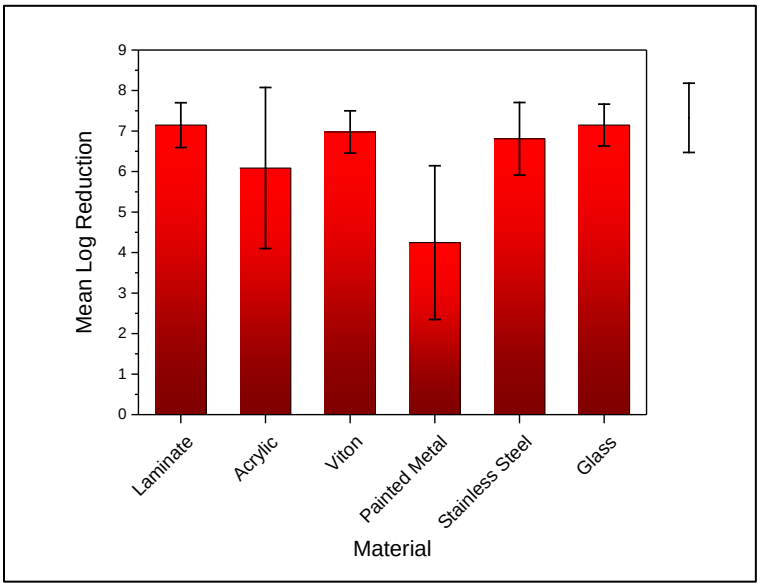


Figure 4. Log reduction at the hot spot location following wiping of a 28” x 28” surface with the Hype-Wipe® disinfecting towel.

A limited redistribution of viable spores to adjacent areas was observed with the lowest number of spores (less than 10) redistributed on Viton™ fluoroelastomer, painted metal, stainless steel, and glass while more than 10 spores, but less than 40 spores, were redistributed on laminate and acrylic. The combination of observed high efficacy and minimal redistribution of spores on a 28” x 28” surface following wiping of such surface size indicates that a single sporicidal wipe can be used to achieve a 6 $\log_{10}$ reduction in viable spores although some material dependence was observed. The research did not address whether a second sporicidal wipe would have inactivated all residual spores.

CONCLUSIONS

Many types of disinfectant and/or sporicidal wipes exist and are commercially available. Of the wipes tested here, only the sporicidal wipes were effective against *B. atrophaeus*. There are, however, significant limitations on the area that can be wiped with such sporicidal wipes. Based on the laboratory testing, an area of up to approximately four square foot resulted in a 6 $\log_{10}$ reduction in viable spores on several common material types, while wiping of larger areas resulted in poor efficacy (less than 6 $\log_{10}$ reduction) and redistributing spores (spreading contamination). The permeable painted surfaces were the most challenging surfaces encountered here. This may be due to loss of sporicidal liquid due to permeation into the paint layer or degradation of the hypochlorite solution on these wipes when in contact with the painted surface.

These data will help individuals such as incident commanders and remediation personnel make informed decisions about surface decontamination options after a biological contamination incident.

Ultimately, decontamination performance of wipes, sprayed liquids/gels, or fumigants, along with the cost and effort will dictate their use in a remediation response.

DISCLAIMER

The U.S. Environmental Protection Agency through its Office of Research and Development funded and managed the research described herein under several contractual agreements listed in the references. This summary has been subjected to the Agency's review and has been approved for publication. Note that approval does not signify that the contents reflect the views of the Agency. Mention of trade names, products, or services does not convey official EPA approval, endorsement, or recommendation.

REFERENCES

[1] U.S. EPA. 2011. Comparative Efficacy of Sporicidal Technologies for the Decontamination of *Bacillus anthracis, B. atrophaeus, and Clostridium difficile* Spores on Building Materials. EPA/600/R14/405.

[2] Calfee, M.W., Y. Choi, J. Rogers, T. Kelly, Z. Willenberg, and K. Riggs. 2011. Lab-Scale Assessment to Support Remediation of Outdoor Surfaces Contaminated with *Bacillus anthracis* Spores. Journal of Bioterrorism & Biodefense, (2): 110

[3] Meyer, K.M., J.A. Tufts, M.W. Calfee, and L. Oudejans. 2014. Efficacy of Sporicidal Wipes for Inactivation of a *Bacillus anthracis* Surrogate. Journal of Applied Microbiology, (117): 1634-1644.

[4] U.S. EPA. 2015. Operational Testing of Sporicidal Wipes for Decontamination of Surfaces Contaminated with *Bacillus anthracis* Surrogate Spores. Research Triangle Park, NC: U.S. Environmental Protection Agency. EPA/600/R15/188.

[5] ASTM International. 2012. ASTM E2896-12 Standard Test Method for Quantitative Petri Plate Method (QPM) for Determining the Effectiveness of Antimicrobial Towelettes. West Conshohocken, PA: ASTM International.

CONTACT INFORMATION

For more information, visit the EPA Web site at http://www2.epa.gov/homeland-security-research.

Technical Contact: Lukas Oudejans (oudejans.lukas@epa.gov)
General Feedback/Questions: Kathy Nickel (nickel.kathy@epa.gov)

U.S. EPA's Homeland Security Research Program (HSRP) develops products based on scientific research and technology evaluations. Our products and expertise are widely used in preventing, preparing for, and recovering from public health and environmental emergencies that arise from terrorist attacks or natural disasters. Our research and products address biological, radiological, or chemical contaminants that could affect indoor areas, outdoor areas, or water infrastructure. HSRP provides these products, technical assistance, and expertise to support EPA's roles and responsibilities under the National Response Framework, statutory requirements, and Homeland Security Presidential Directives.