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Decontamination of Subway Railcar and Related Materials Contaminated with *Bacillus anthracis* Spores via the Fogging of Peracetic Acid and Aqueous Hydrogen Peroxide

Office of Research and Development
Homeland Security Research Program

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U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

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Disclaimer

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Executive Summary

The Department of Homeland Security's (DHS's) Underground Transport Restoration (UTR) Program was established to identify potential methods for rapid characterization, cleanup, and clearance of biological contamination in an underground transit system. This would include physical structures (tunnels and stations) and rolling stock (railcars). The UTR Project is expected to improve the capability for transit systems to recover rapidly from a biological release event and thereby addresses a high-priority need expressed by the Transportation Security Administration (TSA) and local transit systems. As part of this UTR Project, the U.S. Environmental Protection Agency (EPA) is evaluating multiple methodologies for the decontamination of subway and railcar materials contaminated by a biological agent.

This project supports the U.S. EPA Office of Research and Development's (ORD's) Homeland Security Research Program (HSRP) mission of helping protect human health and the environment from adverse impacts resulting from the release of chemical, biological, or radiological agents. With an emphasis on decontamination and consequence management, water infrastructure protection, and threat and consequence assessment, the HSRP is working to develop technology and information that will help detect the intentional introduction of chemical or biological contaminants in buildings or water systems; contain these contaminants; decontaminate buildings, water systems, or other infrastructure; and facilitate the disposal of material resulting from restoration activities.

In the event of a biological incident in a transportation hub such as a subway system, effective remediation of railcars, subway tunnels and stations will require the use of various decontamination approaches. One potential decontamination tool that could be used in such an event is the fogging of sporicidal liquids. The study described in this report builds on previous fogging decontamination research, but with a focus on decontaminating subway railcars and related materials. More precisely, the purpose of this study was to evaluate the efficacy of fogging to decontaminate a variety of subway railcar materials contaminated with *Bacillus anthracis* (*B.a.*; Ames strain) spores. Multiple variables were investigated to assess their effect on decontamination efficacy, including spore species (*B.a.* and *Bacillus atrophaeus* aka *Bacillus globigii*, or *B.g.*), railcar or tunnel material, fogger types, air temperature, sporicidal liquid (peracetic acid [PAA] or aqueous hydrogen peroxide [H_2O_2]), quantity of liquid fogged, and location within the test chamber.

Summary of Major Findings

This evaluation focused on the decontamination of eleven types of subway railcar materials and a common subway tunnel material (unpainted concrete). Decontamination efficacy tests were conducted with spores of virulent *B.a.* Ames and non-virulent *B.g.*, to assess the potential use of *B.g.* as a surrogate for future studies with fogging equipment of sporicidal liquids. A summary of the decontamination efficacy results, in terms of average $\log_{10}$ reduction (LR) by material and microorganism, is shown in Table ES-1. A decontaminant product is considered to be an effective sporicide or sporicidal decontaminant if a 6 LR or greater is achieved based upon appropriate laboratory testing.

The data and statistical analyses generated from this evaluation suggest that *B.g.* may be a suitable surrogate for *B.a.* Ames for future tests assessing the decontamination efficacy of PAA or H_2O_2 using fogging equipment.

Many of the subway railcar materials were effectively decontaminated (achieved a 6LR or greater) by fogging PAA. These materials include the rubber flooring, seat upholstery, aluminum seat backing, Mylar® glass coating, and both new and used cabin air filters. Fogging of PAA was ineffective for the carpet, concrete, and grease (with spores mixed in/encapsulated into grease); and moderately effective (approximately 3-6 LR) for the interior fiberglass side panel material, and the clean and dirty railcar grease (spores left on top of grease).

With respect to the effect of air temperature, while the higher temperature (20 °C) resulted in a greater probability of complete spore population kill and greater LR values compared to the results at 10 °C (an average of 1-2 LR better), many of these differences were not statistically significant.

The two types of foggers yielded similar LR values when compared at 20 °C. Testing conducted using the same parameters but at 10 °C generally yielded higher LR for the Sani-Tizer™ fogger as compared to the Minncare equipment. Overall, however, statistical analysis using the logistic regression model indicated that the type of fogger did not have a significant effect on LR.

There was minimal effect of location within the test chamber on decontamination efficacy. However, as would be expected, coupons stationed horizontally on a cart facing upward, in the center of the chamber, were more likely to show a complete kill compared to the other four locations in the chamber *(i.e.*., vertical orientation on wall, in the duct, underneath the table, and on the floor near the corner).

Table ES-1. Summary of *B.a.* Ames and *B. atrophaeus* Log Reductions by Material Type

Material Type	Log Reduction Across All Tests					
	Minimum		Maximum		Average ± SD	
	B. anthracis	***B. atrophaeus***	***B. anthracis***	***B. atrophaeus***	***B. anthracis***	***B. atrophaeus***
Rubber Flooring	7.11	6.12	8.07	7.31	7.76 ± 0.35	6.92 ± 0.46
Seat Upholstery	7.12	6.23	8.08	7.56	7.79 ± 0.45	6.96 ± 0.57
Aluminum Seat Back	7.38	7.06	8.01	7.65	7.81 ± 0.29	7.30 ± 0.25
Mylar® Glass Window Coating	7.56	6.89	8.06	7.39	7.83 ± 0.17	7.10 ± 0.17
Fiberglass Side Panel	3.39	3.21	7.58	6.84	5.82 ± 1.15	5.65 ± 1.06
Railcar Carpet	0.39	0.41	6.26	5.71	2.43 ± 1.64	1.91 ± 1.20
Unpainted Concrete	0.60	0.30	2.70	2.21	1.62 ± 0.60	1.36 ± 0.65
New Grease (Spores on Top of Grease)	0.21	0.92	7.61	6.41	4.45 ± 2.62	4.70 ± 1.90
New Grease (Spores mixed in to grease aka encapsulated)	0.33	0.80	2.77	4.83	1.59 ± 0.85	2.24 ± 1.02
Used Grease (Spores on Top of Grease)	0.24	0.91	7.91	6.92	5.00 ± 2.29	5.34 ± 1.58
Railcar Air Filter (New)	5.85	6.38	7.99	6.64	6.77 ± 1.10	6.54 ± 0.14
Railcar Air Filter (Used)	2.10	2.63	7.92	7.20	7.10 ± 1.70	6.41 ± 1.30
New Industrial Carpet	4.32	4.81	4.32	4.81	4.32 ± 0.0	4.81 ± 0.0

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Abbreviations/Acronyms

AOAC	Association of Official Analytical Chemists (now AOAC International)
ARCA	Aerosol Research and Component Assessment Chamber
ASTM	American Society of Testing and Materials
B. anthracis	*Bacillus anthracis* Ames
BART	Bay Area Rapid Transit
BBRC	Battelle Biomedical Research Center
B.g.	*Bacillus globigii*, aka *Bacillus atrophaeus*
BSC	biological safety cabinet
CFU	colony forming units
CI	confidence interval
cm	centimeter(s)
°C	degree(s) Celsius
DHS	Department of Homeland Security
DNA	Deoxyribonucleic Acid
E-beam	electron beam
EPA	U.S. Environmental Protection Agency
h	hour
HCl	hydrochloric acid
HSRP	Homeland Security Research Program
HVAC	Heating, ventilation, and air conditioning
kGy	kilogray(s)
L	liter(s)
LAL	Limulus Amebocyte Lysate
LPM	liters per minute
LR	$\log_{10}$ reduction
µg	microgram(s)
µL	microliter(s)
m	meter
mg	milligram(s)
mL	milliliter(s)
mil	thousandth of an inch
min	minute(s)
mm	millimeter(s)
µm	micrometer(s)
MMD	Mass Median Diameter
NA	not applicable
NHSRC	National Homeland Security Research Center
ORD	Office of Research and Development
PAA	peracetic acid

PBS	phosphate buffered saline
PBST	PBS + 0.1% Triton X-100
PCR	polymerase chain reaction
PE	Performance evaluation
PDI	phase Doppler interferometer
psi	Pounds per square inch
PVC	probe volume corrected
QA	quality assurance
QAPP	Quality Assurance Project Plan
QC	quality control
QMP	Quality Management Plan
RH	relative humidity
rpm	revolution(s) per minute
s	second(s)
SD	standard deviation
SE	standard error
SFW	sterile filtered water (cell-culture grade)
SOT	Spores on top
SSE	Sum of squares due to error
SSM	Sum of squares about the mean
STREAMS II	Scientific, Technical, Research, Engineering, and Modeling Support Contract
T0	time zero
TSA	technical systems audit
UTR	Underground Transport Restoration
V	Volt
VMD	Volume Median Diameter

1.0 Introduction

The U.S. Environmental Protection Agency's (EPA's) Homeland Security Research Program (HSRP) is helping protect human health and the environment from adverse impacts resulting from the release of chemical, biological, or radiological agents. With an emphasis on decontamination and consequence management, water infrastructure protection, and threat and consequence assessment, the HSRP is working to develop technology and information that will help detect the intentional introduction of chemical or biological contaminants into buildings or water systems; contain these contaminants; decontaminate buildings, water systems, or other infrastructure; and facilitate the disposal of material resulting from restoration activities.

In the event of a biological incident in a transportation hub such as a subway system, effective remediation of railcars, subway tunnels and stations will require the use of various decontamination approaches. One potential decontamination tool that could be used in such an event is the fogging of sporicidal liquids. The study described in this report builds on previous fogging decontamination research[(1)], but with a focus on decontaminating subway railcar and related materials. More precisely, the purpose of this study was to evaluate the efficacy of fogging to decontaminate a variety of subway railcar materials contaminated with *Bacillus anthracis* (*B.a.*) spores. Over the course of 21 tests, multiple variables were investigated to assess their effect on decontamination efficacy, including spore species, material, fogger type, air temperature, sporicidal liquid, quantity of liquid fogged, and location within the test chamber.

Many of the materials used in the study originated from actual in-use subway railcars, and include carpet, aluminum seat back, seat upholstery, rubber flooring, Mylar® coating (from a glass window), fiberglass interior siding, railcar axle grease, new cabin air filter, and a used cabin air filter. Unpainted concrete was also included in the majority of tests, as this is a common subway tunnel material. Most of the decontamination efficacy tests were conducted using peracetic acid (PAA) fog, based on its use in a previous fog decontamination study[(1)], and PAA's relatively high efficacy against *B.a.* spores on many materials when applied as a spray. However, a few tests were conducted with the fogging of aqueous hydrogen peroxide (H_2O_2) solutions. Tests were conducted with spores of *B.a.* Ames and a potential surrogate, *Bacillus atrophaeus* (aka *Bacillus globigii, or B.g.*). Testing was conducted in a pilot-scale chamber at either 20 °C or 10 °C, the latter to better represent the temperature of underground subway tunnels and stations. Finally, two different foggers were used in the test program: a relatively expensive fogger comprised of primarily stainless steel parts, and a less expensive fogger constructed of primarily plastic components.

Decontamination efficacy was determined based on the $\log_{10}$ reduction (LR) in viable spores recovered from the inoculated samples, with and without exposure to the sporicidal fog. A decontaminant is considered to be an effective sporicide if a 6 LR or greater is achieved in appropriate laboratory testing on the materials tested for a given set of conditions.

The results of this investigation provide decontamination stakeholders and decision-makers with high quality, peer-reviewed data to evaluate the use of fogging equipment to disperse sporicidal liquids in a subway railcar and related environment as a function of the spore type, the material the spore is associated with, temperature, equipment type and sporicidal liquid used.

2.0 Procedures

This section provides an overview of the procedures used for the pilot-scale evaluation of fogging sporicidal liquids to inactivate *B.a.* and *B.g.* spores on 13 different materials. Testing was performed in accordance with the peer-reviewed and EPA-approved Quality Assurance Project Plan (QAPP) for the *Decontamination of Subway and Other Materials through the Fogging of Sporicidal Liquids* and associated amendments.[(2)] The QAPP provides additional procedural details that are not included in this report.

2.1 Test Matrix

The test matrix for the study is shown in Table 2-1. Each of the 21 tests was performed using a subset of six materials (chosen from a total of 13 materials) inoculated with spores of *B.a.* and the same six materials inoculated with *B.g*. Operational parameters such as type of fogger, air temperature, decontaminant chemical, decontaminant volume fogged, and contact time were varied to assess effect on decontamination efficacy. Each of these test variables is further described below.

Table 2-1 Decontamination Test Matrix

Test Number	Operational Parameters					Materials
	Fogging Equipment	Air Temperature (°C)	Sporicidal Liquid	Decontamination Volume Fogged (mL)	Contact Time (h)	
1	Sani-Tizer*	20	PAA	160	18	R, U, A, M, F, Ca
2			8% H_2O_2	2365	168	
3			PAA	160	1-5 Days	Ca
4				78	18	R, M, F, Ca, NGSOT, NF
5			22% H_2O_2			
6			PAA		8	R, M, F, Co, NGSOT, UF
7				160	18	Ca, Co, NGSOT, NGM, UGSOT, UF
8	MinnCare					
9						R, U, A, M, F, Ca
10				500		F, Ca, Co, NGM, UGSOT, UF
11	Sani-Tizer*	10		160		R, U, A, M, F, Ca
12				78		R, M, F, Ca, NG, NF
13				160		Ca, Co, NGSOT, NGM, UGSOT, UF
14	MinnCare			500		F, Ca, Co, NGM, UGSOT, UF
15	Sani-Tizer*	20				
16	MinnCare		35% H_2O_2	500		F, Ca, Co, NGM, UGSOT, UF
17	Sani-Tizer*			1000		F, Ca, Co, NGM, UGSOT, UF
18			PAA			F, Ca, Co, NGSOT, NGM, UGSOT
19		10	PAA			F, Ca, Co, NGM, UGSOT, IC
20			35% H_2O_2			F, Ca, Co, NGM, UGSOT, UF

21	Minncare	10	PAA	500		Ca, Co, NGSOT, NGM, UGSOT, UF

Material Key: R=Rubber, U=Upholstery, A=Aluminum, M=Mylar, F=Fiberglass, Ca=Carpet, Co=Concrete, NGSOT=New Grease Spores on Top, NGM=New Grease Spores Mixed (i.e., encapsulated), UGSOT=Used Grease Spores on Top, NF=New Filter, UF=Used Filter, IC=Industrial Carpet (new).

2.2 Biological Agents

The virulent *B.a.* spores used for this testing were prepared from a qualified stock of the Ames strain at the Battelle Biomedical Research Center (BBRC, Lot B21, West Jefferson, OH) using a BioFlo 3000 fermenter (New Brunswick Scientific Co., Inc., Edison, NJ). The spore lot was subject to a stringent characterization and qualification process required by the Battelle standard operating procedure for spore production. Specifically, the spore lot was characterized prior to use by observation of colony morphology, direct microscopic observation of spore morphology, and size and determination of percent refractivity and percent encapsulation. In addition, the number of viable spores was determined by colony count and expressed as colony forming units per milliliter (CFU/mL). Theoretically, once plated onto bacterial growth media, each viable spore germinates and can yield one CFU. Variations in the expected colony phenotypes were recorded. Endotoxin concentration of each spore preparation was determined by the Limulus Amebocyte Lysate (LAL) assay to assess whether contamination from Gram-negative bacteria occurred during the propagation and purification process of the spores. Genomic deoxyribonucleic acid (DNA) was extracted from the spores and DNA fingerprinting by polymerase chain reaction (PCR) was done to confirm the genotype. This work was confirmed by an independent third party. The virulence of the spore lot was measured by challenging guinea pigs intradermally with a dilution series of spore suspensions, and virulence was expressed as the intradermal median lethal dose. (Note, the tests with guinea pigs were conducted previously and not conducted under this study.) In addition, testing was conducted for robustness of the spores via hydrochloric acid (HCl) resistance.

The *B.g.* spores (Lot 19076-03268) were supplied in powder form by the US EPA, and were originally obtained from Dugway Proving Ground (Tooele County, UT). The *B.g.* stock spore suspensions were prepared in sterile phosphate-buffered saline containing 0.1% Triton X-100 surfactant (PBST; Sigma, St. Louis, MO) at the same concentration as the *B.a.* stock and stored at 2 to 8 degrees Celsius (°C). No further activities were performed to verify the identity of the organism.

The *B.a.* stock spore suspension was prepared in sterile filtered water (SFW) at an approximate concentration of 1×10^9 CFU/mL and stored at 2 to 8 °C. This buffer was chosen to be consistent with previous work conducted with the same *B.g.* spores at EPA.

2.3 Test Materials

Decontamination testing was conducted using a number of materials removed from an actual subway railcar and a common subway tunnel material. These materials are listed in table 2-2.. In one test (Test 19), we included new industrial carpet in order to compare with the used/dirty railcar carpet, to assess whether the dirt and grime was a factor in decontamination efficacy of the railcar carpet. In addition, both new and used railcar grease were used as a “coupon” when applied to glass. The grease coupons were tested in two configurations: 1) spores dried on top of the grease, and 2) dried spores mixed (encapsulated) into the grease. Information on all of these

materials is presented in Table 2-2, and a picture of each is presented in Figure 2-1. Coupons used for testing were cut to uniform length and width (Table 2-2) from the larger pieces of stock material. Coupons materials were prepared for testing by either sterilization via electron beam (E-beam) irradiation at ~200 kilogray (kGy; E-beam Services Inc., Lebanon, OH) or autoclaved at 121 °C for 15 minutes (min). E-beam-irradiated material coupons were sealed in 6 mil (0.006 inch) Uline Poly Tubing (Cat. No. S-2940, Uline, Chicago, IL), and autoclaved coupons were sealed in sterilization pouches (Cat. No. 01-812-50, Fisher, Pittsburgh, PA) to preserve sterility until the coupons were ready for use. Sterilization was intended to eliminate contamination by endogenous microorganisms.

Figure 2-1. Coupon Materials from Left to Right: Railcar Carpet, Mylar, Aluminum, Rubber Flooring, New Filter, Used Filter, Fiberglass, Upholstery, Encapsulated New Grease, New Grease SOT, Used Grease SOT, Unpainted Concrete, Industrial Carpet

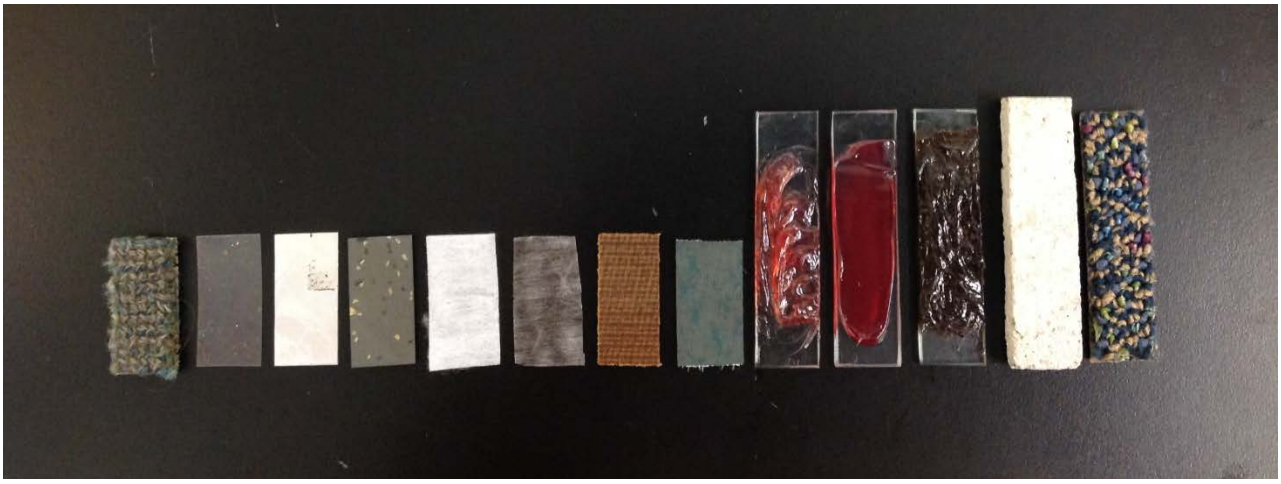

Table 2-2. Test Materials

Material (abbrevia-tion)	Lot, Batch, or ASTM No., or Observation	Manufacturer/ Supplier Name Location	Approximate Coupon Size, Width x Length x Thickness	Material Preparation
Used Carpet (CA)	Received from Bay Area Rapid Transit (BART)	U.S. EPA	1.9 cm x 3.8 cm x 0.2 cm	E-Beam
Aluminum seat backing (A)	Received from BART	U.S. EPA	1.9 cm x 3.8 cm x 0.2 cm	Autoclave
Seat Upholstery (U)	Received from BART	U.S. EPA	1.9 cm x 3.8 cm x 0.2 cm	Autoclave
Rubber Flooring (R)	Received from BART	U.S. EPA	1.9 cm x 3.8 cm x 0.2 cm	Autoclave
Mylar® glass window coating (M)	Received from BART	U.S. EPA	1.9 cm x 3.8 cm x 0.2 cm	Autoclave
Fiberglass interior siding (F)	Received from BART	U.S. EPA	1.9 cm x 3.8 cm x 0.2 cm	Autoclave

New Cabin Air Filter (NF)	Received from BART	U.S. EPA	1.9 cm x 3.8 cm x 0.2 cm	Autoclave
Used Cabin Air Filter (UF)	Received from BART	U.S. EPA	1.9 cm x 3.8 cm x 0.2 cm	Autoclave
New Grease (NG)	Ultra-Duty EP, NLGI 2	Chevron, San Ramon, CA	1 mL of grease onto glass 1.9 cm x 7.6 cm x 0.2 cm	Autoclave
Grimy Used Grease (UG)	Received from BART	U.S. EPA	1 mL of grease onto glass 1.9 cm x 7.6 cm x 0.2 cm	Autoclave
Glass (used only with grease samples)	C1036	Brooks Brothers, Columbus, OH	1.9 cm x 7.6 cm x 0.2 cm	Autoclave
Unpainted Concrete (Co)	ASTM C90 cinder block	Wellnitz Columbus, OH	1.9 cm x 7.6 cm x 0.2 cm	Autoclave
Industrial carpet (IC)	Shaw Swizzle EcoWorx, Style: 10401 Color: Jacks	Shaw Industries, Dalton, GA	1.9 cm × 7.5 cm × 0.3 cm	E-beam

2.4 Inoculation of Coupons

Test and positive control coupons were placed on a flat surface within a Class II biological safety cabinet (BSC) and inoculated with approximately 1×10^8 CFU of viable *B.a.* Ames or surrogate *B.g.* spores per coupon. A 100 microliter (µL) aliquot of a stock suspension of approximately 1×10^9 CFU/mL was dispensed using a micropipette applied as 10 µL droplets across the coupon surface (see Figure 2-2). This approach provided a more uniform distribution of spores across the coupon surface than would be obtained through a single drop of the suspension. Although application of the inoculum onto each material was uniform, the behavior of the inoculum droplets was not. Droplets beaded on the surface of the glass (nonporous material) while they soaked into the other porous materials after producing a liquid bead for a short period of time. The difference in the behavior of the inoculum droplets on each material could lead to a variance in microorganism distribution across coupons; however, this effect was not studied in this evaluation. After inoculation, the coupons were transferred to a Class III BSC and left undisturbed overnight to dry under ambient conditions, approximately 22 °C and 40 % relative humidity (RH).

The grease test materials were prepared by first applying 1 mL of grease using a 3 mL syringe at one end of the glass material. The grease was then spread across the test material using a sterile colony spreader, creating a thin film, and then the target organism was applied in a manner identical to the other test materials. For the “coupon” where the spores were mixed (encapsulated) into the clean grease, after the spore inoculum was dried, a sterile glass rod was used to mix the dried spores into the grease using a circular motion across the glass.

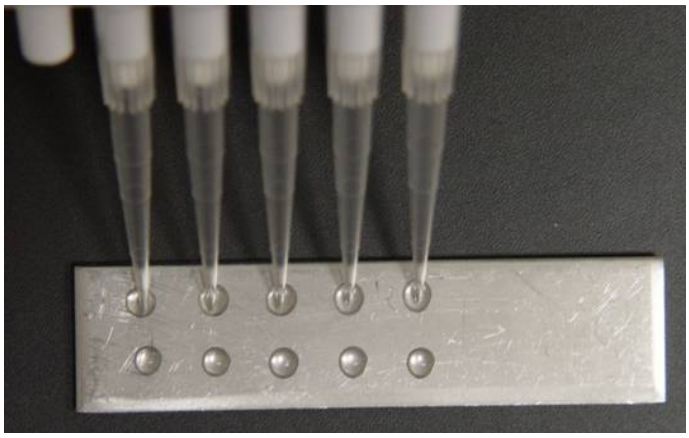

Figure 2-2. Liquid Inoculation of Coupon Using a Micropipette

The number and type of replicate coupons used for each combination of material, decontaminant, concentration, fogger type, and environmental condition included:

- Five test coupons (inoculated with *B. anthracis* or surrogate spores and exposed to sporicidal fog)
- Five positive controls (inoculated with *B. anthracis* or surrogate spores but not exposed to sporicidal fog)
- One laboratory blank (not inoculated and not exposed to sporicidal fog)
- One procedural blank (not inoculated and exposed to sporicidal fog).

On the day following inoculation, coupons intended for decontamination (including blanks) were transferred into the aerosol research and component assessment (ARCA) test chamber, placed in one of five designated positions, and exposed to the sporicidal liquid fog using the apparatus and application conditions specified in Section 2.5. Control coupons remained in the BSC III chamber where they were dried and collected for processing at the conclusion of the test chamber contact time.

2.5 ARCA Test Chamber and Procedures

Decontamination testing was conducted inside the ARCA test chamber with an approximate internal volume of 16 cubic meters (m^3). Figure 2-3 shows a schematic of the ARCA test chamber as well as fogging equipment and test coupon locations. This BSC III chamber is hard-ducted to the facility exhaust system ventilation, but during each test, valves on both the exhaust and supply were closed to create a sealed enclosure. Once test contact duration had concluded, the exhaust and supply valves were opened to allow for any residual fumigant to be removed.

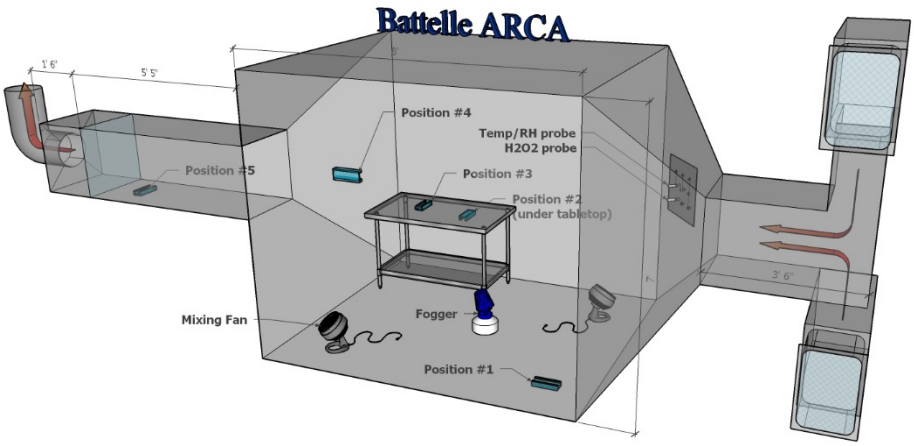

Figure 2-3. Schematic of ARCA Test Chamber

For testing targeting 10 °C conditions, the temperature was controlled using a Krack HTSS-0100MSD air cooled condensing unit and KR26A-089EB low profile evaporator (Krack, Bolingbrook, IL) refrigerant system. Temperature and RH in the test chamber were measured using an HMT368 temperature and humidity probe (Vaisala, Inc., Woburn, MA). The RH of the chamber was not controlled during testing, and rapidly increased with the onset of fogging. Since the PAA solution contains hydrogen peroxide, hydrogen peroxide vapor was measured as an indicator of the fog process using an ATI B12 2-wire gas transmitter (Analytical Technology, Inc., Collegeville, PA). All parameters were recorded every minute during the experimental exposure time using a UX120-006M HOBO data logger and associated HOBOware software (Onset, Bourne, MA).

Five test positions were selected within the ARCA chamber, including three horizontal positions (1, 3, and 5), one vertical (4), and one inverted position (2). One replicate of each coupon material was placed at each location. In addition, at each chamber location, wetness was measured in terms of total percent coverage by using a HOBO S-LWA-M003 leaf wetness sensor connected to a HOBO H21-002 micro station data logger that recorded wetness measurements every minute for the duration of each test. Test locations 1-4 were all located within the main chamber of the ARCA, while test position 5 was located approximately five feet off the main chamber in a 2'x 2' duct. This location was selected to challenge the ability of the fog to disseminate through a more complex area. A representative graph of the ARCA chamber test conditions (from Test 17) data collection can be seen in Figure 2-4.

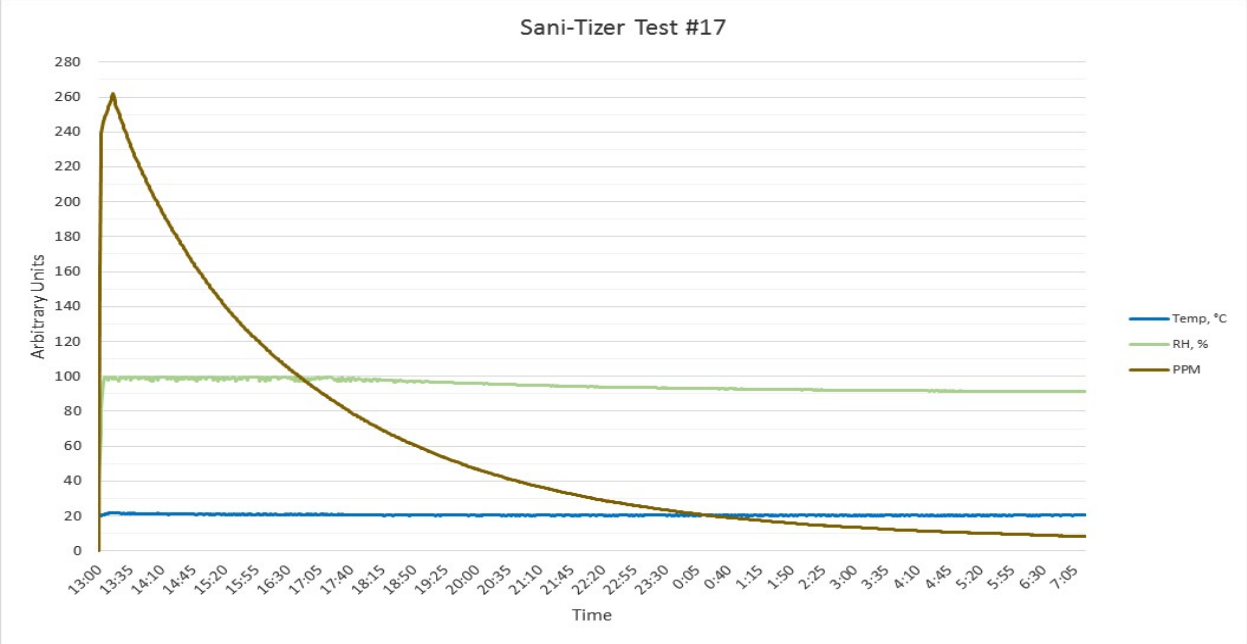

Figure 2-4. Representative Graph of Temperature, RH, and H_2O_2 Vapor Concentration (ppm) During Fogging

2.6 Fogging Equipment

Figure 2.5 is a photo of the ARCA chamber being fogged with a sporicidal liquid. Two fogger technologies were tested for the ability to disseminate fogged sporicidal liquids throughout the large test chamber. The first technology tested was the Sani-Tizer 3001-1 (Curtis Dyna-Fog Ltd., Jackson, GA). This fogger was constructed largely of plastic parts and required a 120 volt (V) circuit to operate. Figure 2-6 shows the Curtis Dyna-Fog Sani-Tizer fogger. The unit was equipped with a one gallon tank, three spray nozzles, and a rotary knob for control of liquid flow rates that are listed as 0 to 4.5 gallons per hour. The median droplet size of 31 microns, generated using PAA (method used to measure droplet size distribution (by volume) is discussed in Section 2.8), was within the published particle size distribution of 5-50 microns as listed in the product manual[(3)]. All testing conducted with this fogger used the low flow setting as indicated on the rotary knob and resulted in flow rates ranging from 63-187 mL/min.

Figure 2-5. ARCA During Fog Generation

Figure 2-6. Sani-Tizer Fogger

The second fogger tested was the Mini Dry Fog System (Mar Cor Purification, Plymouth, MN). Figure 2-7 shows the Mini Dry Fog system which was made entirely of stainless steel and required a controlled outside air source as its means of generating the aerosol droplets using an air atomizing nozzle. The unit was equipped with one spray nozzle, a 500 mL liquid reservoir, and an in-line regulator to maintain pressure at the nozzle. The measured median droplet size (by volume) of 12.4 microns for this evaluation, as described below in Section 2.8, was slightly larger than the 7.5 microns listed in the product literature[(4)]. This device required a controlled

pressure of 75 pounds per square inch (psi) as well as minimum flow rate of 56 liters per minute (LPM). Pressure was measured using a Dwyer DPG-205-NIST (Dwyer, Michigan City, IN). Flow rate was measured using an Aalborg GFM47 flow meter (Aalborg Instruments and Controls, Orangeburg, NY). Data from these devices were recorded every minute during operation using a UX120-006M HOBO data logger.

Figure 2-7. Mini Dry Fog System

2.7 Sporicidal Liquids

Two types of sporicidal liquids were examined in this study (PAA and H_2O_2). PAA was used as received (Minncare Cold Sterilant, Cat. No. 78325-150, Mar Cor Purification, Plymouth, MN) and consisted of 22 % hydrogen peroxide, 9 % acetic acid, and 4.5 % peroxyacetic acid. Three concentrations of aqueous H_2O_2 were used (35 %, 22 % and 8 %). The 35 % solution was used as received (Cat. No. HPV-AQ, Horsham, PA) which consisted of 35 % w/w aqueous hydrogen peroxide solution. This stock solution was diluted to target concentrations of 22 % and 8 % using sterile water. These concentrations were verified by permanganate titration and resulted in final concentrations of 22.4 % and 8.6 % respectively.

2.8 Fog Droplet Size Characterization

During this evaluation, droplet size measurements were made using an Artium Phase Doppler Interferometer (PDI; Model 200MD, Sunnyvale, CA). These tests were conducted to confirm droplet size information reported by the vendors. Fogger droplet size distribution is important, since smaller droplets tend to remain aloft in the air longer and therefore are more easily and widely distributed throughout the volume being decontaminated. The techniques used by the PDI to measure droplet size have previously been described in literature [5,6]. The probe volume corrected (PVC) fluxes were also recorded in order to accurately assess the overall spray plume size distribution. The Artium PDI PVC flux measurements have already been shown to be in good agreement with traditional mechanical patternation local volume flux measurements[7].

The two-dimensional Artium Technologies PDI – 200MD instrument was used to acquire droplet size measurements across the spray plume. The PDI system was operated in a 1-D orientation for

these measurements, resulting in a purely stream-wise velocity component. The transmitter and receiver were mounted on a rail assembly with rotary plates at a 40 degree forward scattering collection angle. The 500 millimeter (mm) lenses were used for both the transmitter and receiver, which allowed for measurement of droplets in the range of 1.5 to 160 micrometers (µm) [8].

The spray nozzles were placed 21 centimeters (cm, 8.25 inches) from the PDI measurement location. The nozzle was sprayed horizontally into a chemical fume hood while affixed to a traversing system. The nozzle traversed both the x and y directions (always 21 cm from the PDI measurement volume) to fully analyze the spray plume.

The nozzle was moved in both the positive and negative x- and y-directions, by 2 cm increments, until the edge of the spray plume was reached. For each test configuration, the spray plume was measured at ~35 measurement locations.

On average, a total of 10,000 droplets were measured at each measurement location as they passed through the PDI laser intersection. Towards the edge of the spray, the PDI was operated for a total of 15 seconds and collected as many droplets as possible during that time. The PVC distribution is used to provide the DV0.1, DV0.5, and DV0.9 diameters as well as the volume flux. The DV0.1 diameter is the value where 10 % of the total volume fogged is made up of drops with diameters less than or equal to the DV0.1. The DV0.5, or volume/mass median diameter (VMD/MMD), is the diameter where 50 % of the total volume of liquid fogged is made up of droplets with diameters smaller than the DV0.5. Finally, the DV0.9 is the value where 90% of the total volume of liquid fogged is made up of droplets with diameters smaller than the DV0.9. The volume flux ($cm^3/cm^2/s$) is a measurement of the liquid volume (cm^3) that passes through the probe volume (cm^2) per unit time (s).

To determine the overall flux of the entire spray plume, a surface was fitted to the volume fluxes measured at each x,y location. The surface was integrated over the measurement range to provide the overall flux, which was then compared to the known liquid flow rate.

The DV0.1, DV0.5, and DV0.9 diameters measured at each x,y location were then multiplied by the volume flux measured at the same measurement location. A surface was also fitted to the flux*diameter values and integrated over the same range. The resulting values were then divided by the calculated volume flux for the entire spray plume to provide the DV0.1, DV0.5, and DV0.9 diameters for the entire spray. Refer to Table 2-3 for these results.

Table 2-3. Measured Droplet Size and Flux Using the PDI.

Test	Fogger Device	Solution	PDI Measurements			
			$D_{V0.1}$, µm	$D_{V0.5}$, µm	$D_{V0.9}$, µm	Flux, mL/min
1	Minncare	Water	8.1	15.7	26.2	13.2
2	Minncare	PAA	6.5	12.4	19.5	1.2
3	Sani-Tizer	Water	18.2	39.9	65.9	380.9
4	Sani-Tizer	PAA	13.4	31.0	58.3	246.8

2.9 Coupon Extraction and Biological Agent Quantification

Spore extraction was achieved by placing test, positive control, and blank coupons in 50 mL polypropylene conical tubes containing 10 mL of sterile PBST. The vials were capped, placed on their side and agitated on an orbital shaker for 15 min at approximately 200 revolutions per minute (rpm) at room temperature.

The amount of residual viable spores was determined using a dilution plating approach. Following extraction, the extract was removed, and a series of tenfold dilutions was prepared in SFW. An aliquot (0.1 mL) of either the undiluted extract and/or each serial dilution was plated onto tryptic soy agar in triplicate and incubated for 18 to 24 h at 37 ± 2 ºC. Colonies were counted manually and CFU/mL was determined by multiplying the average number of colonies per plate by the reciprocal of the dilution. Dilution data representing the greatest number of individually definable colonies were expressed as arithmetic mean ± standard deviation (SD) of the numbers of CFU observed. Laboratory blanks controlled for sterility and procedural blanks controlled for viable spores inadvertently introduced to test coupons. The target acceptance criterion for extracts of laboratory or procedural blanks was zero CFU.

After each decontamination test, the ARCA and control chambers were thoroughly cleaned (using separate steps involving bleach, ethanol, water, then drying). This involved, but was not limited to, removal/bleaching of waste materials and test coupon racks. The immediate test area was wiped with bleach followed by water rinse. Negative control samples (which were negative for all tests) assured we were not getting any cross contamination within the chamber.

2.10 Decontamination Efficacy

The mean percent spore recovery from each coupon was calculated using results from positive control coupons (inoculated, not decontaminated), by means of the following equation:

$$\text{Mean \% Recovery} = [\text{Mean } CFU_{pc}/CFU_{spike}] \times 100 \quad (1)$$

where Mean CFU_{pc} is the mean number of CFU recovered from five replicate positive control coupons of a single material, and CFU_{spike} is the number of CFU spiked onto each of those coupons. The value of CFU_{spike} was known from enumeration of the stock spore suspension. One aliquot of the stock suspension was plated and enumerated on each day of testing to confirm CFU_{spike} concentration. Spore recovery was calculated for *B.a.* Ames or surrogate on each coupon, and the results are included in Section 4 and Appendix A.

The performance or efficacy of the sporicidal liquid fog was assessed by determining the number of viable organisms remaining on each test coupon after decontamination. Those numbers were compared to the number of viable organisms extracted from the positive control coupons.

The number of viable spores of *B.a.* Ames or surrogate organism in extracts of test and positive control coupons was determined to calculate efficacy of the decontaminant. Efficacy is defined as the extent (as $\log_{10}$ reduction or LR) to which viable spores extracted from test coupons after decontamination were less numerous than the viable spores extracted from positive control coupons. The logarithm of the CFU abundance from each coupon extract was determined, and the mean of those logarithm values was then determined for each set of controls and associated test coupons, respectively. Efficacy of a decontaminant for a test organism/test condition on the i^{th} coupon material was calculated as the difference between those mean log values, i.e.:

$$Efficacy\ (LR) = \overline{(\log_{10} CFUc_{ij})} - \overline{(\log_{10} CFUt_{ij})} \quad (2)$$

where $\log_{10} CFUc_{ij}$ refers to the *j* individual logarithm values obtained from the positive control coupons and $\log_{10} CFUt_{ij}$ refers to the *j* individual logarithm values obtained from the individual corresponding test coupons, and the overbar designates a mean value. In tests conducted under this plan, there were five positive controls and five corresponding test coupons (i.e., $j = 5$) for each coupon. A decontaminant or fumigant technology is considered to be an effective sporicide via (AOAC International) AOAC method 966.04 if a 6 LR or greater is achieved.(2)

In the case where no viable spores were found in any of the five test coupon extracts after decontamination, a CFU abundance of 1 was assigned, resulting in a $\log_{10}$ CFU of 0 for that material. This situation occurred when the decontaminant was highly effective, and no viable spores were found on the decontaminated test coupons. In such cases, the final efficacy on that material was reported as greater than or equal to (≥) the value calculated by Equation 2.

The variances (i.e., the square of the SD) of the $\log_{10} CFUc_{ij}$ and $\log_{10} CFUt_{ij}$ values were also calculated for both the control and test coupons (i.e., S^2c_{ij} and S^2t_{ij}), and were used to calculate the pooled standard error (SE) for the efficacy value calculated in Equation 2, as follows:

$$SE = \sqrt{\frac{S^2 c_{\mathrm{ij}}}{5} + \frac{S^2 t_{ij}}{5}} \quad (3)$$

where the number 5 again represents the number *j* of coupons in both the control and test data sets. Each efficacy result is reported as an LR value with an associated 95 % confidence interval (CI), calculated as follows:

$$95\ \%\ \mathrm{CI} = Efficacy\ (LR) \pm (1.96 \times \mathrm{SE}) \quad (4)$$

The significance of differences in efficacy across different test conditions and spore types was assessed based on the 95 % CI of each efficacy result. Differences in efficacy were judged to be significant if the 95 % CIs of the two efficacy results did not overlap. Any results based on this formula are hereafter noted as significantly different. Note that this comparison is not applicable when the two efficacy results being compared are both reported with LRs as ≥ some value.

The average difference in efficacy was determined when comparing the results of two tests and reported as an LR value. This difference in efficacy was calculated as follows:

$$Avg\ Difference\ in\ Efficacy\ (LR) = \frac{\sum_{a=1}^{n} LR_{a,2} - LR_{a,1}}{n} \quad (5)$$

where the letters *a* through *n* represent the material types, the number *1* represents *B.a.* Ames, and the number *2* represents the surrogate microorganism (*B.g.*) for which results are being compared. The letter *n* represents the number of materials tested. When both values were ≥LR (indicating complete inactivation), these were not included in the formula. A positive value indicates that the avirulent organism was inactivated on average to a higher degree (i.e., it was less resistant) across the materials tested compared to *B.a.* Ames.

In some instances, significant differences in average efficacy for a material between tests were assessed with a t-test using Microsoft® Excel, according to the formula below:

$$t = \frac{\overline{X_1} - \overline{X_2}}{S_{\overline{X_1} - \overline{X_2}}} \quad (6)$$

where $\overline{X_1}$ and $\overline{X_2}$ are the means of Tests 1 and 2, respectively. $S_{\overline{X_1} - \overline{X_2}}$ is the standard error of the difference between Tests 1 and 2. This formula compares the averages of two tests to see if they are reliably different from each other. Using this formula, a p-value was assigned where indicated. If the calculated p-value was <0.05, then the two sets of data were considered to be significantly different.

2.11 Statistical Analysis

The mean and 95 percent confidence intervals on the percent recovery for the positive control coupons were calculated by agent and material. For each agent separately, Kruskal-Wallis tests[9] were performed to compare whether percent recovery differs by material. Kruskal-Wallis tests also were performed to compare whether percent recovery differs by agent for each material. No adjustment for multiple tests was applied.

All test results were transformed to binary measurement of either successful decontamination (pass) or fail. A trial was recorded as a success if either: 1) the LR is greater than or equal to 6, LR; or 2) the LR is equal to the average control recovery (e.g., no spores recovered from test coupons, i.e., complete inactivation). The proportion of tests that pass (successful decontamination) and 95 percent Clopper-Pearson[10] confidence intervals were computed by agent, material, equipment, decontaminant, temperature, decontamination volume, and contact time. A chi-squared test for association was performed to test whether the *B.a.* decontamination success proportion was significantly different from the *B.g.* decontamination success proportion across all test conditions.

For *B.a.*, a logistic regression model with main effects for material, fog equipment, decontaminant, temperature, decontaminant quantity, and contact time was fitted to the data to compare the proportions of success. Statistically significant two-factor interactions were added to the model. Models were fitted to the full data set and to a more balanced subset of the data.

All statistical analyses were performed using SAS (Version 9.4, Cary, NC). All results are reported at the 0.05 level of significance.

2.12 Surface Damage

The physical effect of the sporicidal liquids as delivered by the fogging equipment to the materials was qualitatively monitored during the evaluation. This approach provided a gross visual assessment of whether the environmental state changed the appearance of the test materials. The procedural blank was visually compared to a laboratory blank coupon.

3 Quality Assurance/Quality Control

Quality assurance (QA)/quality control (QC) procedures were performed in accordance with the Scientific, Technology, Research, Engineering, and Modeling Support (STREAMS II) Program Quality Management Plan (QMP), Version 3 and the quality assurance project plan (QAPP)[(2)]. The QA/QC procedures and results are summarized below.

3.1 Equipment Calibration

All equipment (e.g., pipettes, incubators, pressure sensor, PDI, Vaisala, biological safety cabinets) and monitoring devices (e.g., thermometer, hygrometer) used at the time of the evaluation were verified as being certified, calibrated, or validated.

3.2 QC Results

QC efforts conducted during decontaminant testing included positive control samples, procedural blanks, laboratory blanks, and inoculation control samples.

Most positive control results were within the target recovery range of 5 to 120 % of the inoculated spores, except for a few instances. The average percent recoveries of both *B.a.* and *B.g.* spores, by material, for positive controls are detailed in Table D-1 and Figure D-1 of the Appendices. Recoveries of spores from positive controls were significantly higher for *B.a.* than for *B.g.* (See Table D-2 and D-3). Generally lower recoveries of spores occurred with materials such as unpainted concrete, fiberglass, and spores encapsulated in grease. Despite the low recoveries of spores from some of these materials, in most cases recoveries were greater than 6 log CFU. All procedural and laboratory blanks met the criterion of no observed CFU for both organisms.

Inoculation control samples were taken from the spore suspension on the day of testing and serially diluted, plated, and counted to establish the spore density used to inoculate the samples. The spore density levels met the QA target criterion of 1×10^9 CFU/mL (±1 log) for all tests.

3.3 Operational Parameters

The temperature, RH, and H_2O_2 vapor concentration during each test were monitored as described in Section 2.0. For select tests, the temperature was actively controlled by using the Krack evaporator as described in Section 2.5. This device was set to the target conditions and allowed to cool the ARCA chamber as needed to stay within target ranges of 10 °C ± 20 % (when required). Readings were taken once every minute for the duration of the contact time. The volume of liquid introduced into the test chamber via the fogger was measured after each test by volumetric pipette to determine residual volume which was subtracted from total volume added to the fogger. The actual operational parameters for each test are shown in Table 3-1 and reported as the average value ± SD. Note that the RH for both the test chamber during fogging and the control chamber for the positive controls were uncontrolled. The average RH for the controls was left at laboratory ambient conditions, while the RH during fogging was typically much higher due to the increase in water vapor released during the fog process.

Table 3-1. Actual Fog Conditions for Tests

Test Number	Sporicidal Liquid Volume Fogged (mL)		H_2O_2 Vapor Concentration (ppm)	Temperature (°C)			RH (%)	
	Target	Actual		Target	Fogging Actual	Control Actual	Fogging Actual	Control Actual
1	160	154	53.94 ± 51.46	20	20.58 ± 0.14	18.10 ± 0.13	80.73 ± 5.25	60.49 ± 0.52
2	2365	2627	12.37 ± 12.38	20	21.18 ± 0.28	18.05 ± 0.37	94.83 ± 1.10	60.89 ± 0.22
3	160	162	4.96 ± 20.96	20	20.45 ± 0.24	18.48 ± 0.38	72.37 ± 4.05	61.36 ± 0.21
4	78	82	16.26 ± 38.25	20	20.45 ± 0.23	19.14 ± 0.25	69.86 ± 4.94	59.11 ± 0.24
5	78	78	13.55 ± 38.51	20	20.67 ± 0.34	18.60 ± 0.15	68.49 ± 4.20	57.57 ± 0.15
6	78	78	35.34 ± 52.98	20	20.96 ± 0.12	19.43 ± 0.05	70.08 ± 7.10	54.20 ± 0.18
7	160	166	39.06 ± 53.57	20	21.46 ± 0.18	18.75 ± 0.35	78.46 ± 5.17	59.57 ± 0.19
8	160	161	40.55 ± 72.09	20	20.96 ± 0.27	20.44 ± 0.22	66.30 ± 11.61	58.20 ± 0.24
9	160	160	41.66 ± 65.91	20	20.32 ± 0.10	19.83 ± 0.03	67.24 ± 13.31	53.16 ± 0.16
10	500	497	68.72 ± 92.47	20	21.73 ± 0.17	19.53 ± 0.36	76.09 ± 9.52	21.31 ± 1.51
11	160	187	12.05 ± 10.52	10	9.70 ± 0.71	19.67 ± 0.41	82.19 ± 7.63	43.31 ± 1.17
12	78	104	4.86 ± 4.97	10	9.88 ± 0.54	19.87 ± 0.12	80.69 ± 6.83	44.21 ± 0.16
13	160	166	7.69 ± 8.38	10	9.87 ± 0.57	19.78 ± 0.15	79.42 ± 7.18	44.63 ± 0.31
14	500	497	14.47 ± 15.09	10	9.64 ± 0.59	19.96 ± 0.74	84.57 ± 8.98	48.62 ± 2.18
15	500	506	38.23 ± 12.52	20	20.18 ± 0.21	19.7 ± 0.15	90.75 ± 3.79	55.05± 0.31
16	500	500	16.51 ±42.08	20	20.04 ± 0.26	20.05 ± 0.13	48.32 ± 10.83	47.58 ± 0.36
17	1000	998	58.85 ± 62.97	20	20.60 ± 0.32	19.51 ± 0.25	94.83 ± 3.69	53.85 ± 0.27
18	1000	1001	24.97 ±20.30	20	21.80 ± 0.22	19.86 ± 0.35	92.93 ± 2.32	56.16 ± 1.32
19	1000	1001	15.69 ± 13.23	10	9.32 ± 0.63	19.95 ± 0.26	78.87 ± 10.13	44.77 ± 1.96
20	1000	1000	23.42 ± 25.37	10	9.64 ± 0.57	18.65 ± 0.26	82.23 ± 10.37	59.62 ± 0.04
21	160	161	1.36 ± 0.51	10	9.64 ± 0.68	20.17 ± 0.49	57.08 ± 5.66	56.52 ± 0.92

Data reported as average ± SD.

3.4 Audits

3.4.1 Performance Evaluation Audit

Performance evaluation (PE) audits were conducted to assess the quality of the results obtained during these experiments. Table 3-2 summarizes the PE audits that were performed.

No PE audits were performed for confirmation of the concentration and purity of *B.a.* or surrogate spores because quantitative standards do not exist for these organisms. The titer enumerations and the control and blank test coupons support the spore measurements.

Table 3-2. Performance Evaluation Audits

Measurement	Audit Procedure	Allowable Tolerance	Actual Tolerance
Volume of liquid from micropipettes	Gravimetric evaluation	± 10 %	± 0.14 % to 5.89 %
Time	Compared to independent clock	± 2 seconds/hour	0 seconds/hour
Temperature	Compared to independent calibrated thermometer	± 2 °C	± 0.29 to 0.39 °C
Relative Humidity	Compare to independent calibrated hygrometer	± 10 %	± 3.52 to 3.63 %

3.4.2 Technical Systems Audit

Observations and findings from the technical system audit (TSA) were documented and submitted to the laboratory technical lead for response. TSAs were conducted on July 13 and 14, 2015 to ensure that tests were being conducted in accordance with the appropriate QAPP and QMP. As part of the audit, test procedures were compared to those specified in the QAPP and data acquisition and handling procedures were reviewed. None of the findings of the TSA required corrective action.

3.4.3 Data Quality Audit

At least 10 % of the data acquired during the evaluation were audited. Data were reviewed in five separate batches from August 2015 through July 2016. A QA auditor traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were verified. Only minor issues were noted with the data, mostly data transcription errors that were corrected.

3.5 QA/QC Reporting

Each assessment and audit was documented in accordance with the QAPP and QMP. For these tests, findings were noted (none significant) in the data quality audit, and no follow-up corrective action was necessary. The findings were mostly minor data transcription errors requiring some recalculation of efficacy results, but none were gross errors in recording. QA/QC procedures were performed in accordance with the QAPP.

3.6 Data Review

Records and data generated in the evaluation received a QC/technical review before they were utilized in calculating or evaluating results and prior to incorporation in this report.

4 Summary of Results and Discussion

The decontamination efficacy of fogged PAA and three concentrations of H_2O_2 (8 %, 22 %, and 35 %) against virulent *B.a.* Ames and *B.g.* was evaluated at target delivery volumes of 78, 160, 500, 1000, and 2365 mL; target temperatures of 10 or 20 °C; and contact times ranging from 8 to 168 hours, for a total of 21 tests. Actual operational parameters as measured are detailed in Section 3. The detailed decontamination efficacy results, showing average CFU recovery from each material for both positive controls and test coupons, for all tests, are found in Appendix A. This chapter of the report discusses decontamination efficacy results as a function of some of the variables that were tested in the study. Some statistical results are also presented to indicate whether test variables significantly affected efficacy.

4.1 Comparing Efficacy for the Different Species

The average difference in decontamination efficacy for each test for the two microorganisms is shown in Table 4-1. These results indicate that *B.g.* had resistance similar to *B.a.* Ames, with average differences ranging from -1.32 to 1.02 LR. (A positive difference in result indicates that *B.g.* was inactivated to a higher degree, i.e., was less resistant, than *B.a.* Ames.) Overall, in 12 tests, *B.g.* was inactivated to a higher degree, and in nine tests, *B.a.* was inactivated to a higher degree.

Estimates with exact 95 percent confidence intervals for the proportion of successes (as defined in Section 2.10) are presented in Table D-4 of Appendix D. Estimates for *B.a.* and *B.g.* are presented side-by-side for comparison. The chi-squared test of statistical dependence between agent and success failed to reject the null hypothesis ($p = 0.1119$); thus, *B.a.* and *B.g.* are not statistically significantly different with respect to the proportion of successes across all test conditions. These results suggest *B.g.* may be a suitable surrogate for *B.a.* Ames when conducting similar types of testing (i.e., fogging with PAA or H_2O_2). Detailed comparison results are found in Appendix B.

Table 4-1. Summary of Average Differences in Efficacy between *B.a.* Ames and *B atrophaeus

Test Number	Equipment	Sporicidal Liquid	Target Temperature (°C)	Contact Time (hours)	Average Difference in Efficacy Between Species*
1	Sani-Tizer	PAA	20	18	0.12
2	Sani-Tizer	8% H_2O_2	20	168	0.22
3	Sani-Tizer	PAA	20	1-7 Days	0.56
4	Sani-Tizer	PAA	20	18	-0.45
5	Sani-Tizer	22% H_2O_2	20	18	0.26
6	Sani-Tizer	PAA	20	8	-0.03
7	Sani-Tizer	PAA	20	18	0.08
8	MinnCare	PAA	20	18	0.14
9	MinnCare	PAA	20	18	-0.73
10	MinnCare	PAA	20	18	-0.75
11	Sani-Tizer	PAA	10	18	-0.18
12	Sani-Tizer	PAA	10	18	0.15
13	Sani-Tizer	PAA	10	18	1.02
14	MinnCare	PAA	10	18	0.50
15	Sani-Tizer	PAA	20	18	-1.32
16	MinnCare	35% H_2O_2	20	18	0.37
17	Sani-Tizer	35% H_2O_2	20	18	-0.06
18	Sani-Tizer	PAA	20	18	-0.79
19	Sani-Tizer	PAA	10	18	0.27
20	Sani-Tizer	35% H_2O_2	10	18	-1.23
21	MinnCare	PAA	10	18	0.44

* Results shown as average difference in efficacy (log reduction). A positive result indicates that the avirulent microorganism *(B.g.*) was inactivated to a higher degree (less resistant) than *B.a.* Ames

4.2 Effects of Test Materials on PAA and H_2O_2 Efficacy for *B.a.* Ames

The LR results for each material, by test, are shown in Figures 4-1 through 4-12, in terms of the average LR ± 95% CI. Differences in efficacy between two materials for the same test are significant if the 95 % CIs of the two efficacy results do not overlap. Table 4-2 shows the average LR for each material for the tests that used that material. Note that only six materials were used during each test, and some of the materials tested initially in the study that were relatively easy to decontaminate (readily achieved ≥ 6 LR) were dropped from further testing. Materials that were harder to decontaminate (e.g., railcar carpet) were included in more tests to find conditions in which decontamination would be successful.

Materials such as such rubber flooring, seat upholstery, aluminum, Mylar, and both air filter types exhibited ≥6 LR for *B.a.* in all or nearly all conditions tested. The carpet, concrete, and encapsulated grease were the most difficult materials to decontaminate (lowest average LR values), with the latter two materials having no test conditions resulting in ≥6 LR. In Test 19, in which clean industrial carpet was tested to compare with the used railcar carpet, there was no significant difference in decontamination efficacy results for the two materials. *B.a.* spores inoculated onto the interior fiberglass siding, and the clean and dirty railcar grease (spores left on top of grease) were moderately inactivated compared to the other materials.

Further details on the decontamination efficacy results and statistical analyses are found in Appendix A.

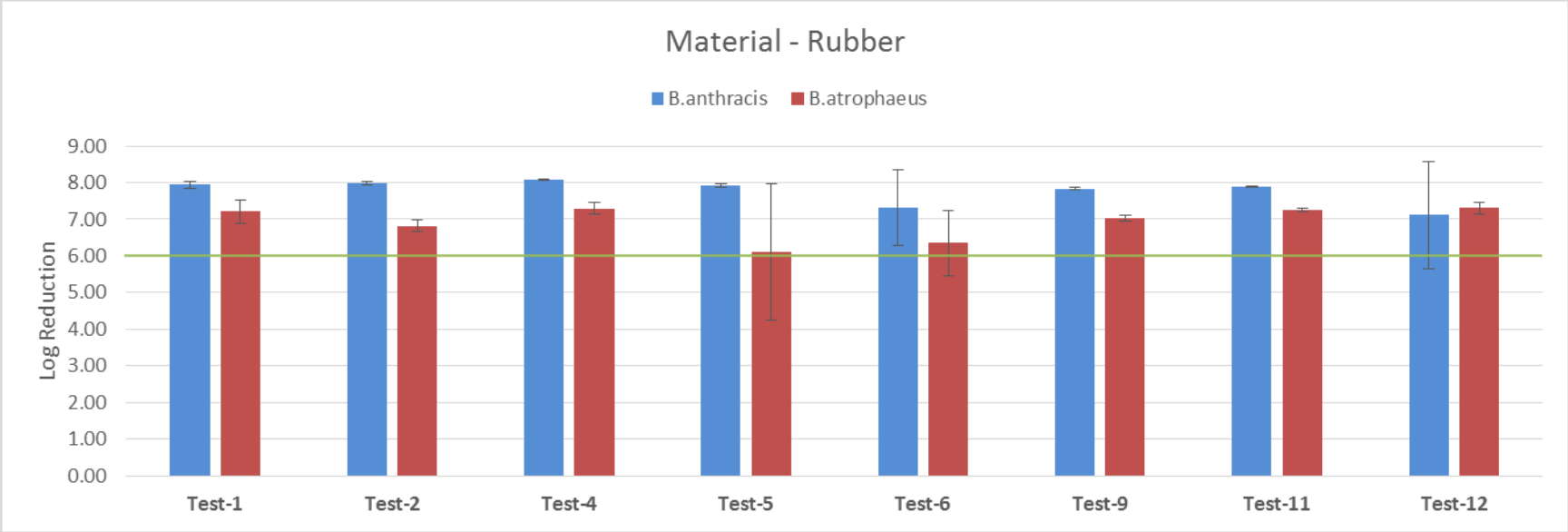

Figure 4-1. Summary of Decontamination Efficacy Results on Rubber against *B. anthracis* Ames and *B. atrophaeus*.

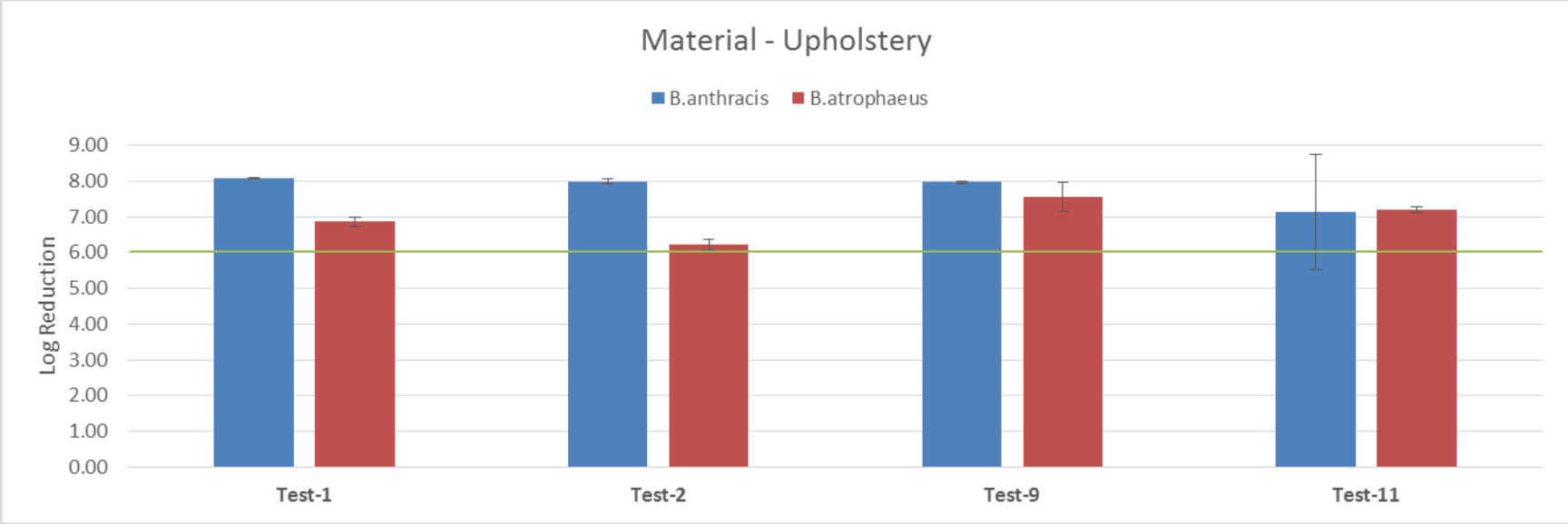

Figure 4-2. Summary of Decontamination Efficacy Results on Upholstery against *B. anthracis* Ames and *B. atrophaeus*.

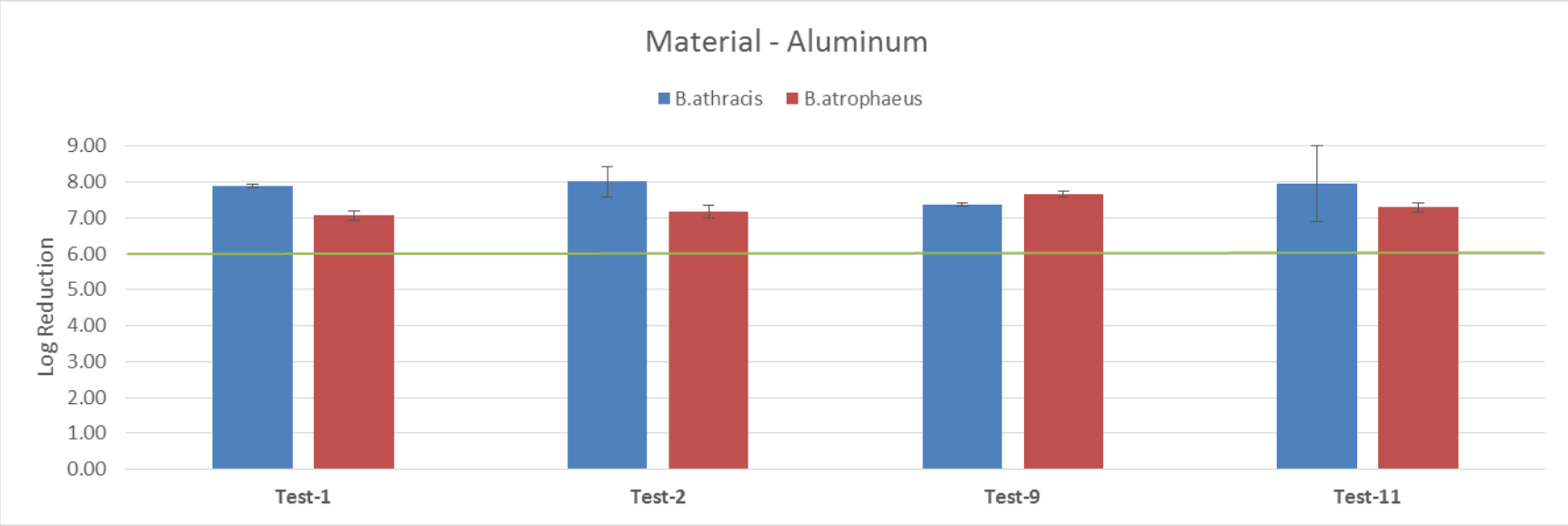

Figure 4-3. Summary of Decontamination Efficacy Results on Aluminum against *B. anthracis* Ames and *B. atrophaeus*.

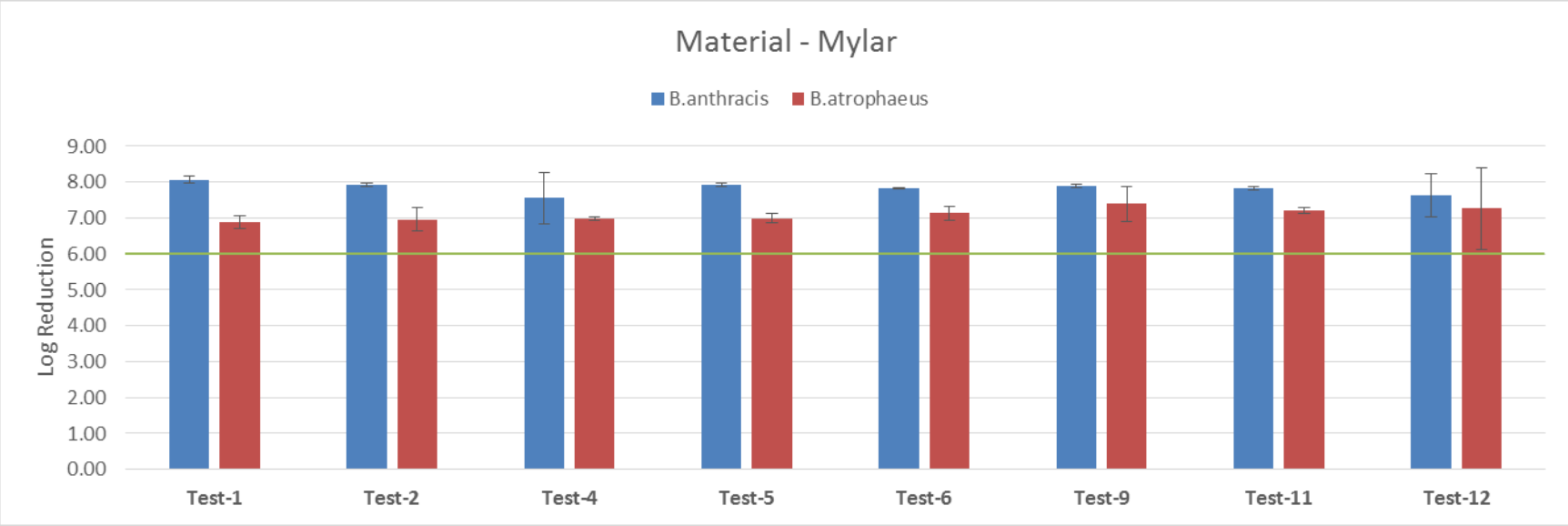

Figure 4-4. Summary of Decontamination Efficacy Results on Mylar against *B. anthracis* Ames and *B. atrophaeus*.

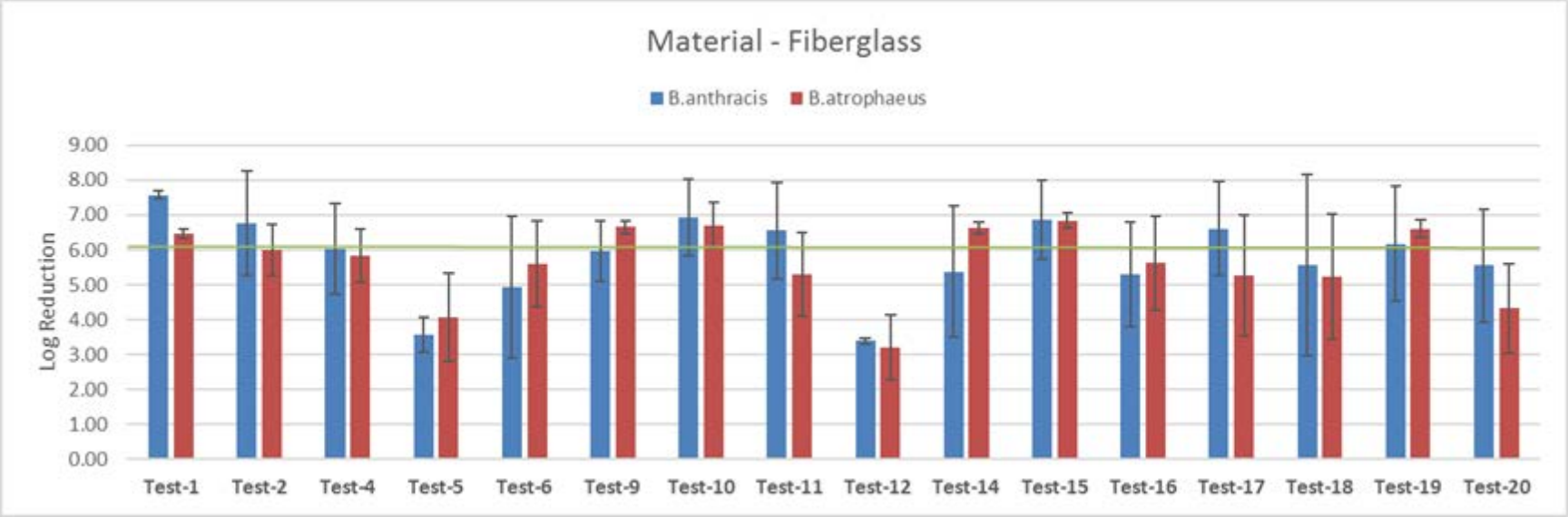

Figure 4-5. Summary of Decontamination Efficacy Results on Fiberglass against *B. anthracis* Ames and *B. atrophaeus*.

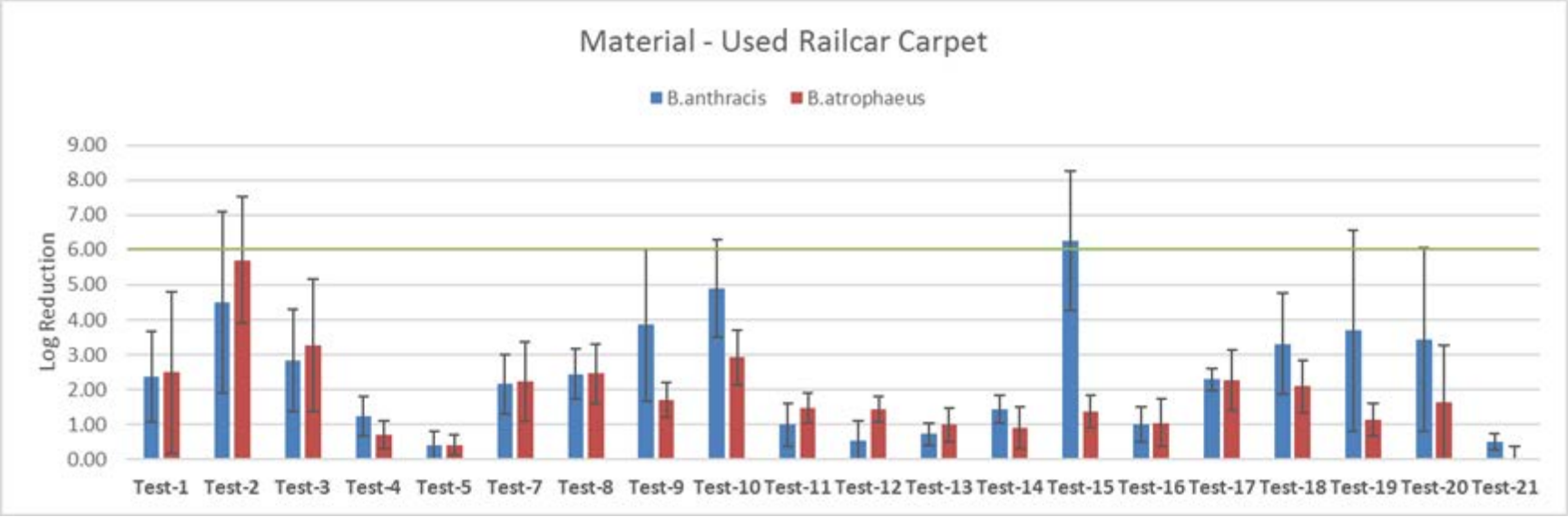

Figure 4-6. Summary of Decontamination Efficacy Results on Railcar Carpet against *B. anthracis* Ames and *B. atrophaeus*.

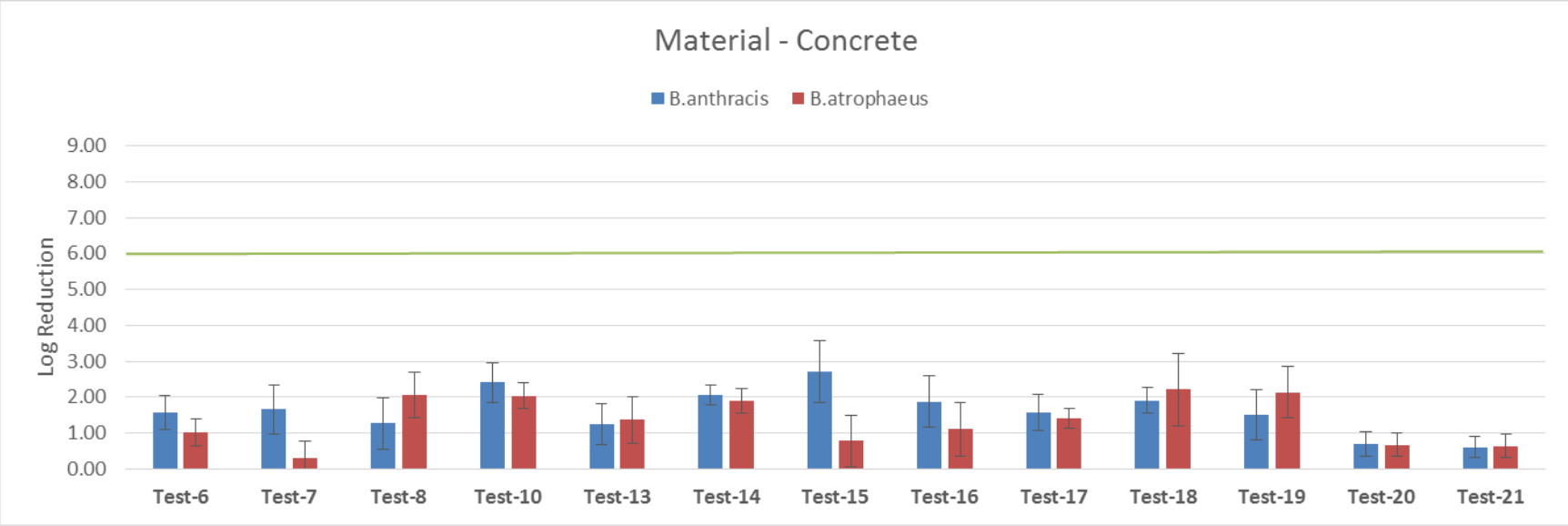

Figure 4-7. Summary of Decontamination Efficacy Results on Concrete against *B. anthracis* Ames and *B. atrophaeus*.

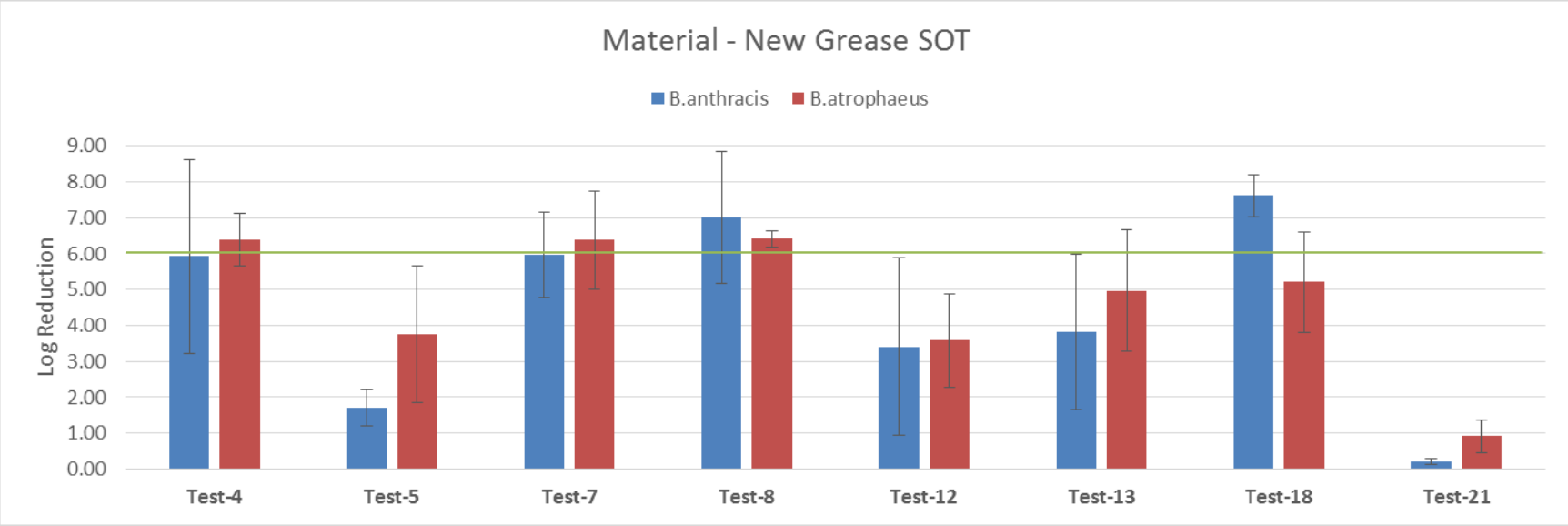

Figure 4-8. Summary of Decontamination Efficacy Results on New Grease SOT against *B. anthracis* Ames and *B. atrophaeus*.

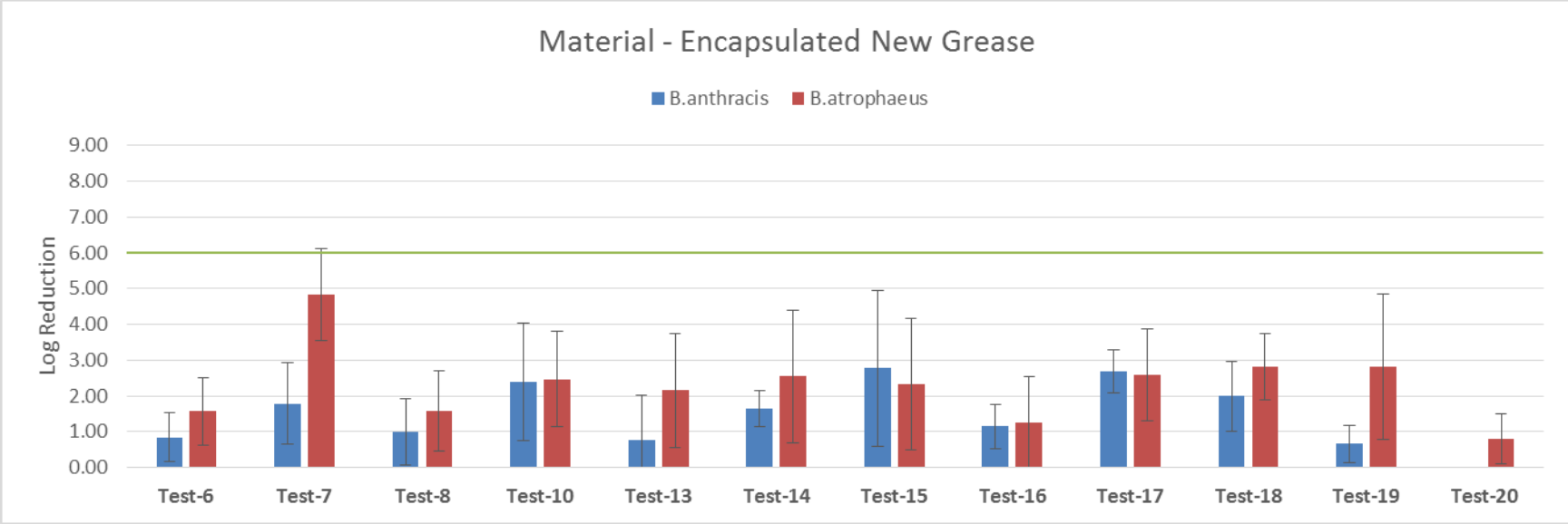

Figure 4-9. Summary of Efficacy Results on Encapsulated NG against *B. anthracis* Ames and *B. atrophaeus*.

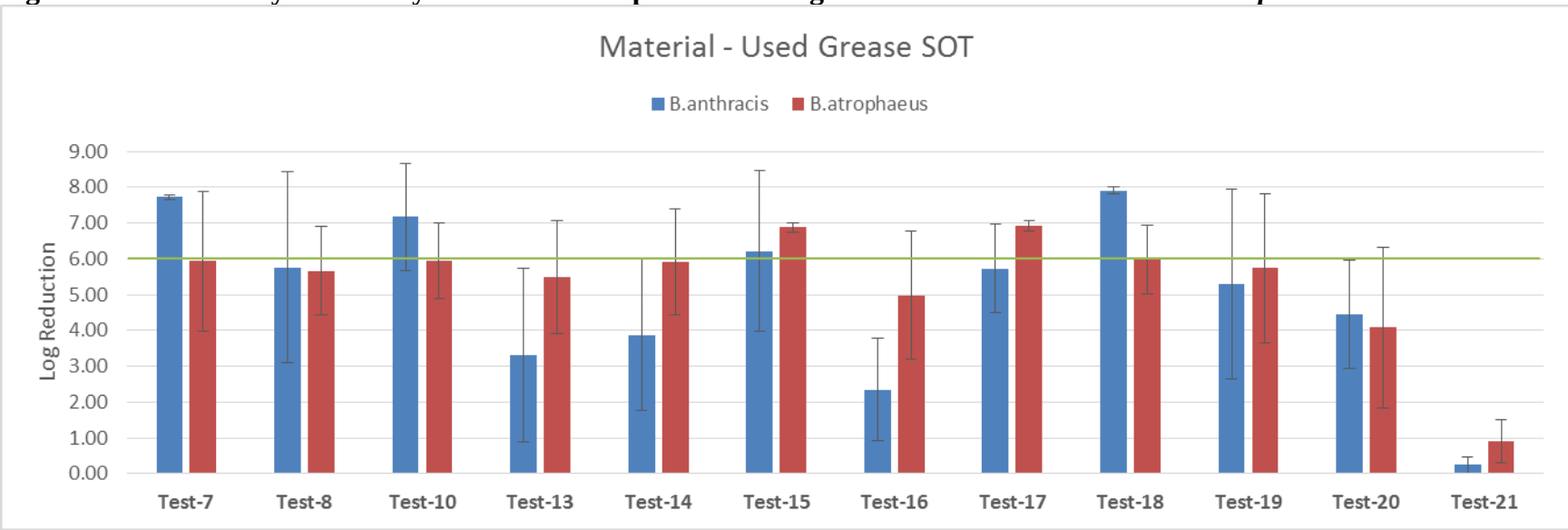

Figure 4-10. Summary of Efficacy Results on Used Grease SOT against *B. anthracis* Ames and *B. atrophaeus*.

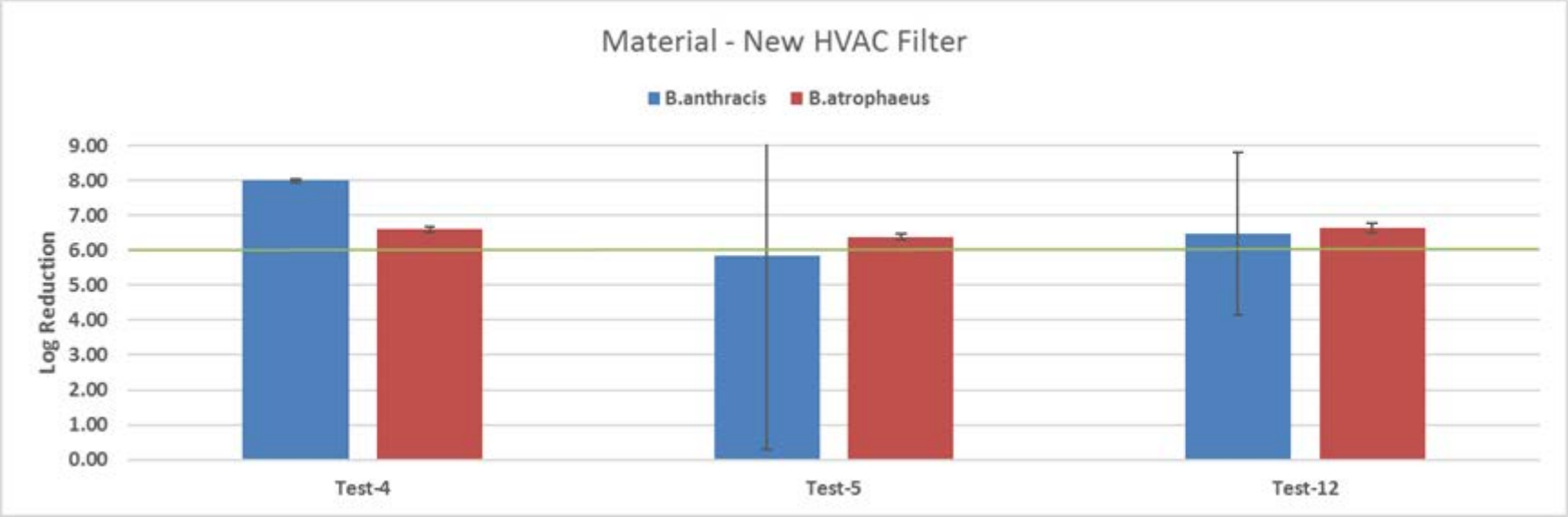

Figure 4-11. Summary of Decontamination Efficacy Results on New Filter against *B. anthracis* Ames and *B. atrophaeus*.

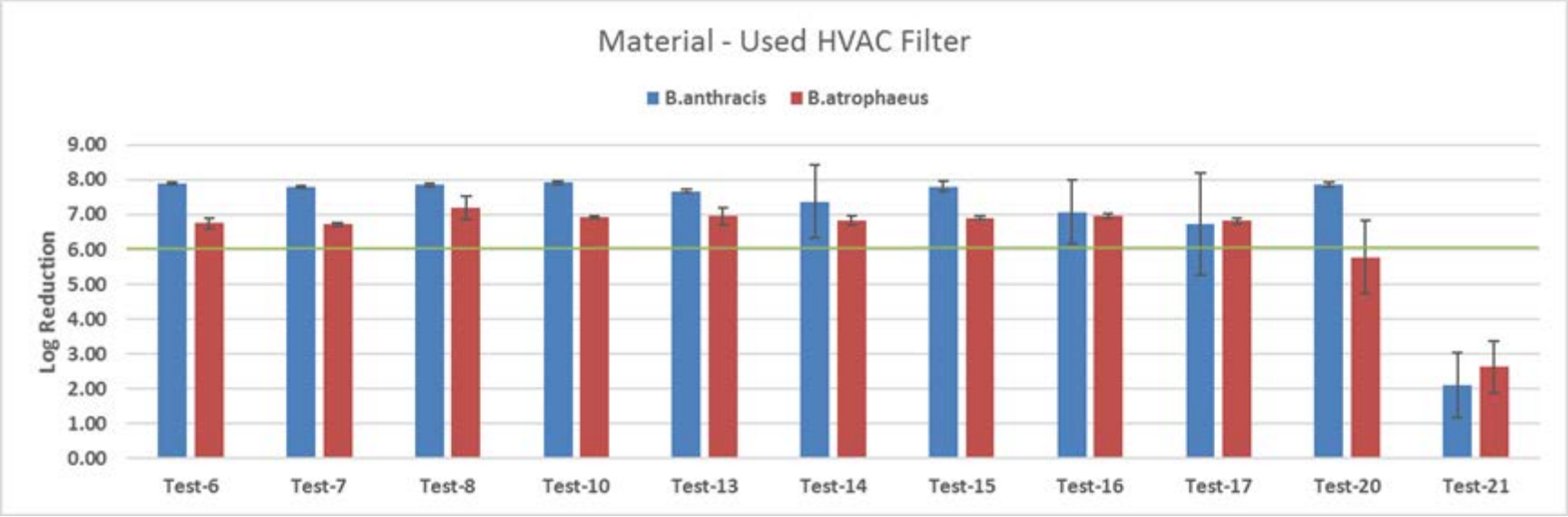

Figure 4-12. Summary of Decontamination Efficacy Results on Used Filter against *B. anthracis* Ames and *B. atrophaeus*.

Table 4-2. Summary of *B.a.* Ames and *B.g.* Log Reductions by Material Type

Material	Number of Tests	Average *B.a.* LR ± SD	Average *B.g.* LR ± SD
Mylar	8	7.83 ± 0.17	7.10 ± 0.17
Aluminum	4	7.81 ± 0.29	7.30 ± 0.25
Upholstery	4	7.79 ± 0.45	6.96 ± 0.57
Rubber	8	7.76 ± 0.35	6.92 ± 0.46
Used Air Filter	11	7.10 ± 1.70	6.41 ± 1.30
New Air Filter	3	6.77 ± 1.10	6.54 ± 0.14
Fiberglass Interior Siding	16	5.82 ± 1.15	5.65 ± 1.06
Used Grease SOT	12	5.00 ± 2.29	5.34 ± 1.58
New Grease SOT	8	4.45 ± 2.62	4.70 ± 1.90
New Industrial Carpet	1	4.32	4.81
Used railcar Carpet	20	2.43 ± 1.64	1.91 ± 1.20
Unpainted Concrete	13	1.62 ± 0.60	1.36 ± 0.65
Encapsulated New Grease	13	1.59 ± 0.85	2.24 ± 1.02

4.3 Effects of Temperature on Decontamination Efficacy

The decontamination efficacy of fogging PAA or H_2O_2 was evaluated at target temperatures of 10 or 20 °C. The tests conducted at 20 °C are representative of the ambient environmental conditions that would be expected at an above ground subway platform, while tests conducted at 10 °C are representative of the underground temperatures that may be encountered in the platforms and tunnels.

The effect of temperature on decontamination efficacy may be assessed when all other test variables are kept constant. Refer to Figures 4-13 through 4-17, in which the LR results are shown in terms of average LR ± 95% CI. Five identical conditions were tested in which only the temperature varied (compare Tests 1 and 11; 4 and 12; 7 and 13; 10 and 14; and 17 and 20). In general, while the higher 20 °C temperature resulted in a greater probability of complete kill and greater LR values compared to the results at 10 °C, many of these differences were not significant or not conclusive (exemplified by overlapping confidence intervals). Indeed, there were many cases in which 6 LR was achieved when fogging at 10 °C. Overall, average differences in LR values for the two temperatures for the comparable tests ranged between 1-2 LR. For further details, refer to Tables C-1 and C-2, and the statistical analyses in Appendix D.

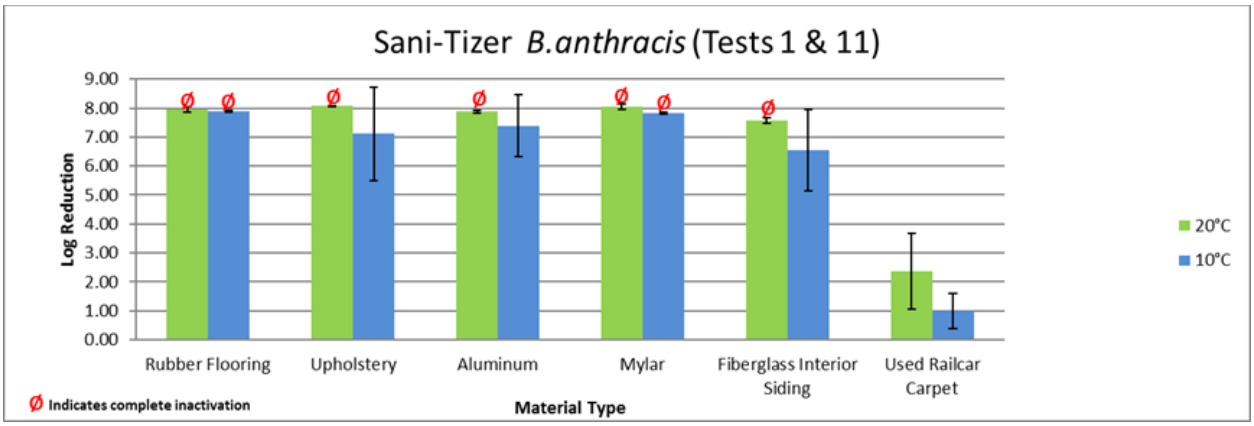

Figure 4-13. Effect of Temperature Against *B. anthracis* Ames: Tests 1 and 11. Results are shown in terms of average LR ± 95% CI.

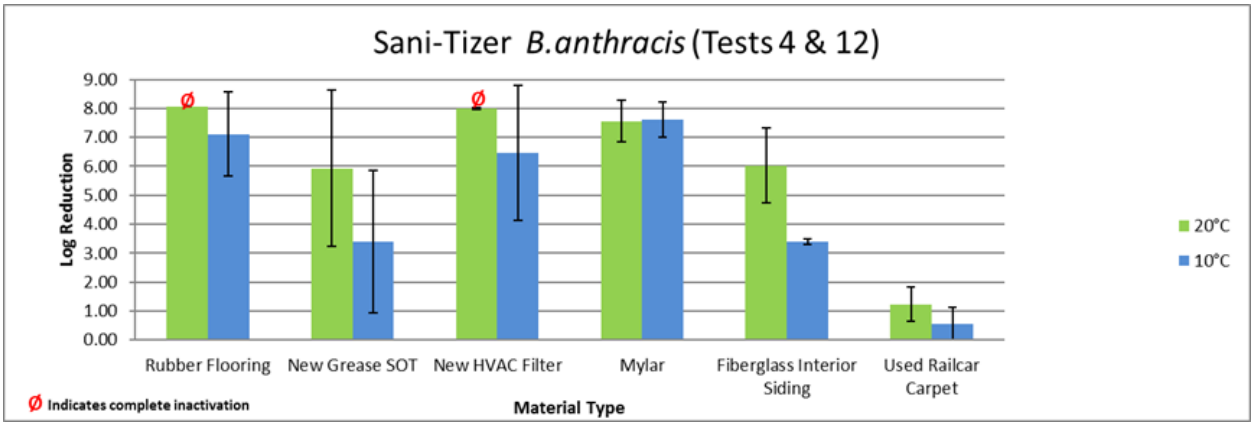

Figure 4-14. Effect of Temperature Against *B. anthracis* Ames: Tests 4 and 12. Results are shown in terms of average LR ± 95% CI.

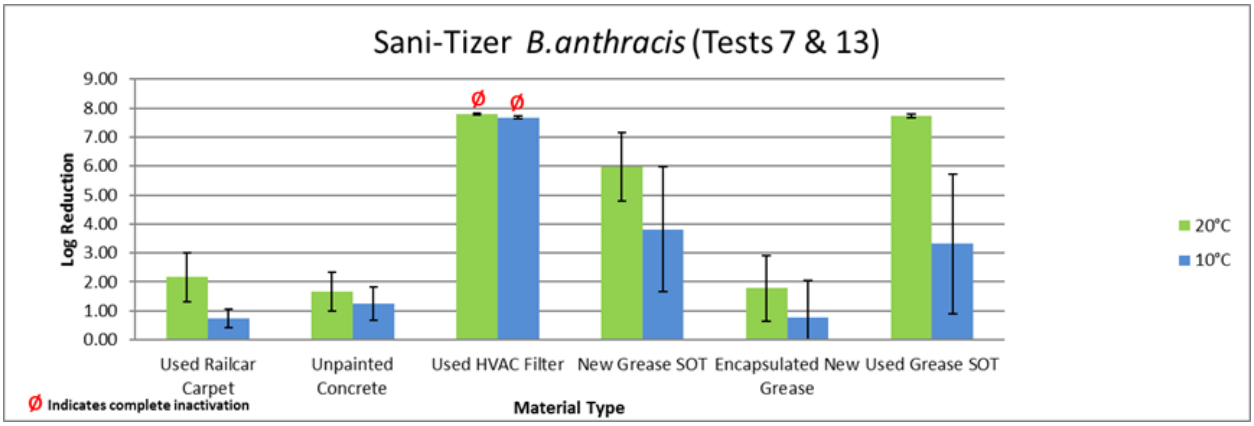

Figure 4-15. Effect of Temperature Against *B. anthracis* Ames: Tests 7 and 13. Results are shown in terms of average LR ± 95% CI.

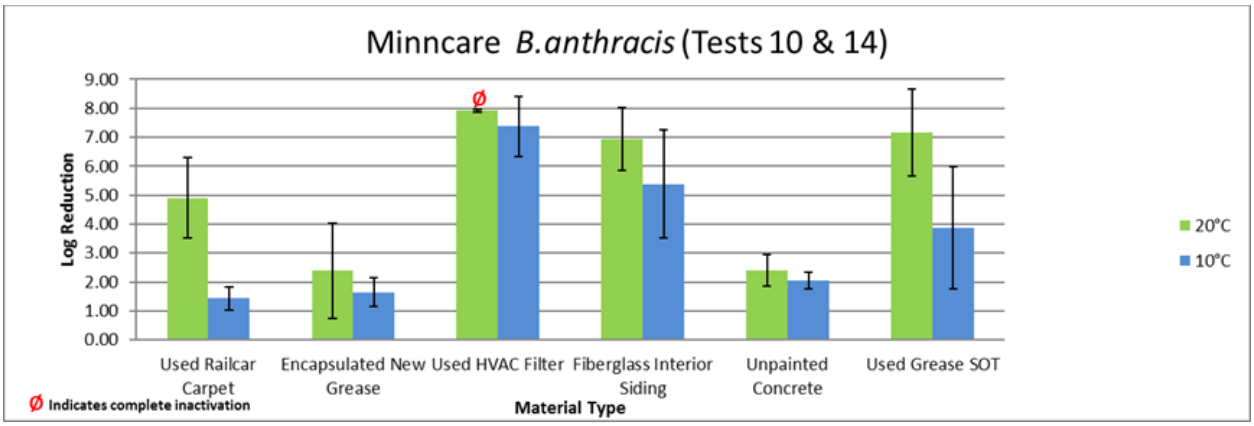

Figure 4-16. Effect of Temperature Against *B. anthracis* Ames: Tests 10 and 14. Results are shown in terms of average LR ± 95% CI.

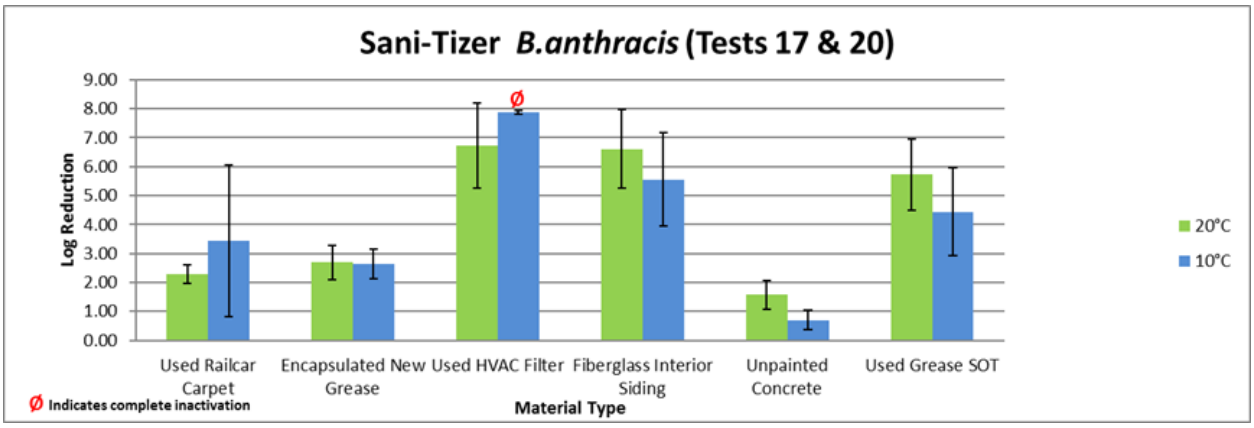

Figure 4-17. Effect of Temperature Against *B. anthracis* Ames: Tests 17 and 20. Results are shown in terms of average LR ± 95% CI.

4.4 Effect of Fogging Equipment on Decontamination Efficacy

The decontamination efficacy of fogging PAA or aqueous H_2O_2 against *B.a.* and *B.g.* was evaluated using two types of fog generating equipment as previously described in Section 2.6. The Minncare cold fogger generated a mean droplet size of 12.4 µm, while the Sani-Tizer generated a larger mean droplet size of 31.0 when spraying PAA solution. The two types of equipment yielded similar LR values when compared under identical test conditions at 20 °C (Figure 4-18). Testing conducted using the same parameters but at 10 °C generally yielded higher LR for the Sani-Tizer as compared to the Minncare equipment (Figure 4-19). That a somewhat higher LR is associated with the fogger producing larger size droplets is an unexpected result, since the larger droplets would tend to settle out sooner. Overall, however, statistical analysis using the logistic regression model indicated that the type of fogger did not have a significant effect on LR. Additional analyses of the effect of equipment type are included in Appendices A and D.

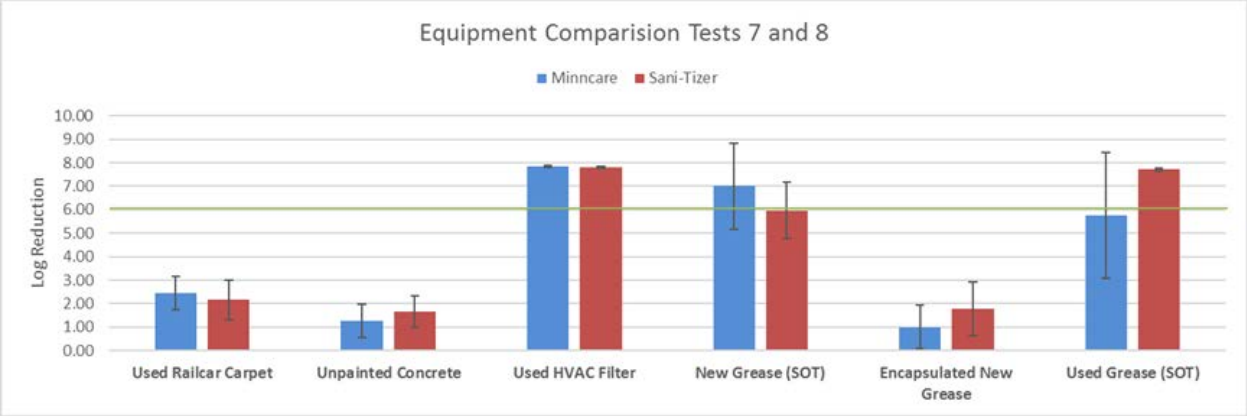

Figure 4-18. Effect of Fogger Equipment Type Against *B. anthracis* Ames at 20°C. Results are shown in terms of average LR ± 95% CI.

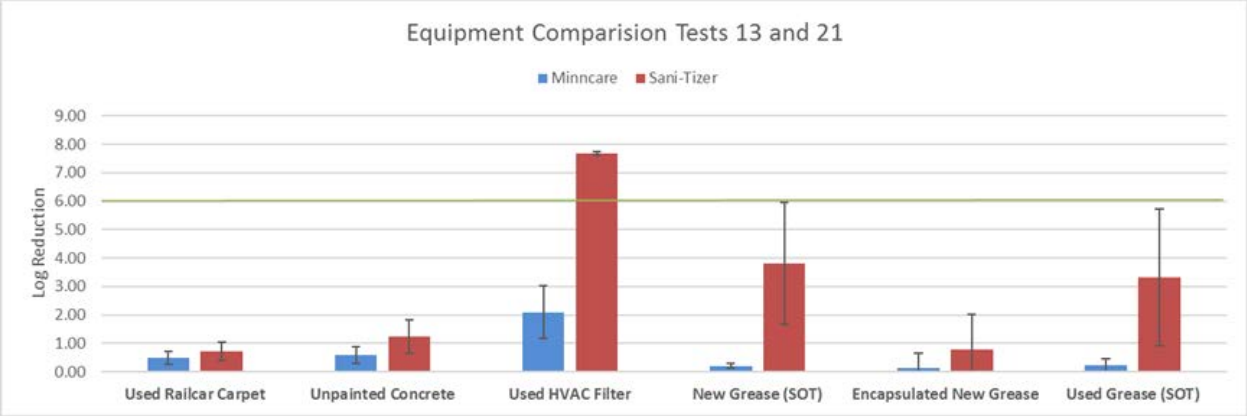

Figure 4-19. Effect of Fogger Equipment Type Against *B. anthracis* Ames at 10°C. Results are shown in terms of average LR ± 95% CI.

4.5 Effect of Sporicidal Liquid and Quantity Fogged on Decontamination Efficacy

The decontamination efficacy of fogging PAA was evaluated for 16 tests, while the fogging of aqueous H_2O_2 was evaluated for five tests. Three of the H_2O_2 tests were conducted under the same operational conditions as three PAA tests, and thus allow us to compare results. For example, in Tests 4 and 5, both tests were conducted with the same fogger, temperature, and volume of sporicidal liquid. Similarly, in Tests 17 and 18, both tests were conducted at the same temperature (20 °C), using the same fogger, and using the same quantity of sporicidal liquid. The results of the comparisons are shown in Figures 4-20 to 4-23, and indicate that while the aqueous H_2O_2 solutions were in most cases less effective than the PAA, there were only a few cases in which there was a significant difference in efficacy. In addition, the lower concentration H_2O_2 solution (22 %) appears to be somewhat less effective than the higher concentration (35 %) H_2O_2 solution.

With respect to the quantity of sporicidal liquid fogged, within the parameters assessed in the statistical analyses, the probability of a complete kill increases as a function of the $\log_{10}$ of the volume of the sporicidal liquid (further discussed in Appendix D).

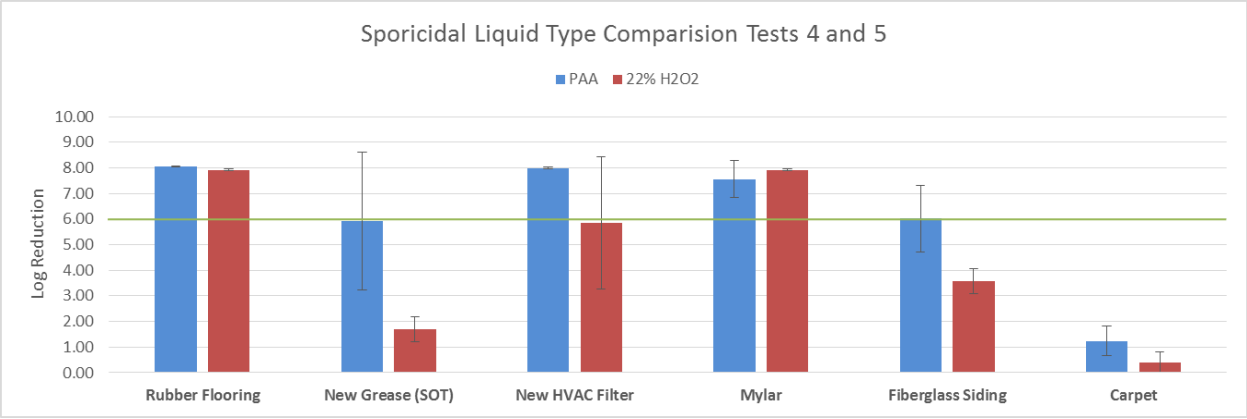

Figure 4-20. Effect of Sporicidal Liquid Type Against *B. anthracis* Ames Tests 4 and 5. Results are shown in terms of average LR ± 95 % CI.

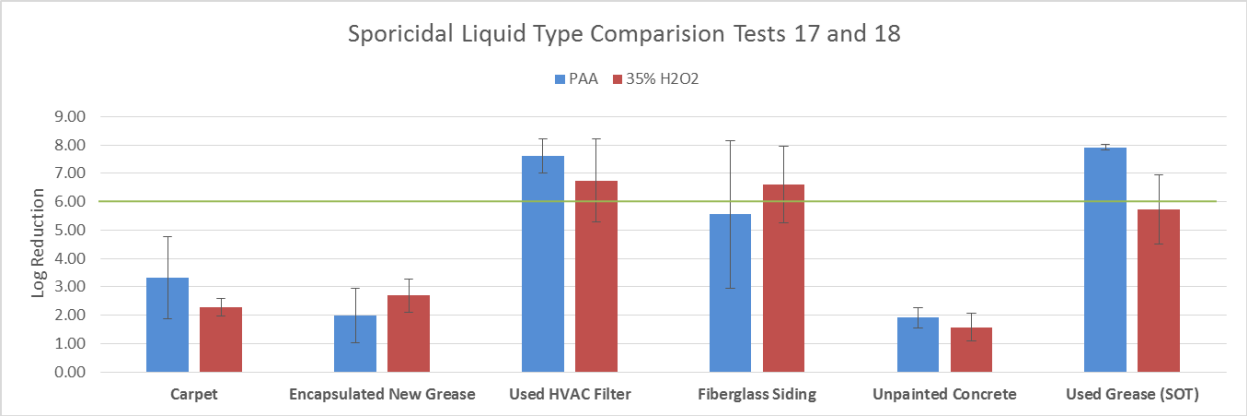

Figure 4-21. Effect of Sporicidal Liquid Type Against *B. anthracis* Ames Tests 17 and 18. Results are shown in terms of average LR ± 95 % CI.

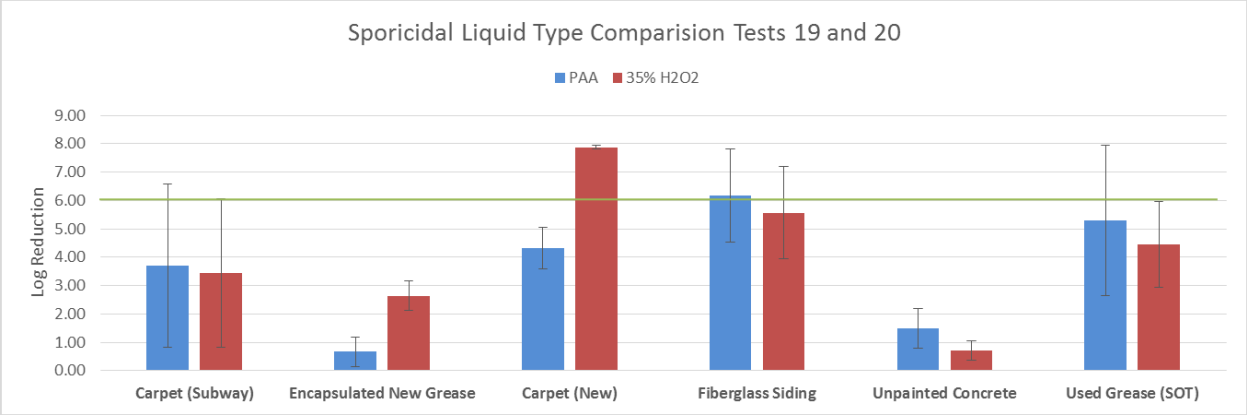

Figure 4-22. Effect of Sporicidal Liquid Type Against *B. anthracis* Ames Tests 19 and 20. Results are shown in terms of average LR ± 95 % CI.

4.6 Effects of Test Location on Efficacy

The decontamination efficacy of fogging was evaluated at five locations within the test chamber (refer to Figure 2-3), as previously described in Section 2.5. Log reductions for *B.a.* Ames were averaged across all 21 tests per location (Figure 4-23). These results showed minimal difference in LR by location. However, the logistic model indicates that the probability of complete kill is significantly different for each location compared to location 3 (coupons placed on the cart, horizontally facing upward, in the center of the chamber), with all locations less likely to result in a complete kill compared to location 3. See Table D7 in the Appendix.

When average percent wetness per location was examined (Figure 4-24), apparent differences existed between horizontal upward facing locations (location 1 and 3, more wet) and inverted, vertical, or offset locations (location 2, 4, and 5, less wet, respectively).

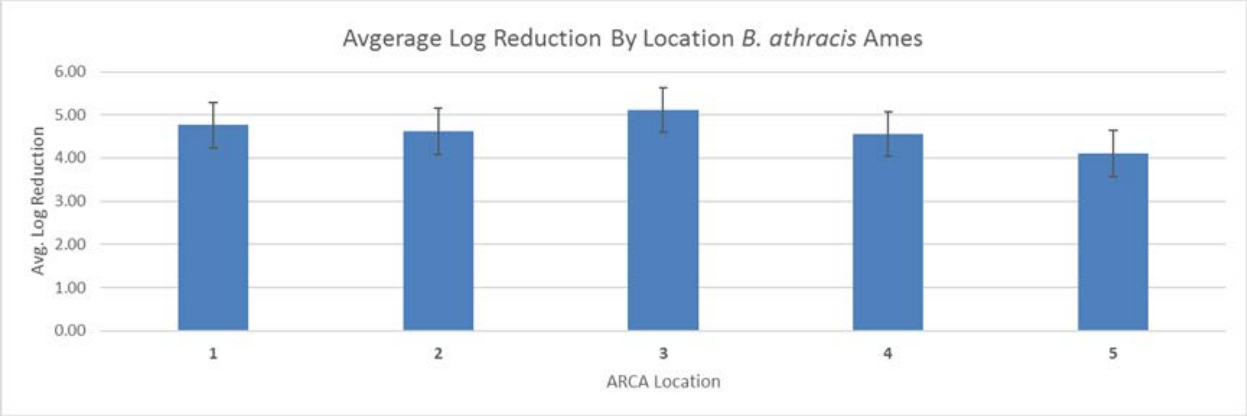

Figure 4-23. Summary of Effect of Location Against *B. anthracis* Ames as Average of All Tests. Results are shown in terms of average LR ± 95% CI.

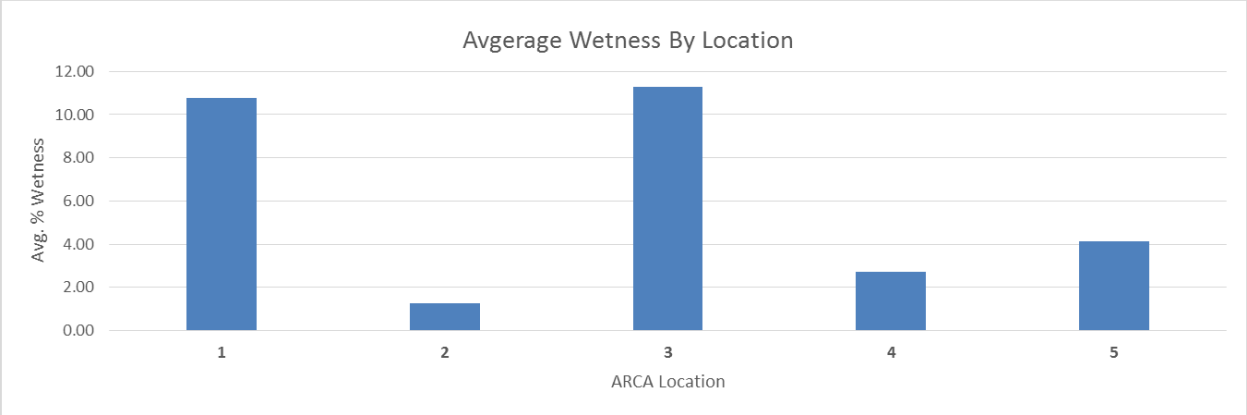

Figure 4-24. Summary of Wetness per Location in Chamber as Average of All Tests

4.7 Surface Damage to Materials

At the end of each decontamination test, the procedural blanks were visually compared to the laboratory blanks, and test coupons were visually compared to positive controls to assess any impact the PAA or H_2O_2 fog may have had on each material type. Based on the visual appearance of the decontaminated coupons, there were no apparent changes in the color, reflectivity, or roughness of the thirteen material surfaces after being exposed to the sporicidal fogs. While not a test material, copper tubing installed in the test chamber as part of the cooling equipment exhibited severe corrosion when exposed to the PAA sporicidal liquid.

4.8 Summary

This evaluation focused on the decontamination of eleven types of subway railcar materials (carpet, aluminum, upholstery, rubber flooring, Mylar® coating, fiberglass, new cabin filter, used cabin filter, new grease with spores mixed (encapsulated) into the grease, new grease with spores

left on top of the grease, and used grease (spores left on top of the grease) and a common subway tunnel material (unpainted concrete). Decontamination efficacy tests were conducted with spores of virulent *B.a.* Ames and non-virulent *B.g.*, to assess the potential use of *B.g.* as a surrogate for future studies with the fogging of sporicidal liquids. Other fogger operational and environmental variables were evaluated for their effect on decontamination efficacy, such as air temperature, location, sporicidal liquid chemical and quantity fogged, and fogging equipment.

The data generated from this evaluation suggest that *B.g.* may be a suitable surrogate for *B.a.* Ames for future tests assessing the decontamination efficacy of PAA or H_2O_2 using fogging equipment.

Many of the subway railcar materials were effectively decontaminated with fogging PAA. These materials include the rubber flooring, seat upholstery, aluminum seat backing, Mylar glass coating, and both new and used cabin air filters. Fogging of PAA was ineffective for the carpet (both the dirty railcar carpet and the new, clean industrial carpet), concrete, and grease (with spores mixed in/encapsulated into the grease); and moderately effective for the interior fiberglass siding, and the clean and dirty railcar grease (spores left on top of grease).

With respect to the effect of air temperature, while the higher temperature (20 °C) resulted in a greater probability of complete spore population kill and greater LR values compared to the results at 10 °C (an average of 1-2 LR better), many of these differences were not statistically significant.

The two types of foggers yielded similar LR values when compared at 20°C. Testing conducted using the same parameters but at 10°C generally yielded higher LR for the Sani-Tizer as compared to the Minncare equipment. Overall, however, statistical analysis using the logistic regression model indicated that the type of fogger did not have a significant effect on LR.

In terms of the effect of chamber location on efficacy, there was minimal difference in average LR by location within the test chamber. However, as would be expected, coupons stationed at location 3 (coupons placed horizontally on a cart facing upward, in the center of the chamber), were more likely to result in a complete kill compared to the other four locations in the chamber.

5 References

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Appendix A
Detailed Test Results

Efficacy Results

The detailed decontamination efficacy results for sporicidal liquids fogged against *B.a.* Ames *and B. atrophaeus* on up to thirteen material types are shown in Tables A-1 through A-3. Zero CFU were observed on all laboratory and procedural blanks.

Table A-1. Inactivation of *B. anthracis* Ames Spores using Fogged Sporicidal Liquids[a]

Test Number	Decon Solution (mL)	Equipment	Contact Time (hour)	Temp (°C)	Material	Inoculum (CFU/coupon)	Mean Recovered *B. anthracis* (CFU/coupon)		Efficacy ± CI[d]
							Positive Control[b]	Test Coupon[c]	
1	PAA (160)	Sani-Tizer	18	20	Rubber Flooring	1.35E+08	$8.95 \pm 2.14 \times 10^{7}$	0.00 ± 0.00	≥7.94 ± 0.10
					Upholstery		$1.20 \pm 0.04 \times 10^{8}$	0.00 ± 0.00	≥8.08 ± 0.01
					Aluminum		$7.65 \pm 0.85 \times 10^{7}$	0.00 ± 0.00	≥7.88 ± 0.04
					Mylar		$1.17 \pm 0.31 \times 10^{8}$	0.00 ± 0.00	≥8.06 ± 0.10
					Fiberglass Siding		$3.90 \pm 1.08 \times 10^{7}$	0.00 ± 0.00	≥7.58 ± 0.10
					Railcar Carpet		$4.60 \pm 2.19 \times 10^{7}$	$1.06 \pm 1.71 \times 10^{6}$	2.37 ± 1.30
2	8% H_2O_2 (2635)	Sani-Tizer	168	20	Rubber Flooring	1.26E+08	$9.71 \pm 1.40 \times 10^{7}$	0.00 ± 0.00	≥7.98 ± 0.05
					Upholstery		$9.87 \pm 1.70 \times 10^{7}$	0.00 ± 0.00	≥7.99 ± 0.06
					Aluminum		$1.02 \pm 0.12 \times 10^{8}$	0.00 ± 0.00	≥8.01 ± 0.04
					Mylar		$8.61 \pm 1.05 \times 10^{7}$	0.00 ± 0.00	≥7.93 ± 0.05
					Fiberglass Siding		$4.05 \pm 2.08 \times 10^{7}$	$9.95 \pm 22.2 \times 10^{2}$	6.77 ± 1.49
					Railcar Carpet		$5.63 \pm 2.17 \times 10^{7}$	$1.95 \pm 2.60 \times 10^{5}$	4.51 ± 2.59
3	PAA (160)	Sani-Tizer	24hr	20	Railcar Carpet	1.17E+08	$2.49 \pm 1.00 \times 10^{7}$	$9.90 \pm 12.1 \times 10^{5}$	1.71 ± 0.67
			48 hr				$2.49 \pm 1.00 \times 10^{7}$	$1.53 \pm 1.64 \times 10^{5}$	2.69 ± 0.94
			120 hr				$2.49 \pm 1.00 \times 10^{7}$	$4.27 \pm 2.77 \times 10^{5}$	1.84 ± 0.36
			144 hr				$2.49 \pm 1.00 \times 10^{7}$	$1.74 \pm 3.08 \times 10^{6}$	1.66 ± 0.68
			168 hr				$2.49 \pm 1.00 \times 10^{7}$	$5.13 \pm 6.71 \times 10^{5}$	2.84 ± 1.47
4	PAA (78)	Sani-Tizer	18	20	Rubber Flooring	1.72E+08	$1.18 \pm 0.06 \times 10^{8}$	0.00 ± 0.00	≥8.07 ± 0.02
					New Grease (SOT)		$1.06 \pm 0.10 \times 10^{8}$	$1.27 \pm 2.85 \times 10^{6}$	5.93 ± 2.70
					New HVAC Filter		$9.83 \pm 1.02 \times 10^{7}$	0.00 ± 0.00	≥7.99 ± 0.04
					Mylar		$8.39 \pm 1.24 \times 10^{7}$	1.41 ± 2.94 x 10	7.56 ± 0.72
					Fiberglass Siding		$3.99 \pm 0.86 \times 10^{7}$	$3.80 \pm 5.54 \times 10^{2}$	6.03 ± 1.30
					Railcar Carpet		$6.42 \pm 3.09 \times 10^{7}$	$4.85 \pm 3.24 \times 10^{6}$	1.24 ± 0.58
5	22% H_2O_2 (78)	Sani-Tizer	18	20	Rubber Flooring	1.09E+08	$8.52 \pm 1.05 \times 10^{7}$	0.00 ± 0.00	≥7.93 ± 0.05
					New Grease (SOT)		$1.07 \pm 0.09 \times 10^{8}$	$3.98 \pm 4.53 \times 10^{6}$	1.70 ± 0.50
					New HVAC Filter		$9.79 \pm 1.20 \times 10^{7}$	$9.04 \pm 12.6 \times 10^{4}$	5.85 ± 2.57
					Mylar		$8.51 \pm 1.09 \times 10^{7}$	0.00 ± 0.00	≥7.93 ± 0.05
					Fiberglass Siding		$4.01 \pm 0.95 \times 10^{7}$	$2.40 \pm 3.96 \times 10^{4}$	3.58 ± 0.50
					Railcar Carpet		$4.17 \pm 2.52 \times 10^{7}$	$1.90 \pm 1.80 \times 10^{7}$	0.39 ± 0.42

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual samples and decontamination efficacy (log reduction).

[b] Positive Controls = samples inoculated, not decontaminated.

[c] Test Coupons = samples inoculated, decontaminated.

[d] CI = confidence interval (± 1.96 × SE).

Table A-1. Inactivation of *B. anthracis Ames* using Fogged Sporicidal Liquids [a] *(Continued)*

Test Number	Decon Solution (mL)	Equipment	Contact Time (hour)	Temp (°C)	Material	Inoculum (CFU/coupon)	Mean Recovered *B. anthracis* (CFU/coupon)		Decontamination Efficacy ± CI[d]
							Positive Control[b]	Test Coupon[c]	
6	PAA (78)	Sani-Tizer	8	20	Rubber Flooring	1.09E+08	$7.13 \pm 0.16 \times 10^{7}$	$8.74 \pm 19.3 \times 10^{6}$	7.33 ± 1.03
					Encapsulated New Grease		$7.87 \pm 4.33 \times 10^{6}$	$1.88 \pm 1.45 \times 10^{6}$	0.85 ± 0.68
					Used HVAC Filter		$7.75 \pm 0.70 \times 10^{7}$	0.00 ± 0.00	≥7.89 ± 0.04
					Mylar		$6.73 \pm 0.45 \times 10^{7}$	0.00 ± 0.00	≥7.83 ± 0.03
					Fiberglass Siding		$2.98 \pm 1.27 \times 10^{7}$	$1.34 \pm 2.20 \times 10^{4}$	4.93 ± 2.03
					Unpainted Concrete		$6.13 \pm 4.81 \times 10^{6}$	$1.72 \pm 1.26 \times 10^{5}$	1.57 ± 0.49
7	PAA (160)	Sani-Tizer	18	20	Railcar Carpet	1.03E+08	$2.10 \pm 1.81 \times 10^{7}$	$3.53 \pm 4.11 \times 10^{5}$	2.16 ± 0.85
					Unpainted Concrete		$2.81 \pm 2.38 \times 10^{6}$	$5.97 \pm 4.82 \times 10^{4}$	1.66 ± 0.68
					Used HVAC Filter		$6.27 \pm 0.58 \times 10^{7}$	0.00 ± 0.00	≥7.80 ± 0.04
					New Grease (SOT)		$5.06 \pm 0.58 \times 10^{6}$	$1.31 \pm 2.86 \times 10^{3}$	5.97 ± 1.19
					Encapsulated New Grease		$6.95 \pm 6.34 \times 10^{6}$	$2.16 \pm 3.55 \times 10^{5}$	1.78 ± 1.14
					Used Grease (SOT)		$5.35 \pm 0.85 \times 10^{7}$	0.00 ± 0.00	≥7.72 ± 0.06
8	PAA (160)	Minncare	18	20	Railcar Carpet	1.04E+08	$2.08 \pm 1.32 \times 10^{7}$	$2.42 \pm 4.36 \times 10^{5}$	2.44 ± 0.71
					Unpainted Concrete		$3.00 \pm 2.96 \times 10^{6}$	$2.00 \pm 2.13 \times 10^{5}$	1.27 ± 0.72
					Used HVAC Filter		$7.17 \pm 0.72 \times 10^{7}$	0.00 ± 0.00	≥7.85 ± 0.04
					New Grease (SOT)		$8.60 \pm 0.80 \times 10^{7}$	$9.46 \pm 21.2 \times 10^{3}$	7.00 ± 1.83
					Encapsulated New Grease		$4.32 \pm 3.80 \times 10^{6}$	$9.27 \pm 13.1 \times 10^{5}$	1.00 ± 0.93
					Used Grease (SOT)		$9.04 \pm 1.47 \times 10^{7}$	$3.95 \pm 8.58 \times 10^{5}$	5.76 ± 2.67
9	PAA (160)	Minncare	18	20	Rubber Flooring	8.40E+07	$6.98 \pm 0.59 \times 10^{7}$	0.00 ± 0.00	≥7.84 ± 0.03
					Upholstery		$9.44 \pm 0.69 \times 10^{7}$	0.00 ± 0.00	≥7.97 ± 0.03
					Aluminum		$8.91 \pm 1.18 \times 10^{7}$	0.00 ± 0.00	≥7.95 ± 0.05
					Mylar		$7.97 \pm 1.06 \times 10^{7}$	0.00 ± 0.00	≥7.90 ± 0.05
					Fiberglass Siding		$3.74 \pm 0.42 \times 10^{7}$	$1.34 \pm 1.90 \times 10^{2}$	5.97 ± 0.87
					Railcar Carpet		$5.31 \pm 2.37 \times 10^{7}$	$4.39 \pm 7.19 \times 10^{5}$	3.85 ± 2.17
10	PAA (500)	Minncare	18	20	Railcar Carpet	1.01E+08	$4.84 \pm 1.94 \times 10^{7}$	$2.82 \pm 3.25 \times 10^{3}$	4.90 ± 1.39
					Encapsulated New Grease		$1.78 \pm 1.63 \times 10^{6}$	$2.11 \pm 4.27 \times 10^{5}$	2.40 ± 1.65
					Used HVAC Filter		$8.39 \pm 0.89 \times 10^{7}$	0.00 ± 0.00	≥7.92 ± 0.04
					Fiberglass Siding		$3.25 \pm 0.90 \times 10^{7}$	$1.21 \pm 2.68 \times 10^{2}$	6.94 ± 1.10
					Unpainted Concrete		$2.04 \pm 1.55 \times 10^{7}$	$1.01 \pm 1.07 \times 10^{5}$	2.41 ± 0.55
					Used Grease (SOT)		$8.62 \pm 0.63 \times 10^{7}$	$1.33 \pm 2.98 \times 10^{3}$	7.17 ± 1.50
11	PAA (160)	Sani-Tizer	18	10	Rubber Flooring	9.67E+07	$7.85 \pm 0.57 \times 10^{7}$	0.00 ± 0.00	≥7.89 ± 0.03
					Upholstery		$8.83 \pm 1.22 \times 10^{7}$	$2.54 \pm 5.68 \times 10^{3}$	7.12 ± 1.61
					Aluminum		$8.50 \pm 0.85 \times 10^{7}$	$1.07 \pm 2.38 \times 10^{2}$	7.38 ± 1.07
					Mylar		$6.13 \pm 1.44 \times 10^{7}$	0.00 ± 0.00	≥7.78 ± 0.10
					Fiberglass Siding		$4.40 \pm 0.76 \times 10^{7}$	$7.08 \pm 15.4 \times 10^{2}$	6.56 ± 1.40
					Railcar Carpet		$3.39 \pm 1.64 \times 10^{7}$	$6.43 \pm 6.96 \times 10^{6}$	0.99 ± 0.61
12	PAA (78)	Sani-Tizer	18	10	Rubber Flooring	1.04E+08	$7.14 \pm 0.65 \times 10^{7}$	$1.07 \pm 2.40 \times 10^{3}$	7.11 ± 1.46
					New Grease (SOT)		$1.07 \pm 0.16 \times 10^{8}$	$2.04 \pm 2.95 \times 10^{6}$	3.40 ± 2.47
					New HVAC Filter		$1.04 \pm 0.09 \times 10^{8}$	$3.06 \pm 6.84 \times 10^{5}$	6.47 ± 2.35
					Mylar		$8.41 \pm 0.72 \times 10^{7}$	7.46 ± 14.4	7.62 ± 0.60
					Fiberglass Siding		$3.39 \pm 0.38 \times 10^{7}$	$5.63 \pm 7.14 \times 10^{4}$	3.39 ± 0.87
					Railcar Carpet		$2.80 \pm 2.33 \times 10^{7}$	$9.70 \pm 7.79 \times 10^{6}$	0.54 ± 0.58
13	PAA (160)	Sani-Tizer	18	10	Railcar Carpet	1.01E+08	$4.04 \pm 1.73 \times 10^{7}$	$7.90 \pm 5.97 \times 10^{6}$	0.73 ± 0.32
					Unpainted Concrete		$5.66 \pm 3.12 \times 10^{6}$	$4.85 \pm 3.97 \times 10^{5}$	1.24 ± 0.58
					Used HVAC Filter		$4.87 \pm 0.71 \times 10^{7}$	0.00 ± 0.00	≥7.68 ± 0.06
					New Grease (SOT)		$7.30 \pm 0.67 \times 10^{7}$	$4.27 \pm 7.72 \times 10^{5}$	3.81 ± 2.16
					Encapsulated New Grease		$1.32 \pm 1.41 \times 10^{6}$	$3.96 \pm 3.87 \times 10^{5}$	0.77 ± 1.26
					Used Grease (SOT)		$7.67 \pm 0.24 \times 10^{7}$	$2.32 \pm 4.32 \times 10^{6}$	3.31 ± 2.41

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual samples and decontamination efficacy (log reduction).

[b] Positive Controls = samples inoculated, not decontaminated.

[c] Test Coupons = samples inoculated, decontaminated.

[d] CI = confidence interval (± 1.96 × SE).

Table A-1. Inactivation of *B. anthracis Ames* using Fogged Sporicidal Liquids [a] *(Continued)*

Test Number	Decon Solution (mL)	Equipment	Contact Time (hour)	Temp (°C)	Material	Inoculum (CFU/coupon)	Mean Recovered *B. anthracis* (CFU/coupon)		Decontamination Efficacy ± CI[d]
							Positive Control[b]	Test Coupon[c]	
14	PAA (500)	Minncare	18	10	Railcar Carpet	1.13E+08	$4.75 \pm 2.57 \times 10^7$	$1.89 \pm 1.46 \times 10^6$	1.43 ± 0.41
					Encapsulated New Grease		$1.38 \pm 0.40 \times 10^7$	$5.81 \pm 7.65 \times 10^5$	1.65 ± 0.51
					Used HVAC Filter		$8.11 \pm 1.04 \times 10^7$	9.42 ± 20.8 x 10	7.37 ± 1.05
					Fiberglass Siding		$3.08 \pm 1.05 \times 10^7$	$1.12 \pm 2.23 \times 10^4$	5.38 ± 1.87
					Unpainted Concrete		$1.23 \pm 0.54 \times 10^7$	$9.76 \pm 2.70 \times 10^4$	2.06 ± 0.29
					Used Grease (SOT)		$6.65 \pm 2.24 \times 10^7$	$4.89 \pm 10.1 \times 10^5$	3.87 ± 2.11
15	PAA (500)	Sani-Tizer	18	20	Railcar Carpet	1.00E+08	$2.09 \pm 1.31 \times 10^7$	$2.22 \pm 4.96 \times 10^4$	6.26 ± 1.99
					Encapsulated New Grease		$4.75 \pm 0.83 \times 10^6$	$3.41 \pm 4.95 \times 10^5$	2.77 ± 2.19
					Used HVAC Filter		$6.72 \pm 2.14 \times 10^7$	0.00 ± 0.00	≥7.80 ± 0.15
					Fiberglass Siding		$3.12 \pm 2.08 \times 10^7$	$1.27 \pm 2.83 \times 10^2$	6.86 ± 1.13
					Unpainted Concrete		$1.48 \pm 1.15 \times 10^7$	$7.27 \pm 8.64 \times 10^4$	2.70 ± 0.86
					Used Grease (SOT)		$8.74 \pm 1.20 \times 10^7$	1.16 ± 2.59 x 105	6.22 ± 2.25
16	35% H_2O_2 (500)	Minncare	18	20	Railcar Carpet	9.03E+07	$2.98 \pm 1.76 \times 10^7$	$3.62 \pm 3.59 \times 10^6$	1.01 ± 0.49
					Encapsulated New Grease		$5.05 \pm 2.17 \times 10^6$	$5.83 \pm 4.84 \times 10^5$	1.15 ± 0.61
					Used HVAC Filter		$6.98 \pm 0.76 \times 10^7$	3.39 ± 4.66 x 10	7.08 ± 0.92
					Fiberglass Siding		$3.54 \pm 2.10 \times 10^7$	$3.06 \pm 5.59 \times 10^3$	5.30 ± 1.50
					Unpainted Concrete		$7.10 \pm 12.8 \times 10^7$	$5.37 \pm 4.89 \times 10^5$	1.87 ± 0.72
					Used Grease (SOT)		$6.49 \pm 3.63 \times 10^7$	$1.02 \pm 1.56 \times 10^6$	2.34 ± 1.43
17	35% H_2O_2 (1000)	Sani-Tizer	18	20	Railcar Carpet	1.03E+08	$2.92 \pm 0.79 \times 10^7$	$1.73 \pm 0.79 \times 10^5$	2.29 ± 0.31
					Encapsulated New Grease		$8.55 \pm 0.60 \times 10^7$	$4.24 \pm 5.96 \times 10^5$	2.69 ± 0.59
					Used HVAC Filter		$7.20 \pm 0.39 \times 10^7$	$1.16 \pm 2.55 \times 10^3$	6.74 ± 1.47
					Fiberglass Siding		$5.37 \pm 4.39 \times 10^7$	$4.74 \pm 1.02 \times 10^2$	6.61 ± 1.35
					Unpainted Concrete		$6.87 \pm 1.46 \times 10^6$	$3.00 \pm 2.78 \times 10^5$	1.57 ± 0.49
					Used Grease (SOT)		$7.92 \pm 0.46 \times 10^7$	$8.14 \pm 8.30 \times 10^2$	5.73 ± 1.23
18	PAA (1000)	Sani-Tizer	18	20	Railcar Carpet	6.60E+07	$5.28 \pm 1.85 \times 10^7$	$3.43 \pm 5.65 \times 10^5$	3.31 ± 1.45
					Encapsulated New Grease		$1.22 \pm 6.53 \times 10^6$	$1.46 \pm 3.15 \times 10^5$	1.99 ± 0.97
					New Grease (SOT)		$8.27 \pm 1.40 \times 10^7$	7.46 ± 1.44	7.61 ± 0.60
					Fiberglass Siding		$4.18 \pm 0.85 \times 10^7$	$5.67 \pm 12.6 \times 10^5$	5.56 ± 2.60
					Unpainted Concrete		$1.38 \pm 0.70 \times 10^7$	$1.95 \pm 1.79 \times 10^5$	1.91 ± 0.36
					Used Grease (SOT)		$8.39 \pm 2.09 \times 10^7$	0.00 ± 0.00	≥7.91 ± 0.10
19	PAA (1000)	Sani-Tizer	18	10	Carpet (Subway)	7.17E+07	2.46 ± 2.56 x 107	6.07 ± 6.71 x 105	3.69 ± 2.88
					Encapsulated New Grease		4.18 ± 3.01 x 106	9.35 ± 6.76 x 105	0.67 ± 0.52
					Industrial Carpet		7.01 ± 0.50 x 107	1.30 ± 2.21 x 104	4.32 ± 0.74
					Fiberglass Siding		3.47 ± 0.35 x 107	1.16 ± 1.82 x 103	6.17 ± 1.65
					Unpainted Concrete		4.38 ± 4.77 x 106	1.85 ± 2.38 x 105	1.50 ± 0.70
					Used Grease (SOT)		8.50 ± 0.55 x 107	8.50 ± 5.45 x 107	5.29 ± 2.65
20	35% H_2O_2 (1000)	Sani-Tizer	18	10	Railcar Carpet	9.60E+07	4.70 ± 3.75 x 107	2.28 ± 2.42 106	3.23 ± 2.75
					Encapsulated New Grease		3.86 ± 2.23 x 108	1.26 ± 1.73 x 106	2.64 ± 0.51
					Used HVAC Filter		7.58 ± 1.39 x 107	0.00 ± 0.00	≥7.87 ± 0.07
					Fiberglass Siding		3.47 ± 1.18 x 107	2.12 ± 3.80 x 103	5.56 ± 1.62
					Unpainted Concrete		4.90 ± 3.98 x 106	8.11 ± 3.07 x 105	0.70 ± 0.34
					Used Grease (SOT)		7.62 ± 1.01 x 107	2.17 ± 4.77 x 105	4.44 ± 1.51
21	PAA (160)	Minncare	18	10	Railcar Carpet	8.03E+07	7.45 ± 0.64 x 107	2.78 ± 1.64 x 107	0.49 ± 0.23
					Unpainted Concrete		4.64 ± 3.48 x 106	1.00 ± 0.32 x 106	0.60 ± 0.29
					Used HVAC Filter		6.47 ± 6.25 x 107	5.16 ± 10.9 x 106	2.10 ± 0.92
					New Grease (SOT)		8.07 ± 0.97 x 107	5.01 ± 1.06 x 106	0.21 ± 0.09
					Encapsulated New Grease		2.59 ± 2.34 x 106	9.03 ± 5.57 x 105	0.33 ± 0.51
					Used Grease (SOT)		6.95 ± 0.57 x 107	4.49 ± 2.17 x 107	0.24 ± 0.23

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual samples and decontamination efficacy (log reduction).

[b] Positive Controls = samples inoculated, not decontaminated.

[c] Test Coupons = samples inoculated, decontaminated.

[d] CI = confidence interval (± 1.96 × SE).

Table A-2. Inactivation of *B. atrophaeus* Spores using Fogged Sporicidal Liquids[a]

Test Number	Decon Solution (mL)	Equipment	Contact Time (hour)	Temp (°C)	Material	Inoculum (CFU/coupon)	Mean Recovered *B. atrophaeus* (CFU/coupon)		Efficacy ± CI[d]
							Positive Control[b]	Test Coupon[c]	
1	PAA (160)	Sani-Tizer	18	20	Rubber Flooring	8.50E+07	$2.30 \pm 2.57 \times 10^7$	0.00 ± 0.00	≥7.21 ± 0.33
					Upholstery		$7.61 \pm 2.49 \times 10^6$	0.00 ± 0.00	≥6.86 ± 0.12
					Aluminum		$1.20 \pm 0.39 \times 10^6$	0.00 ± 0.00	≥7.06 ± 0.13
					Mylar		$8.38 \pm 3.62 \times 10^6$	0.00 ± 0.00	≥6.89 ± 0.17
					Fiberglass Siding		$3.11 \pm 1.31 \times 10^6$	0.00 ± 0.00	≥6.47 ± 0.14
					Railcar Carpet		$1.75 \pm 1.27 \times 10^7$	$6.05 \pm 7.00 \times 10^5$	2.49 ± 2.30
2	8% H_2O_2 (2635)	Sani-Tizer	168	20	Rubber Flooring	1.11E+08	$6.89 \pm 2.77 \times 10^6$	0.00 ± 0.00	≥6.81 ± 0.16
					Upholstery		$1.82 \pm 0.75 \times 10^6$	0.00 ± 0.00	≥6.23 ± 0.14
					Aluminum		$1.68 \pm 1.02 \times 10^7$	0.00 ± 0.00	≥7.18 ± 0.18
					Mylar		$1.35 \pm 1.56 \times 10^7$	0.00 ± 0.00	≥6.96 ± 0.33
					Fiberglass Siding		$2.37 \pm 0.62 \times 10^6$	1.41 ± 2.94 x10	6.00 ± 0.72
					Railcar Carpet		$4.36 \pm 1.03 \times 10^6$	$7.86 \pm 17.6 \times 10^3$	5.71 ± 1.80
3	PAA (160)	Sani-Tizer	24hr	20	Railcar Carpet	1.19E+08	$1.31 \pm 0.96 \times 10^7$	$1.36 \pm 1.63 \times 10^5$	2.54 ± 1.07
			48 hr				$1.31 \pm 0.96 \times 10^7$	$1.67 \pm 2.83 \times 10^5$	2.68 ± 1.35
			120 hr				$1.31 \pm 0.96 \times 10^7$	$1.62 \pm 2.64 \times 10^5$	2.71 ± 1.46
			144 hr				$1.31 \pm 0.96 \times 10^7$	$4.99 \pm 1.54 \times 10^4$	2.34 ± 0.31
			168 hr				$1.31 \pm 0.96 \times 10^7$	$6.19 \pm 6.51 \times 10^4$	3.27 ± 1.91
4	PAA (78)	Sani-Tizer	18	20	Rubber Flooring	1.06E+08	$2.13 \pm 0.81 \times 10^7$	0.00 ± 0.00	≥7.30 ± 0.16
					New Grease (SOT)		$6.30 \pm 2.81 \times 10^6$	1.41 ± 2.94 x 10	6.39 ± 0.74
					New HVAC Filter		$3.97 \pm 1.04 \times 10^6$	0.00 ± 0.00	6.59 ± 0.10
					Mylar		$9.75 \pm 1.60 \times 10^6$	0.00 ± 0.00	6.98 ± 0.06
					Fiberglass Siding		$3.12 \pm 1.50 \times 10^6$	1.39 ± 1.77 x 10	5.84 ± 0.76
					Railcar Carpet		$1.26 \pm 0.88 \times 10^7$	$2.96 \pm 2.43 \times 10^6$	0.70 ± 0.40
5	22% H_2O_2 (78)	Sani-Tizer	18	20	Rubber Flooring	1.09E+08	$1.38 \pm 0.93 \times 10^7$	$9.94 \pm 2.22 \times 10^4$	6.12 ± 1.86
					New Grease (SOT)		$6.60 \pm 4.97 \times 10^6$	$6.31 \pm 12.2 \times 10^4$	3.74 ± 1.90
					New HVAC Filter		$2.44 \pm 0.51 \times 10^3$	0.00 ± 0.00	≥6.38 ± 0.08
					Mylar		$1.02 \pm 0.33 \times 10^7$	0.00 ± 0.00	≥6.99 ± 0.13
					Fiberglass Siding		$2.08 \pm 0.93 \times 10^6$	$1.11 \pm 1.45 \times 10^3$	4.07 ± 1.28
					Railcar Carpet		$1.03 \pm 0.61 \times 10^7$	$3.69 \pm 1.29 \times 10^6$	0.41 ± 0.29

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual samples and decontamination efficacy (log reduction).

[b] Positive Controls = samples inoculated, not decontaminated.

[c] Test Coupons = samples inoculated, decontaminated.

[d] CI = confidence interval (± 1.96 × SE).

Table A-2. Inactivation of *B. atrophaeus* Spores using Fogged Sporicidal Liquids [a] *(Continued)*

Test Number	Decon Solution (mL)	Equipment	Contact Time (hour)	Temp (°C)	Material	Inoculum (CFU/coupon)	Mean Recovered *B. trophaeus* (CFU/coupon)		Decontamination Efficacy ± CI[d]
							Positive Control[b]	Test Coupon[c]	
6	PAA (78)	Sani-Tizer	8	20	Rubber Flooring	1.02E+08	$2.74 \pm 1.29 \times 10^7$	$4.03 \pm 5.43 \times 10$	6.35 ± 0.89
					Encapsulated New Grease		$9.89 \pm 8.05 \times 10^5$	$3.08 \pm 3.88 \times 10^4$	1.57 ± 0.94
					Used HVAC Filter		$5.89 \pm 1.99 \times 10^6$	0.00 ± 0.00	$\geq 6.75 \pm 0.14$
					Mylar		$1.52 \pm 0.94 \times 10^7$	0.00 ± 0.00	$\geq 7.13 \pm 0.20$
					Fiberglass Siding		$4.77 \pm 3.01 \times 10^6$	$1.41 \pm 2.08 \times 10^2$	5.61 ± 1.24
					Unpainted Concrete		$2.50 \pm 1.42 \times 10^5$	$2.53 \pm 1.49 \times 10^4$	1.03 ± 0.38
7	PAA (160)	Sani-Tizer	18	20	Railcar Carpet	1.39E+08	$2.31 \pm 1.42 \times 10^7$	$5.55 \pm 6.55 \times 10^5$	2.23 ± 1.14
					Unpainted Concrete		$2.39 \pm 2.05 \times 10^5$	$1.12 \pm 1.07 \times 10^5$	0.30 ± 0.46
					Used HVAC Filter		$5.31 \pm 0.59 \times 10^6$	0.00 ± 0.00	$\geq 6.72 \pm 0.04$
					New Grease (SOT)		$1.74 \pm 1.74 \times 10^7$	$4.35 \pm 9.70 \times 10^2$	6.38 ± 1.37
					Encapsulated New Grease		$1.12 \pm 1.37 \times 10^6$	$1.54 \pm 2.10 \times 10^2$	4.83 ± 1.29
					Used Grease (SOT)		$1.40 \pm 1.96 \times 10^7$	$1.50 \pm 3.35 \times 10^4$	5.94 ± 1.95
8	PAA (160)	Minncare	18	20	Railcar Carpet	1.16E+08	$9.32 \pm 3.42 \times 10^7$	$7.32 \pm 6.64 \times 10^5$	2.46 ± 0.85
					Unpainted Concrete		$7.92 \pm 7.66 \times 10^5$	$6.82 \pm 8.71 \times 10^3$	2.07 ± 0.63
					Used HVAC Filter		$2.04 \pm 1.32 \times 10^7$	0.00 ± 0.00	$\geq 7.20 \pm 0.33$
					New Grease (SOT)		$2.90 \pm 1.70 \times 10^6$	0.00 ± 0.00	$\geq 6.41 \pm 0.22$
					Encapsulated New Grease		$1.08 \pm 1.17 \times 10^5$	$3.59 \pm 3.37 \times 10^3$	1.57 ± 1.21
					Used Grease (SOT)		$1.09 \pm 1.64 \times 10^7$	$4.73 \pm 8.65 \times 10$	5.67 ± 1.22
9	PAA (160)	Minncare	18	20	Rubber Flooring	1.05E+08	$1.11 \pm 0.24 \times 10^7$	0.00 ± 0.00	$\geq 7.04 \pm 0.08$
					Upholstery		$5.23 \pm 4.09 \times 10^7$	0.00 ± 0.00	$\geq 7.56 \pm 0.40$
					Aluminum		$4.50 \pm 0.94 \times 10^7$	0.00 ± 0.00	$\geq 7.65 \pm 0.08$
					Mylar		$4.75 \pm 5.32 \times 10^7$	0.00 ± 0.00	$\geq 7.39 \pm 0.50$
					Fiberglass Siding		$4.95 \pm 2.34 \times 10^6$	0.00 ± 0.00	$\geq 6.66 \pm 0.18$
					Railcar Carpet		$1.09 \pm 0.46 \times 10^7$	$3.54 \pm 3.95 \times 10^5$	1.70 ± 0.49
10	PAA (500)	Minncare	18	20	Railcar Carpet	1.07E+08	$2.58 \pm 1.59 \times 10^7$	$9.26 \pm 14.8 \times 10^4$	2.92 ± 0.79
					Encapsulated New Grease		$5.69 \pm 5.35 \times 10^5$	$7.15 \pm 15.7 \times 10^4$	2.47 ± 1.33
					Used HVAC Filter		$8.60 \pm 0.82 \times 10^6$	0.00 ± 0.00	$\geq 6.93 \pm 0.04$
					Fiberglass Siding		$1.20 \pm 0.84 \times 10^7$	7.46 ± 14.4	6.71 ± 0.64
					Unpainted Concrete		$3.73 \pm 1.73 \times 10^5$	$4.16 \pm 3.21 \times 10^3$	2.04 ± 0.36
					Used Grease (SOT)		$1.60 \pm 0.16 \times 10^7$	$1.14 \pm 1.57 \times 10^2$	5.94 ± 1.06
11	PAA (160)	Sani-Tizer	18	10	Rubber Flooring	9.13E+07	$2.11 \pm 1.15 \times 10^7$	0.00 ± 0.00	$\geq 7.28 \pm 0.17$
					Upholstery		$1.62 \pm 0.29 \times 10^7$	0.00 ± 0.00	$\geq 7.20 \pm 0.07$
					Aluminum		$2.35 \pm 0.73 \times 10^7$	0.00 ± 0.00	$\geq 7.35 \pm 0.12$
					Mylar		$1.59 \pm 0.37 \times 10^7$	0.00 ± 0.00	$\geq 7.19 \pm 0.08$
					Fiberglass Siding		$5.44 \pm 2.00 \times 10^6$	$2.20 \pm 3.52 \times 10^2$	5.30 ± 1.19
					Railcar Carpet		$2.35 \pm 1.13 \times 10^7$	$9.36 \pm 5.01 \times 10^5$	1.48 ± 0.44
12	PAA (78)	Sani-Tizer	18	10	Rubber Flooring	1.22E+08	$2.19 \pm 0.83 \times 10^7$	0.00 ± 0.00	$\geq 7.31 \pm 0.16$
					New Grease (SOT)		$6.41 \pm 0.84 \times 10^6$	$7.02 \pm 15.5 \times 10^4$	3.58 ± 1.29
					New HVAC Filter		$4.57 \pm 1.86 \times 10^6$	0.00 ± 0.00	$\geq 6.64 \pm 0.13$
					Mylar		$1.90 \pm 0.73 \times 10^7$	0.00 ± 0.00	$\geq 7.26 \pm 0.14$
					Fiberglass Siding		$5.11 \pm 3.35 \times 10^6$	$1.35 \pm 1.78 \times 10^4$	3.21 ± 0.94
					Railcar Carpet		$1.09 \pm 1.13 \times 10^7$	$3.41 \pm 1.71 \times 10^5$	1.43 ± 0.36
13	PAA (160)	Sani-Tizer	18	10	Railcar Carpet	1.22E+08	$2.21 \pm 2.76 \times 10^7$	$1.52 \pm 0.94 \times 10^5$	0.99 ± 0.48
					Unpainted Concrete		$3.52 \pm 2.45 \times 10^5$	$2.93 \pm 4.50 \times 10^4$	1.36 ± 0.64
					Used HVAC Filter		$1.08 \pm 0.64 \times 10^7$	0.00 ± 0.00	$\geq 6.96 \pm 0.25$
					New Grease (SOT)		$1.05 \pm 0.12 \times 10^7$	$2.86 \pm 4.69 \times 10^3$	4.97 ± 1.69
					Encapsulated New Grease		$2.60 \pm 2.96 \times 10^5$	$8.39 \pm 9.23 \times 10^3$	2.16 ± 1.59
					Used Grease (SOT)		$7.40 \pm 5.30 \times 10^6$	$7.47 \pm 11.2 \times 10^2$	5.50 ± 1.58

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual samples and decontamination efficacy (log reduction).

[b] Positive Controls = samples inoculated, not decontaminated.

[c] Test Coupons = samples inoculated, decontaminated.

[d] CI = confidence interval (± 1.96 × SE).

Table A-2. Inactivation of *B. atrophaeus* Spores using Fogged Sporicidal Liquids [a] *(Continued)*

Test Number	Decon Solution (mL)	Equipment	Contact Time (hour)	Temp (°C)	Material	Inoculum (CFU/coupon)	Mean Recovered *B. atrophaeus* (CFU/coupon)		Decontamination Efficacy ± CI[d]
							Positive Control[b]	Test Coupon[c]	
14	PAA (500)	Minncare	18	10	Railcar Carpet	8.53E+07	$2.18 \pm 2.01 \times 10^7$	$3.03 \pm 3.52 \times 10^6$	0.90 ± 0.59
					Encapsulated New Grease		$7.28 \pm 7.55 \times 10^5$	$2.88 \pm 6.22 \times 10^4$	2.55 ± 1.86
					Used HVAC Filter		$7.23 \pm 2.48 \times 10^6$	0.00 ± 0.00	≥6.84 ± 0.13
					Fiberglass Siding		$4.63 \pm 2.02 \times 10^6$	0.00 ± 0.00	≥6.63 ± 0.16
					Unpainted Concrete		$7.10 \pm 3.88 \times 10^5$	$1.00 \pm 0.68 \times 10^4$	1.90 ± 0.35
					Used Grease (SOT)		$1.01 \pm 0.30 \times 10^7$	$1.29 \pm 2.86 \times 10^3$	5.92 ± 1.47
15	PAA (500)	Sani-Tizer	18	20	Railcar Carpet	1.00E+08	$1.05 \pm 0.50 \times 10^7$	$6.04 \pm 4.18 \times 10^5$	1.36 ± 0.47
					Encapsulated New Grease		$4.04 \pm 5.50 \times 10^5$	$3.18 \pm 4.33 \times 10^4$	2.33 ± 1.83
					Used HVAC Filter		$8.26 \pm 1.21 \times 10^6$	0.00 ± 0.00	≥6.91 ± 0.06
					Fiberglass Siding		$7.99 \pm 5.09 \times 10^6$	0.00 ± 0.00	≥6.84 ± 0.21
					Unpainted Concrete		$5.32 \pm 7.14 \times 10^5$	$4.71 \pm 2.51 \times 10^4$	0.78 ± 0.72
					Used Grease (SOT)		$8.17 \pm 3.77 \times 10^6$	0.00 ± 0.00	≥6.88 ± 0.14
16	35% H_2O_2 (500)	Minncare	18	20	Railcar Carpet	1.03E+08	$8.22 \pm 1.76 \times 10^6$	$1.32 \pm 0.84 \times 10^6$	1.05 ± 0.69
					Encapsulated New Grease		$6.29 \pm 6.68 \times 10^5$	$1.89 \pm 2.49 \times 10^5$	1.25 ± 1.28
					Used HVAC Filter		$9.25 \pm 1.70 \times 10^6$	0.00 ± 0.00	≥6.96 ± 0.06
					Fiberglass Siding		$2.37 \pm 1.65 \times 10^6$	$5.01 \pm 11.2 \times 10^2$	5.63 ± 1.35
					Unpainted Concrete		$9.54 \pm 4.46 \times 10^5$	$1.38 \pm 1.25 \times 10^5$	1.10 ± 0.75
					Used Grease (SOT)		$1.20 \pm 0.55 \times 10^7$	$6.51 \pm 12.5 \times 10^3$	4.98 ± 1.79
17	35% H_2O_2 (1000)	Sani-Tizer	18	20	Railcar Carpet	1.11E+08	$1.08 \pm 0.69 \times 10^7$	$1.71 \pm 1.83 \times 10^5$	2.27 ± 0.881
					Encapsulated New Grease		$8.01 \pm 1.99 \times 10^6$	$1.87 \pm 3.05 \times 10^5$	2.59 ± 1.27
					Used HVAC Filter		$6.85 \pm 1.89 \times 10^6$	0.00 ± 0.00	≥6.82 ± 0.09
					Fiberglass Siding		$4.77 \pm 2.37 \times 10^6$	$3.33 \pm 7.20 \times 10^3$	5.26 ± 1.73
					Unpainted Concrete		$2.10 \pm 1.02 \times 10^5$	$8.14 \pm 4.21 \times 10^3$	1.41 ± 0.27
					Used Grease (SOT)		$8.93 \pm 4.44 \times 10^6$	0.00 ± 0.00	≥6.92 ± 0.16
18	PAA (1000)	Sani-Tizer	18	20	Railcar Carpet	1.08E+08	$1.25 \pm 0.93 \times 10^7$	$3.28 \pm 6.00 \times 10^5$	2.09 ± 0.75
					Encapsulated New Grease		$4.66 \pm 4.65 \times 10^5$	$1.32 \pm 1.74 \times 10^3$	2.83 ± 0.93
					New Grease (SOT)		$2.40 \pm 2.23 \times 10^6$	$5.88 \pm 12.8 \times 10^2$	5.21 ± 1.40
					Fiberglass Siding		$7.50 \pm 3.02 \times 10^6$	$1.88 \pm 4.20 \times 10^4$	5.24 ±1.79
					Unpainted Concrete		$1.18 \pm 0.81 \times 10^6$	$2.54 \pm 3.23 \times 10^4$	2.21 ± 1.01
					Used Grease (SOT)		$6.11 \pm 1.48 \times 10^6$	$2.02 \pm 4.46 \times 10^3$	5.58 ± 1.57
19	PAA (1000)	Sani-Tizer	18	10	Carpet (Subway)	9.33E+07	1.73 ± 1.58 x 107	1.07 ± 0.94 106	1.15 ± 0.48
					Encapsulated New Grease		1.16 ± 6.55 x 106	6.26 ± 10.80 x 104	2.82 ± 2.03
					Industrial Carpet		3.65 ± 1.13 x 107	7.85 ± 6.09 x 102	4.81 ± 0.43
					Fiberglass Siding		4.87 ± 2.93 x 106	0.00 ± 0.00	≥6.61 ± 0.26
					Unpainted Concrete		5.87 ± 4.74 x 105	5.34 ± 7.97 x 103	2.13 ± 0.71
					Used Grease (SOT)		8.09 ± 7.22 x 106	3.70 ± 8.27 x 104	5.75 ± 2.08
20	35% H_2O_2 (1000)	Sani-Tizer	18	10	Railcar Carpet	1.29E+08	1.36 ± 0.99 x 107	1.68 ± 1.43 x 106	1.62 ± 1.65
					Encapsulated New Grease		5.26 ± 5.93 x 105	5.88 ± 5.74 x 104	0.80 ± 0.70
					Used HVAC Filter		4.08 ± 1.14 x 106	8.07 ± 16.1 x 10	5.78 ± 1.04
					Fiberglass Siding		3.35 ± 0.99 x 106	1.15 ± 1.66 x 103	4.32 ± 1.27
					Unpainted Concrete		5.07 ± 3.41 x 105	9.22 ± 3.39 x 104	0.67 ± 0.32
					Used Grease (SOT)		6.09 ± 1.99 x 106	9.31 ± 20.0 x 104	4.08 ± 2.24
21	PAA (160)	Minncare	18	10	Railcar Carpet	9.83E+07	1.65 ± 1.39 x 107	7.71 ± 0.79 x 107	-0.04 ± 0.37
					Unpainted Concrete		6.66 ± 3.42 x 106	1.33 ± 0.56 x 105	0.70 ± 0.31
					Used HVAC Filter		3.49 ± 0.78 x 106	4.48 ± 8.91 x 104	2.63 ± 0.75
					New Grease (SOT)		6.22 ± 5.17 x 106	8.16 ± 5.90 x 105	0.92 ± 0.46
					Encapsulated New Grease		1.60 ± 1.64 x 106	1.17 ± 1.74 x 105	1.36 ± 0.89
					Used Grease (SOT)		6.90 ± 4.80 x 106	1.55 ± 2.17 x 106	0.91 ± 0.59

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual samples and decontamination efficacy (log reduction).

[b] Positive Controls = samples inoculated, not decontaminated.

[c] Test Coupons = samples inoculated, decontaminated.

[d] CI = confidence interval (± 1.96 × SE).

Appendix B
Comparing Efficacy for the Different Microorganisms

All 21 tests were conducted using *B. anthracis* Ames *and B. atrophaeus* (*B.g.*). The results showed that *B. atrophaeus* has resistance similar to *B.a.* Ames when exposed to PAA and H_2O_2 fog at both the ambient (20°C) and lower simulated subway (10°C) conditions. The detailed differences in efficacy by material type and test number are shown in Tables B-1 and B-2.

Table B-1. Difference in Efficacy between *B. anthracis* Ames and *B. atrophaeus**

Test Number	Decon Solution (mL)	Equipment	Contact Time (hour)	Temp (°C)	Material	*B.a.* Ames Efficacy		*B.g.* Efficacy		Average Difference in Efficacy
1	PAA (160)	Sani-Tizer	18	20	Rubber Flooring	≥	7.94	≥	7.21	0.12
					Upholstery	≥	8.08	≥	6.86	
					Aluminum	≥	7.88	≥	7.06	
					Mylar	≥	8.06	≥	6.89	
					Fiberglass Siding	≥	7.58	≥	6.47	
					Railcar Carpet		2.37		2.49	
2	8% H_2O_2 (2635)	Sani-Tizer	168	20	Rubber Flooring	≥	7.98	≥	6.81	0.22
					Upholstery	≥	7.99	≥	6.23	
					Aluminum	≥	8.01	≥	7.18	
					Mylar	≥	7.93	≥	6.96	
					Fiberglass Siding		6.77		6.00	
					Railcar Carpet		4.51		5.71	
3	PAA (160)	Sani-Tizer	168† 24hr, 48hr, 120hr, 144hr, 168hr)	20	Railcar Carpet		1.71		2.54	0.56
							2.69		2.68	
							1.84		2.71	
							1.66		2.34	
							2.84		3.27	
4	PAA (78)	Sani-Tizer	18	20	Rubber Flooring	≥	8.07	≥	7.30	-0.45
					New Grease (SOT)		5.93		6.39	
					New HVAC Filter	≥	7.99		6.59	
					Mylar		7.56		6.98	
					Fiberglass Siding		6.03		5.84	
					Railcar Carpet		1.24		0.70	
5	22% H_2O_2 (78)	Sani-Tizer	18	20	Rubber Flooring	≥	7.93		6.12	0.26
					New Grease (SOT)		1.70		3.75	
					New HVAC Filter		5.85	≥	6.38	
					Mylar	≥	7.93	≥	6.99	
					Fiberglass Siding		3.58		4.07	
					Railcar Carpet		0.39		0.41	
6	PAA (78)	Sani-Tizer	8	20	Rubber Flooring		7.33		6.35	-0.03
					Encapsulated New Grease		0.85		1.57	
					Used HVAC Filter	≥	7.89	≥	6.75	
					Mylar	≥	7.83	≥	7.13	
					Fiberglass Siding		4.93		5.61	
					Unpainted Concrete		1.57		1.03	
7	PAA (160)	Sani-Tizer	18	20	Railcar Carpet		2.16		2.23	0.08
					Unpainted Concrete		1.66		0.3	
					Used HVAC Filter	≥	7.80	≥	6.72	
					New Grease (SOT)		5.97		6.38	
					Encapsulated New Grease		1.78		4.83	
					Used Grease (SOT)	≥	7.72		5.94	

* Results shown as average difference in efficacy (log reduction). A positive result indicates that the avirulent microorganism *(B.g.*) was inactivated to a higher degree (less resistant) than *B.a.* Ames

Table B-2. Difference in Efficacy between *B. anthracis* Ames and *B. atrophaeus**

Test Number	Decon Solution (mL)	Equipment	Contact Time (hour)	Temp (°C)	Material	*B.a.* Ames Efficacy		*B.g.* Efficacy		Average Difference in Efficacy
8	PAA (160)	Minncare	18	20	Railcar Carpet		2.44		2.46	0.14
					Unpainted Concrete		1.27		2.07	
					Used HVAC Filter	≥	7.85	≥	7.20	
					New Grease (SOT)		7.00		6.41	
					Encapsulated New Grease		1.00		1.57	
					Used Grease (SOT)		5.76		5.67	
9	PAA (160)	Minncare	18	20	Rubber Flooring	≥	7.84	≥	7.04	-0.73
					Upholstery	≥	7.97	≥	7.56	
					Aluminum	≥	7.95	≥	7.65	
					Mylar	≥	7.90	≥	7.39	
					Fiberglass Siding		5.97		6.66	
					Railcar Carpet		3.85		1.70	
10	PAA (500)	Minncare	18	20	Railcar Carpet		4.90		2.92	-0.75
					Encapsulated New Grease		2.40		2.47	
					Used HVAC Filter	≥	7.92	≥	6.93	
					Fiberglass Siding		6.94		6.71	
					Unpainted Concrete		2.41		2.04	
					Used Grease (SOT)		7.17		5.94	
11	PAA (160)	Sani-Tizer	18	10	Rubber Flooring	≥	7.89	≥	7.28	-0.18
					Upholstery		7.12	≥	7.20	
					Aluminum		7.38	≥	7.35	
					Mylar	≥	7.78	≥	7.19	
					Fiberglass Siding		6.56		5.30	
					Railcar Carpet		0.99		1.48	
12	PAA (78)	Sani-Tizer	18	10	Rubber Flooring		7.11	≥	7.31	0.15
					New Grease (SOT)		3.40		3.58	
					New HVAC Filter		6.47	≥	6.64	
					Mylar		7.62	≥	7.26	
					Fiberglass Siding		3.39		3.21	
					Railcar Carpet		0.54		1.43	
13	PAA (160)	Sani-Tizer	18	10	Railcar Carpet		0.73		0.99	1.02
					Unpainted Concrete		1.24		1.36	
					Used HVAC Filter	≥	7.68	≥	6.96	
					New Grease (SOT)		3.81		4.97	
					Encapsulated New Grease		0.77		2.16	
					Used Grease (SOT)		3.31		5.50	
14	PAA (500)	Minncare	18	10	Railcar Carpet		1.43		0.90	0.50
					Encapsulated New Grease		1.65		2.55	
					Used HVAC Filter		7.37	≥	6.84	
					Fiberglass Siding		5.38	≥	6.63	
					Unpainted Concrete		2.06		1.90	
					Used Grease (SOT)		3.87		5.92	
15	PAA (500)	Sani-Tizer	18	20	Railcar Carpet		6.26		1.36	-1.32
					Encapsulated New Grease		2.77		2.33	
					Used HVAC Filter	≥	7.80	≥	6.91	
					Fiberglass Siding		6.86	≥	6.84	
					Unpainted Concrete		2.70		0.78	
					Used Grease (SOT)		6.22	≥	6.88	

* Results shown as average difference in efficacy (log reduction). A positive result indicates that the avirulent microorganism *(B.g.*) was inactivated to a higher degree (less resistant) than *B.a.* Ames

Table B-3. Difference in Efficacy between *B. anthracis* Ames and *B. atrophaeus**

Test Number	Decon Solution (mL)	Equipment	Contact Time (hour)	Temp (°C)	Material	*B.a.* Ames Efficacy		*B.g.* Efficacy	Average Difference in Efficacy
16	35% H_2O_2 (500)	Minncare	18	20	Railcar Carpet	1.01		1.05	0.37
					Encapsulated New Grease	1.15		1.25	
					Used HVAC Filter	7.08	≥	6.96	
					Fiberglass Siding	5.30		5.63	
					Unpainted Concrete	1.87		1.10	
					Used Grease (SOT)	2.34		4.98	
17	35% H_2O_2 (1000)	Sani-Tizer	18	20	Railcar Carpet	2.29		2.27	-0.06
					Encapsulated New Grease	2.69		2.59	
					Used HVAC Filter	6.74	≥	6.82	
					Fiberglass Siding	6.61		5.26	
					Unpainted Concrete	1.57		1.41	
					Used Grease (SOT)	5.73	≥	6.92	
18	PAA (1000)	Sani-Tizer	18	20	Railcar Carpet	3.31		2.09	-0.79
					Encapsulated New Grease	1.99		2.83	
					New Grease (SOT)	7.61		5.21	
					Fiberglass Siding	5.56		5.24	
					Unpainted Concrete	1.91		2.21	
					Used Grease (SOT)	7.91	≥	5.58	
19	PAA (1000)	Sani-Tizer	18	10	Carpet (Subway)	3.69		1.15	0.27
					Encapsulated New Grease	0.67		2.82	
					Industrial Carpet	4.32		4.81	
					Fiberglass Siding	6.17	≥	6.61	
					Unpainted Concrete	1.5		2.13	
					Used Grease (SOT)	5.29		5.75	
20	35% H_2O_2 (1000)	Sani-Tizer	18	10	Railcar Carpet	3.23		1.62	-1.23
					Encapsulated New Grease	2.64		0.8	
					Used HVAC Filter	7.87	≥	5.78	
					Fiberglass Siding	5.56		4.32	
					Unpainted Concrete	0.7		0.67	
					Used Grease (SOT)	4.44		4.08	
21	PAA (160)	Sani-Tizer	18	10	Railcar Carpet	0.49		-0.04	0.44
					Unpainted Concrete	0.6		0.7	
					Used HVAC Filter	2.1		2.63	
					New Grease (SOT)	0.21		0.92	
					Encapsulated New Grease	0.33		1.36	
					Used Grease (SOT)	0.24		0.91	

* Results shown as average difference in efficacy (log reduction). A positive result indicates that the avirulent microorganism *(B.g.*) was inactivated to a higher degree (less resistant) than *B.a.* Ames

Appendix C
Effects of Materials and Operational Parameters on Decontamination Efficacy

Effects of Temperature on Efficacy

The decontamination efficacy of PAA and H_2O_2 fog against *B.a*. Ames and *B. g.* was evaluated at target temperatures of 10 or 20 °C. These temperatures were tested at uncontrolled RH and volumes of sporicidal liquid ranging from 78 to 500 mL PAA and 500 mL H_2O_2. Results are summarized in Table C-1 and C-2. The comparisons are made for two test conditions that share the same fogging parameters except temperature. A negative result for the average difference in efficacy indicates a higher efficacy at the higher temperature.

Table C-1. Difference in Efficacy Between *B. anthracis* Ames[a] at 10°C and 20°C

Material Type	Test 1 PAA 160 mL; Sani-Tizer; 20 °C; 18 hr	Test 11 PAA 160 mL; Sani-Tizer; 10 °C; 18 hr	Average Difference in Efficacy
Rubber Flooring	≥ 7.94	≥ 7.89	-0.97
Upholstery	≥ 8.08	7.12	
Aluminum	≥ 7.88	7.38	
Mylar	≥ 8.06	≥ 7.83	
Fiberglass Interior Siding	≥ 7.58	6.55	
Railcar Carpet	2.37	0.99	
Material Type	**Test 4** **PAA 78 mL; Sani-Tizer; 20 °C; 18 hr**	**Test 12** **PAA 78 mL; Sani-Tizer; 10 °C; 18 hr**	**Average Difference in Efficacy**
Rubber Flooring	≥ 8.07	7.11	-1.38
New Grease SOT	5.93	3.40	
New Filter	≥ 7.99	6.47	
Mylar	7.56	7.62	
Fiberglass Interior Siding	6.03	3.39	
Railcar Carpet	1.24	0.54	
Material Type	**Test 7** **PAA 160 mL; Sani-Tizer; 20 °C; 18 hr**	**Test 13** **PAA 160 mL; Sani-Tizer; 10 °C; 18 hr**	**Average Difference in Efficacy**
Railcar Carpet	2.16	0.73	-1.89
Unpainted Concrete	1.66	1.24	
Used Filter	≥ 7.80	≥ 7.68	
New Grease SOT	5.97	3.81	
Encapsulated New Grease	1.78	0.77	
Used Grease SOT	7.72	3.31	
Material Type	**Test 10** **PAA 500 mL; Minncare; 20 °C; 18 hr**	**Test 14** **PAA 500 mL; Minncare; 10 °C; 18 hr**	**Average Difference in Efficacy**
Railcar Carpet	4.90	1.43	-1.66
Encapsulated New Grease	2.40	1.65	
Used Filter	≥ 7.92	7.37	
Fiberglass Interior Siding	6.94	5.38	
Unpainted Concrete	2.41	2.06	
Used Grease SOT	7.17	3.87	
Material Type	**Test 17** **35% H_2O_2 500 mL; Sani-Tizer; 20 °C; 18 hr**	**Test 20** **35% H_2O_2 500 mL; Sani-Tizer; 10 °C; 18 hr**	**Average Difference in Efficacy**
Railcar Carpet	2.29	3.23	-0.20
Encapsulated New Grease	2.69	2.64	
Used HVAC Filter	6.74	≥ 7.87	
Fiberglass Interior Siding	6.61	5.56	
Unpainted Concrete	1.57	0.70	
Used Grease SOT	5.73	4.44	

[a] Data are expressed as decontamination efficacy (log reduction).

Table C-2. Difference in Efficacy Between *B. atrophaeous*[a] at 10°C and 20°C

Material Type	Test 1 PAA 160 mL; Sani-Tizer; 20 °C; 18 hr	Test 11 PAA 160 mL; Sani-Tizer; 10 °C; 18 hr	Average Difference in Efficacy
Rubber Flooring	≥ 7.21	≥ 7.25	-1.09
Upholstery	≥ 6.86	≥ 7.20	
Aluminum	≥ 7.06	≥ 7.29	
Mylar	≥ 6.89	≥ 7.20	
Fiberglass Interior Siding	≥ 6.47	5.30	
Railcar Carpet	2.49	1.48	
Material Type	**Test 4** **PAA 78 mL; Sani-Tizer; 20 °C; 18 hr**	**Test 12** **PAA 78 mL; Sani-Tizer; 10 °C; 18 hr**	**Average Difference in Efficacy**
Rubber Flooring	≥ 7.30	≥ 7.31	-1.57
New Grease SOT	6.39	3.58	
New Filter	≥ 6.59	≥ 6.64	
Mylar	≥ 6.98	≥ 7.26	
Fiberglass Interior Siding	5.84	3.21	
Railcar Carpet	0.70	1.43	
Material Type	**Test 7** **PAA 160 mL; Sani-Tizer; 20 °C; 18 hr**	**Test 13** **PAA 160 mL; Sani-Tizer; 10 °C; 18 hr**	**Average Difference in Efficacy**
Railcar Carpet	2.23	0.99	-0.94
Unpainted Concrete	0.30	1.36	
Used Filter	≥ 6.72	≥ 6.96	
New Grease SOT	6.38	4.97	
Encapsulated New Grease	4.83	2.16	
Used Grease SOT	5.94	5.50	
Material Type	**Test 10** **PAA 500 mL; Minncare; 20 °C; 18 hr**	**Test 14** **PAA 500 mL; Minncare; 10 °C; 18 hr**	**Average Difference in Efficacy**
Railcar Carpet	2.92	0.90	-0.44
Encapsulated New Grease	2.47	2.55	
Used Filter	≥ 6.93	≥ 6.84	
Fiberglass Interior Siding	6.71	≥ 6.63	
Unpainted Concrete	2.04	1.90	
Used Grease SOT	5.94	5.92	
Material Type	**Test 17** **35% H_2O_2 500 mL; Sani-Tizer; 20 °C; 18 hr**	**Test 20** **35% H_2O_2 500 mL; Sani-Tizer; 10 °C; 18 hr**	**Average Difference in Efficacy**
Railcar Carpet	2.27	1.62	-1.39
Encapsulated New Grease	2.59	0.80	
Used HVAC Filter	≥ 6.82	≥ 5.78	
Fiberglass Interior Siding	5.26	4.32	
Unpainted Concrete	1.41	0.67	
Used Grease SOT	≥ 6.92	4.08	

[a] Data are expressed as decontamination efficacy (log reduction).

Effects of Fogger Equipment Type on Sporicidal Liquid Efficacy

The decontamination efficacy of PAA and H_2O_2 against *B.a*. Ames and B. g. was evaluated using two types of fogging equipment (Minncare Mini Dry Fogger and Curtis Dynafogger Sani-Tizer). These pieces of equipment were tested at uncontrolled RH and volumes of sporicidal liquid ranging from 78 to 500 mL PAA and 500 mL H_2O_2. Results are summarized in Table C-3 and C-6. The comparisons are made for two test conditions that share the same fumigation parameters except equipment.

Table C-3. Difference in *B. anthracis* Ames[a] Efficacy Between Equipment Type

Material Type[a]	Sani-Tizer (Tests 1 and 2)	Minncare (Tests 8 and 9)	Average Difference in Efficacy
	PAA 160 mL; 20 °C; 18 hr		
Rubber Flooring	≥ 7.94	≥ 7.84	
Upholstery	≥ 8.08	≥ 7.97	
Aluminum	≥ 7.88	≥ 7.95	
Mylar	≥ 8.06	≥ 7.90	
Fiberglass Interior Siding	≥ 7.58	5.97	
Railcar Carpet	2.37	2.44	-0.28
Railcar Carpet (other test)	2.16	3.85	
Unpainted Concrete	1.66	1.27	
Used HVAC Filter	≥ 7.80	≥ 7.85	
New Grease (SOT)	5.97	7.00	
Encapsulated New Grease	1.78	1.00	
Used Grease (SOT)	≥ 7.72	5.76	

[a] Data are expressed as decontamination efficacy (log reduction).

Table C-4. Difference in *B. anthracis* Ames[a] Efficacy Between Equipment Type

Material Type[a]	Sani-Tizer (Test 13)	Minncare (Test 21)	Average Difference in Efficacy
	PAA 160 mL; 10 °C; 18 hr		
Railcar Carpet	0.73	0.49	
Unpainted Concrete	1.24	0.6	
Used HVAC Filter	≥ 7.68	2.1	-2.26
New Grease (SOT)	3.81	0.21	
Encapsulated New Grease	0.77	0.33	
Used Grease (SOT)	3.31	0.24	

[a] Data are expressed as decontamination efficacy (log reduction).

Table C-5. Difference in *B. atrophaeus*[a] Efficacy Between Equipment Type

Material Type[a]	Sani-Tizer (Tests 1 and 7)	Minncare (Tests 8 and 9)	Average Difference in Efficacy
	PAA 160 mL; 20 °C; 18 hr		
Rubber Flooring	≥ 7.21	≥ 7.04	-0.30
Upholstery	≥ 6.86	≥ 7.56	
Aluminum	≥ 7.06	≥ 7.65	
Mylar	≥ 6.89	≥ 7.39	
Fiberglass Interior Siding	≥ 6.47	6.66	
Railcar Carpet	2.49	1.7	
Carpet	2.23	2.46	
Unpainted Concrete	0.3	2.07	
Used HVAC Filter	≥ 6.72	≥ 7.2	
New Grease (SOT)	6.38	6.41	
Encapsulated New Grease	4.83	1.57	
Used Grease (SOT)	5.94	5.67	

[a] Data are expressed as decontamination efficacy (log reduction).

Table C-6. Difference in *B. atrophaeus*[a] Efficacy Between Equipment Type

Material Type[a]	Sani-Tizer	Minncare	Average Difference in Efficacy
	PAA 160 mL; 10 °C; 18 hr		
Railcar Carpet	0.99	-0.04	-2.58
Unpainted Concrete	1.36	0.7	
Used HVAC Filter	≥ 6.96	2.63	
New Grease (SOT)	4.97	0.92	
Encapsulated New Grease	2.16	1.36	
Used Grease (SOT)	5.5	0.91	

[a] Data are expressed as decontamination efficacy (log reduction).

Effects of Sporicidal Liquid on *B.a.* Ames Efficacy

The decontamination efficacy of PAA and H_2O_2 against *B.a*. Ames and *B. g*. was evaluated using two types of sporicidal chemicals. These sporicidal liquids were tested at 10°C and 20°C, uncontrolled RH, and volumes of sporicidal liquid ranging from 78 to 100 mL. Results are summarized in Table C-7 and C-10. The comparisons are made for two test conditions that share the same fogging operational parameters except sporicidal liquid type.

Table C-7. Difference in *B. anthracis* Ames[a] Efficacy Between Liquid Type (Tests 4/5)

Material Type[a]	PAA 78 mL; Sani-Tizer; 20 °C; 18 hr	22%H_2O_2 78 mL; Sani-Tizer; 20 °C; 18 hr	Average Difference in Efficacy
Rubber Flooring	≥ 8.07	≥ 7.93	-1.86
New Grease (SOT)	5.93	1.70	
New HVAC Filter	≥ 7.99	5.85	
Mylar	7.56	≥ 7.93	
Fiberglass Siding	6.03	3.58	
Railcar Carpet	1.24	0.39	

[a] Data are expressed as decontamination efficacy (log reduction).

Table C-8. Difference in *B. anthracis* Ames[a] Efficacy Between Liquid Type (Tests 15/16)

Material Type[a]	PAA 500 mL; Sani-Tizer; 20 °C; 18 hr	35%H_2O_2 500 mL; Sani-Tizer; 20 °C; 18 hr	Average Difference in Efficacy
Railcar Carpet	6.26	1.01	-2.31
Encapsulated New Grease	2.77	1.15	
Used HVAC Filter	≥ 7.80	7.08	
Fiberglass Siding	6.86	5.30	
Unpainted Concrete	2.70	1.87	
Used Grease (SOT)	6.22	2.34	

[a] Data are expressed as decontamination efficacy (log reduction).

Table C-9. Difference in *B. anthracis* Ames[a] Efficacy Between Liquid Type (Tests 17/18)

Material Type[a]	PAA 1000 mL; Sani-Tizer; 20 °C; 18 hr	35%H_2O_2 1000 mL; Sani-Tizer; 20 °C; 18 hr	Average Difference in Efficacy
Railcar Carpet	3.31	2.29	-0.44
Encapsulated New Grease	1.99	2.69	
Used HVAC Filter	7.61	6.74	
Fiberglass Siding	5.56	6.61	
Unpainted Concrete	1.91	1.57	
Used Grease (SOT)	≥ 7.91	5.73	

[a] Data are expressed as decontamination efficacy (log reduction).

Table C-10. Difference in *B. anthracis* Ames[a] Efficacy Between Liquid Type (Tests 19/20)

Material Type[a]	PAA 1000 mL; Sani-Tizer; 10 °C; 18 hr		35% H_2O_2 1000 mL; Sani-Tizer; 10 °C; 18 hr	Average Difference in Efficacy
Carpet (Subway)	3.69		3.43	0.50
Encapsulated New Grease	0.67		2.64	
Industrial Carpet (New)	4.32	≥	7.87	
Fiberglass Siding	6.17		5.56	
Unpainted Concrete	1.5		0.7	
Used Grease (SOT)	5.29		4.44	

[a] Data are expressed as decontamination efficacy (log reduction).

Appendix D
Detailed Statistical Analysis

Introduction

This report contains the statistical analysis of *B. anthracis* (*B.a.*) and *B. atrophaeus* (*B.g.*) decontamination data for different decontamination methods on a variety of materials and location of the materials in the decontamination chamber.

Results

Positive controls. Table D1 contains the mean percent recoveries for the positive controls for each spore species and material with 95 percent confidence intervals on the means; percent recoveries for each positive control coupon are plotted in Figure D-1. The Kruskal-Wallis tests to compare materials by agent were statistically significant for both *B.a.* ($p < 0.001$) and *B.g.* ($p < 0.001$) (Table D-2). The p-values for each Kruskal-Wallis test to compare *B.a.* vs *B.g.* for each material are presented in Table D-3. The percent recovery for *B.a.* is statistically significantly different from the percent recovery for *B.g.* for all materials.

Comparing decontamination efficacy for *Ba* and *Bg*. Estimates with exact 95 percent confidence intervals for the proportion of successes (complete inactivation or ≥ 6 LR) are presented in D-4. Estimates for *B.a.* and *B.g.* are presented side-by-side for comparison. The chi-squared test of statistical dependence between agent and success failed to reject the null hypothesis ($p = 0.1119$); thus, we conclude that *B.a.* and *B.g.* are not statistically significantly different with respect to the proportion of successes across all test conditions.

Assessing the effect of parameters on efficacy. The main effects logistic regression model could not be fitted to the complete data set as planned due to quasi-complete separation of the data. Three materials that had successes for all tests or failures for all tests were removed from the data set to allow the model to be fitted: clean carpet (no successes), Mylar (all successes), and unpainted concrete (no successes). In addition, a more balanced data set was constructed by removing the following records:

- Materials: new HVAC filter, aluminum, and upholstery (in addition to clean carpet, Mylar, and unpainted concrete already removed)
- Decontaminant liquids: 22% H_2O_2 and 8% H_2O_2
- Decontamination Volume: 2635 mL
- Time: 8, 168, and 1-5 days

The main effects logistic regression model was fitted to the full dataset with the three materials removed and the more balanced subset of the data. Conclusions from the two models were equivalent, and time was not found to be statistically significant in the full data model. Therefore, two-factor interactions were considered for the model of the more balanced subset of data. Two of the two-factor interactions were found to be statistically significant and were added

to the model: Temperature x $\log_{10}$ Decontamination Volume and Equipment x Temperature. Parameter estimates for the final logistic regression model are presented in Table D-5.

Odds ratios for all pairwise material comparisons, comparisons of all locations with location 3 (center of room), and decontamination sporicidal liquid comparison are presented in Tables D-6, D-7, and D-8.

Based on the parameters of the logistic model, materials can be grouped by decontamination effectiveness. The following groups are suggested:

- Rubber Flooring, Used HVAC Filter – Highly effective decontamination
- Clean Grease SOT, Fiberglass Interior Siding, Used Grease SOT – Moderately effective decontamination
- Used Carpet, Encapsulated Clean Grease – Ineffective decontamination

Though not included in the model due to quasi-complete separation, decontamination is highly effective for Mylar (100% success) and highly ineffective for Clean Carpet and Unpainted Concrete (0% complete kills). New HVAC filter, aluminum, and upholstery were also not included in the logistic model and were not perfectly separated. However, all but one test was successful for aluminum and upholstery and all but three were successful for new HVAC filter. These limited number of results suggest that aluminum and upholstery group in the highly effective decontamination category, and new HVAC filter group in the moderately or highly effective decontamination category.

The logistic model indicates that the probability of a complete kill is different for each location compared to location 3, with all locations less likely to result in a complete kill compared to location 3.

The two decontamination sporicidal liquids are shown to be statistically significantly different, with PAA more likely to result in a complete kill.

Temperatures are also statistically significant, with higher temperature having a greater probability of a complete kill.

Finally, the probability of a complete kill increases as a function of the $\log_{10}$ of the volume of the decontaminant liquid.

Conclusions

Analysis of the percent recovery showed statistically significant differences in percent recovery for different materials for each agent and for different agents for each material. We conclude that *B.a.* and *B.g.* are not statistically significantly different with respect to the proportion of successes (complete kills) across all test conditions. For *B.a.*, materials can be grouped with respect to effectiveness as follows:

- Rubber Flooring, Used HVAC Filter, Mylar – Highly effective decontamination
- Clean Grease SOT, Fiberglass Interior Siding, Used Grease SOT – Moderately effective decontamination

- Used Carpet, Encapsulated Clean Grease, Clean Carpet, Unpainted Concrete – Ineffective decontamination

Decontamination time is not statistically significant, nor is equipment. Location 3 has the highest probability of observing a complete kill. Higher temperature and greater volume of decontamination SL both increase the probability of a complete kill. Use of PAA increases the probability of complete kill compared to 35 % H_2O_2.

Table D-1. Mean Percent Recovery for Control Coupons for Each Agent and Material with 95 Percent Confidence Intervals

Agent	Material	N	Mean Percent Recovery (95% Confidence Interval)
B. anthracis	New HVAC Filter	15	82.30 (70.94,93.67)
B. anthracis	Aluminum	20	82.93 (73.42,92.45)
B. anthracis	Clean Carpet	5	97.82 (89.17,100.0)*
B. anthracis	Clean Grease SOT	40	86.52 (78.30,94.75)
B. anthracis	Encapsulated Clean Grease	65	41.50 (11.35,71.65)
B. anthracis	Fiberglass Interior Siding	80	37.55 (33.63,41.47)
B. anthracis	Mylar	40	72.88 (67.11,78.66)
B. anthracis	Rubber Flooring	40	73.58 (70.28,76.88)
B. anthracis	Unpainted Concrete	65	14.30 (4.23,24.36)
B. anthracis	Upholstery	20	92.77 (85.39,100.0)*
B. anthracis	Used Carpet	100	40.47 (35.15,45.79)
B. anthracis	Used Grease SOT	60	83.89 (77.17,90.61)
B. anthracis	Used HVAC Filter	55	70.73 (67.14,74.32)
B. atrophaeus	New HVAC Filter	15	3.14 (2.40, 3.88)
B. atrophaeus	Aluminum	20	24.45 (17.95,30.94)
B. atrophaeus	Clean Carpet	5	39.14 (24.15,54.14)
B. atrophaeus	Clean Grease SOT	40	6.07 (4.26, 7.88)
B. atrophaeus	Encapsulated Clean Grease	65	1.18 (0.69, 1.67)
B. atrophaeus	Fiberglass Interior Siding	80	4.71 (3.91, 5.51)
B. atrophaeus	Mylar	40	16.54 (9.90,23.18)
B. atrophaeus	Rubber Flooring	40	17.84 (13.32,22.36)
B. atrophaeus	Unpainted Concrete	65	0.53 (0.41, 0.65)
B. atrophaeus	Upholstery	20	19.53 (7.34,31.71)
B. atrophaeus	Used Carpet	100	17.47 (13.54,21.40)
B. atrophaeus	Used Grease SOT	60	8.81 (7.04,10.59)
B. atrophaeus	Used HVAC Filter	55	7.46 (6.03, 8.89)

* Confidence limits less than 0 or greater than 100 truncated to 0 or 100 to reflect valid range of percent recovery values.

Table D-2. Kruskal-Wallis Tests of Differences among Materials for Each Agent

Agent	DF	p value
B. anthracis	12	< 0.001
B. atrophaeus	12	< 0.001

Table D-3. Kruskal-Wallis Tests of *B.a.* vs *B.g.* for Each Material

Agent	DF	p value
New HVAC Filter	1	< 0.001
Aluminum	1	< 0.001
Clean Carpet	1	0.0088
Clean Grease SOT	1	< 0.001
Encapsulated Clean Grease	1	< 0.001
Fiberglass Interior Siding	1	< 0.001
Mylar	1	< 0.001
Rubber Flooring	1	< 0.001
Unpainted Concrete	1	< 0.001
Upholstery	1	< 0.001
Used Carpet	1	< 0.001
Used Grease SOT	1	< 0.001
Used HVAC Filter	1	< 0.001

Table D-4. Proportion Success (≥ 6 LR or Total Kill) for *B.a.* and *B.g.* with Exact 95 Percent Confidence Intervals

Material	Equipment	Decon liquid	Temp °C	Decon Volume (mL)	Time (Hours)	*B.a.*		*B.g.*	
						Number Success/N	Proportion Success (Exact 95% Confidence Interval)	Number Success/ N	Proportion Success (Exact 95% Confidence Interval)
New HAVC Filter	Sani-Tizer	22% H_2O_2	20	78	18	3/5	0.60 (0.15, 0.95)	5/5	1.00 (0.48, 1.00)
New HVAC Filter	Sani-Tizer	PAA	10	78	18	4/5	0.80 (0.28, 0.99)	5/5	1.00 (0.48, 1.00)
New HVAC Filter	Sani-Tizer	PAA	20	78	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Aluminum	MinnCare	PAA	20	160	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Aluminum	Sani-Tizer	8% H_2O_2	20	2635	168	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Aluminum	Sani-Tizer	PAA	10	160	18	4/5	0.80 (0.28, 0.99)	5/5	1.00 (0.48, 1.00)
Aluminum	Sani-Tizer	PAA	20	160	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Clean Carpet	Sani-Tizer	PAA	10	1000	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Clean Grease SOT	MinnCare	PAA	10	160	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Clean Grease SOT	MinnCare	PAA	20	160	18	4/5	0.80 (0.28, 0.99)	5/5	1.00 (0.48, 1.00)
Clean Grease SOT	Sani-Tizer	22% H_2O_2	20	78	18	0/5	0.00 (0.00, 0.52)	1/5	0.20 (0.01, 0.72)
Clean Grease SOT	Sani-Tizer	PAA	10	78	18	1/5	0.20 (0.01, 0.72)	0/5	0.00 (0.00, 0.52)
Clean Grease SOT	Sani-Tizer	PAA	10	160	18	1/5	0.20 (0.01, 0.72)	2/5	0.40 (0.05, 0.85)
Clean Grease SOT	Sani-Tizer	PAA	20	78	18	3/5	0.60 (0.15, 0.95)	4/5	0.80 (0.28, 0.99)
Clean Grease SOT	Sani-Tizer	PAA	20	160	18	3/5	0.60 (0.15, 0.95)	4/5	0.80 (0.28, 0.99)
Clean Grease SOT	Sani-Tizer	PAA	20	1000	18	5/5	1.00 (0.48, 1.00)	3/5	0.60 (0.15, 0.95)
Encapsulated Clean Grease	MinnCare	35% H_2O_2	20	500	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Encapsulated Clean Grease	MinnCare	PAA	10	160	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Encapsulated Clean Grease	MinnCare	PAA	10	500	18	0/5	0.00 (0.00, 0.52)	1/5	0.20 (0.01, 0.72)
Encapsulated Clean Grease	MinnCare	PAA	20	160	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Encapsulated Clean Grease	MinnCare	PAA	20	500	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Encapsulated Clean Grease	Sani-Tizer	35% H_2O_2	10	1000	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Encapsulated Clean Grease	Sani-Tizer	35% H_2O_2	20	1000	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Encapsulated Clean Grease	Sani-Tizer	PAA	10	160	18	0/5	0.00 (0.00, 0.52)	1/5	0.20 (0.01, 0.72)
Encapsulated Clean Grease	Sani-Tizer	PAA	10	1000	18	0/5	0.00 (0.00, 0.52)	1/5	0.20 (0.01, 0.72)
Encapsulated Clean Grease	Sani-Tizer	PAA	20	78	8	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)

Material	Equipment	Decon liquid	Temp °C	Decon Volume (mL)	Time (Hours)	*B.a.*		*B.g.*	
						Number Success/N	Proportion Success (Exact 95% Confidence Interval)	Number Success/ N	Proportion Success (Exact 95% Confidence Interval)
Encapsulated Clean Grease	Sani-Tizer	PAA	20	160	18	0/5	0.00 (0.00, 0.52)	3/5	0.60 (0.15, 0.95)
Encapsulated Clean Grease	Sani-Tizer	PAA	20	500	18	1/5	0.20 (0.01, 0.72)	1/5	0.20 (0.01, 0.72)
Encapsulated Clean Grease	Sani-Tizer	PAA	20	1000	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Fiberglass Interior Siding	MinnCare	35% H_2O_2	20	500	18	1/5	0.20 (0.01, 0.72)	4/5	0.80 (0.28, 0.99)
Fiberglass Interior Siding	MinnCare	PAA	10	500	18	2/5	0.40 (0.05, 0.85)	5/5	1.00 (0.48, 1.00)
Fiberglass Interior Siding	MinnCare	PAA	20	160	18	2/5	0.40 (0.05, 0.85)	5/5	1.00 (0.48, 1.00)
Fiberglass Interior Siding	MinnCare	PAA	20	500	18	4/5	0.80 (0.28, 0.99)	4/5	0.80 (0.28, 0.99)
Fiberglass Interior Siding	Sani-Tizer	22% H_2O_2	20	78	18	0/5	0.00 (0.00, 0.52)	1/5	0.20 (0.01, 0.72)
Fiberglass Interior Siding	Sani-Tizer	35% H_2O_2	10	1000	18	2/5	0.40 (0.05, 0.85)	1/5	0.20 (0.01, 0.72)
Fiberglass Interior Siding	Sani-Tizer	35% H_2O_2	20	1000	18	3/5	0.60 (0.15, 0.95)	3/5	0.60 (0.15, 0.95)
Fiberglass Interior Siding	Sani-Tizer	8% H_2O_2	20	2635	168	4/5	0.80 (0.28, 0.99)	4/5	0.80 (0.28, 0.99)
Fiberglass Interior Siding	Sani-Tizer	PAA	10	78	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Fiberglass Interior Siding	Sani-Tizer	PAA	10	160	18	3/5	0.60 (0.15, 0.95)	2/5	0.40 (0.05, 0.85)
Fiberglass Interior Siding	Sani-Tizer	PAA	10	1000	18	3/5	0.60 (0.15, 0.95)	5/5	1.00 (0.48, 1.00)
Fiberglass Interior Siding	Sani-Tizer	PAA	20	78	8	2/5	0.40 (0.05, 0.85)	3/5	0.60 (0.15, 0.95)
Fiberglass Interior Siding	Sani-Tizer	PAA	20	78	18	2/5	0.40 (0.05, 0.85)	3/5	0.60 (0.15, 0.95)
Fiberglass Interior Siding	Sani-Tizer	PAA	20	160	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Fiberglass Interior Siding	Sani-Tizer	PAA	20	500	18	4/5	0.80 (0.28, 0.99)	5/5	1.00 (0.48, 1.00)
Fiberglass Interior Siding	Sani-Tizer	PAA	20	1000	18	3/5	0.60 (0.15, 0.95)	2/5	0.40 (0.05, 0.85)
Mylar	MinnCare	PAA	20	160	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Mylar	Sani-Tizer	22% H_2O_2	20	78	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Mylar	Sani-Tizer	8% H_2O_2	20	2635	168	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Mylar	Sani-Tizer	PAA	10	78	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Mylar	Sani-Tizer	PAA	10	160	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Mylar	Sani-Tizer	PAA	20	78	8	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Mylar	Sani-Tizer	PAA	20	78	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Mylar	Sani-Tizer	PAA	20	160	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Rubber Flooring	MinnCare	PAA	20	160	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)

Material	Equipment	Decon liquid	Temp °C	Decon Volume (mL)	Time (Hours)	*B.a.*		*B.g.*	
						Number Success/N	Proportion Success (Exact 95% Confidence Interval)	Number Success/ N	Proportion Success (Exact 95% Confidence Interval)
Rubber Flooring	Sani-Tizer	22% H_2O_2	20	78	18	5/5	1.00 (0.48, 1.00)	4/5	0.80 (0.28, 0.99)
Rubber Flooring	Sani-Tizer	8% H_2O_2	20	2635	168	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Rubber Flooring	Sani-Tizer	PAA	10	78	18	4/5	0.80 (0.28, 0.99)	5/5	1.00 (0.48, 1.00)
Rubber Flooring	Sani-Tizer	PAA	10	160	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Rubber Flooring	Sani-Tizer	PAA	20	78	8	4/5	0.80 (0.28, 0.99)	2/5	0.40 (0.05, 0.85)
Rubber Flooring	Sani-Tizer	PAA	20	78	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Rubber Flooring	Sani-Tizer	PAA	20	160	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Unpainted Concrete	MinnCare	35% H_2O_2	20	500	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Unpainted Concrete	MinnCare	PAA	10	160	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Unpainted Concrete	MinnCare	PAA	10	500	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Unpainted Concrete	MinnCare	PAA	20	160	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Unpainted Concrete	MinnCare	PAA	20	500	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Unpainted Concrete	Sani-Tizer	35% H_2O_2	10	1000	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Unpainted Concrete	Sani-Tizer	35% H_2O_2	20	1000	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Unpainted Concrete	Sani-Tizer	PAA	10	160	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Unpainted Concrete	Sani-Tizer	PAA	10	1000	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Unpainted Concrete	Sani-Tizer	PAA	20	78	8	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Unpainted Concrete	Sani-Tizer	PAA	20	160	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Unpainted Concrete	Sani-Tizer	PAA	20	500	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Unpainted Concrete	Sani-Tizer	PAA	20	1000	18	0/5	0.00 (0.00, 0.52)	1/5	0.20 (0.01, 0.72)
Upholstery	MinnCare	PAA	20	160	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Upholstery	Sani-Tizer	8% H_2O_2	20	2635	168	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Upholstery	Sani-Tizer	PAA	10	160	18	4/5	0.80 (0.28, 0.99)	5/5	1.00 (0.48, 1.00)
Upholstery	Sani-Tizer	PAA	20	160	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Used Carpet	MinnCare	35% H_2O_2	20	500	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Used Carpet	MinnCare	PAA	10	160	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Used Carpet	MinnCare	PAA	10	500	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Used Carpet	MinnCare	PAA	20	160	18	1/10	0.10 (0.00, 0.45)	0/10	0.00 (0.00, 0.31)

Material	Equipment	Decon liquid	Temp °C	Decon Volume (mL)	Time (Hours)	*B.a.*		*B.g.*	
						Number Success/N	Proportion Success (Exact 95% Confidence Interval)	Number Success/ N	Proportion Success (Exact 95% Confidence Interval)
Used Carpet	MinnCare	PAA	20	500	18	1/5	0.20 (0.01, 0.72)	0/5	0.00 (0.00, 0.52)
Used Carpet	Sani-Tizer	22% H_2O_2	20	78	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Used Carpet	Sani-Tizer	35% H_2O_2	10	1000	18	1/5	0.20 (0.01, 0.72)	0/5	0.00 (0.00, 0.52)
Used Carpet	Sani-Tizer	35% H_2O_2	20	1000	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Used Carpet	Sani-Tizer	8% H_2O_2	20	2635	168	2/5	0.40 (0.05, 0.85)	4/5	0.80 (0.28, 0.99)
Used Carpet	Sani-Tizer	PAA	10	78	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Used Carpet	Sani-Tizer	PAA	10	160	18	0/10	0.00 (0.00, 0.31)	0/10	0.00 (0.00, 0.31)
Used Carpet	Sani-Tizer	PAA	10	1000	18	2/5	0.40 (0.05, 0.85)	0/5	0.00 (0.00, 0.52)
Used Carpet	Sani-Tizer	PAA	20	78	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Used Carpet	Sani-Tizer	PAA	20	160	18	0/10	0.00 (0.00, 0.31)	1/10	0.10 (0.00, 0.45)
Used Carpet	Sani-Tizer	PAA	20	160	24	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Used Carpet	Sani-Tizer	PAA	20	160	48	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Used Carpet	Sani-Tizer	PAA	20	160	120	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Used Carpet	Sani-Tizer	PAA	20	160	144	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Used Carpet	Sani-Tizer	PAA	20	160	168	0/5	0.00 (0.00, 0.52)	1/5	0.20 (0.01, 0.72)
Used Carpet	Sani-Tizer	PAA	20	500	18	4/5	0.80 (0.28, 0.99)	0/5	0.00 (0.00, 0.52)
Used Carpet	Sani-Tizer	PAA	20	1000	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Used Grease SOT	MinnCare	35% H_2O_2	20	500	18	0/5	0.00 (0.00, 0.52)	2/5	0.40 (0.05, 0.85)
Used Grease SOT	MinnCare	PAA	10	160	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Used Grease SOT	MinnCare	PAA	10	500	18	1/5	0.20 (0.01, 0.72)	3/5	0.60 (0.15, 0.95)
Used Grease SOT	MinnCare	PAA	20	160	18	3/5	0.60 (0.15, 0.95)	3/5	0.60 (0.15, 0.95)
Used Grease SOT	MinnCare	PAA	20	500	18	4/5	0.80 (0.28, 0.99)	2/5	0.40 (0.05, 0.85)
Used Grease SOT	Sani-Tizer	35% H_2O_2	10	1000	18	1/5	0.20 (0.01, 0.72)	2/5	0.40 (0.05, 0.85)
Used Grease SOT	Sani-Tizer	35% H_2O_2	20	1000	18	2/5	0.40 (0.05, 0.85)	5/5	1.00 (0.48, 1.00)
Used Grease SOT	Sani-Tizer	PAA	10	160	18	1/5	0.20 (0.01, 0.72)	3/5	0.60 (0.15, 0.95)
Used Grease SOT	Sani-Tizer	PAA	10	1000	18	3/5	0.60 (0.15, 0.95)	4/5	0.80 (0.28, 0.99)
Used Grease SOT	Sani-Tizer	PAA	20	160	18	5/5	1.00 (0.48, 1.00)	4/5	0.80 (0.28, 0.99)
Used Grease SOT	Sani-Tizer	PAA	20	500	18	3/5	0.60 (0.15, 0.95)	5/5	1.00 (0.48, 1.00)

Material	Equipment	Decon liquid	Temp °C	Decon Volume (mL)	Time (Hours)	*B.a.*		*B.g.*	
						Number Success/N	Proportion Success (Exact 95% Confidence Interval)	Number Success/ N	Proportion Success (Exact 95% Confidence Interval)
Used Grease Sot	Sani-Tizer	PAA	20	1000	18	5/5	1.00 (0.48, 1.00)	3/5	0.60 (0.15, 0.95)
Used HVAC Filter	MinnCare	35% H_2O_2	20	500	18	4/5	0.80 (0.28, 0.99)	5/5	1.00 (0.48, 1.00)
Used HVAC Filter	MinnCare	PAA	10	160	18	0/5	0.00 (0.00, 0.52)	0/5	0.00 (0.00, 0.52)
Used HVAC Filter	MinnCare	PAA	10	500	18	4/5	0.80 (0.28, 0.99)	5/5	1.00 (0.48, 1.00)
Used HVAC Filter	MinnCare	PAA	20	160	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Used HVAC Filter	MinnCare	PAA	20	500	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Used HVAC Filter	Sani-Tizer	35% H_2O_2	10	1000	18	5/5	1.00 (0.48, 1.00)	3/5	0.60 (0.15, 0.95)
Used HVAC Filter	Sani-Tizer	35% H_2O_2	20	1000	18	4/5	0.80 (0.28, 0.99)	5/5	1.00 (0.48, 1.00)
Used HVAC Filter	Sani-Tizer	PAA	10	160	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Used HVAC Filter	Sani-Tizer	PAA	20	78	8	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Used HVAC Filter	Sani-Tizer	PAA	20	160	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)
Used HVAC Filter	Sani-Tizer	PAA	20	500	18	5/5	1.00 (0.48, 1.00)	5/5	1.00 (0.48, 1.00)

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Table D-5. Parameter Estimates for Final Selected Model Fit to More Balanced Data Subset.

Variable	Variable Level	DF	Estimate	Standard Error	Wald Statistic	p value
Intercept	--	1	3.0269	1.9476	2.4156	0.1201
MATERIAL	Clean Grease SOT	1	-4.1773	1.2164	11.7930	0.0006*
MATERIAL	Encapsulated Clean Grease	1	-9.6562	1.6205	35.5091	0.0000*
MATERIAL	Fiberglass Interior Siding	1	-4.3792	1.2067	13.1705	0.0003*
MATERIAL	Used Carpet	1	-7.3149	1.2727	33.0323	0.0000*
MATERIAL	Used Grease SOT	1	-4.6608	1.2267	14.4358	0.0001*
MATERIAL	Used HVAC Filter	1	-1.4842	1.2654	1.3756	0.2409
EQUIPMENT	MinnCare	1	-0.5114	0.4247	1.4501	0.2285
DECON	35% H_2O_2	1	-2.5871	0.5243	24.3506	0.0000*
TEMP	10	1	-7.2662	2.3292	9.7323	0.0018*
Log DeconVol	--	1	1.5852	0.6384	6.1657	0.0130*
LOCATION	1	1	-1.0337	0.5177	3.9871	0.0458*
LOCATION	2	1	-1.1647	0.5205	5.0075	0.0252*
LOCATION	4	1	-1.6939	0.5342	10.0558	0.0015*
LOCATION	5	1	-1.9625	0.5425	13.0848	0.0003*
logDeconVol*TEMP	10	1	2.3514	0.8896	6.9859	0.0082*
EQUIPMENT*TEMP	MinnCare / 10	1	-2.1316	0.8192	6.7701	0.0093*

-- There is no variable level for intercept of continuous variables.
* Statistically significant at α = 0.05 level.

Table D-6. Odds Ratio Estimates for Pairwise Material Comparisons.

Material	Rubber Flooring	Clean Grease SOT	Encapsulated Clean Grease	Fiberglass Interior Siding	Used Carpet	Used Grease SOT
	Odds Ratio Estimate (p-value)#					
Clean Grease SOT	0.02 (0.0006*)					
Encapsulated Clean Grease	0.00 (<0.0001*)	239.59 (<0.0001*)				
Fiberglass Interior Siding	0.01 (0.0003*)	1.22 (0.7235)	0.01 (<0.0001*)			
Used Carpet	0.00 (<0.0001*)	23.05 (<0.0001*)	0.10 (0.0347*)	18.84 (<0.0001*)		
Used Grease SOT	0.01 (0.0001*)	1.62 (0.4203)	0.01 (<0.0001*)	1.33 (0.5345)	0.07 (<0.0001*)	
Used HVAC Filter	0.23 (0.2409)	0.07 (0.0003*)	0.00 (<0.0001*)	0.06 (<0.0001*)	0.00 (<0.0001*)	0.04 (<0.0001*)

Odds ratios greater (less) than one indicate that the odds of a success for row label material are greater (less) than for the column label material.
* Statistically significant at α = 0.05 level.

Table D-7. Odds Ratio Estimate for Comparisons of Locations within Chamber.

Contrast	Estimate# (p-value)
Location 1 vs. Location 3	0.36 (0.0458*)
Location 2 vs. Location 3	0.31 (0.0252*)
Location 4 vs. Location 3	0.18 (0.0015*)
Location 5 vs. Location 3	0.14 (0.0003*)

Odds ratios greater (less) than one indicate that the odds of a success for first location are greater (less) than for Location 3.
* Statistically significant at α = 0.05 level.

Table D-8. Odds Ratio Estimates for Decontamination SL Comparisons.

Contrast	Estimate (p-value)
35 % H_2O_2 vs. PAA	0.08 (0.0000*)

Odds ratios less than one indicate that the odds of a success for first sporicidal liquid are greater (less) than for second SL.
* Statistically significant at α = 0.05 level.

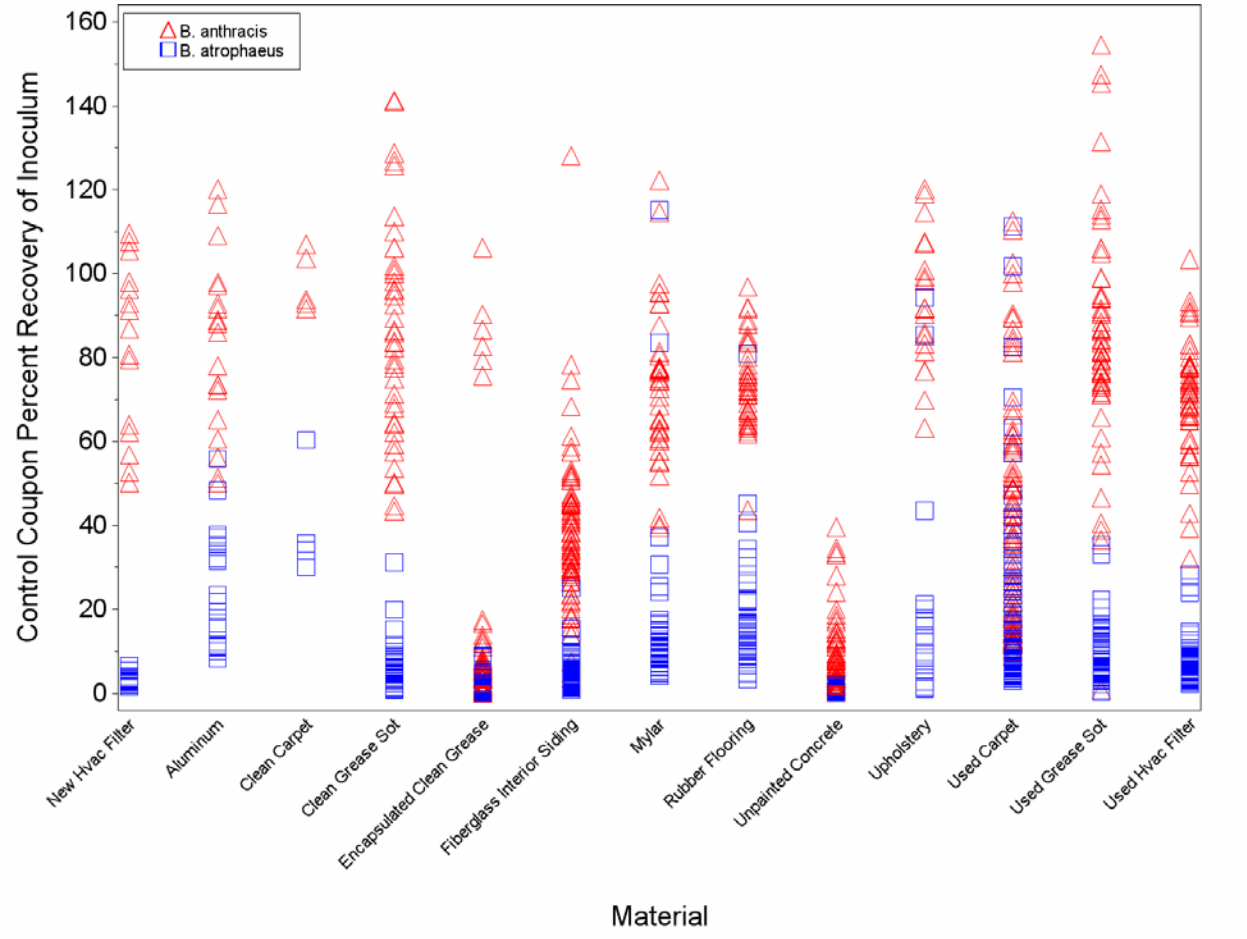

Figure D-1. Plot of Control Coupon Percent Recovery of Inoculum by Material and Agent. Note That Percent Recovery Values Greater than 200 % Are Not Included in the Plot.

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