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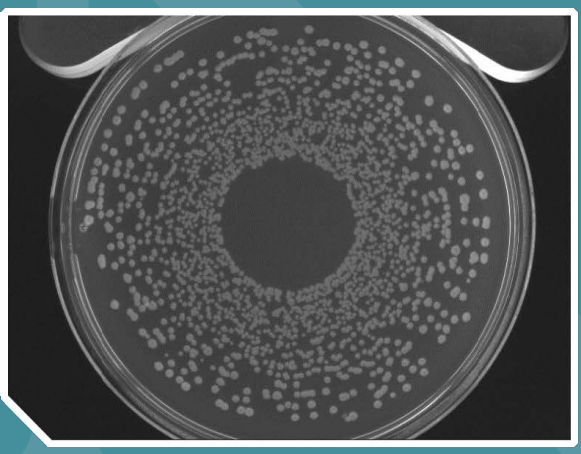
Operational Testing of Floor Cleaning Cloths for Household Remediation Following a Large-Scale Biological Contamination Incident



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Operational Testing of Floor Cleaning Cloths for Household Remediation Following a Large-Scale Biological Contamination Incident

Assessment and Evaluation Report

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Disclaimer

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This research effort is part of the U.S. Environmental Protection Agency's (EPA's) Homeland Security Research Plan (HSRP) to evaluate low tech/self-help techniques for *Bacillus (B.) anthracis* remediation operations. Self-help actions may be undertaken at the edge of a contamination zone of a wide-area *B. anthracis* spore release to reduce indoor exposure risk outside the contamination zone. Here, a floor cleaning/dust removal system using disposable dust cloths was evaluated to determine their propensity to remove spores from indoor flooring materials. The results of this work would inform responders, governments, and health departments in their guidance development for self-self-help recommendations to the general public.

This effort was directed by the principal investigator from the Office of Research and Development's (ORD's) NHSRC, with support of a project team consisting of staff from across EPA. The contributions of the following individuals have been a valued asset throughout this effort:

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Acronyms and Abbreviations

ADA	aerosol deposition apparatus
ATCC	American Type Culture Collection
B.	*Bacillus*
Biolab	NHSRC Biocontaminant Laboratory
°C	Degree(s) Celsius
CFU	colony-forming unit(s)
cm	centimeter(s)
DI	deionized
DQI	data quality indicator
EPA	U.S. Environmental Protection Agency
ft	foot/feet
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
H_2O_2	hydrogen peroxide
HSRP	Homeland Security Research Program
in.	inch(es)
Lpm	liter(s) per minute
LR	log reduction
µm	micrometer(s)
MDI	metered-dose inhaler
min	minute(s)
mL	milliliter(s)
mm	millimeter(s)
NHSRC	National Homeland Security Research Center
NIST	National Institute of Standards and Technology
ORD	Office of Research and Development
PBS	phosphate-buffered saline
PBST	phosphate-buffered saline with 0.05% Tween® 20
PRB	polyester-rayon blend
PVC	polyvinyl chloride
QA	quality assurance
QAPP	quality assurance project plan
QC	quality control
RH	relative humidity
rpm	revolution(s) per minute
RSD	relative standard deviation
SSFMS	Swiffer® Sweeper® floor mop system
SD	standard deviation
STS	sodium thiosulfate
TSA	tryptic soy agar
VHP	vaporous hydrogen peroxide
WA	work assignment

Executive Summary

The U.S. Environmental Protection Agency (EPA), Office of Research and Development (ORD), Homeland Security Research Program (HSRP) continues to strive to protect human health and the environment from the adverse impacts resulting from acts of terror by investigating the effectiveness and applicability of technologies for homeland security-related remediation applications.

EPA's HSRP evaluates remediation operations for *Bacillus (B.) anthracis* contamination, including low tech/self-help actions that may be undertaken at the edge of a contamination zone of a wide-area *B. anthracis* spore release as to reduce indoor exposure risks. Here, a floor cleaning/dust removal system using disposable dust cloths was evaluated to determine their propensity to remove spores from indoor flooring materials. The Swiffer® Sweeper® system was selected as a representative method of commonly encountered dusting approaches that are readily available. The objective of this study was to evaluate the Swiffer® Sweeper® floor mop system (SSFMS) as a low-tech method to clean indoor residential floors contaminated with *B. anthracis* spores (the causative agent of anthrax). The SSFMS can be used with dry sweeper heads or wet mopping heads.

Two types of flooring surfaces, vinyl and laminate, were contaminated with *B. atrophaeus* spores (used as a surrogate for *B. anthracis* spores). The surfaces were inoculated using a metered-dose inhaler that dispensed an aerosolized spore preparation (approximately 3×10^7 spores) onto a 12-inch (in.) x 12-in. center surface area. Three replicate tests were performed on a total of six test combinations consisting of the dry sweeping, wet mopping, and dry sweeping followed by wet mopping of vinyl and laminate flooring. Samples were collected from the following:

- Three contaminated 35-in. x 35-in. flooring coupons, after cleaning (wipe sampled in four different areas to assess cleaning efficacy and to measure potential redistribution of spores).
- Supplied chamber air (filter sampled to assess aerosol formation cleaning of the flooring coupon).
- Swiffer® Sweeper® cloths used to clean the coupon surfaces.

PHYSICAL REMOVAL RESULTS

Decontamination efficacy for a test combination was expressed as the average log reduction (LR) in the number of viable spore colony-forming units (CFU) before cleaning as compared with CFU after cleaning. Results for each SSFMS operation mode/flooring combination are shown in Table ES-1. These data represent the LR for the inoculated 12-in. x 12-in. center of the coupon ("hot spot").

Table ES-1: Summary of Swiffer® Sweeper® Cleaning Tests

Swiffer® Cleaning Method	Flooring Material	CFU LR	
		Average (n=3)	Standard Deviation
Dry sweeping	Laminate	2.1	0.4
	Vinyl	2.1	0.6
Wet mopping	Laminate	3.3	0.3
	Vinyl	3.0	0.2
Dry sweeping / wet mopping	Laminate	3.4	0.2
	Vinyl	3.4	0.2

One Swiffer® cleaning treatment reduced the concentration of spores on the coupon by at least two orders of magnitude, independent of flooring type or Swiffer® cloth type (wet versus dry). However, results within each test (three replicates) were highly variable, and none of the three cleaning methods produced results that were significantly different from each other. Further, the wet methods (wet mopping and dry sweeping/wet mopping) caused greater spore redistribution across the coupon, i.e., a greater spread of contamination. Decontamination efficacy was not significantly different for vinyl and laminate flooring. Sampling of contaminated stainless steel (reference coupons) and flooring coupons yielded spore counts that were not significantly different from each other.

SPORES ON CLOTHS

The Swiffer® Sweeper® cloths were analyzed to determine the amount of spores collected following a cleaning treatment. The collection efficiencies of the Swiffer® Sweeper® cloths are shown in Table ES-2. Recoveries are with respect to the CFU recovered from the same material without the Swiffer cleaning action (i.e., positive controls; n=3).

Table ES-2: Recoveries from Swiffer® Cloths following Cleaning Treatment

Swiffer® Cloth Condition	Flooring Material	% Recovery	
Dry	Laminate	89 ± 62	
	Vinyl	46 ± 7	
Wet	Laminate	82 ± 37	
	Vinyl	47 ± 30	
Dry and Wet sweep	Laminate	67 ± 9 [dry]	2.0 ± 0.9 [wet]
	Vinyl	40 ± 4 [dry]	3.7 ± 1.2 [wet]

Although the results suggest no statistical difference between recoveries from vinyl versus laminate, numerically more spores were recovered from the laminate surface than from the vinyl surface, independent of treatment type. However, significant differences were observed in the average number of spores recovered on the Swiffer® cloths (wet or dry) used during the first treatment compared to the wet Swiffer® cloths used in a subsequent treatment. Spore recovery from the wet Swiffer® cloth used during a dry sweeping/wet mopping operation was less than 4% of the initial spore count inoculated on the coupon prior to treatment, independent of material type.

The % recoveries for both the wet and dry Swiffer® Sweeper® cloth at the 10^7 spores level (40-89% across both materials) are comparable to currently used sponge wipe surface sampling methods [1,2] with the significant benefit of the ability to sample a larger (here, 35-in. x 35-in.) surface area than the 12-in. x 12-in.) sponge wipe reference method. As such, Swiffer® Sweeper® cloths may facilitate composite sampling from wide areas. Such sampling assessment was not part of this study which focused on the intended physical removal / decontamination of spores from surfaces.

SPORES REAEROSOLIZATION

Aerosol samples were collected to estimate the occurrence and magnitude of aerosolization of viable spores during each SSFMS treatment process. Less than 0.002% of the surface load was found to be aerosolized during any of the treatment processes applied, independent of the type of material or treatment. This study did not address the possible additional spores collected due to reaerosolization by movements of a person who is cleaning the floor surface.

IMPACT OF STUDY

Floor sweeper and mop combinations such as the Swiffer® Sweeper® are most likely to be used in areas that are not heavily contaminated. The observed 2.1–3.4 LR in viable spores across all tested cleaning approaches indicates a reduced indoor exposure risk. A large number of spores, however, remained on the surface after these cleaning approaches. The presence of a significant number of spores (amounts similar to the initial spore counts) indicates that these cloths are heavily contaminated following this cleaning approach. Reuse requires thorough inactivation of spores and may not be suitable for these intentionally disposable cloths. A homeowner would need to dispose of not only the cloths but presumably also the mop to avoid cross-contamination of less contaminated areas. Recommended disposal steps should include inactivation of spores by e.g., soaking of the cloths in diluted bleach prior to disposal.

LIMITATIONS OF STUDY

The reported sweeping and mopping effectiveness should not be compared directly to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) requirement for registration of a technology as a sporicide, which requires demonstration of a greater than or equal to 6 LR in viable spores. Instead, the sweeping and mopping effectiveness should be associated with other low-tech methods that could be used to reduce indoor exposure potential in less contaminated areas. For example, the use of a robotic cleaner[3] on a laminate surface was reported with a similar number of spores recovered from a hot spot location as in this study. The data in this report can assist responders, governments, and health departments in deciding whether to recommend these common cleaning approaches.

1 Introduction

This project evaluated the effectiveness of floor cleaning/dust removal system using disposable dust cloths such as the Swiffer® Sweeper® floor mop system (SSFMS) as a potential low tech/self-help method for the removal of spores from hard-surface floors. This research supports the mission of the U.S. Environmental Protection Agency's (EPA's) Homeland Security Research Program (HSRP) to supply data, performance measures, and considerations that may be useful during wide-area remediation following a biological contamination incident. The project addresses HSRP strategic goals as described in detail in the Homeland Security Strategic Research Action Plan.[4]

1.1 Background

EPA's HSRP recognizes that the environmental remediation following a chemical, biological, or radiological event involves an interconnected system of activities that requires coordinated efforts to optimize cleanup effectiveness, minimize cost and recovery time, and reduce unintended consequences. Currently, EPA's National Homeland Security Research Center (NHSRC) is evaluating decontamination technologies for *Bacillus (B.) anthracis* as part of remediation operations. Following a wide-area contamination incident, it is likely that homeowners at the edge of an exclusion zone, as advised by their local governments, may consider taking self-help actions using low tech approaches that would reduce their indoor exposure risk to biological spores migrated into their residential property. Self-help actions could include efforts to transfer biological spores from indoor surfaces to waste collection bins by vacuuming or dust sweeping utilizing disposable cloths or pads. Here, the Swiffer® Sweeper® was evaluated for use in the physical removal of spores from indoor flooring materials.

1.2 Project Description and Objectives

The purpose of this project was to assess the impact of dusting/sweeping or mopping a hard, nonporous surface as a potential self-help practice to reduce surface-bound contamination levels for the ultimate purpose of reducing indoor exposures of home or building owners following a wide-area *B. anthracis* spore release. The objectives of this study were to determine the efficacy of a common off-the-shelf dust cleaner, the Swiffer® Sweeper® system, in removing surrogate spores for *B. anthracis* from hard floor surfaces and to determine the extent of redistribution of spores from a contaminated area during a cleaning event. Redistributed spores were considered to be either spores moved from a highly concentrated surface area to adjacent sterile surfaces or spores present in air samples due to reaerosolization.

2 Experimental Approach

2.1 Test Matrix

The SSFMS was evaluated for removing spores from surfaces contaminated with *B. atrophaeus* spores, used as surrogate spores for *B. anthracis*. Air samples were also collected during the SSFMS operation to determine if the SSFMS process created a potential inhalation hazard due to the reaerosolization of spores.

Two surface types were investigated: laminate wood flooring and vinyl flooring. Known quantities of spores were deposited on coupons made from these surfaces, and spore removal was performed using dry sweeping, wet mopping, and dry sweeping followed by wet mopping. The laminate and vinyl surfaces were sampled for spores, and Swiffer® Sweeper® cloths were also evaluated for viable spores as a measure of spore removal capability.

The experimental approach used to meet the project objectives is described below:

1. **Preparation of representative coupons of test materials:**

   Large coupons were made from laminate or vinyl materials with dimensions of 35 inches (in.) x 35 in. for test samples and 14 in. x 14 in. for positive controls.

2. **Sterilization of the coupon materials:**

   Prior to use, laminate and vinyl coupons were sterilized using a hydrogen peroxide (H_2O_2) vapor cycle.

3. **Contamination of coupons:**

   Coupons were contaminated using an aerosol deposition method, as described in Section 2.2.6. Briefly, a known quantity of the surrogate organism (1×10^7 colony-forming units [CFU] of *B. atrophaeus* spores) was deposited onto the center of a coupon using a metered-dose inhaler (MDI) fitted into a prefabricated template. The strategy was to inoculate the center of each coupon with a known concentration of surrogate spores.

4. **Decontamination effectiveness:** The spore decontamination approach consisted of cleaning the entire 35-in. x 35-in. surface of a contaminated coupon with a SSFMS (not sterilized). The overall contamination reduction achieved with the Swiffer® Sweeper® was then characterized by sampling for viable spores on the Swiffer cloth (by extraction of the cloth), the coupon surface (by surface wipe sampling), and the chamber headspace (by air sampling). Decontamination effectiveness was measured as log reduction (LR) in viable spores for the contaminated center 12-in. x 12-in. area. A summary of the test matrix is presented in Table 2-1.

Table 2-1: Test Matrix for Surface Decontamination Studies

Test ID	Mop System	Material	Replicates	Blanks	Samples
1	Dry sweeping	Laminate	3	1	Surface, air, and Swiffer® cloth
2		Vinyl	3	1	Surface, air, and Swiffer® cloth
3	Wet mopping	Laminate	3	1	Surface, air, and Swiffer® cloth
4		Vinyl	3	1	Surface, air, and Swiffer® cloth
5	Dry sweeping followed by wet mopping	Laminate	3	1	Surface, air, and Swiffer® cloth
6		Vinyl	3	1	Surface, air, and Swiffer® cloth

2.2 Experimental Methods and Materials

This section describes the experimental methods, including the preparation of coupons and application of the test organism.

2.2.1 Swiffer® Sweeper® Floor Mop System

A hard surface sweeping and mopping tool, the Swiffer® Sweeper® floor mop system http://swiffer.com/en-us/shop-products/sweeping/swiffer-sweeper-floor-mop-starter-kit), shown in Figure 2-1, was evaluated for its ability to remove *B. atrophaeus* spores from two hard, nonporous floor materials: laminate and vinyl. Swiffer® Sweeper® is a sweeping and mopping system made by Procter and Gamble (Cincinnati, OH) that consists of a handle and sweeping/mopping head with a disposable cloth attached. The disposable cloths are used to remove dust and dirt from a flooring surface. According to the manufacturer, the dry cloth has deep ridges and grooves that conform to the surface of a floor to trap and lock dirt, dust and hair. The wet cloth dissolves dirt and grime and traps it away. A combination of both, dry sweeping and then wet mopping, is recommended for extra dirty floor areas. Here, the SSFMS was evaluated for its ability to remove spores from the flooring surfaces.

Both dry sweeping cloths (Swiffer® Sweeper® dry cloth refills – unscented, 26.5 × 20.3 cm [10.4 × 8.0 in.]) and wet mopping cloths (Swiffer® wet mopping cloth refills – Gain original scent, 25.4 × 20.3 cm [10.0 × 8.0 in.]) were used for spore removal.

http://swiffer.com/en-us/shop-products/sweeping

Figure 2-1: Swiffer® Sweeper® floor mop system

2.2.2 Test Chambers

The 35-in. x 35-in. vinyl or laminate flooring test coupons were retained in test chambers (91 centimeters [cm] [length] × 91 cm [width] × 46 cm [height]) constructed of clear acrylic material (5-millimeters [mm] thickness), with the inside surface coated with chemical-resistant polyvinyl chloride (PVC) Type I antistatic film (Model # 7524T15, McMaster-Carr, Elmhurst, IL). The top of the test chamber was outfitted with a grommet port designed to hold the Swiffer® handle and a circular sampling port for a Via-Cell® bioaerosol sampling cassette (product no. VIA010, Zefon International, Ocala, FL). Another port, located on the front of the chamber, was outfitted with a 0.2-micrometer (µm) sterilizing-grade filter (Aervent™, Millipore, Molsheim, France) so that clean make-up air could be supplied during air sampling. The test chamber with Swiffer® unit, sampling ports, and coupon is shown in Figure 2-2. Four test chambers (three for test coupons and one for a procedural blank) were used for each material type/Swiffer® mode combination.

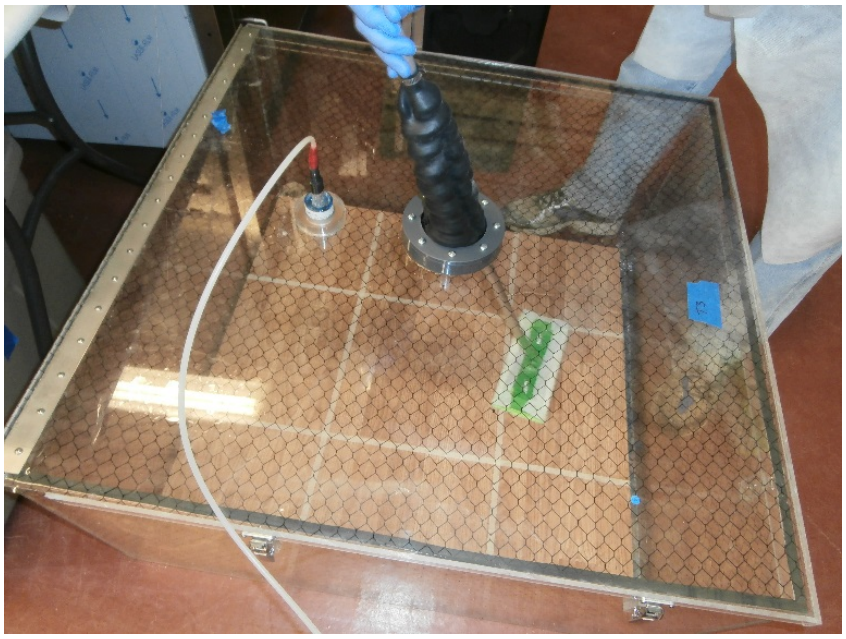

Figure 2-2: Laminate coupon located in acrylic testing chamber with Swiffer® and Via-Cell® ports

2.2.3 Test Coupon Preparation

Two types of coupons, vinyl and laminate flooring, with dimensions of 35 in. x 35 in. for test samples and 14 in. x 14 in. for positive controls, were used in this study (Table 2-2). Thin painter's tape was used to section the large coupons into nine areas. A 14-in. x 14-in. center sampling area (Figure 2-3) was designated as the placement area for the aerosol deposition apparatus (ADA), as described in Section 2.2.6. Both the test coupons and the positive control coupons had an effective inoculation area of 12 in. × 12 in. Only the center area was inoculated to allow for measurement of cross contamination due to the cleaning from a local hot spot to the surrounding area.

Table 2-2: Building Materials

Material	Description	Manufacturer/ Supplier Name (location)	Coupon Surface Size, Length x Width (in.)	Inoculated Surface Size, Length x Width (in.)	Material Preparation and Sterilization
Vinyl flooring	Vinyl flooring 8 feet (ft) x 12 ft Casa Grande beige, precut sheet vinyl, residential grade, low gloss, stain resistant, scratch resistant, 0.157 in. (4 mm) thick	Casa Grande, item #L91118X12, Lowe's Home Improvement, Morrisville, NC	35 x 35 or 14 X 14	12 x 12	Remove incidental dust and grime with alcohol wipes (P/N 21910-110, VWR International, LLC, Radnor, PA). Sterilize (vaporous hydrogen peroxide, VHP).
Laminate flooring	Laminate wood locking flooring 7-5/8 in. x 50-3/4 in.	Item #103553, Lowe's Home Improvement, Morrisville, NC	35 x 35 or 14 x 14	12 x 12	Fabricate coupons using established procedures. Remove incidental dust and grime with alcohol wipes. Sterilize (VHP).

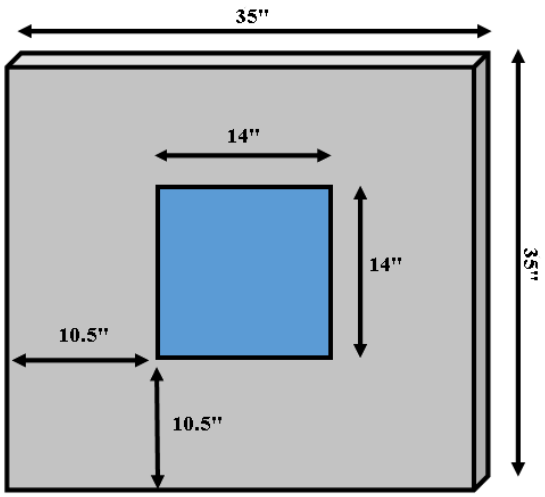

Figure 2-3: Coupon dimensions

2.2.4 Coupon and Equipment Sterilization

Prior to use, laminate and vinyl coupons were sterilized with 400 parts per million hydrogen peroxide (H_2O_2) vapor for four hours using a STERIS VHP® ED1000 generator (STERIS, Mentor, OH) and then held at room temperature for a minimum of 2–3 days to allow off-gassing of H_2O_2. Stainless steel coupons were sterilized with a 250 degrees Celsius (°C) gravity cycle using an autoclave (Amsco Century SV 120 Scientific prevacuum sterilizer, STERIS, Mentor, OH). Sterility was evaluated by swab sampling one coupon from each sterilization batch. Prior to each test, the test chambers were sterilized using the following procedure:

1. Don sterile gloves, wipe the inner surfaces using a bleach towel (Dispatch® hospital cleaner disinfectant towels with bleach, concentration of sodium hypochlorite: 0.65%; model number 69260; Caltech Industries, Inc., Midland, MI).
2. Wait for at least 5 minutes (min) and then wipe the surface again using a new bleach towel.
3. Discard the bleach towels and wipe the surface using 3% sodium thiosulfate (STS) wipes. These wipes are prepared in-house by adding 90 milliliters (mL) of sterile 1N STS and 310 mL of sterile deionized (DI) water to a canister of clean wipes (Fisherbrand™ dry Clean-Wipes™, cat. no. 06-664-14, Fisher Scientific Pittsburgh, PA).
4. Immediately follow by wiping the surface using alcohol wipes (premoistened alcohol/DI water Clean-Wipes™, cat. no. 06-665-24, Fisher Scientific, Pittsburgh, PA).

2.2.5 Spore Preparation

The test organism for this work was a powdered spore preparation of *B. atrophaeus* American Type Culture Collection (ATCC) 9372 mixed with silicon dioxide particles. This preparation was obtained from the U.S. Army Dugway Proving Ground Life Sciences Division. The preparation for this procedure is described in Brown et al.[5] In short, after 80–90% sporulation, the suspension was centrifuged to generate a preparation of approximately 20% solids. A preparation resulting in a powdered matrix containing

approximately 1 × 10^{11} viable spores per gram was prepared by dry blending and jet milling the dried spores with fumed silica particles (Degussa, Frankfurt am Main, Germany).

2.2.6 Coupon Inoculation

Coupons (test and positive controls) were inoculated with *B. atrophaeus* spores using an MDI.[6] Briefly, each coupon was contaminated independently with a separate ADA designed to fit one 14 in. x 14 in. coupon. The MDI and actuator were attached to an ADA through an opening in the center top of the ADA's stainless steel hood, which is sized to cover the square inoculation area exactly. The MDI was discharged one single time into the ADA. The spores were allowed to settle on the coupon surfaces for a minimum of 18 hours. At the end of the minimum 18-hour period, the ADA was removed immediately before sampling. Photographs of an ADA, an MDI, and an MDI actuator used in this project are shown in Figures 2-4 and 2-5.

Figure 2-4: Aerosol deposition apparatus

Figure 2-5: MDI and actuator

2.3 Operational Testing of Swiffer® Sweeper® System

A sampling team of three people, designated as one sampler and two support persons, was used. The sampler was responsible for taking the mop and wipe samples, while a support person was responsible for assembling the sampling equipment and receiving and securing samples taken by the sampler. Another support person handled testing materials such as contaminated coupons and ADAs and operated the air sampling equipment.

2.3.1 Operational Testing Modes

The Swiffer® Sweeper® was evaluated for spore removal using three operation modes: sweeping, mopping, and a combination of sweeping followed by mopping.

2.3.1.1 Dry Sweeping Mode

The following procedure was used for evaluation of the Swiffer® in dry sweeping mode:

1. The support person sets the inoculated coupon, including the ADA, inside the test chamber prior to sampling.
2. The support person opens the bag containing a dry Swiffer® cloth (Swiffer® Sweeper® dry cloth).
3. Using aseptic technique, the sampler removes the cloth from the bag and assembles it on the Swiffer® handle, which is already inserted into the sampling port of the test chamber.
4. The support person then removes the ADA and simultaneously starts the air sampling period of 25 min, corresponding to one full air exchange, and the sweeping of the coupon surface.
5. When the air sampling is completed, the support person holds open the lid of the chamber, and the sampler retrieves the mop cloth.

2.3.1.2 Wet Mopping Mode

Wet mopping was conducted as outlined above with the following exception: the dry sweeping cloth (Swiffer® Sweeper® dry cloth) was replaced with a wet mopping cloth (Swiffer® Sweeper® wet mopping cloth). Sampling and collection of the mop cloth were conducted as before.

2.3.1.3 Dry Sweeping/Wet Mopping Mode

After conducting testing using either dry or wet mop Swiffer® cloths, surface cleaning was conducted using a combination of the two methods. In this approach, the coupon surface is first cleaned using the dry cloth and then cleaned using the wet cloth as follows:

1. The support person sets the inoculated coupon, including the ADA, inside the test chamber prior to sampling.
2. The support person opens the bag containing a dry Swiffer® cloth (Swiffer® Sweeper® dry cloth).
3. Using aseptic technique, the sampler removes the dry cloth from the bag and assembles it on the Swiffer® handle, which is already inserted into the sampling port of the chamber.
4. The support person then removes the ADA and simultaneously starts the air sampling period of 25 min, corresponding to one full air exchange, and the sampler begins to sweep the surface of the coupon.
5. When the air sampling period is completed, the support person holds open the lid of the chamber and the sampler retrieves the dry Swiffer® Sweeper® cloth.
6. At the end of the sweeping phase sampling period, both the support person and the sampler change their gloves and repeat steps 2 through 5 using a wet mopping cloth (Swiffer® Sweeper® wet mopping cloth).

The order of dry sweeping followed by wet mopping is the recommended order according to the manufacturer (http://swiffer.com/en-us/tips-and-articles/how-to-use-swiffer-sweeper).

2.3.2 Sample Coupon Sweeping Pattern

All sample coupons, 35 in. × 35 in., were swept using an up and down and left to right movement (see Figure 2-6) in the following sweeping pattern:

1. Place the loaded Swiffer® Sweeper® in the lower left-hand corner of the sample coupon.

2. Start sweeping with a stroke straight toward the top of the coupon. The Swiffer® head will be parallel with the top of the coupon. After reaching the top of the coupon, pull the sweeper straight down, retracing the path swept in the first stroke. The Swiffer® head will be parallel with the bottom of the coupon. This set of strokes sweeps the non-inoculated area.
3. In the next stroke, overlap the previous stroke approximately 5 in. and go diagonally from left to right. When the top of the coupon is reached, sweep the return stroke straight down with the sweeper head parallel to the top edge of the coupon until the bottom of the coupon is reached.
4. Repeat the coupon sweeping strokes as in step 3 until the end of the test coupon is reached.
5. When the right side of the coupon is reached, perform a straight sweeping stroke toward the top. The Swiffer® head on this stroke is parallel to the top edge. When the top is reached, pull the sweeper straight down toward the bottom of the coupon, retracing the path of the up stroke. The Swiffer® head on this stroke is parallel to the bottom edge. This completes the coupon sweeping.

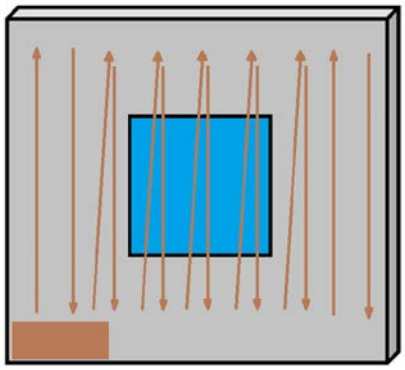

Figure 2-6: Swiffer® Sweeper® sampling pattern.

The brown rectangle in the lower left corner represents the Swiffer® mop. The blue area is the 12-in. x 12-in. area inoculated with spores. Brown lines represent movements of the Swiffer® mop head.

3 Sampling Approach

This section discusses the sampling approach, including sampling site environmental conditions, sampling media, sampling frequency, wipe sampling procedures, and prevention of cross-contamination during sampling.

For each sampling event (or test), a sampling event log sheet was maintained that included each sampling team member's name, the date, the run number, and all sample codes with corresponding coupon codes. The coupon codes were preprinted on the sampling event log sheet before sampling began. The following sections discuss the sampling strategy and methods used for sampling coupon surfaces and the test chamber air.

3.1 Sampling Site Environmental Conditions

Ambient environmental conditions such as temperature and relative humidity (RH) can affect the evaporation rate of liquids from surfaces. All tests were conducted at room temperature and ambient RH. Three strategically placed HOBO® temperature and RH sensors (HOBO® data logger U12 series, Onset

Computers, Bourne, MA) recorded RH and temperature. The devices are calibrated by the on-site Metrology Laboratory (Metlab) at EPA -RTP. All coupons were conditioned at ambient conditions for one week before use.

3.2 Sampling Strategy

Each test consisted of four large material coupons (three test coupons and one procedural blank) and three 14-in. x 14-in. material coupons (positive controls). Additionally, three 14-in. x 14-in. stainless steel coupons were inoculated with the test set and served as inoculation control coupons. Swabs were taken of each surface type prior to each test to monitor for contamination. The following sections discuss the sample types and frequency of sampling and monitoring events.

3.2.1 Sample Types

Each testing sequence resulted in the following samples:

- **Wipe samples:** Polyester-rayon blend (PRB) wipe samples were collected from each material in sets of four, positive control surface samples were collected in sets of three for both stainless steel and material type, and one was collected for a procedural blank surface sample.
- **Swiffer® Sweeper® cloth (dry and wet):** cloth samples were collected for each material type/Swiffer® Sweeper® operational mode, including a procedural blank.
- **Aerosol samples**: Aerosol samples were collected using Via-Cell® bioaerosol cassettes during each decontamination and procedural blank test. Results for these samples were used to estimate the occurrence and magnitude of fugitive emissions of viable *B. atrophaeus* spores during the sweeping and/or mopping process.

- **Swab Samples**: BactiSwab™ samples were collected to check the sterility of the material and equipment prior to sampling.

3.2.2 Sampling Frequency

The Swiffer® dry cloth tests and the Swiffer® wet cloth tests each generated 20 samples. The test using dry and then wet cloths generated 34 samples. One air sample was generated during the Swiffer® testing sequence for a sampling period of 25 min at an air sampling flow rate of 15.3 L/min, corresponding to one full air exchange. Samples were recovered, extracted, and plated. Plates were incubated to grow the *B. atrophaeus* spores, and the resulting colonies were counted.

Table 3-1 shows the sampling frequencies for each material type/Swiffer® mode combination test (as shown in Table 2-1) required for this project. Tests 5 and 6 samples were doubled since these tests involved a combination of sweeping and mopping operations.

Table 3-1: Sampling Measurements/Frequency for Swiffer® Evaluation Testing

Testing Sequence	Measurement	Sample Type	Frequency
For each material type/ Swiffer® dry, wet and combination test	Procedural blank coupon CFU	Swiffer® cloth	1 (2 for Tests 5 and 6)
		Wipe	4 (8 for Tests 5 and 6)
	Laboratory blank solution CFU	Swiffer® cloth	1 (2 for Tests 5 and 6)
	Test coupons post-sweep/mop CFU	Swiffer® cloth	1 (2 for Tests 5 and 6)
		Wipe	4 (8 for Tests 5 and 6)
	Positive control CFU	Wipe	6 (3 stainless steel and 3 test material)
	Air sampling	Via-Cell®	3 (6 for Tests 5 and 6)

3.3 Sampling Methods

The following sections discuss the methods used for wipe sampling and air sampling.

3.3.1 Wipe Sampling

The general approach for the wipe sampling was to use a moistened, sterile, non-cotton PRB gauze wipe to wipe an area to recover spores. A three-person team (consisting of a sampler, a coupon handler, and a support person) was used to collect wipe samples. Aseptic techniques were used throughout.

3.3.1.1 Wipe Sampling Preparation

All materials needed for sampling were prepared before the sampling event began. Table 3-2 lists the materials used for sampling. Team members wore non-powdered surgical gloves during sampling. Individually wrapped premoistened bleach wipes, used for sample bag decontamination, were placed in sterile sampling bags. A sampling material bin was stocked for each sampling event based on the sample quantity needed for that test. The bin contained enough wipe-sampling kits to accommodate all required samples for a specific test event.

Table 3-2: Sampling Materials and Equipment

Material or Equipment	Description
Non-powdered, sterile surgical gloves	KIMTECH PURE* G3 Sterile STERLING™ Nitrile Gloves, Kimberly-Clark Professional® (VWR P/N HC61110 for extra-large, VWR P/N HC61190 for large, and VWR P/N HC61180 for medium)
Non-powdered, non-sterile surgical gloves	Examination gloves (Fisherbrand™ Powder-Free Nitrile Exam Gloves (Fisher Scientific P/N 19-130-1597D for large and 19-130-1597C for medium sizes)
Disposable laboratory coats	Kimberly-Clark Kleenguard A10 light-duty apparel (P/N 40105)
Phosphate-buffered saline (PBS)	PBS with PBST (Sigma Aldrich USA, P/N: P3563-10PAK)
50-mL conical tubes	BD Falcon™ BlueMax graduated tubes, 15-mL (Fisher Scientific P/N 14-959-70C)
Sterile sampling bags	Fisherbrand™ Sterile Sampling Bags (TWIRL'EM) Overpack size 10-in. by 14-in. Inner bag size: 5.5-in. by 9-in. (for wipe) Sample bag size: 5.5-in. by 9-in.
Bleach wipes	Dispatch® bleach wipes (Clorox® Co., Oakland, CA) or Hype-Wipes (Current Technologies, Indianapolis, IN)
Wipes for sampling	Kendall Curity Versalon absorbent gauze sponge, 2-in. by 2-in., sterile packed (rayon-polyester blend), http://www.mfasco.com/ (last accessed December 5, 2016)
Swabs	BactiSwab®, http://www.remelinc.com/Industrial/CollectionTransport/BactiSwab.aspx (last accessed December 5, 2016)
Analytical filter units	150-mL Nalgene analytical filter units, 0.2-µm cellulose acetate (Fisher Scientific cat. no. 130-4020)
Aerosol sampling cassettes	Via-Cell® VIA010 bioaerosol sampling cassette, http://www.zefon.com/store/via-cell-bioaerosol-sampling-cassette.html (last accessed December 5, 2016)
Sterilizing-grade filter	Aervent™ 0.2-micron sterilizing-grade cartridge filter (Millipore SAS, Molsheim, France)
Sampling pump	Isokinetic Method 5 Source Sampling Console, http://www.apexinst.com/product/xc-50-method-5-source-sampling-console (last accessed April 25, 2017)

3.3.1.2 Wipe Sampling Procedure

After the coupon surface was swept with the SSFMS, wipe samples were collected at four locations to determine the level of spores remaining on the center of the coupon and spore redistribution across the coupon by the Swiffer® cloth. The four locations sampled are indicated in Figure 3-1 and consist of the following surface areas (corresponding to the numbers shown in ascending order): 147, 110, 147, and 196 in.^{2}. The wipe sampling started at the lowest anticipated spore concentration area (Location 1) and proceeded to the highest anticipated spore concentration (Location 4). The wipe sampling for both the dry and the wet sweeping mode started when the aerosol sampling was completed (25 min). This sampling procedure was used also for sampling the procedural negative coupons following a Swiffer® Sweeper® treatment. Wipe samples were extracted in 20 mL of phosphate-buffered saline with 0.05% TWEEN® 20 (PBST), sonicated, vortexed, and subjected to serial 10-fold dilution and plating.

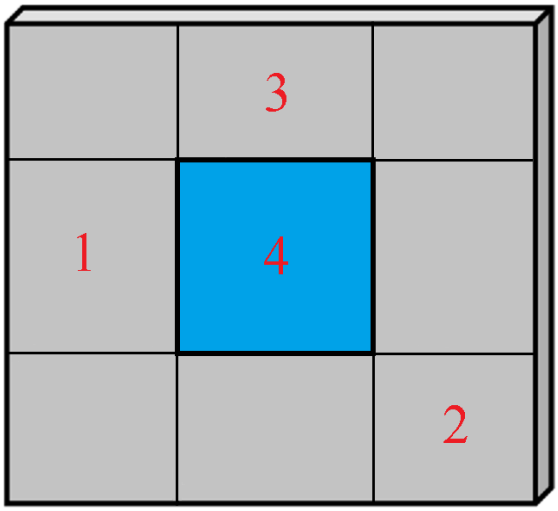

Figure 3-1: Wipe sampling grid, locations, and order

The sampling team used aseptic techniques and followed a strict sampling protocol to avoid any cross-contamination among coupons or among samples. The support person logged observations into the laboratory notebook and made sampling kit items (premoistened all-purpose sponge, conical tube, sampling bags, etc.) available to the other team members.

The following sequence was followed for obtaining a sample:

- The support person opened the test chamber to allow access to the coupon and removed the Swiffer® head, being careful not to touch the coupon.
- The sampler removed the Swiffer® cloth and put it in the sample bag that was handed to the sampler by the support person.
- The support person handed a sampling sponge to the sampler.
- The sampler wiped the surface of the coupon horizontally, using a consistent amount of pressure and using S-strokes to cover the designated sample area of the coupon.
- The sampler folded the sponge in half, concealing the exposed side and then wiped the same surface vertically using the same S-stroke technique.
- The sampler folded the sponge again and rolled up the folded sponge so that it would fit into a conical tube.
- The support person opened a conical tube, holding the tube inside a sterile sampling bag.
- The sampler placed the folded sponge into the conical tube that the support person was holding, being careful not to touch the surface of the tube or the plastic sterile sampling bag.

For each separate test (each of the two flooring types and each of the three Swiffer® methods), surface sampling of the materials was completed for all procedural blank coupons before sampling of any test material. Positive controls were sampled last.

3.3.2 Air Sampling with Via-Cell®

Air sampling was conducted concurrently with Swiffer® sweeping using an EPA Method 5 dry gas meter box to measure the volume of air added, a Method 5 pump to force air flow (at 15 liters per minute, Lpm), and Via-Cell® bioaerosol sampling cassettes to sample for aerosolized spores. Air sampling events were conducted for one full air exchange of the sampling chamber (13.45 ft^3, added to the test chamber in approximately 25 min). A Millipore filter was installed on the air inlet of each chamber to prevent any contamination present in the laboratory air from contaminating the air introduced into the chamber.

4 Testing and Measurements

4.1 Analytical Procedure

Spores were extracted from the wipe samples, swabs, aerosol filters, and Swiffer® Sweeper® cloths. Spores in these extracts were assayed by growth on nutrient agar plates in the NHSRC Biocontaminant Laboratory (Biolab). The wipe samples and aerosol filters were extracted in 20 mL of PBST and vortexed for two minutes in 10-second bursts. The Swiffer® Sweeper® cloths were extracted in 80 mL of PBST and stomached at 230 revolutions per minute (rpm) for two minutes. The samples were analyzed either qualitatively for spore presence (quality control [QC] swab samples) or quantitatively for the number of viable spores recovered per sample (CFU). Details of the analysis procedures are provided below.

The extracts for all sample types were plated in triplicate using a spiral plater (Autoplate® spiral plating system, Advanced Instruments Inc., Norwood, MA), which deposits a known volume of sample in three 10-fold serial dilutions on each plate. Plates were then incubated at 35 ± 2 °C for 16 to 19 hours. During incubation, the colonies develop along the lines where the liquid was deposited on the rotating plate in decreasing amounts from the center to the edge of the rotating agar plate (see Figure 4-1). The number of spores was estimated using a QCount colony counter (Advanced Instruments Inc., Norwood, MA).

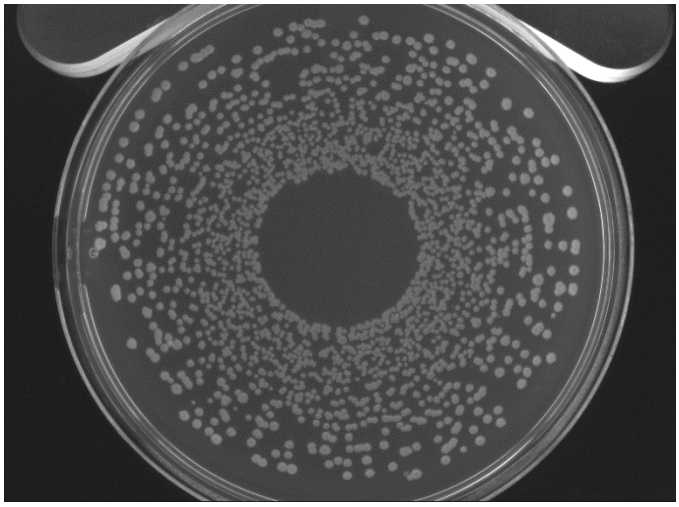

Figure 4-1: Bacterial colonies on a spiral-plated agar plate

Positive control samples were diluted 100-fold (10^{-2}) in PBST prior to spiral plating, and samples with unknown concentrations were plated with no dilution and following a 100-fold dilution. Samples with known low concentrations were plated with no dilution. The QCount instrument automatically calculates the CFU/mL in a sample based on the dilution plated and the number of colonies that develop on the plate and records the data in an MS Excel spreadsheet. Only plates containing between 30 and 300 CFU were used for spore recovery estimates.

If a low count (< 30 CFU per plate) occurs for a sample that was spiral plated with a neat (undiluted) aliquot, then two options are available:

1. Spread plate an undiluted aliquot with a larger volume (100, 200, and 400 microliters, each in triplicate).
2. Filter plate an undiluted aliquot with a larger volume (e.g., 1, 2, or 10 mL).

Filter plating is done using the Pall MicroFunnel unit with 0.45 µm GN-6 Metricel white membrane (P/N 4804, Pall Corporation, Port Washington, NY). The sample aliquot is added to 10 mL DI water, which is then poured over the filter. The vacuum system is opened and the liquid is funneled through the filter, trapping the spores on the filter/membrane. The filter is then washed with another 10-mL aliquot of sterile DI water. The filter is removed from the plastic housing and placed onto a tryptic soy agar (TSA) plate. Plates are incubated at 35 ± 2 °C for 18–24 hours prior to manual enumeration.

The swabs are removed from sample packaging and held by the wooden end while the inoculated end is rolled across a fresh TSA plate in an S-motion. The swab is rolled over the TSA plate so that the entire circumference of the inoculated tip touches the plate. Plates are incubated overnight at the appropriate temperature and then evaluated for growth.

4.2 Data Reduction

Each test series was composed of a flooring type (vinyl or laminate) and a Swiffer® Sweeper® mode (dry, wet, or dry/wet), positive controls and procedural blanks (negative controls). The raw data for this study are the colony counts from each of the three test coupons, each of the six positive control coupons (three flooring coupons and three stainless steel coupons), and each procedural blank (one negative control coupon) from each of the six test series (flooring type/Swiffer® Sweeper® mode combination).

Average and standard deviation (SD) were calculated for counts from the replicate test coupons and positive control coupons. The LR of spores was calculated for the inoculated center area only; the redistribution of the spores to other areas outside the center of the coupon was not included in this calculation. LR is defined in this project as the difference in the average of the logarithm of the number of viable spores (determined by CFU) recovered on the material control coupons minus the average of the logarithm of the number of viable spores (determined by CFU) recovered on the center, 12 by 12 in. area of the test coupons.

Efficacy, defined as LR in CFU count after a Swiffer® Sweeper® treatment, was calculated using Equation 4-1 for each material within each combination of decontamination procedure (*i*) and test material (*j*) as follows:

$$LR_{ijk} = \frac{\sum_{c=1}^{c} (\log_{10} C_{ijc})}{N_{ijc}} - \log_{10}\left(N_{ijk} \right) \qquad (4\text{-}1)$$

where

C_{ijc} is the number of viable organisms recovered from C control coupons for the i^{th} decontamination procedure and j^{th} test material,

N_{ijc} is the number of control coupons for the i^{th} decontamination procedure and j^{th} test material,

N_{ijk} is number of viable organisms recovered on the k^{th} replicate of the inoculated center area (Location 4) of the test coupon for the i^{th} decontamination procedure and j^{th} test material.

If no viable spores were detected, then the detection limit of the sample was used and the efficacy reported as greater than or equal to the value calculated by Equation 4-1. The detection limit of a sample depends on the analysis method and so might vary. The detection limit of a plate was assigned a value of 0.5 CFU, but the fraction of the sample plated varies. For instance, the detection limit of a 0.1-mL plating of a 20-mL sample suspension is 100 CFU (0.5 CFU/0.1 mL * 20 mL), but if all 20 mL of the sample is filter-plated, the detection limit would be 0.5 CFU.

The standard deviation (SD) of LR_i is calculated by Equation 4-2:

$$SD_{\eta_{ij}} = \sqrt{\frac{\sum_{k=1}^{N_s}\left(x_{ijk} - LR_{ij}\right)^2}{N_{ijk} - 1}} \qquad (4\text{-}2)$$

where $SD_{\eta i}$ is standard deviation of ηi,

LR_{ij} is the average LR of spores on a specific material surface, and

X_{ikj} is the average of the LR of each from the surface of a decontaminated coupon (Equation 4-3):

$$x_{ijk} = \frac{\sum_{k}\{\sum_{C=1}\log(CFU_{ijc})/N_C - \log(CFU_{ijk})\}}{N_{ijk}} \qquad (4\text{-}3)$$

where $\sum_{c}\log(CFU_{ijc})/N_{ijc}$ represents the "mean of the logs," the average of the logarithm transformed number of viable spores (determined by CFU) recovered on the control coupons (C is control, j is coupon number, and N_C is number of coupons [*1, j*]); and CFU is number of CFU on the surface (Location 4) of the k^{th} decontaminated material surface.

5 Results and Discussion

This section discusses the results of the three different Swiffer® operations—sweeping, mopping, or a combination of sweeping and mopping—for spore removal from the two flooring material types, laminate and vinyl.

5.1 Post-Decontamination Recoveries

The post-decontamination surface sampling recoveries (CFU) from the test materials are shown in Figure 5-1. The figure depicts the locations of the sampling surfaces for each test, along with the number of spores recovered from each sampling location. The colors illustrate the relative concentrations of recovered spores at various spots on the coupon, with red indicating high concentration and green indicating low concentration. The figure shows the cross-contamination of the areas adjacent to the hot spot (center of coupon; Location 4 in Figure 3-1) during the sweeping/mopping process. As discussed previously, the surface of the coupon was sampled in ascending order (Location 1 was wipe sampled prior to Location 2, etc.), whereas the Swiffer® wiping pattern occurred from left to right across the surface of the coupon through a series of vertical wipe strokes.

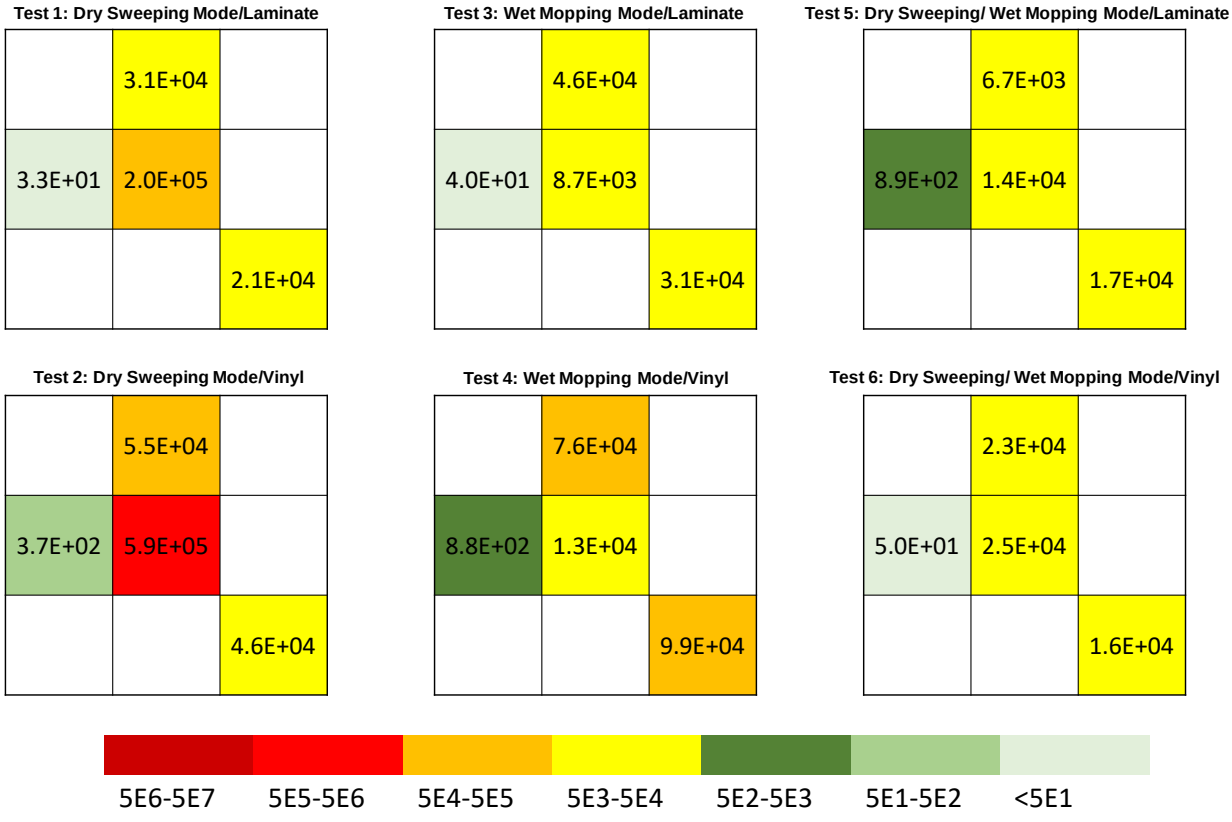

Figure 5-1: Average spore recovery (CFU) after Swiffer® treatment

Redistribution of spores was observed for every Swiffer® cleaning treatment with a significantly lower number of spores recovered at Location 1, directly to the left of the inoculated center area compared to Locations 2 and 3. The vertical strokes from left to right do not cause a redistribution of spores until the cloth makes contact with the inoculated center. Hence, areas to the left of the center are less contaminated due to redistribution than those to the right of the inoculated center.

The dry mopping and wet mopping (Tests 1-4) resulted in higher redistribution in total number of spores recovered outside the center area (5.3×10^4-1.8×10^5 range) as compared with the dry/wet sweeping combination approach (Tests 5-6; 2.4×10^4-3.9×10^4 range). This distribution occurs because the double sweeping/mopping effort removes more spores from the center surface during the second round of mopping, rather than redistributing more spores from the inoculated center. Location 1 to the left of the center, which was always swept or mopped before contact occurred with the contaminated center location, had the lowest of number of spores recovered (3.3×10^1-8.9×10^2 range) across all six tests. Locations 2 and 3 contained a higher number of spores and were within one order of magnitude (1 log) of the remaining number of spores in the center position.

5.2 Swiffer® Sweeper® Decontamination Efficacy

Decontamination efficacy is represented as the LR in viable spores (CFU) for the inoculated center following sweeping and/or mopping and is presented as the average LR across the three replicates in a particular test (compared to positive controls). The results for the decontamination efficacy of each Swiffer® Sweeper® operation mode/material type combination are presented in Tables 5-1 through 5-6. For better comparison, all recoveries from slightly different sampling area sizes were normalized to a one square foot area. Each coupon inoculation was confined to the center of the coupon (Location 4). Considering this center location only, the dry sweeping resulted in an average LR of 2.1 for both materials (Tables 5-1 and 5-2). The wet mopping resulted in slightly higher average LRs of 3.3 and 3.0 (Tables 5-3 and 5-4) for laminate and vinyl, respectively. The combination approach of dry sweeping/wet mopping resulted in an average LR of 3.4 for both material types (Tables 5-5 and 5-6).

The post-treatment wipe sampling of the center area (Location 4) yielded a higher CFU count than samples from all other areas of the coupon (Locations 1-3). Dry sweeping, wet mopping and dry/wet mopping caused spore redistribution, as discussed above, which resulted in a more even distribution of spores across the whole coupon surface.

Dry mopping left approximately 10^5 spores in the center of the coupon (depending on the material), whereas wet mopping and dry/wet mopping left approximately 10^4 spores in this same location. Despite the statistically equivalent recovery of spores from wet and dry mopping methods, these results suggest that the higher log spore reduction, reported above for the wet Swiffer® cloths, can be attributed to a better pick up/removal of spores when compared to the dry Swiffer® cloths. Further, the results suggest that the material effects on the decontamination efficacy for all the Swiffer® Sweeper® operations are minimal, and the sample recoveries for each Swiffer® Sweeper® operation mode are not statistically significantly different (Student's t-test: $p > 0.078$ at 95% confidence interval).

Table 5-1: Test 1 – Dry Sweeping/Laminate

Replicate No.	Spore Recovery (CFU/ft^2)						LR
	Test Sample Location*				Control Samples		
	1	2	3	4	Stainless Steel Samples	Positive Controls	
Replicate 1	4.90×10^1	9.59×10^3	3.92×10^4	2.35×10^5	3.24×10^7	1.10×10^7	1.76
Replicate 2	3.31×10^1	1.68×10^4	6.26×10^3	2.82×10^4	2.68×10^7	8.12×10^6	2.68
Replicate 3	1.44×10^1	5.62×10^4	4.70×10^4	1.78×10^5	2.94×10^7	2.14×10^7	1.88
Average	**3.21×10^1**	**2.75×10^4**	**3.08×10^4**	**1.47×10^5**	**2.95×10^7**	**1.35×10^7**	**2.11**
SD	**1.73×10^1**	**2.51×10^4**	**2.16×10^4**	**1.07×10^5**	**2.80×10^7**	**6.99×10^6**	**0.41**

*See Figure 3-1.

Table 5-2: Test 2 – Dry Sweeping/Vinyl

Replicate No.	Spore Recovery (CFU/ft^2)						LR
	Test Sample Location*				Control Samples		
	1	2	3	4	Stainless Steel Samples	Positive Controls	
Replicate 1	1.48×10^2	5.72×10^4	2.64×10^4	5.04×10^4	2.52×10^7	2.50×10^7	2.78
Replicate 2	9.40×10^2	5.85×10^4	3.04×10^4	7.61×10^5	4.08×10^7	3.54×10^7	1.60
Replicate 3	3.92×10^0	6.58×10^4	1.05×10^5	4.85×10^5	2.84×10^7	3.00×10^7	1.79
Average	**3.64×10^2**	**6.05×10^4**	**5.39×10^4**	**4.32×10^5**	**3.15×10^7**	**3.01×10^7**	**2.06**
SD	**5.04×10^2**	**4.65×10^3**	**4.43×10^4**	**3.58×10^5**	**8.24×10^6**	**5.20×10^6**	**0.63**

*See Figure 3-1.

Table 5-3: Test 3 – Wet Mopping/Laminate

Replicate No.	Spore Recovery (CFU/ft^2)						LR
	Test Sample Location*				Control Samples		
	1	2	3	4	Stainless Steel Samples	Positive Controls	
Replicate 1	ND	3.84×10^4	4.64×10^4	2.34×10^3	4.18×10^7	1.02×10^7	3.69
Replicate 2	7.84×10^1	5.98×10^4	1.41×10^4	9.60×10^3	2.88×10^7	9.26×10^6	3.08
Replicate 3	3.92×10^1	2.38×10^4	7.54×10^4	7.26×10^3	3.32×10^7	1.49×10^7	3.20
Average	**3.92×10^1**	**4.07×10^4**	**4.53×10^4**	**6.40×10^3**	**3.46×10^7**	**1.15×10^7**	**3.32**
SD	**3.92×10^1**	**1.81×10^4**	**3.07×10^4**	**3.70×10^3**	**6.61×10^6**	**3.02×10^6**	**0.32**

*See Figure 3-1.
ND: Non-detect

Table 5-4: Test 4 – Wet Mopping Mode/Vinyl

Replicate No.	Spore Recovery (CFU/ft^2)						LR
	Test Sample Location*				Control Samples		
	1	2	3	4	Stainless Steel Samples	Positive Controls	
Replicate 1	1.83×10^2	5.77×10^4	6.11×10^4	3.04×10^4	2.92×10^7	2.10×10^7	3.02
Replicate 2	2.25×10^3	1.05×10^5	7.82×10^4	2.01×10^4	4.86×10^7	3.84×10^7	3.20
Replicate 3	1.57×10^2	2.23×10^5	8.48×10^4	3.97×10^4	5.36×10^7	3.56×10^7	2.90
Average	$\mathbf{8.64 \times 10^2}$	$\mathbf{1.29 \times 10^5}$	$\mathbf{7.47 \times 10^4}$	$\mathbf{3.01 \times 10^4}$	$\mathbf{4.38 \times 10^7}$	$\mathbf{3.17 \times 10^7}$	**3.04**
SD	$\mathbf{1.20 \times 10^3}$	$\mathbf{8.51 \times 10^4}$	$\mathbf{1.22 \times 10^4}$	$\mathbf{9.78 \times 10^3}$	$\mathbf{1.29 \times 10^7}$	$\mathbf{9.34 \times 10^6}$	**0.15**

*See Figure 3-1.

Table 5-5: Test 5 – Dry Sweeping/Wet Mopping/Laminate

Replicate No.	Spore Recovery (CFU/ft^2)						LR
	Test Sample Location*				Control Samples		
	1	2	3	4	Stainless Steel Samples	Positive Controls	
Replicate 1	8.16×10^0	2.39×10^4	2.51×10^3	6.40×10^3	2.98×10^7	2.26×10^7	3.58
Replicate 2	9.45×10^1	2.28×10^4	2.14×10^2	1.09×10^4	2.50×10^7	1.81×10^7	3.35
Replicate 3	2.51×10^3	1.87×10^4	1.69×10^4	1.26×10^4	3.58×10^7	3.20×10^7	3.28
Average	$\mathbf{8.70 \times 10^2}$	$\mathbf{2.18 \times 10^4}$	$\mathbf{6.55 \times 10^3}$	$\mathbf{9.97 \times 10^3}$	$\mathbf{3.02 \times 10^7}$	$\mathbf{2.42 \times 10^7}$	**3.40**
SD	$\mathbf{1.42 \times 10^3}$	$\mathbf{2.75 \times 10^3}$	$\mathbf{9.05 \times 10^3}$	$\mathbf{3.21 \times 10^3}$	$\mathbf{5.41 \times 10^6}$	$\mathbf{7.08 \times 10^6}$	**0.16**

*See Figure 3-1.

Table 5-6: Test 6 – Dry Sweeping/Wet Mopping/Vinyl

Replicate No.	Spore Recovery (CFU/ft^2)						LR
	Test Sample Location*				Control Samples		
	1	2	3	4	Stainless Steel Samples	Positive Controls	
Replicate 1	5.71×10^1	1.13×10^4	1.65×10^4	9.99×10^3	3.48×10^7	3.06×10^7	3.64
Replicate 2	1.24×10^1	1.78×10^4	1.66×10^4	2.04×10^4	2.60×10^7	5.74×10^7	3.33
Replicate 3	8.49×10^1	3.21×10^4	3.47×10^4	2.42×10^4	4.62×10^7	4.16×10^7	3.25
Average	$\mathbf{5.15 \times 10^1}$	$\mathbf{2.04 \times 10^4}$	$\mathbf{2.26 \times 10^4}$	$\mathbf{1.82 \times 10^4}$	$\mathbf{3.57 \times 10^7}$	$\mathbf{4.32 \times 10^7}$	**3.40**
SD	$\mathbf{3.66 \times 10^1}$	$\mathbf{1.07 \times 10^4}$	$\mathbf{1.05 \times 10^4}$	$\mathbf{7.3 \times 10^3}$	$\mathbf{1.01 \times 10^7}$	$\mathbf{1.35 \times 10^7}$	**0.20**

*See Figure 3-1.

5.3 Post-Decontamination Swiffer® Sweeper® Cloth Recovery

The Swiffer® Sweeper® cloths were analyzed to determine the number of spores collected following a treatment event. The results for the two types of Swiffer® Sweeper® cloths (dry and wet) are presented in Table 5-7. No statistical difference was observed between the spores recovered on the Swiffer® Sweeper® cloths as a function of material type (Student's t-test, p-value 0.44) or Swiffer® Sweeper® cloth type (Student's t-test, p-value 0.34). Although these results suggest no statistical difference between recoveries from vinyl versus laminate, numerically more spores were recovered from the cloth that treated a vinyl surface (80% ± 37%) than from a cloth that treated a laminate surface (45 ± 16%). Significant differences in average spore recoveries were observed between the first and second treatments of Tests 5 and 6

(Student's t-test, p-value 0.000, or zero probability under the null hypothesis). The spore recoveries from the dry Swiffer® cloth following the first treatment approach (dry cloth) resulted in significantly higher recoveries ($1.69 \times 10^7 \pm 7.1 \times 10^5$, average over laminate and vinyl) than recoveries on the wet cloth following the second treatment ($1.04 \times 10^6 \pm 7.7 \times 10^5$), independent of material type.

Variations in number of spores recovered across replicates were occasionally high (e.g., Tests 1 and 3) which may be attributed to poor contact with the laminated surface. Lower (Test 1) or higher (Test 3) spore recoveries from the cloths do not correlate with higher (Test 1) or lower (Test 3) total number of spores recovered from the treated surface (Tables 5-1 and 5-3, respectively).

Table 5-7: Post Decontamination Swiffer® Sweeper® Cloth Spore Recoveries

Test ID	Material	Swiffer® Sweeper® Cloth Post decon Recovery				
		Replicate	Dry Cloth		Wet Cloth	
			CFU	% Recovered	CFU	% Recovered
1	Laminate	1	1.58×10^7	117		
		2	2.39×10^6	18		
		3	1.77×10^7	131		
		Average	**1.20×10^7**	**89**		
		SD	**6.81×10^6**	**62**		
2	Vinyl	1	1.16×10^7	39		
		2	1.53×10^7	51		
		3	1.51×10^7	50		
		Average	**1.40×10^7**	**46**		
		SD	**1.67×10^6**	**7**		
3	Laminate	1			5.00×10^6	44
		2			9.84×10^6	86
		3			1.34×10^6	117
		Average			**9.42×10^6**	**82**
		SD			**3.45×10^6**	**37**
4	Vinyl	1			4.08×10^6	13
		2			1.88×10^7	59
		3			2.22×10^7	70
		Average			**1.50×10^7**	**47**
		SD			**7.87×10^6**	**30**
5	Laminate	1	1.54×10^7	64	3.28×10^5	1.4
		2	1.89×10^7	78	7.27×10^5	3.0
		3	1.47×10^7	61	4.20×10^5	1.7
		Average	**1.64×10^7**	**67**	**4.92×10^5**	**2.0**
		SD	**1.83×10^6**	**9**	**1.71×10^5**	**0.9**
6	Vinyl	1	1.52×10^7	35	2.05×10^6	4.8
		2	1.88×10^7	43	1.02×10^6	2.4
		3	1.83×10^7	42	1.71×10^6	3.9
		Average	**1.74×10^7**	**40**	**1.59×10^6**	**3.7**
		SD	**1.58×10^6**	**4**	**4.30×10^5**	**1.2**

5.4 Spore Aerosolization

Aerosol samples were collected to estimate the occurrence and magnitude of fugitive emissions of viable spores during each SSFMS treatment process. Spores were observed in most of the air samples, as shown in Table 5-8. Less than 0.002% of the surface load was found to be aerosolized during any of the treatment processes applied, independent of material type or treatment type.

Table 5-8: Spore Recoveries in the Aerosol Samples

Test ID	Material	Cloth Type	Aerosolized Spores		
		Wet/Dry	Replicate	(CFU)/Sample	Concentration (CFU/ft^3)
1	Laminate	Dry	1	2	0.17
			2	1	0.08
			3	13	0.93
			Average	**5**	**0.40**
			SD	**6**	**0.45**
2	Vinyl	Dry	1	1	0.08
			2	19	1.39
			3	10	0.71
			Average	**10**	**0.74**
			SD	**9**	**0.65**
3	Laminate	Wet	1	41	3.03
			2	228	16.85
			3	24	1.81
			Average	**98**	**7.22**
			SD	**113**	**8.35**
4	Vinyl	Wet	1	16	1.16
			2	118	8.72
			3	120	8.83
			Average	**85**	**6.23**
			SD	**60**	**4.42**
5a	Laminate	Dry	1	53	3.88
			2	26	1.93
			3	78	5.72
			Average	**52**	**3.82**
			SD	**26**	**1.90**
5b		Wet	1	8	0.59
			2	1	0.08
			3	35	2.61
			Average	**15**	**1.10**
			SD	**18**	**1.33**
6a	Vinyl	Dry	1	ND	ND
			2	2	0.17
			3	ND	ND
			Average	**1**	**0.06**
			SD	**1**	**0.11**
6b		Wet	1	52	3.82
			2	2	0.17
			3	69	5.13
			Average	**41**	**3.03**
			SD	**35**	**2.58**

6 Quality Assurance and Quality Control

6.1 Project Documentation

This project was performed under a Category III quality assurance project plan (QAPP), approved August 2015. All test activities were documented via narratives in laboratory notebooks and the use of digital video and photography. The documentation included a record for each sampling procedure, any deviations from the QAPP, and physical impacts on materials. All tests were conducted in accordance with established operating procedures to ensure repeatability and adherence to the data quality validation criteria set for this project.

6.2 Integrity of Samples and Supplies

Samples were carefully maintained and preserved to ensure their integrity. Samples were stored away from standards or other samples that could possibly cross-contaminate them.

Supplies and consumables were acquired from reputable sources and were National Institute of Standards and Technology (NIST)-traceable whenever possible. Supplies and consumables were examined for evidence of tampering or damage upon receipt and prior to use, as appropriate. Supplies and consumables showing evidence of tampering or damage were discarded. All examinations were documented and supplies were appropriately labeled.

6.3 Instrument Calibrations

The project used established and approved operating procedures for the maintenance and calibration of all laboratory equipment. All laboratory measuring devices used in this project were certified as having been recently calibrated or were calibrated by the on-site EPA Metrology Laboratory at the time of use. Calibration of instruments was done at the frequency shown in Table 6-1. Any deficiencies were noted, and the instrument was replaced to meet calibration tolerances.

Table 6-1: Instrument Calibration Frequency

Equipment	Calibration/Certification	Expected Tolerance
Stopwatch	Compare against NIST Official U.S. time at http://nist.time.gov/timezone.cgi?Eastern/d/-5/java once every 30 days	±1 min/30 days
Clock	Compare to office U.S. time @ time.gov every 30 days	± 1 min/30 days
Scale	Check calibration with Class 2 weights	± 0.1% weight
Pipettes	Certified as calibrated at time of use/recalibrated by gravimetric evaluation of pipette performance to manufacturer's specifications every year.	± 5%
Meter boxes	Pretest calibration and post-test check for bias	± 5% bias

6.4 Critical Measurements

The following measurements were deemed critical to accomplish project objectives:

- Volume of air sampled
- Sampling time
- Incubation temperature
- Plated volume
- CFU counts.

The data quality indicators (DQIs) for the critical measurements are listed in Table 6-2. DQIs were used to determine if the collected data met the quality assurance (QA) objectives. Decisions to accept or reject test results were based on engineering judgment used to assess the likely impact of the failed criterion on the conclusions drawn from the data. The acceptance criteria were set at the most stringent levels that can routinely be achieved. All DQIs were within the target acceptance criteria set for this project as shown in Table 6-2.

Several QC checks were used for measurement instruments to ensure the data collected met the criteria listed in Table 6-2. The integrity of the samples during collection and analysis was evaluated. Validated operating procedures using qualified, trained, and experienced personnel were used to ensure data collection consistency. When necessary, training sessions were conducted by knowledgeable parties, and in-house practice runs were used to gain expertise and proficiency prior to initiating the research. The QC checks that were performed in this project are detailed in the following sections.

Table 6-2: DQIs and Acceptance Criteria for Critical Measurements

Measurement Parameter	Analysis Method	Accuracy	Acceptance Criteria	Mean Value Pass/Fail
Time	NIST-calibrated stopwatch	± 1 min per hour	± 2 min	Pass
Volumes	Serological pipette tips	0.1 mL	± 10% of target value	Pass
Counts of CFU per plate	QCount	1.82×10^4 < QC Plate < 2.3×10^4	Within range of QC plate	Pass
Plated volume (liquid)	Pipette	2%	± 1%	Pass
Temperature of incubation chamber	NIST-traceable thermometer (daily)	± 2 °C	± 2 °C	Pass
Sample volume (gas)	EPA Method 5 gas meter	Leak check before and after test	5%	Pass

In addition to the measurement instrument checks, positive control samples and procedural blanks were included along with the test samples so that optimal spore recovery and unintentional contamination of test coupons could be assessed. Replicate coupons were included for each set of test conditions to assess the variability of each test procedure.

6.5 NHSRC Biolab Quality Checks

Quantitative standards do not exist for biological agents. An Advanced Instruments QCount system was used to count viable spores. Counts generated that were either greater than 300 or less than 30 were considered outside of the targeted range. If the CFU count for bacterial growth did not fall within the target range, the sample was either filtered or replated. Replates and filter plates were enumerated manually.

A QC plate was analyzed before each batch of plates and was enumerated on the QCount. The result was verified to be within the range indicated on the back of the QC plate. As the plates were being counted, a visual inspection of colony counts made by the QCount software was performed. Obvious count errors made by the software were corrected by adjusting the settings (e.g., colony size, light, field of view) and recounting or by manually removing or adding colonies as needed.

The acceptance criteria for the critical CFU measurements were set at the most stringent level that could be achieved routinely. Positive controls were included along with the test samples in the experiments so that spore recovery from the different surface types could be assessed. Background checks were also included as part of the standard protocol to check for unanticipated contamination. Replicate coupons were included for each set of test conditions to characterize the variability of the test procedures.

Further QC samples were collected and analyzed to check the ability of the NHSRC Biolab to culture the test organism, as well as to demonstrate that materials used in this effort did not themselves contain spores. The checks included the following:

- **Field blank wipes:** wipes and Swiffer® cloths transferred between sampler and sample handler but not used to sample a material coupon.
- **Procedural blank coupons:** material coupons sampled in the same fashion as test coupons but not contaminated with surrogate organism prior to sampling.
- **Swabs of laboratory material coupons:** sterility swabs taken of the surfaces of all representative materials prior to the setup of a given test.
- **Stainless steel positive control coupons:** coupons inoculated in tandem with the test coupons and meant to demonstrate the highest level of contamination recoverable from a particular inoculation event.

Additional QC checks for Biolab procedures are shown in Table 6-3. These provide assurances against cross-contamination and other biases in microbiological samples.

Table 6-3: Additional Quality Checks for Biological Measurements

Sample Type	Frequency	Acceptance Criteria	Information Provided	Corrective Action
Inoculum control coupon: sample from stainless steel coupon contaminated with biological agent and sampled using wipe method	Three per test	1 x 10^7 for *B. atrophaeus* 50% relative standard deviation (RSD) between CFU recovered from first and last of each test set	Used to determine drift in the MDI	If outside range, identify and remove source of variability if possible
Positive control coupon: sample from material coupon contaminated with biological agent and sampled using wipe method	Three per test	1 x 10^7 for *B. atrophaeus* 50% RSD between coupons in each test set	Used to determine the extent of inoculation on the target coupon type	If outside range, identify and remove source of variability if possible
Procedural blank: coupon without biological agent that underwent the sampling procedure	One per test	Non-detect	Controls for sterility of materials and methods used in the procedure	Analyze extracts from procedural blank without dilution; identify and remove source of contamination if possible
Swab	One swab per test coupon	Non-detect	Controls for sterility of materials and methods used in the procedure	Analyze extracts without dilution to assess growth or no growth on the plate
Blank TSA sterility control: plate incubated but not inoculated	Each plate	No observed growth after incubation	Controls for sterility of plates	All plates incubated before use, so contaminated plates discarded before use
Replicate plating of diluted microbiological samples	Each sample	Reportable CFU of triplicate plates must be within 100%. Reportable CFU are between 30 and 300 CFU per plate	Used to determine the precision of the replicate plating	Replate sample
Unexposed field blank samples; a wipe kit will be transferred without handling	One per test	Non-detect	Level of contamination present during sampling	Clean up environment; sterilize sampling materials before use

6.6 QA Assessments and Response Actions

The QA assessment and corrective action procedures in this project were intended to provide rapid detection of data quality problems. However, some contamination in QC samples was observed after the completion of testing, as shown in Table 6-4. The few contaminations observed in the procedural blanks and the Swiffer® cloths resulted from intrinsic bacteria in the laboratory space. Swiffer® cloths were used out of the box and were not sterilized prior to experimentation.

The research team was unable to address the QC contamination issues prior to the completion of the experimental testing but does not believe they had a significant impact on the results. Project personnel were intimately involved with the data on a daily basis so that any data quality issue became apparent soon after it occurred. Corrective actions were taken as soon as practical when and if a problem was observed. The nature of the problem and corrective steps taken were noted in the project notebook of record.

Table 6-4: QA/QC Assessment

Spore Recoveries for the Various Sample Types (CFU per Sample)								
Test ID	Inoculum Controls		Positive Controls		Procedural Blanks			Field Blank
	Average	SD	Average	SD	Coupon	Sample Cloth	Via Cell®	
1	2.95E+07	2.29E+06	1.35E+07	5.70E+06	ND	5	1	ND
2	3.15E+07	6.73E+06	3.01E+07	4.25E+06	ND	ND	ND	ND
3	3.05E+07	9.67E+05	1.15E+07	2.46E+06	ND	ND	ND	ND
4	4.38E+07	1.05E+07	3.17E+07	7.63E+06	ND	ND	27	ND
5 (dry cloth)	3.02E+07	4.42E+06	2.42E+07	5.78E+06	1	220	ND	ND
5 (wet cloth)						ND	ND	ND
6 (dry cloth)	3.57E+07	8.27E+06	4.32E+07	1.10E+07	1	ND	ND	2
6 (wet cloth)						ND	ND	ND
ND: non-detect								

7 Summary

The objective of this study was to assess the impact of an off-the-shelf floor sweep/mop system as a potentially effective self-help approach for homeowners to reduce indoor exposure potential following a wide-area *B. anthracis* spore (anthrax) release. The results of this study are summarized as follows:

- The post-treatment sample from the inoculated area ("hot spot" location) in the center of a coupon yielded higher CFU/viable spore count than samples from all other areas adjacent to it.
- Swiffer® dry sweeping resulted in surface LR values between 2.06 and 2.11; the wet mopping operation resulted in a slightly higher LR, between 3.04, and 3.32; and the combination of dry sweeping/wet mopping resulted in the highest LR of the approaches evaluated, namely, 3.4, independent of type of material.
- The dry sweeping and wet mopping resulted in higher redistribution of the spores beyond the "hot spot" area as compared with the dry/wet sweep/mop approach.
- The effects of material (vinyl versus laminate) on the decontamination efficacy for all SSFMS modes (dry and wet operations) are minimal, and the sample recoveries are not statistically significantly different.
- The total recoveries (CFU) on the Swiffer® Sweeper® cloths (wet or dry) used during one treatment were within one order of magnitude of the initial spore counts inoculated on the coupons, independent of material (Student's t-test, p-value 0.44) and Swiffer® cloth type (Student's t-test, p-value 0.34). However, significant differences (Student's t-test, p-value 0.000, or zero probability under the null hypothesis) were observed in the average number of spores recovered on the dry Swiffer® cloths ($1.69 \times 10^7 \pm 7.1 \times 10^5$) compared to the spores recovered on the wet Swiffer® cloths ($1.04 \times 10^6 \pm 7.7 \times 10^5$) used during a dry sweeping/wet mopping operation.
- The high total recovery (CFU) is comparable to currently used sponge wipe surface sampling methods with the significant benefit of the ability to sample a larger (here, 35-in. x 35-in.) surface area than the 12-in. x 12-in.) sponge wipe reference method.
- A low level of spore reaerosolization was observed in most of the air samples for all the decontamination treatments, independent of material type or Swiffer® cloth type.

The highest LR of spores was found for wet mopping or a combination of dry/wet mopping, independent of material type. However, due to the high variability of test results, there was no statistically significant difference among treatments. This high variability suggests that cleanup with Swiffer® sweeping and/or mopping systems would give results that would be highly variable in real-world usage.

The presence of spores on the cloths in amounts similar to the initial spore counts indicates that the cloths are heavily contaminated following this cleaning approach. A homeowner would need to dispose of not only the cloths but also the mop to avoid cross-contamination of less contaminated areas. Recommended disposal steps should include inactivation of spores by e.g., soaking of the cloths in diluted bleach prior to disposal.

All approaches leave significant residual amounts of spores on the material surface. In the context of a Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) registration as a sporicide, the Swiffer® Sweeper® approach cannot be considered as an "effective" sporicidal surface decontamination treatment

as a 6 LR of spores has not been achieved. However, the sweeping and mopping effectiveness should be compared to other low-tech decontamination methods that could be used to reduce indoor exposure potential in less contaminated areas. Recently, the use of a robotic cleaner[3] on a laminate surface was reported with a similar number of spores recovered from a hot spot location as in this study. Data in this report will assist responders, governments, and health departments in deciding on recommendations of specific cleaning approaches.

8 References

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