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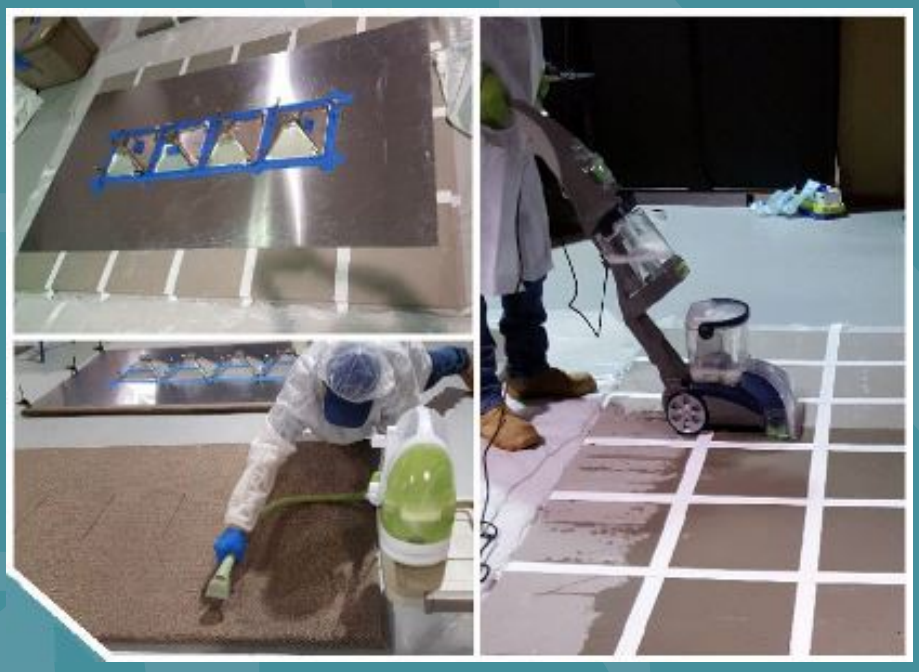
Evaluation of Commercial Wet Vacuums for *Bacillus* Spore Sampling on Surfaces



Office of Research and Development
Homeland Security Research Program

Disclaimer

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Executive Summary

This project supports the mission of the U.S. Environmental Protection Agency's (EPA) Office of Research and Development's (ORD) Homeland Security Research Program (HSRP). EPA's National Homeland Security Research Center (NHSRC) strives to accomplish the HSRP mission by providing information, expertise and products that can be widely used to prevent, prepare for, and recover from public health and environmental emergencies arising from terrorist threats and incidents.

The existing surface sampling strategy for *Bacillus anthracis* (*B. anthracis*) spore attack from a post-terror incident requires the use of various sampling methods, depending on the surface type (porous or nonporous). The established comparative surface sampling methods include wet wipes (for smooth nonporous surfaces), dry vacuuming (for rough and porous surfaces), and wet swabs (for small and/or hard to sample areas such as keyboards). The existing methods may be labor-intensive, costly, and time-consuming for wide area incident response.

The objective of this work was to develop and optimize a wet vacuum cleaner-based sampling method so that this widely-available commercial device could be used for sampling spores on both porous and nonporous surface types. Such a sampling device would use a liquid sampling medium that could be analyzed directly without an extraction step (spore recovery from the sampling medium) required for other surface sampling methods. This direct analysis could potentially increase recovery efficiency while reducing the sample analysis turnaround time and cost.

The main objective of this project was to assess an alternative cost-effective, reliable, commercially available (or built with off-the-shelf materials) wet vacuum cleaner that could be used for sampling *Bacillus* spores (i.e., surrogates of *B. anthracis*) on both porous and nonporous surfaces. The technical approach for this study involved bench-scale research, as part of Phase I tests, on the effectiveness of sampling liquids and operational parameters (such as elapsed time and liquid volume) to sample spores from different surfaces.

A custom-made vacuum sampling device (built with off-the-shelf materials) was used to sample spores from representative flooring surfaces. As part of Phase II tests, four classes of commercially-available wet vacuum, also known as carpet cleaners (portable, residential, commercial and wet/dry cleaners) were evaluated for spore sampling efficiency on nonporous surfaces (vinyl flooring) as well as porous surfaces (concrete and carpet). The evaluation criteria for down-selection of the vacuum cleaners to test included vacuum efficiency, availability, ease of use, ability to access remote areas and cost.

Phase I: Evaluation of Wet Vacuum Cleaner Operational Parameters

The Phase I study evaluated the sampling efficiency of wet vacuum cleaners as a function of liquid agent, elapsed time between liquid application and suction, and liquid volume used to perform the needed wetting process. Three material types were used for this evaluation: carpet, concrete, and laminated wood, inoculated with *Bacillus* spores (surrogates for *Bacillus anthracis*) of 2×10^6 colony forming units (CFU)/ft^2. The Phase I study results showed that

deionized (DI) water amended with Tween® liquid achieved the highest recovery among the tested liquid/material combinations with an average recovery of 53% on laminated wood coupons, while DI water resulted in the lowest average recovery of 9.3% when used with concrete coupons. The spore recoveries were found to be dependent on the recovered volumetric fraction of liquid sprayed onto the coupons. The highest recovered liquid volume was from laminated wood (72-80%), followed by carpet (39-49%), and lastly concrete (16-19%).

Tests were conducted to determine optimal elapsed time between liquid application and suction. The results showed that the elapsed time between the liquid spray application and wet vacuum sampling of the target material had little or no effect on the wet vacuum sampling spore recovery for both laminated wood (29 ± 3.9%), and carpet materials (31 ± 4.9%) over a range of 1 to 300 seconds. Due to rapid liquid absorption into the concrete paver surface, the elapsed time could not be varied for sampling efficiency. Concrete surface tests were conducted by dividing the sampling area per coupon into one (no division), two and four sections. The test results showed the increased spore recovery efficiency with more divided sampling area (quicker liquid retrieval). The spore recovery increased from an average of 15% for one section, 29% for two sections, to 59% for four sections per coupon.

The total volume of liquid sprayed onto carpet material had a strong effect on the overall spore recovery for a constant elapsed time between the time the liquid medium was sprayed and the time the surface was sampled. For carpet, the spore recovery increased from 3.4% to 31% when the liquid application volume was increased from 44 to 111 mL/ft^2. For laminated wood, the effect of the volume sprayed for the spore recovery seemed to be negligible, with an average recovery varying between 31 to 38% when the liquid volume was increased from 8.9 to 22 mL/ ft^2. For concrete, the liquid volume seems to have a negligible effect, if any, on the overall spore recovery with spore recoveries approximately 37-38% when the liquid volume was increased from 70 to 100 mL/ft^2.

The Phase I study concluded that the optimal wet vacuum sampling conditions were DI water amended with Tween® liquid, a short elapsed-time (less than 20 seconds) between liquid application and suction, and a minimum of 100 mL/ft^2 of liquid collected for optimum spore sample recovery.

Phase II: Commercially-Available Wet Vacuum Cleaner Evaluation

The Phase II study evaluated commercially available wet vacuum cleaners for surface spore sampling efficacy. The wet vacuums were selected by the project team considering the information from the Consumer Reports[1]. The selection criteria were ease of use, separate cleaning and recovery of (dirty) tanks, suction power, portability, cost and a heated cleaning option.

[1] Consumer Report. 2015. Carpet Cleaners of 2015. The reader will require subscription to Consumer Reports. https://www.consumerreports.org/products/carpet-cleaner/ratings-overview/

Phase II evaluated three classes (residential, commercial, portable) of commercially-available wet vacuum cleaners as well as a wet/dry vacuum (Shop-Vac) for their effectiveness in sampling *Bacillus* spores. Three material types were used for this evaluation: carpet, concrete, and vinyl flooring, inoculated with either *B. atrophaeus* var*. globigii* (*Bg*), or *B. thuringiensis* subsp. *kurstaki* (*Btk*), both surrogates of *B. anthracis,* at a spore concentration of 10^7-10^8 CFU/ft^2.

Each vacuum cleaner was evaluated based on cleaning patterns and time listed in the ASTM F-1284-09 (ASTM, 2009) standard method for evaluating dirt removal effectiveness of residential vacuum cleaners from carpet surfaces. The operational aspects and ratings were reviewed and the following three commercially available wet vacuum cleaners and one dry vacuum were selected for testing:

- Bissell Little Green portable wet-vacuum cleaner,
- Rug Doctor ProX3 commercial wet-vacuum cleaner,
- Hoover Dual Steam wet-vacuum cleaner,
- Shop-Vac wet/dry vacuum cleaner.

The sampling efficiencies of the selected wet vacuums were assessed by comparing their recoveries to the recoveries obtained by currently available surface sampling methods. Sampling efficiencies for porous surfaces (carpet and concrete) were determined by comparing the recoveries obtained by the four vacuum cleaners to the recoveries obtained by the existing sampling methods such as vacuum sock (carpet) and 37 mm cassette (concrete) (Calfee et al., 2013), respectively. For the nonporous surface (vinyl flooring), the wet vacuum sampling approach was compared to the Polyester Rayon Blend (PRB) wipe sampling method.

The overall results show that sampling via wet vacuum is a viable alternative to these traditional sampling methods. All wet vacuum cleaner spore recoveries were within the order of magnitude of the material-specific EPA-accepted sampling methods (PRB wipe, vacuum sock, and 37-mm cassette).

A two-way analysis of variance (ANOVA) was performed to examine the two different categorically independent variables (material type/sampling method) on the sampling efficacy of the wet vacuum cleaners for spores. The results of the analysis (72 samples: four vacuum cleaners, three materials, two surrogates, sampled in triplicate), demonstrated that the effect of the material type on the mean recoveries is not statistically significant for all the types of vacuum cleaners (F-value = 0.446, *p*-value = 0.642), while the effect of sampling methods on the mean recovery is statistically significant (F-value = 3.03, *p*-value = 0.036). The interaction of the two factors showed no significant difference in the mean recovery (F-value = 2.06, *p*-value = 0.07) at the 0.05 level.

The overall spore recovery efficiencies for the wet vacuum cleaners, independent of material and spore types, varied between 32 $\pm$ 20% for the portable, 25 $\pm$ 26% for Shop-Vac, 33 $\pm$ 17% for the residential, and 55 $\pm$ 52% for the commercial wet vacuum cleaner. In terms of both usability and repeatability, the operators chose the residential wet vacuum cleaner as a better sampling option over other tested cleaners for a wide area sampling of *Bacillus* spores.

This is based on the operators' assessment for its lowest RSD (51%), weight, application speed, and less prone to cross-contamination.

Contents

Figures

List of Tables

Acronyms and Abbreviations

ADA	Aerosol Deposition Apparatus
ANOVA	analysis of variance
B.	*Bacillus*
Ba	*Bacillus anthracis*
Bg	*Bacillus atrophaeus* var. *globigii*
BioLab	NHSRC RTP Microbiology Laboratory
Btk	*Bacillus thuringiensis* subsp. *kurstaki*
cm	centimeter(s)
CFU	Colony Forming Unit(s)
COTS	Commercial Off-The-Shelf
DI	Deionized
DTRL	Decontamination Technologies Research Laboratory
DQI	Data Quality Indicator
DQO	Data Quality Objective
EPA	U.S. Environmental Protection Agency
ft	foot (feet)
g	gravity
HEPA	high-efficiency particulate air
HSRP	Homeland Security Research Program
H_2O_2	Hydrogen Peroxide
in.	inch(es)
L	liter(s)
Lpm	liter(s) per Minute
m	meter(s)
MDI	Metered Dose Inhaler
µm	micrometer(s)
mL	milliliter(s)
mm	millimeter(s)
min	minute(s)_
MOP	Miscellaneous Operating Procedure
NHSRC	National Homeland Security Research Center
NIST	National Institute of Standards and Technology
OP	operating procedure
ORD	Office of Research and Development
OSB	oriented strand board
ppm	part(s) per million
PPE	Personal Protective Equipment
PRB	Polyester Rayon Blend
psi	pound(s) per square inch
QA	quality Assurance
QC	quality control
RMC	Reference Material Coupon

rpm	revolution(s) per minute
RSD	relative standard deviation
RTP	Research Triangle Park
sec	second(s)
TSA	tryptic soy agar
VHP®	Vaporous Hydrogen Peroxide

1. Introduction

The U.S. Environmental Protection Agency's (EPA's) Homeland Security Research Program (HSRP) strives to provide expertise and products that can be widely used to prevent, prepare for, and recover from public health and environmental emergencies arising from terrorist threats and other contamination incidents. HSRP conducts research to provide expertise and guidance on the selection and implementation of surface sampling methods that may ultimately provide the scientific basis for a significant reduction in the time and cost of sampling surfaces contaminated with *Bacillus anthracis*.

The currently-used surface sampling methods for biological agents include swabs, wipes, and vacuums fitted with filter-type collection media. Individual methods are material-dependent for application and limited in sampling area (1 – 4 square feet (ft^2) per sample). These methods may be physically demanding for application in a wide area incident. To improve the sampling capability for responding to a wide area incident, this study evaluated commercial wet vacuums as a sampling tool. The wet vacuums are applicable on both porous and nonporous surfaces, widely available, and easy to operate for collection of biological agents. In addition, the wet vacuum can sample more than 100 ft^2 per sample and generate liquid samples that may reduce the post-collection processing steps.

This study is composed of two phases: Phase I, Evaluation of Wet Vacuum Cleaner Operational Parameters and Phase II, Evaluation of commercially Available Wet Vacuum Cleaners. Phase I optimized the collection efficiency of a custom-made wet vacuum-based surface sampling device on both porous and nonporous surface types through a set of controlled operating parameters. The parameters tested for the wet vacuum sampling were type of liquid (Task 1), temporal lapse between liquid application and suction (Task 2), and liquid volume (Task 3). The vacuum-based sampling technique consisted of an optimized sampling nozzle along with a liquid dispenser and a liquid collection sample vessel that would eliminate post-collection processing since agents were to be captured directly into a liquid that could be analyzed. As part of Phase II tests, commercial wet vacuums were evaluated to determine their effectiveness for spore surface sampling on both porous and nonporous surfaces, using the results of Phase I. A field-usable operating procedure (OP) was developed, based on the results of the Phase II study.

2 Study Approach

The Phase I experimental approach consisted of selecting a sampling liquid, optimizing the temporal lapse between liquid application and suction, and evaluating the volume of liquid collected as a function of surface type. The outcomes of Phase I were assessed to determine the parameters resulting in an optimal sampling approach before moving on to Phase II for evaluating commercially-available wet vacuums. The experimental approach for each phase is presented in the next section.

2.1 Phase I: Evaluation of Wet Vacuum Cleaner Operational Parameters

A custom-made wet vacuum cleaner was designed to collect liquid samples from wet surfaces contaminated with *Bacillus atrophaeus* var. *globigii (Bg),* a surrogate for the spore-forming bacterial agent *Bacillus anthracis*. Three material types were investigated: carpet, concrete, and laminated wood. The parameters tested for the wet vacuum sampling were type of liquid, temporal lapse between liquid application and suction, and liquid volume. Spore recoveries from the tested vacuum samplers were compared to recoveries from Polyester Rayon Blend (PRB) wipe samples collected from stainless steel coupons.

Preliminary work under this project demonstrated that a typical wet vacuum cleaner collects between 1.5 and 2.5 liters per minute (Lpm) of liquid during sampling. Between 20% and 50% of the liquid applied was recoverable from carpet. Typical cleaning operation using a wet vacuum cleaner is performed at a rate of approximately 2.5 seconds per stroke. (ASTM, 2009) The test coupons were sampled at a linear speed of three to five seconds per square foot throughout the entire coupon. A 45-millimeter (mm) vacuum nozzle attached to a wet vacuum adapter was used to sample the coupons. Coupons were vacuumed by traversing the coupon using overlapping strokes in one direction, then again in a second direction at 90-degrees to the first.

2.1.1 Liquid for Sampling

Three types of liquid (deionized (DI) water, phosphate-buffered saline with Tween® 20 (PBST), and DI water with Tween® 20 at 0.05% concentration) were tested for sample collection on three material types (laminated wood, carpet, concrete) inoculated with a target spore surface concentration of 2×10^6 colony forming units (CFU)/square foot (ft^2). The test matrix for this task is shown in Figure 2-1.

Table 2-1. Sampling Liquid Test Matrix (Task 1)

Test ID	Material	Variable: Liquid types (ID code)
1A	Carpet	DI water (W), PBST (P), DI water with Tween® (T)
1B	Concrete	DI water (W), PBST (P), DI water with Tween® (T)
1C	Laminated Wood	DI water (W), PBST (P), DI water with Tween® (T)

2.1.2 Elapsed Time

Three different elapsed times for the liquid application and vacuuming were assessed. Based on the results from Task 1, this optimization test was conducted using only the most efficient extraction liquid, DI water with 0.05% Tween® 20 (DI-Tween). The total volume of liquid applied for sampling was 250 mL for carpet coupons, 400-500 mL for concrete coupons, and 100 mL for laminated wood coupons. Different liquid volumes per surface type were determined based on the collected liquid volume, targeting 50-100 mL for analysis. For carpet and laminated wood, the elapsed time between liquid spraying and collection was tested at 1, 30, 100, and 300 seconds (sec). For the one-sec elapsed time, the liquid was suctioned immediately following spraying.

For the concrete coupons, the surface liquid was retrieved immediately after the liquid spraying was completed. Due to the high liquid-absorptive nature of the material, tests were conducted in which the surface area was partitioned into smaller testing areas to increase the liquid volume for collection. For the first test, the entire 500 milliliter (mL) volume was sprayed onto the concrete coupon and then vacuumed. For the second test, each half of the coupon was sprayed and vacuumed consecutively using half of the volume (250 mL) on each half. In the third test, the coupon was sprayed and vacuumed consecutively in quarters, using 125 mL on each quarter. For each concrete test, each coupon's sample was cumulative (comprised of 1, 2 or 4 sub-samples, respectively) and the same liquid collection system was used. The test matrix is shown in Table 2-2.

Table 2-2. Elapsed Time Test Matrix (Task 2)

Test ID	Material	Volume Sprayed (mL)	Variable: Elapsed Times (sec)
2A	Carpet	250	1[b], 30, 100, 300
2C	Laminated Wood	100	1, 30, 100, 300
2B1	Concrete	1[a] x 500	1
2B2		2 x 250	1
2B3		4 x 125	1

[a]Indicates the number of concrete surface subdivisions to be vacuumed.
[b]1 sec elapsed time represents sampling that started immediately after spraying.

2.1.3 Liquid Volume

The objective of this test was to determine the effect of changing the volume of liquid applied for sampling on the spore recovery at a constant elapsed time. For each test, the volume of DI-Tween liquid was sprayed onto the carpet and the laminated wood coupons and remained for the prescribed elapsed time before vacuuming. For the concrete coupons, with a 1-sec elapsed time, each coupon surface was divided into quarters (2B3 from Table 2-2). The test consisted of four consecutive spraying and vacuuming combinations of each quarter, with the sample being cumulative and collected with the same custom-made vacuum device with a liquid collection system. The test matrix for this test is shown in Table 2-3.

Table 2-3. Liquid Volume Test Matrix (Task 3)

Test ID	Material	Variable: Volume Sprayed (mL)	Elapsed Times (sec)
3A	Carpet	100, 50	30
3C	Laminated Wood	50, 10	30
3B	Concrete	100 (4[a] x 25), 40 (4[a] x 10)	1
[a] Indicates the number of concrete surface subdivisions to be vacuumed.			

2.1.4 Material and Equipment

2.1.4.1 Wet Vacuum Sampling Device

The wet vacuum sampling device used in this evaluation was a prototype apparatus made from off-the-shelf components, including a wet vacuum adapter comprised of 1 meter (m) of latex tubing (Fisher Cat #: 14-178-2BB); a home-made 45-millimeter (mm) × 1-mm cross-sectional area-enhanced vacuum nozzle made of impact-resistant polycarbonate material (McMaster Cat. No. 1749K399) with tube fitting; and a Cord-Grip fitting connected to a 1-liter (L) Nalgene bottle (Fisher Cat. No. 02-543-03) with cap (see Figure 2-1). The wet vacuum sampling assembly was connected to a self-contained service vacuum pump (Omega Plus HEPA Vacuum pump, Atrix International Inc., Burnsville, MN) to provide the suction at 628 watts and a filtration efficiency of 99.9% at 0.3 micrometers (μm).

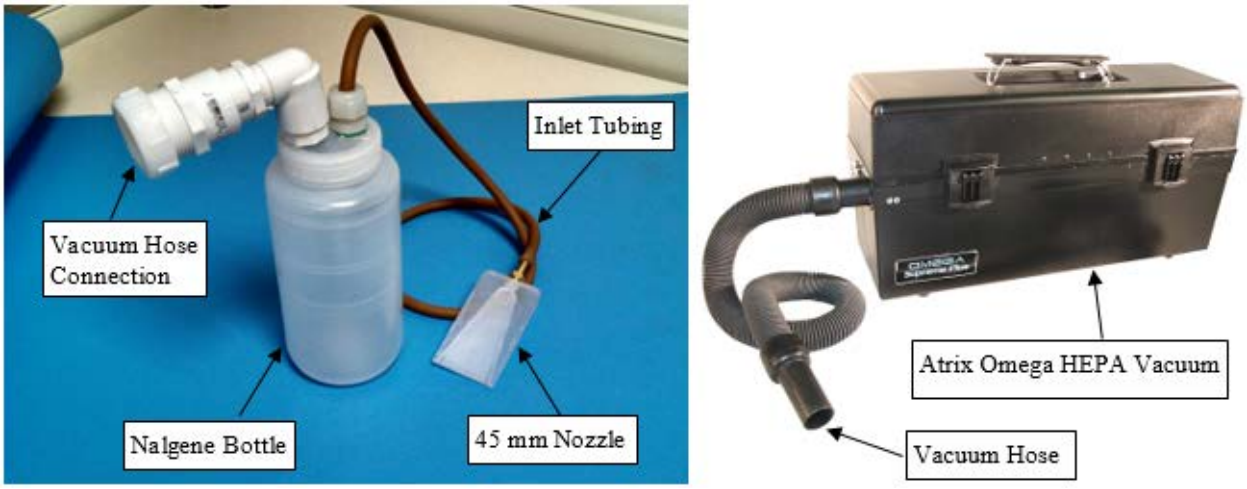

Figure 2-1. Wet Vacuum Sampling Assembly

Sampling liquid was sprayed onto a horizontally placed coupon and then vacuumed through the collection apparatus with the nozzle. The vacuumed liquid was collected in a clean, sterile Nalgene bottle. New, sterile nozzles and inlet tubing were used for each sample and coupon.

2.1.4.2 Liquid Agent Spray

A spray box apparatus (Figure 2-2) was designed to facilitate repeatable spraying of the 28 inches (in.)- x 28 in.-coupons and was equipped with a hinged cover with an 18 in.- x 18 in.-opening and a liquid collection vessel. The spray box lid opening was used as a template to ensure consistent sprays between coupons. The hinged cover was opened following the wetting of the coupons, to allow for vacuum sampling of a larger area.

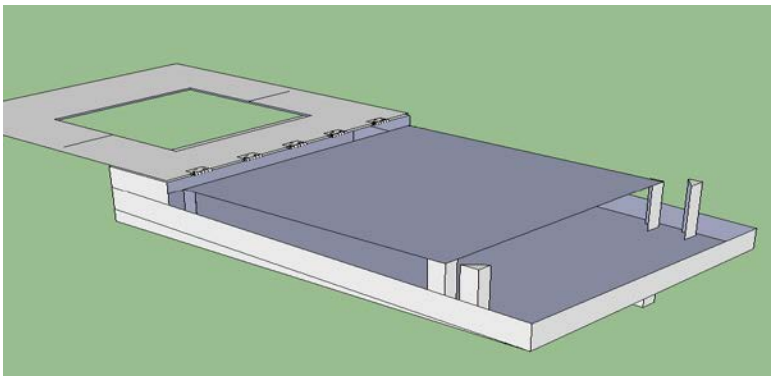

Figure 2-2. Spray Box with Lid in Open Position

Three liquids were evaluated in Phase I: DI water, PBST, and DI-Tween. A High purity DI water system (Dracor Water System, Durham, NC) was used to obtain DI water for each test in this study. The system features 1- to 0.2-µm × 10-in. pre-filters, acid-washed activated carbon, and two mixed-bed deionizers. The PBST solution was prepared by dissolving one packet of PBS with Tween® 20 (Cat. No. P-3563, Sigma Aldrich Corporation, St. Louis, MO) in 1 L of DI water. The final sampling solution, DI-Tween, was prepared by adding 500 µL of Tween® 20 (Cat. No. BP337-100, Fisher Scientific, Hampton, NH) to 1 L of DI water. All three solutions were sterilized and placed into two sterile 500-mL reagent Nalgene bottles using a 500-mL bottle top filter with a 33-mm neck and a 0.22-µm cellulose acetate filter (Part No. 431118, Corning Inc, Corning, NY).

Each sterile sampling solution was aseptically transferred to a clean backpack sprayer vaporous hydrogen peroxide (VHP®)-sterilized). The sampling liquid (~100 mL) was applied onto the center of each test coupon marked by an area of 16 in. × 16 in. This area is demarcated by a stainless-steel template (Figure 2-3). The entire coupon was then vacuumed using the custom-made device shown in Figure 2-1, performing five replicates for each test. The liquid collected was analyzed for spores, and the analytical results were compared to the number of spores recovered from the stainless-steel control coupons.

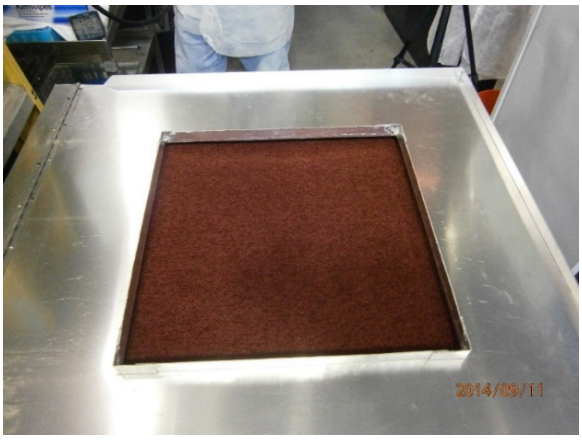

Figure 2-3: 16-in. x 16-in. Stainless-Steel Spray Template.

2.1.4.3 Electric Backpack Sprayer

The material coupons were wetted with the target wetting agent (DI water, PBST, or DI-Tween) using a rechargeable backpack sprayer (SRS-600 ProPack, SHURflo®, Cypress, CA) (Figure 2-4). The sprayer was maintained at a pressure of 35 pounds per square inch (psi) and a flow rate of approximately 1 L/minute (min) for most of the tests. The chemical-resistant backpack sprayer comes with a four-gallon tank and can spray up to 120 gallons on a single battery charge. Four pump speeds allow for adjustable spray patterns. For Phase I tests, the backpack sprayer pump was set at 35 psi and a target flow rate of approximately 1 L/min. The backpack sprayer was decontaminated by soaking the tank with pH-adjusted bleach for 10 min, then rinsing three times with the sterilized wetting agent. Separate backpack sprayers were used for each wetting agent.

Figure 2-4. Electric Backpack Sprayer

2.1.5 Coupon Preparation

Three material types (carpet, concrete, and laminated wood (28-in. × 28-in.) coupons) were investigated in this study. These materials were prepared following standard procedures for representativeness and uniformity so that tests could be reliably reproduced. Control coupons for inoculation of surrogate organism checks were made of stainless steel. Specifications for all test coupon materials and material preparation instructions are detailed in Table 2-4.

Table 2-4. Description of Test Coupon Materials and Material Preparation

Material	Description	Manufacturer/ Supplier Name, Location	Coupon Surface Size L x W x H (in)	Material Preparation
Carpet	100% Nylon Multiplicity Tile #54594	Shaw Industries, Dalton, GA	24 x 24 x 0.25	Remove wood particles using soft-bristle brush. Sterilize (VHP®)*
Concrete	Quikrete Type I & II Portland Cement; Quikrete All Purpose Sand	Lowe's Companies, Inc., Mooresville, NC	28 x 28 x 1	Remove particles by power washing. After power washing, allow to air dry in climate-controlled environment for at least five days. Sterilize (autoclave).
Laminated Wood	Winchester Oak Smooth Laminate Wood Planks	Lowe's Companies, Inc., Mooresville, NC	28 x 28 x 0.276	Remove particles and dust by wiping clean with water and wipe dry. Sterilize (VHP®)
Stainless Steel	Multipurpose Stainless Steel (48 in x 48 in), type 304, #2B mill (unpolished), 0.036 in thick	McMaster-Carr, Atlanta, GA	14 x 14 x 0.036	Remove any lubricant/grease from shearing with acetone and wipe dry. Remove particles and dust by wiping clean with water and wipe dry. Sterilize (autoclave).

*VHP® = Vaporized hydrogen peroxide.

2.1.5.1 Carpet Coupons

The carpet coupons (Figure 2-5) were prefabricated 24-in. x 24-in. (0.61 m by 0.61 m) 100% nylon tile, affixed in the center of a 28- by 28- by 7/16-in Oriented Strand Board (OSB) (Norbord Technology, Ville St. Laurent, Quebec, Canada) using an adhesive caulk (Model LN-601 CP, Liquid Nails® Adhesive, Strongsville, OH, USA). Coupons were clamped together and allowed to dry overnight before use.

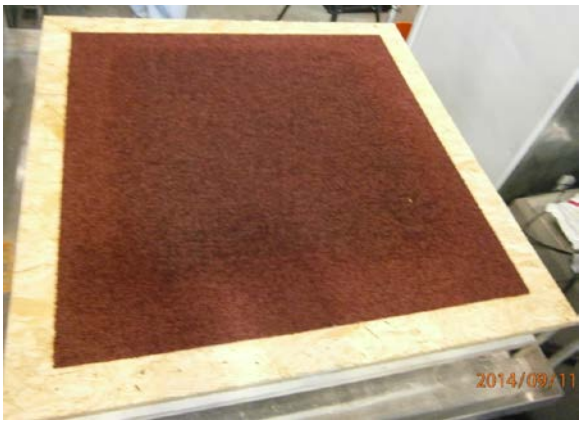

Figure 2-5. Carpet Coupon

2.1.5.2 Concrete Coupons

The concrete coupons were prepared on-site using QUIKRETE® sand/topping mix that consists of a uniformly blended mixture of Portland cement, commercial-grade sands, and other approved ingredients. The concrete coupons were made using custom 28 in.× 28 in. × 1-in. deep forms. The concrete was prepared according to the package instructions, using a trough and a garden hose for the water supply. Following preparation of the concrete, coupons were covered with plastic and allowed to cure for no less than five days before use (Figure 2-6).

Figure 2-6. Concrete Coupons

2.1.5.3 Laminated Wood Coupons

The laminated wood coupons (Figure 2-7) were cut to 28 in. × 28 in. from Project Source 7.6-in. × 4.23-ft Winchester oak smooth laminated wood planks. The coupons were glued to a 7/16-in.-thick OSB using an adhesive caulk (Model No. LN-601 CP, Liquid Nails® Adhesive, Strongsville, OH). Coupons were clamped together and allowed to dry overnight before use.

Figure 2-7. Laminated Wood Coupon

2.1.5.4 Stainless-Steel Coupons

The stainless-steel coupons (Figure 2-8) were cut to 14 in. x 14 in. from 48 in. x 48-in Multipurpose Type 304 stainless steel sheets using heavy duty power hydraulic shear (National Sheet Metal In., Smartt, TN). Disposable Manila paper templates, 12 in. x 12-in. (30.5 centimeters (cm) x 30.5 cm) (Part # 225-2416, SKC Eighty-Four, PA), were used to identify the inoculated area.

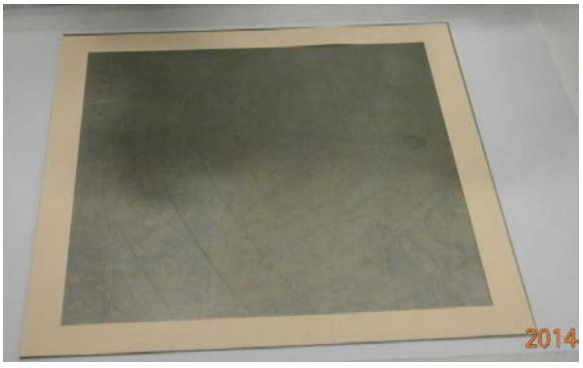

Figure 2-8. Stainless Steel Coupon

2.2 Phase II – Commercially-Available Wet Vacuum Cleaner Evaluation

Phase II evaluated three types (residential, commercial, portable) of commercially-available wet vacuum cleaners as well as a Shop-Vac cleaner for spore sampling. Each vacuum cleaner type was evaluated based on cleaning patterns and time listed in the ASTM F-1284-09 (ASTM, 2009) standard method for evaluating dirt removal effectiveness of residential vacuum cleaners from carpet surfaces.

2.2.1 Wet Vacuum Cleaners

The Phase II study evaluated commercially-available wet vacuum cleaners for surface spore sampling efficacy. The wet vacuums were selected by the project team considering the information from the Consumer Reports. The operational aspects and ratings were reviewed, and three commercially available wet vacuums and one wet/dry vacuum were selected. The wet vacuums were selected based on reviews regarding ease of use, separate clean tank (to

contain liquid before dispensed) and recovery tank (to contain dirty liquid from surface), suction power, portability, cost and a heated cleaning option. The down-selected vacuum cleaners are discussed in the following sections.

2.2.1.1 Residential Cleaners

Based on the Consumer Reports reviews of residential vacuum cleaners, the Hoover Dual V Steam Vac All Terrain with Spinscrub (Model No. F7452900, The Hoover Company, North Canton, OH), shown in Figure 2-9, was chosen for this study. This vacuum cleaner can be used on a variety of surfaces (hardwood flooring, laminated flooring and upholstery) as well as in challenging sampling situations (cold environments) by applying heat directly to the floor.

The Hoover F7452900 has brushes and two nozzles to deliver equal suction power across the width of the nozzle. The cleaning nozzle is approximately 13 in. wide. The wet vacuum cleaner has separate solution (clean) and recovery (dirty) liquid tanks (one-gallon capacity) as well as hand tools for cleaning hard-to-reach areas. The vacuum cleaner was set to "Wash Auto Rinse" mode (a unit function for spraying liquid automatically while vacuuming) during vacuum sampling.

Figure 2-9. Wet Vacuuming with Residential Vacuum Cleaner (Hoover)

2.2.1.2 Commercial Cleaners

Like the Hoover Steam Vac, the Rug Doctor Pro X3 (Rug Doctor, Inc., Plano, TX) commercial vacuum cleaner, Figure 2-10, can be used on a variety of surfaces such as hardwood and vinyl flooring. The Rug Doctor ProX3 comes pre-assembled and thus requires little preparation prior to sampling. The Pro X3 cleaning nozzle is approximately 10 in. wide with a vibrating brush for increased extraction power. It is equipped with separate solution (clean) and recovery (dirty) liquid tanks with a large capacity (four gallons), allowing for sampling larger areas in one sampling event. The vacuum cleaner was chosen for this study due to its wide availability and ease of use.

Figure 2-10. Wet Vacuuming with Commercial Vacuum Cleaner (Rug Doctor)

2.2.1.3 Portable Cleaners

Portable vacuum cleaners are more suitable for harder-to-reach areas. While they are not meant for cleaning entire rooms, portable cleaners are ideal for spot cleaning. The Bissell Little Green ProHeat machine (Model No. 14259, Bissell Corp, Grand Rapids, MI) (Figure 2-11) was chosen for this study. As with the other cleaners used in this study, the ProHeat also has separate clean and dirty tanks (48-ounce capacity) and comes with a built-in water heater for heated cleaning in cold environments.

Figure 2-11 Wet Vacuuming with Portable Vacuum Cleaner (Bissell ProHeat)

2.2.1.4 Wet/Dry Vacuum Cleaners

Wet-dry vacuum cleaners are available in a variety of sizes, ranging from 2.5 gallons to 14 gallons. To aid with portability and ease of use in this study, the mini wet-dry Rigid Pro Pack vacuum (Model No. WD4550, RIGID Tool Company, Elyria, OH) (Figure 2-12) was selected. The ProPack has a 2.5-gallon tank capacity but does not come with a liquid dispenser.

Figure 2-12. Wet Vacuuming with Wet/Dry Vacuum Cleaner (Rigid ProPack)

2.2.2 Phase II Coupon Preparation

Coupons of three materials (vinyl flooring, carpet, and concrete) were used in Phase II. The test coupon sizes were based on carpet test area sizes listed in the ASTM F-1284-09 (ASTM, 2009), standard method for evaluating the effectiveness of residential vacuum cleaners in removing dirt from carpet surfaces. A typical test coupon for Phase II, with the inoculated areas, is illustrated in Figure 2-13.

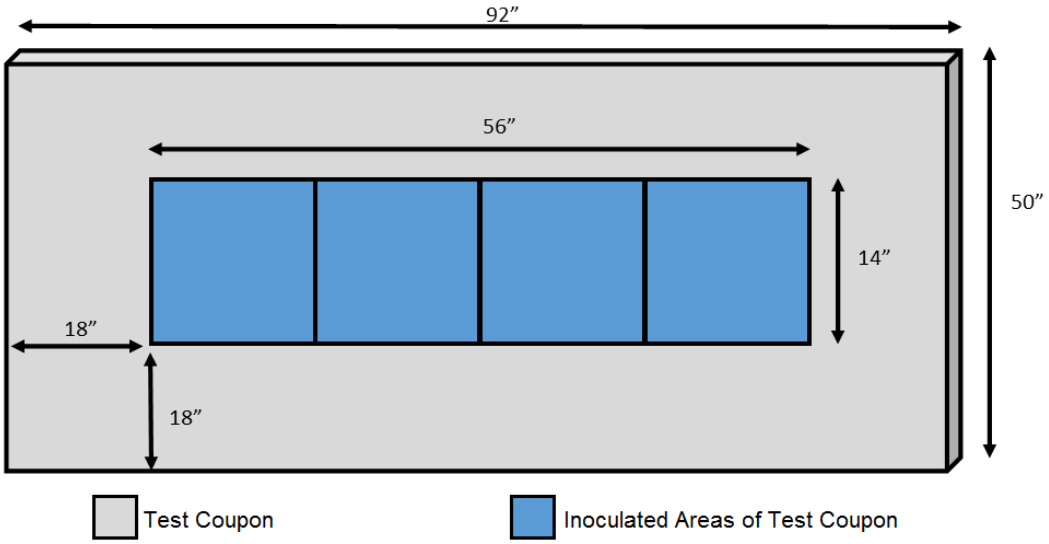

Figure 2-13 Phase II Test Coupon Schematic

Table 2-5 lists the characteristics of the test coupons used in this study.

Table 2-5. Phase II Material Description

Material	Manufacturer/Supplier Name (Location)	Test Coupon Size L x W (in.)	Control Coupon Size L x W (in.)
Concrete	16-in. x 16-in. pewter concrete step stone (Cat. No. 204659, Home Depot, Atlanta, GA)	96 x 48	16 x 16
Carpet	Beaulieu Solutions Laredo Sagebrush loop carpet (Cat. No. 409921, Home Depot, Atlanta, GA)	92 x 50	14 x 14
Vinyl	12-ft.-wide River Park staggered slate brown multi-vinyl sheet (Cat. No. 732233, Home Depot, Atlanta, GA)	92 x 50	14 x 14
Stainless steel	16-gauge type 304 mill-finished stainless steel (Dillon Supply Company, Raleigh, NC)	–	14 x 14

The test coupons were prepared following standard procedures for representativeness and uniformity so that tests could be reliably reproduced. Control coupons for inoculation of surrogate organism checks were made of stainless steel. Preparation of the test coupons for Phase II is summarized in the next sections.

2.2.2.1 Phase II Carpet and Vinyl Coupons

All carpet (Figure 2-14) and vinyl flooring (Figure 2-15) test coupons had a surface area of 32 ft^2 (92 in. x 50 in.) and were cut to size from larger (18 ft x 12 ft) sheets and glued onto plywood sheathing (Plytanium 15/32 CAT PS1-09 pine plywood sheathing, Home Depot, Atlanta, GA) using, respectively, TEC Skill Set carpet flooring adhesive (Model No. 7047485021, Lowe's, Durham, NC) and TEC Multi-Floor flooring adhesive (Model No. 7074255021, Lowe's, Durham, NC). The positive and negative control coupons for the same materials were 14 in. x 14 in. and fabricated in the same way as the test coupons but scaled down to size.

Figure 2-14. Carpet Coupon

Figure 2-15. Vinyl Coupon

2.2.2.2 Phase II Concrete Coupons

Due to their size and difficulty in transporting, concrete test coupons (Figure 2-16) were not fabricated in-house. Instead, 24 concrete pavers sized 16 in. x 16 in. (Cat. No. 204659, Pewter Concrete Step Stone, Home Depot, Atlanta, GA) were aligned to yield a larger test coupon, similar in size (96 in. x 48 in.) to the carpet coupons. The seams between the paver stones were covered with 2 in. duct tape to prevent the sampling liquid from seeping through. Concrete positive and negative control coupons consisted of a single concrete paver that was 16 in. x 16 in.

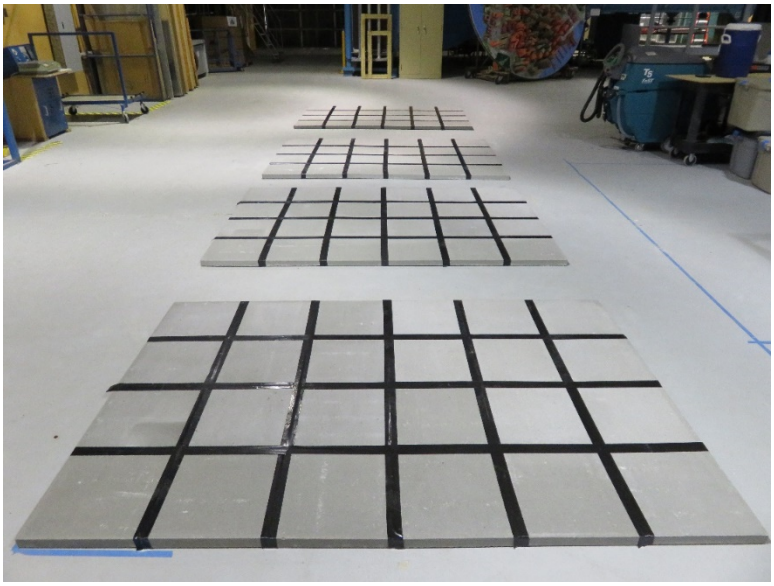

Figure 2-16 Concrete Coupons

All positive control and inoculation control coupons were tested in triplicate (i.e., three replicates per test). A negative control that was not inoculated but was subjected to the same sampling techniques as its inoculated counterpart was also included.

2.2.3 Test Matrix

The Phase II test matrix was developed as the tests progressed based on the results and ease of testing (sampling recovery, sterilization methods, and inoculation methods used). Test conditions were scaled up from Phase I laboratory results. The overall test matrix for commercial wet vacuum cleaner testing is shown in Table 2-6 for each surrogate contaminant (*Bg* and *B. thuringiensis* subsp. *kurstaki* (*Btk*)).

Table 2-6. Phase II Test Matrix

Target Spore (*Bg* and *Btk*) Concentration	Test ID	Material	Vacuum Cleaner Type	Vacuum Cleaner Model
10^7 CFU/ft^2	1	Carpet	Portable	Bissell Little Green
	2		Commercial	Rug Doctor ProX3
	3		Residential	Hoover Dual Steam Vac
	4		Wet/Dry	Shop-Vac
	5	Concrete	Portable	Bissell Little Green
	6		Commercial	Rug Doctor ProX3
	7		Residential	Hoover Dual Steam Vac
	8		Wet/Dry	Shop-Vac
	9	Vinyl	Portable	Bissell Little Green
	10		Commercial	Rug Doctor ProX3
	11		Residential	Hoover Dual Steam Vac
	12		Wet/Dry	Shop-Vac

2.3 Testing and Sampling Approaches

The general testing approach, for both Phase I and Phase II testing, consisted of inoculating coupons with *Bacillus* spores, using an aerosol deposition method that delivered approximately 10^6-10^7 spores of a surrogate organism for *B. anthracis* on a material surface. The inoculated material surfaces underwent the vacuuming process, and recovery of spores from treated surfaces (test samples) was compared to recovery from stainless steel surfaces that were inoculated but not treated (inoculation control samples). Surface sampling efficacy was calculated as the difference between the average inoculation control recoveries and the post-sampling recovery on each treated surface (test sample).

The approach for all tests followed the layout shown in Figure 2-17. Coupons for a selected test material (concrete, carpet, or laminated wood) were inoculated and sampled using the wet vacuuming (Wet Vac) method, yielding liquid samples for microbiological analysis. Wet Vac samples were also collected from blank (un-inoculated) material coupons, and these are referred to as Test Blanks.

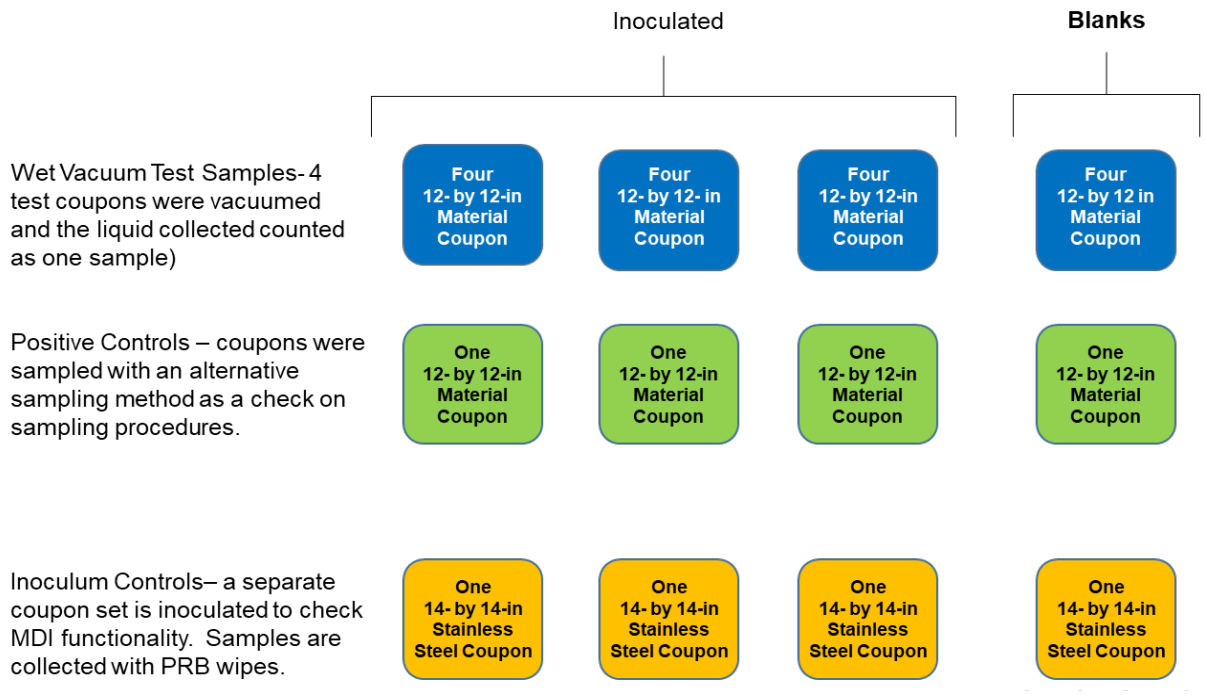

Figure 2-17. Sampling Approach

Positive control coupons that consisted of the selected test material were also inoculated. These samples were collected using an alternative (non-wet vacuum) sampling method, which served as a check on sampling procedures.

Each test included inoculum controls designed to check metered dose inhaler (MDI) performance consistency. All inoculum controls consisted of stainless steel coupons sampled with PRB wipes, which were processed for microbiological analysis. An un-inoculated stainless-steel coupon was also wipe-sampled for sterility, and it is referred to as the Inoculum Control Blank. Blanks were included for each sampling method to check for cross-contamination.

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

- **Preparation of representative coupons of test materials:** Tests used coupons made of carpet, laminated wood or vinyl flooring, and concrete. Coupons for Phase I had a surface area of 784 in^2 (28- by 28-in), described in Section 2.1.5, while Phase II used larger coupons with a surface area of 4600 in^2 (92- x 50-in.), described in Section 2.2.2.
- **Sterilization of the coupon materials:** Prior to use, the coupons were wrapped in Tyvek bags and sterilized using a 4-hour Vaporous Hydrogen Peroxide (VHP®) sterilization cycle at 250 ppm (Section 2.3.1).
- **Inoculation of coupons:** Test coupons were inoculated using an aerosol deposition method, as described in Section 2.2.3. Briefly, a known quantity of the surrogate

organism (10^5 - 10^7 *Bg* or *Btk* spores) was deposited onto a coupon using an MDI. The inoculation occurred a minimum of 18 hours prior to testing.

- **Wet vacuum cleaner sampling process:** The wet vacuum sampling approach for Phase I described in Section 3.1 consisted of applying a sampling liquid to the test coupon via a backpack sprayer and using a custom-made vacuum device to recover the liquid and the spores. For Phase II, the vacuum sampling approach consisted of using commercial off–the-shelf (COTS) wet vacuum cleaners, following the sampling procedure described in Section 3.2. Inoculation control coupons underwent sampling techniques involving PRB wipes, 37 mm cassettes and vacuum socks.
- **Sampling Evaluation:** The spore recovery efficiencies relative to the number of spores deposited was estimated for each of the four wet vacuum cleaners, and for each material-specific traditional surface sampling method. To account for differing inoculation levels achieved across numerous test days and MDIs, recovery was compared to the inoculum control coupon inoculated using the same MDI and collected on the same test day as the test samples.

  Spore Recovery Efficiency = (Test Wet Vacuum Recovery/Inoculum Check Recovery) x 100
- **Sample Sterility Evaluation:** Swab samples collected from materials prior to testing were analyzed (growth/no growth) to demonstrate sterility of the test materials.

2.3.1 Material and Equipment Sterilization

Test coupons were placed in Tyvek® bags (steam component bags, General Econopak, Philadelphia, PA) prior to sterilization. Batches of carpet and laminated wood coupons were exposed to 250 ppm hydrogen peroxide (H_2O_2) vapor for four hours using a STERIS VHP® ED1000 generator (STERIS Corporation, Mentor, OH). Stainless-steel coupons were sterilized for 30 min in an autoclave cycle; the concrete coupons were used as is. After sterilization, the coupons treated with VHP® sat for up to two weeks at room temperature to force off-gassing of H_2O_2 and prevent any biocidal effects. Sterility was evaluated by swab sampling one coupon from each sterilization batch. Any dust or debris on each concrete coupon was removed using a clean RIGID Pro Pack vacuum cleaner (Model No. WD4550, RIGID Tool Company, Elyria, OH).

2.3.2 Spore Preparation

The test organisms for this work were powdered spore preparations of *Bacillus atrophaeus* var*. globigii* (*Bg*), and *Bacillus thuringiensis* subsp*. kurstaki* (*Btk*), obtained from the U.S. Army Dugway Proving Ground Life Sciences Division (Dugway, UT). The powdered spore preparation for this procedure is described elsewhere (Brown et al., 2007).

2.3.2.1 *Bacillus atrophaeus* var. *globigii*

Bg, a surrogate for the spore-forming bacterial agent *Bacillus anthracis,* was used for this project. Like *B. anthracis*, *Bg* is a soil dwelling, Gram-positive, spore forming, aerobic microorganism, but unlike *B. anthracis*, it is non-pathogenic. *Bg* forms an orange pigment when grown on nutrient agar, a desirable characteristic when there is a need to detect viable spores in

environmental samples. *Bg* has a long history of use in the biodefense community as a simulant for anthrax-associated biowarfare and bioterrorism events (Gibbons, et al., 2011).

2.3.2.2 *B. thuringiensis* subsp. *Kurstaki (Btk)*

Btk, another surrogate for *B. anthracis,* was also used for this project. Like *Bg*, *Bacillus thuringiensis* strains are not considered human pathogens, but unlike *Bg*, *Btk* produces no orange pigment when grown on nutrient agar such as tryptic soy agar (TSA). *Btk* colonies are whitish, round to irregular in shape, and have a matte or opaque texture. *Btk* is also known for parasporal crystal formation, which makes it useful as a biopesticide. Multiple *Btk* strains are registered with the USEPA as biopesticides (USEPA, 2017), and strain HD-1 is found in commercial products, like Foray, used to control gypsy moths (Valaderes et al., 2001).

Strain HD-1 has also been used to develop genetically tagged strains, whereby unique and stable genetic signatures or "barcodes" have been integrated into non-protein coding regions of the chromosome (Emanuel et al. 2012; Buckley et al. 2012). This barcoding system facilitates detection of *Btk* by real-time PCR assay, even in the presence of non-tagged background *Btk* or other *Bacillus* species. This project utilized *Btk* with barcode T1B2 (Buckley et al. 2012).

2.3.2.3 MDI Preparation

MDIs were used to inoculate material surfaces with spore preparations of either *Bg* or *Btk*. Dry spores received from Dugway Proving Ground were resuspended in 100% ethanol, then combined into each MDI canister with 1,1,1,2-tetrafluoroethane (HFA-134a), a non-ozone depleting propellant. Each MDI was charged with a volume of spore preparation plus propellant sufficient to deliver 200 discharges of 50 µL per discharge. The number of discharges per MDI was tracked to ensure that the use did not exceed this value.

2.3.2.4 MDI Spore Concentration Validation and Spatial Distribution

Following the manufacturing process, MDIs were tested for concentration and spore quality. MDIs were actuated to deposit either *Bg* or *Btk* spores on five 18-mm aluminum coupons. The MDI canister (Figure 2-18, panel A) was situated inside an actuator (Figure 2-18, panel B) and fitted into an adapter (Figure 2-18, panel C) that securely held the coupon so that each time the actuator was depressed, a repeatable number of spores was deposited on the coupon (Lee et al., 2011).

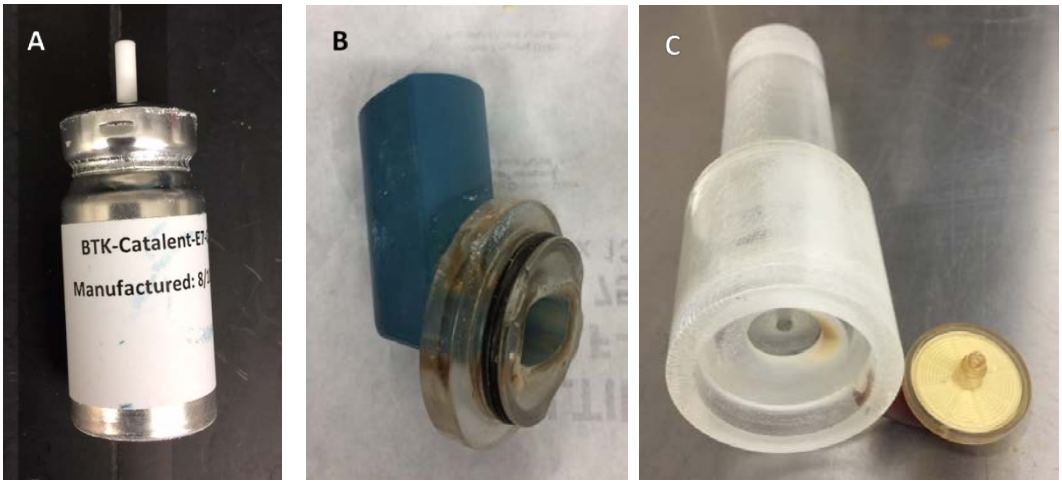

Figure 2-18. MDI Actuator Adapter for Small, 18 mm Coupons (A), Catalent MDI Canister (B), Actuator Adapter (C)

After inoculation, coupons were placed into sterile 50-mL conical tubes, then extracted with 10 mL of sterile phosphate buffered saline with Tween® 20 (PBST) by sonicating for 10 min and vortexing continuously for two min. Following extraction, a 5-mL volume was transferred into a fresh 50-mL conical tube and heat-treated in an 80 °C water bath for 10 minutes. Aliquots from both non-heat-treated and heat-treated tubes were then spiral-plated (Autoplate 5000, Advanced Instruments, Norwood, MA) in triplicate on TSA plates. Plates were enumerated with the QCount (Advanced Instruments, Norwood, MA).

Mean results (CFU/mL) for each of the five 18-mm coupons were averaged and then multiplied by the total volume (10 mL) to determine the MDI concentration per actuation. The percent relative standard deviation (% RSD) was calculated by dividing the standard deviation by the average mean, then multiplying by 100. Spore quality was estimated by comparing heat-treated results to non-heat-treated results for each canister. MDIs with a % RSD less than approximately 50% and a spore quality score of greater than approximately 85% were put into the inventory for testing.

Selected MDIs (*Bg* canister #1 and *Btk* canister #1) were also actuated to determine the spray distribution over the center section (approximately 12 in. x 12 in.) of a 14-in. x 14 in. test coupon. Forty sterile reference material coupons (RMCs, 1” x 2”) were manually arranged in a grid (5 x 8 RMCs) on a sterile 14 in. x 14 in. stainless-steel coupon as shown in Figure 2-19. After RMC placement, the coupon was covered with an Aerosol Deposition Apparatus (ADA) (Calfee et al., 2013a) like the ADA shown in Figure 2-20.

Figure 2-19. RMCs Placed on 14 in. x 14 in. Stainless Steel Coupon

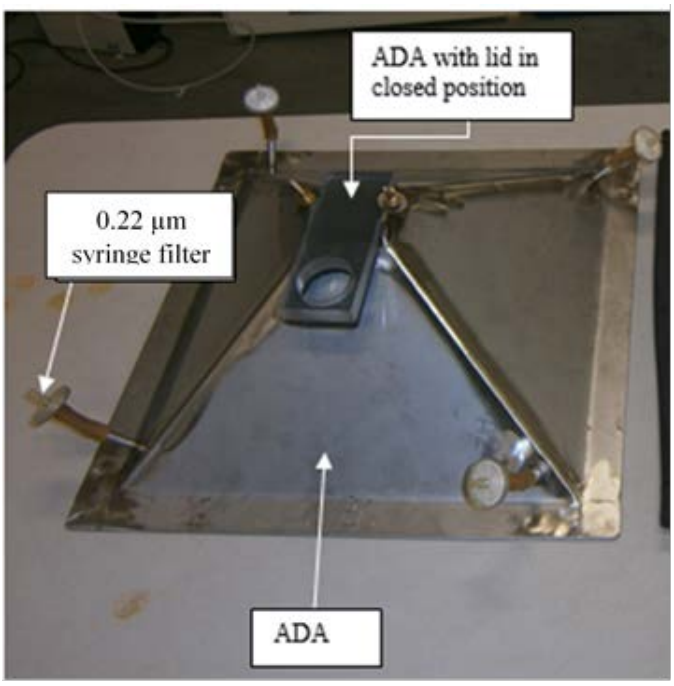

Figure 2-20.ADA Used on 14 in. x 14 in. Stainless Steel Coupon

To inoculate the RMCs, each MDI canister was placed in a 50-mL conical tube adapter that was attached to a Vortex Genie (Part #EF3030A, Daigger Scientific, Vernon Hills, IL), then vortexed (see Figure 2-21, panel A) vertically (stem up) for two min at top speed. The MDI was rotated in the adapter (stem down) and vortexed for another two min, then purged three times with a 10-second side vortex between each purge (see Figure 2-21, panel B).

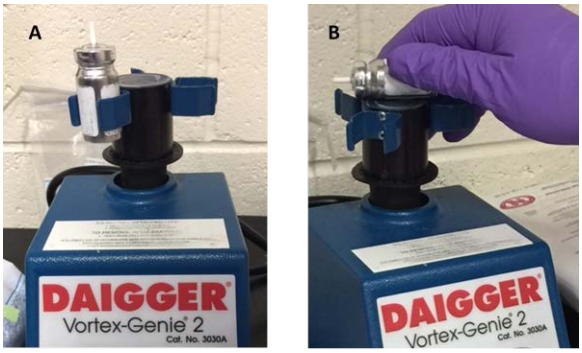

Figure 2-21. MDI Content Mixing and Purging Prior to Inoculation

After purging, the MDI was placed on the ADA and discharged once. Spores were allowed to settle overnight, and the RMCs were collected into individual 50-mL conical tubes. After addition of 20 mL sterile PBST, RMC tubes were sonicated for 10 min, then vortexed continuously for two min. Aliquots were spiral-plated on TSA plates and incubated at 35 °C ± 2 °C for 18 to 20 hours (*Bg*) or 28 °C ± 2 °C for 18 to 22 hours (*Btk*). Plates were enumerated by QCount. Results were used to develop heat maps, like the *Bg* MDI example shown in Figure 2-22.

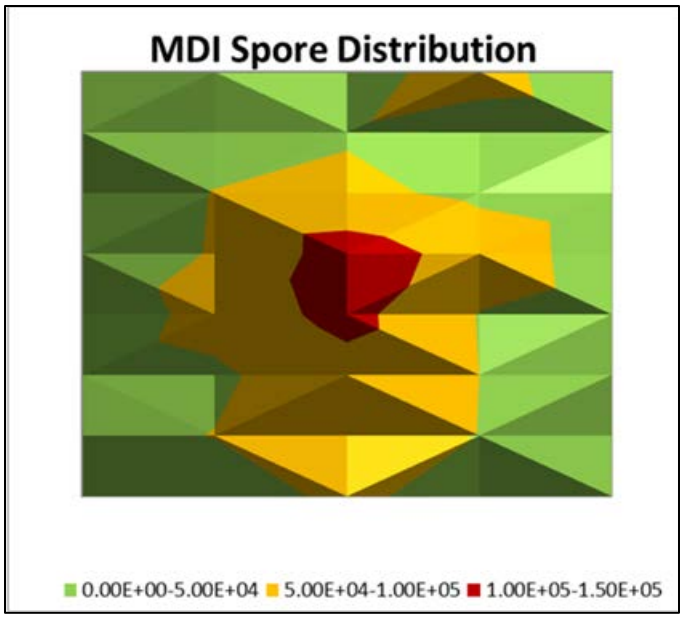

Figure 2-22. Color Coded MDI Spore Distribution Heat Map over 40 RMCs after Actuation of *Bg* Canister #1 (CFUs per RMC coupon, 2 in^2)

While the highest spore counts were observed on RMCs placed near the middle of the test coupon, spores were also distributed along the outer edge of the test area. Cumulative recovery across all RMCs was 1.6 x 10^6 CFU, which was approximately 27.6% of the average 5.79 x 10^6 CFU/actuation observed during the 18-mm aluminum coupon test for *Bg* canister #1. The higher recovery for the 18-mm coupon test, compared to the cumulative RMC recovery, was accounted for as follows: 1) variations in recovery efficiencies for 18-mm coupons and RMCs (i.e., losses to the pyramid surfaces), and 2) losses due to the spaces between the RMCs, which were inoculated but not recovered during the RMC test. Similar results were obtained for the selected *Btk* MDI (canister #1). Cumulative recovery across all *Btk* Canister #1 RMCs was 3.68 x 10^6 CFU, which was approximately 26.9% of the 1.37 x 10^7 CFU/actuation observed during the 18-mm coupon test.

2.2.3 Inoculation of Coupons

Test coupons for Phase I were inoculated with approximately 10^6-10^7 aerosolized spores on the same day using an aerosol deposition method (Figure 2-23). A sterile stainless-steel skirt with the same dimensions as the test coupon, except for a 14-in. x 14-in. area cut open in its center, was placed on each coupon to maintain sterility of the coupon surface that was not inoculated. A single ADA was placed over the open 14-in. x 14-in. area in the center of the skirt of each test coupon.

Figure 2-23. Phase I Carpet Coupon with Skirt and ADA.

Each ADA was designed to cover a 14-in. x 14-in. area of any coupon of any thickness. Just prior to dosing, each ADA lid was opened, and an actuator with the MDI was placed in the opening and depressed to release the spores (Figure 2-24). The ADA lid was closed after inoculation.

The same approach was used for the inoculation of Phase II test coupons. A sterile stainless -steel skirt with the same dimensions as the test coupon, except for a 56-in. x 14-in. area cut open in its center, was placed on each coupon to maintain sterility of parts of the coupon surface that were not inoculated. Four ADAs were placed over the open 56-in. x 14-in. area in the center of the skirt of each test coupon. Skirts were used only for the tests that involved carpet coupons. Vinyl flooring and concrete coupons were covered using only four ADAs per test coupon. The carpet test coupon layout inoculation is shown in Figure 2-24. For

the paver stones, spores were inoculated on the taped surface. The estimated taped surface area is approximately 10% of the entire inoculated paver area.

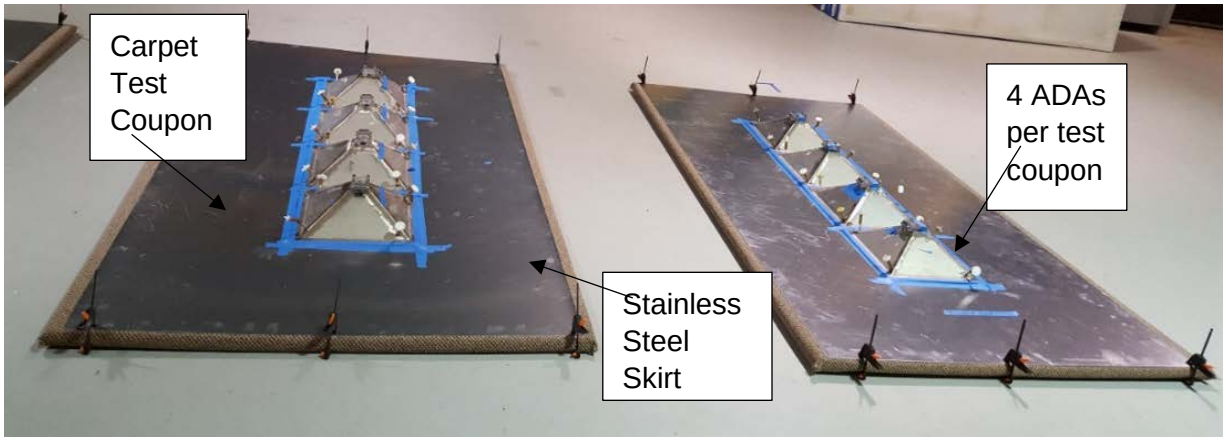

Figure 2-24. Inoculation of Phase II Test Coupons

Stainless steel coupons (14 in. x 14 in.), which served as quality control (QC) checks for the MDI and each inoculation event, were also inoculated as the first, middle, and last coupons during an inoculation event. Coupons were inoculated a maximum of 48 hours and a minimum of 18 hours prior to the sampling event

Positive control coupons were sampled using a non-wet vacuum, an alternative sampling method to check on sampling procedures. Carpet samples were collected using vacuum socks, and concrete samples were collected using 37-mm cassette filters.

3 Sampling Procedures

Prior to the sampling event, all materials needed for sampling were prepared using aseptic A two-person sampling team, using aseptic techniques whenever possible, was designated to perform the sampling procedure following a strict sampling protocol that listed each person's role during the sample test preparation, sampling, and sample handling. The sampling procedure consisted of the operations described below and were performed in sequence.

3.1.1 Wet Vacuum Sampling Kit Preparation

The wet vacuum sampling kit was prepared prior to the start of each testing sequence. The following procedures were followed by a two-person sampling team designated as sampler and support person:

1. The support person wears new gloves, opens the sampling supply bin and removes the sample kit from the bin.

2. The support person opens the outer bag and allows the sampler to remove the nozzle and tubing from the kit.

3. The support person removes the sterile Nalgene bottle from the overpack bag and allows the sampler to remove the cap and place it back in the overpack bag.

4. The support person holds the Wet Vacuum Adapter so the sampler can install the latex tubing from the bottom of the adapter through the Cord-Grip fitting (Figure 3-1). The sampler leaves approximately 3-4 in. of tubing below the cap and connects the tubing to the nozzle fitting once threaded (Figure 3-2).

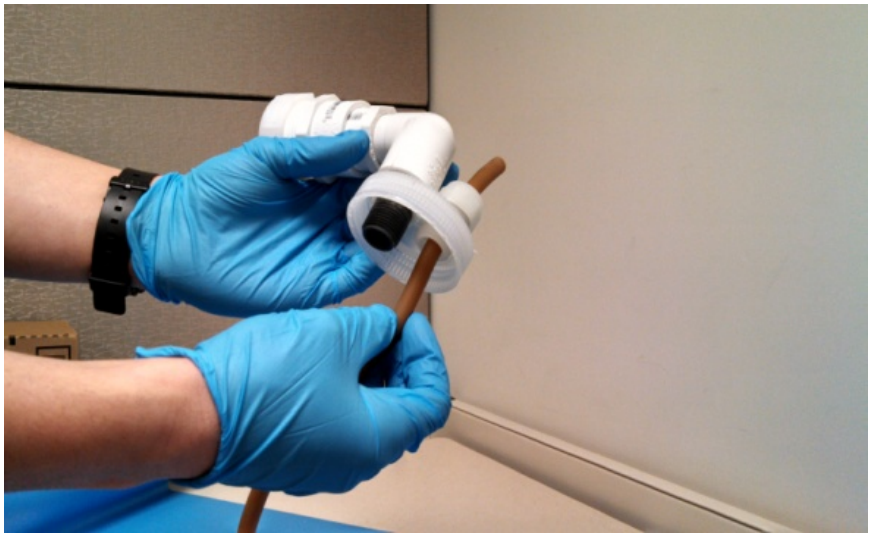

Figure 3-1. Wet Vacuum Adapter

Figure 3-2. Uncapped Wet Vacuum Sampling Kit

5. The support person places the wet vacuum adapter onto the sterile Nalgene bottle, taking care not to touch the inside of the bottle, the latex tubing, or the Cord-Grip fitting used to connect the latex tubing to the apparatus.

6. The support person connects the Atrix Vacuum Nozzle to the Wet Vacuum Adapter fitting. The wet vacuum sampler is then ready for sampling.

The wetting and sampling operations were performed by the same team that prepared the wet sampler kit, with the addition of an assistant on an as-needed-basis. The sampling team followed a strict protocol for sampling (Calfee et al., 2013b) as summarized below.

3.1.2 Spraying Sequence

1. The three-person sampling team members each don new Personal Protective Equipment (PPE) before beginning a sampling set. A single set of clean lab-coat and gloves is worn unless a change is required due to:

    - a material type change (e.g., stainless steel to carpet),
    - going to a lower contamination level (positive controls to test samples),
    - any possible contamination of the current PPE at the sampler's discretion, or
    - when the contamination level is unknown.

2. The support person removes the sterilized test coupon from the Tyvek® bag and places it on the spray box (Figure 2-2) with the lid in the open position, then removes the ADA. The support person then closes the spray box lid.

3. The sampler and support person ensure that the correct sample coupon has been selected, referencing the coupon code on the sampling bag.

4. The support person or assistant records the coupon code (when required) on the sampling log sheet next to the corresponding bag number that was just recorded.

5. With the vacuum nozzle in one hand, the sampler uses their free hand to spray the 18-in. x 18-in. center area of the coupon in an evenly dispersed pattern until the required volume of wetting agent has been dispensed.

    a. The spray wand nozzle is held at a 90° angle and 3 in. above the spray box lid opening. The spray box lid has an 18-in. x 18-in. opening that served as a template to ensure consistent sprays between coupons. Once the spraying is completed, the sprayer wand is holstered in a manner that keeps the nozzle from contacting any surfaces.

    b. Each pass of the nozzle overlaps 50% with the previous pass.

    c. Each coupon is sampled at a rate of approximately three min per coupon.

3.1.3 Sampling Sequence

1. At the end of the spraying sequence, the support person then fully opens the lid of the spray box, exposing the coupon for sampling.

2. The support person or assistant is prepared to record the duration of sampling. Prompts are given to the sampler so that the sample duration is as close to the value indicated in the test plan as possible.

3. The sampler:

    a. Checks the fitting to the nozzle and adjusts, if necessary.

    b. Turns on the vacuum.

    c. Vacuums “horizontally” from one side of the coupon to the other, starting from the lower right corner of the coupon (Figure 3-4). The sampling covers the center 20- by 20-in. area (or 26- by 26-in. area, depending on the coupon) of the material surface, and the nozzle is kept at a 45-degree angle. The nozzle width is used to estimate a 2-in. border around the coupon. The nozzle is pushed forward so that the coupon is sampled in the direction of the larger angle (135 degrees). Each pass of the coupon with the vacuum overlaps 50% with the previous pass.

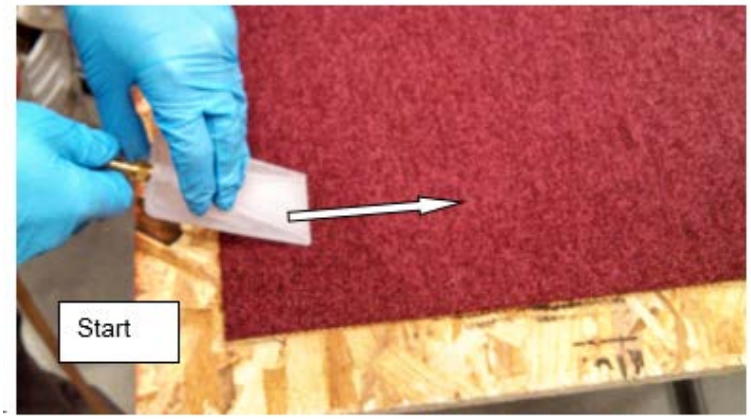

Figure 3-3. Wet Vacuum Sampling

d. The same area is vacuumed “vertically” using the same technique.

e. After sampling, the sampler lifts the nozzle so that the nozzle is one meter directly over the Nalgene bottle, with the tubing taut, so that the tubing is clear of liquid.

3.2 Phase II: Commercially Available Wet Vacuum Cleaner

All test vacuum cleaners, regardless of nozzle width, were moved back and forth in a specified pattern as shown in Figure 3-4. Briefly, each test coupon was divided into N strips, with N being half the width of a vacuum cleaner nozzle. The wet vacuum cleaner was placed on the sampling area so that the front edge of the vacuum cleaner nozzle lip coincided with the line defining the beginning of the sampling area. The first vacuuming stroke was a backward stroke, while DI-Tween was simultaneously sprayed onto the coupon surface. The second stroke was a forward vacuum stroke to vacuum any residual liquid not covered by the first stroke. Next, the vacuum nozzle was moved horizontally by half the size of the width of the nozzle, so that the previous vacuum path was overlapped. This process was repeated until the entire surface of the test coupon was covered.

The wet vacuuming sampling protocol is summarized below:

1. Don a fresh pair of sterile boot covers before stepping back into the sweep area.
2. Charge each vacuum cleaner with approximately 1 L of 0.05% Tween® 20. Record all volumes in the laboratory notebook.
3. Place the vacuum cleaner nozzle so that the front edge of the vacuum cleaner nozzle lip coincides with one edge of the test coupon, as shown in Figure 3-4.
    a. Stroke 1: Backward stroke until reaching the end of the opposite edge of the test coupon.
    b. Stroke 2: Forward stroke until the initial start location is reached.
    c. Move the vacuum cleaner horizontally by half of the nozzle width area.

d. Repeat the process (steps a–c) for the next sampling strip until the entire coupon is reached.

e. Immediately post-sampling, aseptically transfer the liquid from the wet vacuum "dirty" tank to a pre-labeled clean Nalgene bottle. Record the volume collected in the laboratory notebook.

f. Sterilize the outside of the Nalgene bottle using Dispatch® bleach wipes (Caltech Industries, Inc., Midland, MI) and place the bottle in a secondary containment unit such as a Twirl'Em® Sterile Sampling Bags (Labplas, QC Canada). Sterilize the outside of the secondary containment unit using bleach wipes.

g. Place the whole sample into the collection bin.

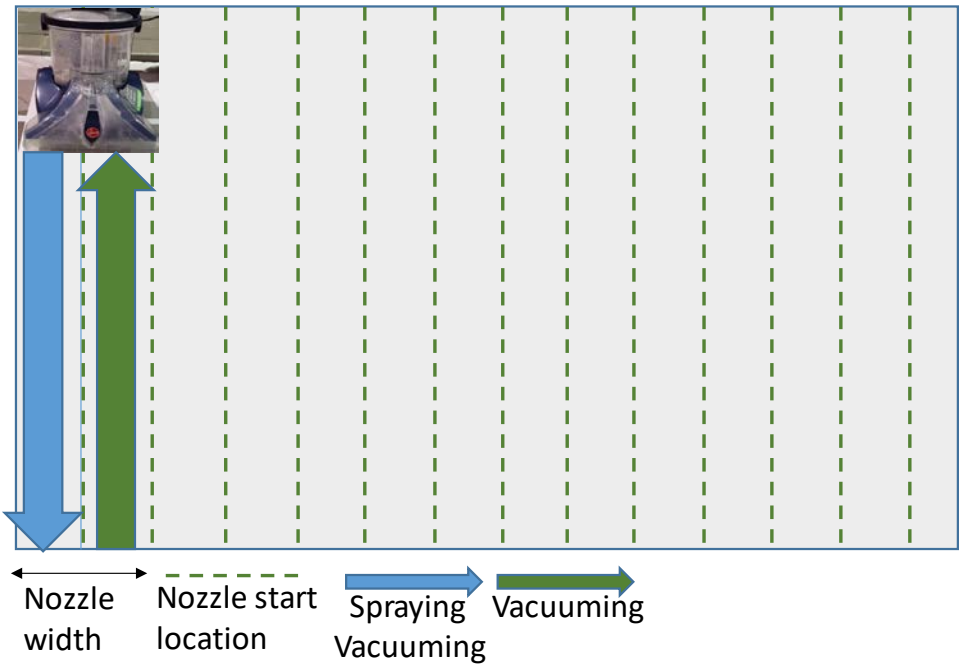

Figure 3-4. Wet Vacuuming Technique

3.3 Wipe Sampling

For Phase II of this study, stainless steel inoculum control coupons and vinyl flooring positive control coupons were wipe-sampled. A moistened sterile noncotton sponge (2 in. x 2 in., 4-ply; Curity all-purpose sponge, Cat. No. 8042, Covidien PLC, Dublin, Ireland) was used to wipe a specified area to recover bacteria, viruses, and biological toxins. Sampling was conducted on one coupon at a time. All coupons were placed horizontally for sampling.

The sponges were prepared in the BioLab by aseptically removing them from their packing and placing them into an unlabeled sterile 50-mL conical tube (Cat. No. 14-959-49A, Fisher Scientific, Waltham, MA) using sterile forceps (Part No. 7190Busse Hospital

Disposables, Hauppauge, NY). Each transferred sponge was then moistened by adding 2.5 mL of sterile PBST and capped. The surface area for all samples was 1 ft^2. A template was used to cover the exterior (1 in.) of each coupon, leaving a square (12 in. x 12 in.) exposed for sampling for all coupons. The outer 1 in. of each coupon was not sampled to avoid edge effects.

3.4 Vacuum Sock Sampling

Recovery efficiencies for carpet material sampling were determined by comparing the recoveries of commercially-available vacuum cleaners to the recovery obtained using a vacuum sock sampling method. During vacuum sock sampling, a 14-in. x 14-in. sterile positive control coupon and a sterile sock/nozzle attachment were used to collect the sample. Holding the nozzle at a 45-degree angle over the sample area, samples were taken using horizontal and vertical S-strokes. This method is a modified version of the method detailed in the study by Brown (Brown et al., 2007)

3.5 Cassette Sampling

Concrete surfaces were sampled using a 37-mm filter cassette. A vacuum pump at the back end of the filter pulled 20 L/min of air through the filter. A 3-cm section of Tygon® tubing was cut to an angle of 45° on one end, the non-angled terminus was attached to the cassette, and the angled end was used as a nozzle. The nozzle and filter were moved along the coupon at approximately 4-in./sec in both directions (i.e., horizontally and vertically). A single coupon per sample was used for these methods. The nozzle was extracted separately, the nozzle extract was then combined with the filter extraction vessel, and filter extraction commenced.

3.6 Swab Sampling

The general approach for swab sampling was to use a swab (BactiSwab® Collection and Transport System, Remel, Thermo Fisher Scientific, Waltham, MA) to wipe a specified area to recover bacterial spores. The liquid in the swab is listed as “Modified Stuart medium” and is part of the BactiSwab® package. Swab samples were collected from all decontamination procedure equipment before use to serve as sterility checks.

3.7 Liquid Collection

Liquids vacuumed from coupons during Phase I tests were collected directly in Nalgene bottles (Cat. No. 02-923-90, Fisher Scientific, Waltham, MA) fitted to the custom-sampling device. Sampling liquids from the vacuum cleaners were aseptically transferred to Nalgene bottles or specimen cups for Phase II tests.

For each test, the total mass of sampling liquid collected was recorded for comparison of the collection vessel final weight to the initial weight value. If a large volume of liquid, i.e., more than 1 L, was collected in the dirty tanks of the vacuum cleaners in Phase II tests, a representative aliquot of 100 mL was obtained for bioanalysis. Each 100-mL aliquot was taken via aseptic technique using a new 100-mL sterile serological pipette and sterile specimen cup (Cat. No. B1202-1O-OR, Starplex Scientific, Cleveland, TN). The liquid in the dirty tanks was homogenized by gently stirring the dirty tank before obtaining an aliquot. The liquid samples

were then double-contained in sterile bags (Cat. No. 01-002-53, Fisher Scientific, Waltham, MA) and transported to the BioLab for analysis.

4 Analytical Procedures

The NHSRC Research Triangle Park (RTP) BioLab analyzed samples either qualitatively for spore presence (quality control, swab samples) or quantitatively for the number of viable spores recovered per sample. Results were reported in colony forming units (CFU) per unit volume. Details of the analytical procedures are provided below. A laboratory notebook was used to document the details of each sampling event (or test).

4.1 Sample Extraction

4.1.1 Wipes

Wipe samples were received in 50-mL conical tubes. Spores were extracted from the wipes by adding 20 mL PBST to each sample, then agitating the tubes using a vortex mixer (set to maximum rotation) for two minutes in 10-second intervals. Aliquots were then removed for plating. (Brown et al., 2007)

4.1.2 Vacuum Socks

Spores were extracted by first cutting the vacuum sock into small pieces with sterile scissors. The vacuum sock pieces and any residual debris were deposited into a specimen cup preloaded with 20 mL sterile PBST. Specimen cups were agitated on the rotary shaker at 300 rpm for 30 min, then aliquots were removed for plating (Brown et al., 2007 and Calfee et al., 2013b).

4.1.3 Small vacuum 37-mm cassettes

Spores were extracted from the 37-mm cassette filter with a total of 10-mL sterile PBST. PBST was pipetted from the 10-mL stock into the cassette to wet the inside contents thoroughly, then the cassettes were opened. The wetted filter and debris were placed in a 120-mL specimen cup. Additional stock volumes of PBST were used to further rinse the inside of the cassette, and all liquid was pipetted into the specimen cup.

The nozzles were extracted separately using 50 mL sterile PBST. After agitation of the PBST over the nozzle, PBST was removed to a sterile 50-mL conical tube and centrifuged for 15 min at 3500 x *g* (gravity) to pellet the spores. Approximately 46 to 47 mL of the PBST was removed and discarded. The remaining liquid was vortexed to re-suspend the spore pellet, then all contents were added to the specimen cup. The total extraction volume was then recorded.

4.2 Spiral Plating and Filter Plating

Sample extracts that required dilution were plated in triplicate using a spiral plater (Autoplate 5000, Advanced Instruments Inc., Norwood, MA). The automated spiral plater deposits the sample in exponentially decreasing amounts across a rotating agar plate in concentric lines to achieve three 10-fold serial dilutions on each plate. Plates with *Bg* samples were incubated at 35 ± 2 °C for 18 to 20 hours. Plates with *Btk* samples were incubated at 28 ± 2 °C for 18 to 22 hours. During incubation, the colonies develop along the lines where the

sample was deposited (see Figure 4-1). Colonies on each plate were enumerated using a QCount® colony counter (Advanced Instruments Inc., Norwood, MA).

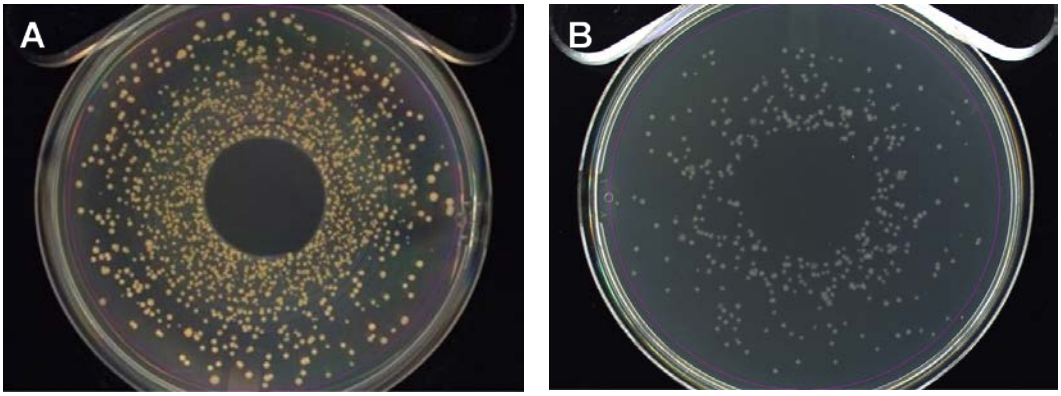

Figure 4-1. *Bg* (panel A) and *Btk* (panel B) Bacterial Colonies (CFU) on a Spiral-plated Agar Plate

Positive control samples were diluted 100-fold (10^{-2}) in PBST before spiral plating; samples of unknown concentration were plated with no dilution and with a 100-fold dilution. Samples with known low concentrations were plated with no dilution. The QCount® colony counter was used to automatically calculate the CFU/mL in a sample based on the dilution plated and the number of colonies that develop on the plate. The QCount® recorded the data in an Microsoft Excel spreadsheet.

Only plates that met the threshold of at least 30 CFU were used for spore recovery estimates. After quantitation with the QCount® colony counter, samples with plate results below the 30-CFU threshold were either re-spiral plated with a more concentrated sample aliquot or filter-plated to achieve a lower detection limit. The filter plate volume was based on the CFU data from the QCount® result. Filter plating was performed using 100-mL capacity Micro-Funnel™ unit with 0.45 µm GN-6 Metricel membranes (Pall Corporation, Port Washington, NY) and a vacuum manifold (Pall Corporation, Port Washington, NY). The filters were placed onto TSA plates and incubated at 35 ± 2 °C for 20 to 24 hours before manual enumeration. Figure 4-2 shows filter plates with colonies of *Bg* (panel A) and *Btk* (panel B).

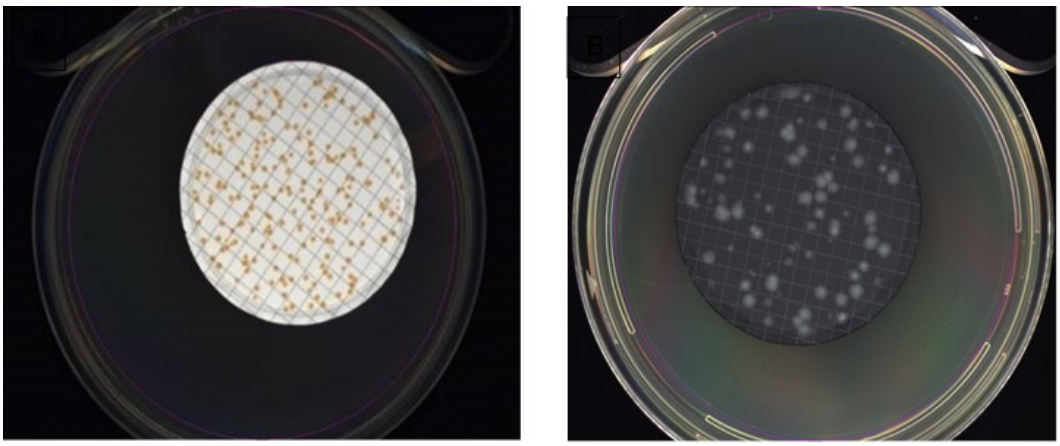

Figure 4-2. *Bg* (A) and *Btk* (B) Bacterial Colonies (CFU) on a Filter Plate

4.3 Wet Vacuum Sample Processing

Samples with large amounts of debris were homogenized by manually shaking the sample by hand followed by spiral plating as described above. Although the debris caused minor discoloration of the TSA plates, the samples still produced reliable and reproducible colony counts and required no further analysis. Spiral-plated colony counts are unreliable for samples with a small number of spores. While the existing protocol was to filter-plate the samples with low colony counts, the debris clogged the filters and prevented any colonies from growing on the plates. A new process was developed to process large sample volumes with large amounts of debris and background contamination while maintaining a high sensitivity to the low concentration of spores.

Sample containers with small numbers of spores and large amounts of debris were continuously shaken by hand for 2 min for thorough mixing. A homogenized aliquot was collected from the bulk volume (aliquot volume determined based on anticipated sample concentration) from each sample and was heat treated at 80 °C for 20 min while being vortexed every 5 min. Following this heat shock, the samples were centrifuged at 5500 x *g* for 15 min at 4 °C. The supernatant was filter-plated, and the pellet was resuspended in PBST and manually spread-plated on TSA plates. The plates were incubated at 35 ± 2 °C for 18 to 24 hours prior to manual enumeration.

5 Results and Discussion

This section discusses the results of the Phase I that consisted of determining the operational parameters that would allow the highest *Bacillus* spore recovery, and Phase II, which consisted of evaluating four types of commercially-available wet vacuum cleaners (portable, residential, commercial and wet/dry cleaners) for spore sampling efficiency, ease of use, and overall reliability.

5.1 Phase I: Evaluation of Wet Vacuum Cleaner Operational Parameters

The Phase I experimental approach consisted of assessment of a sampling liquid, the temporal lapse between liquid application and suction, and volume of liquid collected as a function of surface type.

5.1.1 Selection of Sampling Liquid

5.1.1.1 Liquid Volume Recovery

Three types of liquids were tested for sample collection to determine the proper sampling liquid type. Tables 5-1 through 5-3 show the volumes of liquid applied and volumes recovered for each type of material/liquid type combination. The volumes collected were dependent on material type. The highest volume recovery was from laminated wood (72-80%), followed by carpet (39-49%), and with the lowest recovered collection liquid from concrete (16-19%).

Table 5-1. Volume of Liquid Recovered from Carpet Coupons

Liquid	Coupon	Spray Time	Elapsed Time	Volume Applied	Volume Recovered	Liquid Recovery	Average Recovery	Collection Time
		sec	sec	mL	mL	%	%	sec
DI Water	1	19	160	317	107	34	39	300
	2	19	160	317	108	34		240
	3	19	160	317	157	50		240
	4	19	120	317	121	38		180
	5	19	150	317	117	37		240
PBST	1	20	160	333	139	42	39	180
	2	20	160	333	144	43		240
	3	20	160	333	120	36		180
	4	20	160	333	130	39		180
	5	20	160	333	121	36		150
DI Water with Tween® 20	1	18	160	300	132	44	49	180
	2	17	160	283	143	50		180
	3	18	160	300	153	51		180
	4	18	160	300	165	55		180
	5	17	160	283	122	43		150

Table 5-2. Volume of Liquid Recovered from Concrete Coupons

Liquid	Coupon	Spray Time	Elapsed time	Volume Applied	Volume Recovered	Liquid Recovery	Average Recovery	Collection Time
		sec	Sec	mL	mL	%	%	sec
DI Water	1	30	60	500	96	19	19	150
	2	24	60	400	76	19		60
	3	24	60	400	55	14		60
	4	24	60	400	85	21		60
	5	24	60	400	94	24		60
PBST	1	30	60	500	53	11	16	60
	2	30	60	500	113	23		60
	3	30	60	500	51	10		63
	4	30	60	500	71	14		60
	5	30	60	500	110	22		60
DI Water with Tween® 20	1	25	60	417	56	13	16	60
	2	25	60	417	78	19		60
	3	25	60	417	39	9		112
	4	25	60	417	75	18		60
	5	25	60	417	89	21		60

Table 5-3. Volume of Liquid Recovered from Laminated Wood Coupons

Liquid	Coupon	Spray Time	Elapsed time	Volume Applied	Volume Recovered	Liquid Recovery	Average Recovery	Collection Time
		sec	sec	mL	mL	%	%	sec
DI Water	1	6	60	100	78	78	72	150
	2	6	100	100	66	66		149
	3	6	100	100	70	70		159
	4	6	100	100	75	75		150
	5	6	100	100	73	73		150
PBST	1	7	104	117	73	62	72	149
	2	7	90	117	77	66		151
	3	7	105	117	89	76		143
	4	7	97	117	89	76		138
	5	7	70	117	90	77		143
DI Water with Tween® 20	1	6	120	100	72	72	80	140
	2	6	105	100	86	86		140
	3	6	76	100	78	78		142
	4	6	76	100	84	84		114
	5	6	74	100	78	78		144

5.1.1.2 Spore Recovery as a Function of Liquid Type

The recoveries for each type of material/liquid collection type combination are presented in Table 5-4 and illustrated in Figure 5-1. The stainless-steel recovery results were collected by PRB wipe sampling in Figure 5-1. The percent recoveries for each type of material/liquid collection were calculated as a percent of the total recovery on the stainless-steel control coupons. DI-Tween liquid solution was the highest performing liquid among the three liquid/material combinations tested with an average recovery of 53% with laminated wood

coupons, while DI water had the lowest average recovery of 9 % when used with concrete coupons. Overall, all three solutions performed within one order of magnitude of the stainless-steel coupon average recovery.

Table 5-4. Spore Recovery as a Function of Liquid Collection Type

Spore Recovery (CFU) Summary for Different Liquid Types							
Test ID	Liquid Used	Material	Positive Control Recoveries		Wet Vacuum Recoveries		
			Average	Stdev	Average	Stdev	%
1A	DI Water	Carpet	8.19E+05	2.49E+04	2.03E+05	8.83E+04	25
		Concrete			6.31E+04	2.67E+04	9
		Laminated Wood			2.02E+05	1.20E+05	27
1B	PBST	Carpet	6.80E+05	2.79E+04	1.47E+05	2.34E+04	18
		Concrete			9.62E+04	3.93E+04	14
		Laminated Wood			3.65E+05	1.08E+05	49
1C	DI Water with Tween® 20	Carpet	7.50E+05	1.16E+05	3.02E+05	1.43E+05	37
		Concrete			9.96E+04	7.26E+04	15
		Laminated Wood			3.95E+05	1.87E+05	53

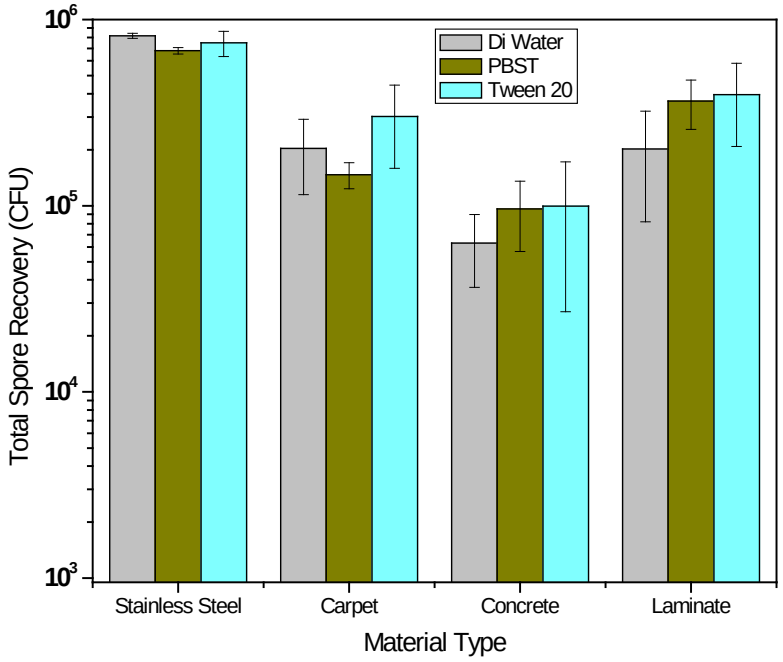

Figure 5-1. Spore Recovery for Material/Liquid Collection Combination

5.1.2 Elapsed Time

5.1.2.1 Sample Liquid Recovery Volume

This test was designed to determine the effect of delay between liquid deposition and retrieval on sampling efficiency. The tests were conducted using the DI water with 0.05% Tween® 20. The elapsed time between the liquid spray application and vacuuming of the target material was evaluated. Concrete paver coupons are highly water-absorptive, so it is difficult to retrieve enough liquid volume for analysis.

In this test, the liquid retrieval from the concrete surfaces was conducted right after the surface spraying. For concrete coupons, the overall contact time that was comprised of the spray time, elapsed time, and sampling time was used as an alternative parameter for the optimization process. The overall contact time was varied by partitioning the surface area of each coupon to enable better collection of the sample liquid.

The results for the total liquid volume applied and total volume recovered for each type of material/elapsed time combination are presented in Table 5-5 for carpet material, Table 5-6 for the concrete material/surface partition combination, and Table 5-7 for laminated wood material. The average liquid agent volumetric ratio was calculated using the actual volume collected over the target volume applied determined by the number of sprays from the backpack sprayer. The volume of liquid sample collected was found to be independent of elapsed time, but dependent on material type.

Recoveries for carpet and laminated wood, averaged over all elapsed times tested, were 52 ± 5.8% and 123 ± 8.8%, respectively. Partitioning the concrete coupon surface area into two halves or four quadrants increased the liquid sample collection from an average of 15 ± 4.3% for an average contact time of 96 secs (see Table 5-2), 29 ± 9.2 % for a contact time of 45 secs, to an average of 33 ± 5.3% for a contact time of 21 sec.

Table 5-5. Volume of Liquid Recovered from Carpet at Various Elapsed times

Elapsed Time	Coupon	Spray Time	Volume Applied	Volume Recovered	Collection Time	Liquid Recovery per Test	Average Liquid Recovery
Sec	ID	Sec	mL	mL	Sec	%	%
1	1	15	250	132	160	53	54
	2	15	250	133		53	
	3	15	250	145		58	
	4	15	250	123		49	
	5	15	250	140		56	
30	1	15	250	107		43	52
	2	15	250	152		61	
	3	15	250	129		52	
	4	15	250	142		57	
	5	15	250	118		47	
100	1	15	250	131		52	54
	2	15	250	146		58	
	3	15	250	126		50	
300	1	15	250	97.0		39	49
	2	15	250	125		50	
	3	15	250	110		44	
	4	15	250	138		55	
	5	15	250	137		55	

Table 5-6. Volume of Liquid Recovered from Different Concrete Coupon Surface Partitions

Coupon Surface Partition	Coupon	Spray Time				Liquid				Contact Time
		Time 1	Time 2	Time 3	Time 4	Applied	Recovered	Recovered per Test	Average	
	ID	sec	sec	sec	sec	mL	mL	%	%	sec
Full	1	25	x	x	x	417	56	13	16	60
	2	25	x	x	x	417	78	19		60
	3	25	x	x	x	417	39	9		112
	4	25	x	x	x	417	75	18		60
	5	25	x	x	x	417	89	21		60
Halves (0.5)	1	15	15	x	x	500	119	24	29	45
	2	15	15	x	x	500	105	21		
	3	15	15	x	x	500	176	35		
	4	15	15	x	x	500	116	23		
	5	15	15	x	x	500	211	42		
Quarters (0.25)	1	6	6	6	6	400	136	34	33	21
	2	6	6	6	6	400	136	34		
	3	6	6	6	6	400	136	34		
	4	6	6	6	6	400	101	25		
	5	6	6	6	6	400	160	40		

Table 5-7. Volume of Liquid Recovered from Laminated Wood at Different Elapsed Times

Elapsed Time	Coupon	Spray Time	Volume Applied	Volume Recovered	Collection Time	Liquid Recovery per Test	Average Liquid Recovery
sec	ID	sec	mL	mL	sec	%	%
1	1	6	100	137	160	137	122
	2	6	100	118		118	
	3	6	100	116		116	
	4	6	100	127		127	
	5	6	100	114		114	
30	1	6	100	117		117	120
	2	6	100	128		128	
	3	6	100	116		116	
	4	6	100	123		123	
	5	6	100	118		118	
100	1	6	100	130		130	124
	2	6	100	116		116	
	3	6	100	125		125	
300	1	6	100	118		118	125
	2	6	100	148		148	
	3	6	100	122		122	
	4	6	100	116		116	
	5	6	100	121		121	

5.1.2.2 Spore Recovery as a Function of Elapsed Time

The spore recoveries as a function of elapsed time for both carpet and laminated wood are presented in Table 5-8 and illustrated in Figure 5-2. The results show that for these two materials, the elapsed time (1-300 sec) between liquid application and suction and the type of material had little or no effect on average recovery. However, for concrete, partitioning the coupon area into halves or quarters had a marked effect on the total spores recovered, as shown in Table 5-9 and illustrated in Figure 5-3.

The spore recovery increased from an average of 16.0 % (see Table 5-2) for one spraying/sampling combination sequence covering the whole coupon surface (96 sec contact time) to 28.7% for two consecutive sequences covering two halves of the coupon (45-sec contact time), and 59.3% for four consecutive sequences covering four quarters of the total area of the coupon (21-sec contact time).

Table 5-8. Spore Recovery as a Function of Elapsed time for Carpet and Laminated Wood Coupons

Spore Recovery (CFU) Summary for Various Elapsed times							
Test ID	Material	Elapsed Time	Positive Control Recovery		Wet Vacuum Recovery		
		Sec	Average (CFU)	Standard Deviation	Average (CFU)	Standard Deviation	%
2A	Carpet	1	6.27×10^5	5.35×10^4	1.61×10^5	2.86×10^4	26
		30			1.95×10^5	6.48×10^4	31
		100			2.35×10^5	5.41×10^4	38
		300			1.91×10^5	4.97×10^4	31
2C	Laminated Wood	1	5.09×10^5	8.79×10^4	1.55×10^5	1.02×10^5	31
		30			1.46×10^5	4.37×10^4	29
		100			1.10×10^5	4.02×10^4	22
		300			1.99×10^5	3.93×10^4	39
Stdev = Standard deviation							

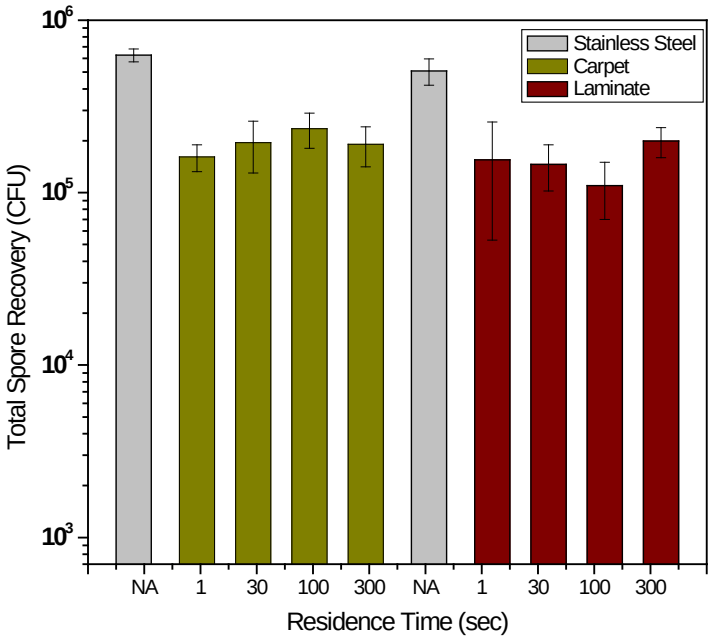

Figure 5-2. Spore Recovery at Different Elapsed Times

Table 5-9. Spore Recovery as a Function of Number of Partitions on the Concrete Coupon

Spore Recovery (CFU) Summary for Various Elapsed Times - Divided Area								
Test ID	Material	Surface (partitions)	Contact Time (sec)	Positive Control Recoveries		Average CFU Recovered		
			sec	Average (CFU)	Standard Deviation	Average (CFU)	Standard Deviation	%
2B	Concrete	2	45	5.96×10^5	2.26×10^5	1.71×10^5	1.09×10^5	29
		4	21			3.54×10^5	3.21×10^5	59

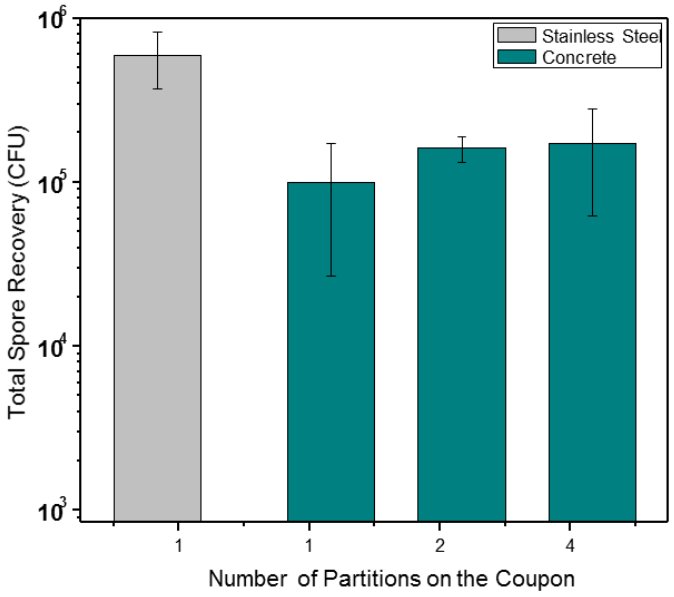

Figure 5-3. Spore Recovery for Different Number of Partitions on the Concrete Coupon

5.1.3 Liquid Volume

5.1.3.1 Sample Liquid Recovery Volume

This test was designed to determine the impact of applied liquid volume on the sampling efficacy, using the most efficient extraction liquid (DI water with 0.05% Tween® 20). The results from these initial tasks determined that the elapsed time had no effect on spore recovery for carpet and laminated wood; therefore, elapsed time was set at a constant value of 30 seconds. For concrete surfaces, a coupon partitioned into quadrants was used for the liquid volume test based on the results in the previous section.

The results for the total volume recovered for each type of material and volume applied combination are presented in Table 5-10 for carpet material, Table 5-11 for the concrete /surface partition combination, and Table 5-12 for laminate wood. The liquid volume recovery

fraction on carpet coupon decreased with less liquid volume sprayed: an average recovery of 5.4% for an average of 107 mL of initial spray, 8.5% for 161 mL, and 49% for 293 mL (Table 5-1). These results suggest that the spraying liquid volume on carpet requires at least 161 mL per ft^2 to generate the target liquid volume (10 mL) for analysis.

Table 5-10. Liquid Volume Recovery from Carpet Coupon

Volume Applied	Coupon	Spray Time	Elapsed Time	Collection Time	Liquid Recovery per Test		Average Liquid Recovery Fraction
mL	ID	sec	sec	sec	mL	%	%
107	1	6	24	100	5.8	5.4	5.4
	2	6	30	99	7.7	7.2	
	3	10	30	107	12	6.7	
	4	6	30	131	4.7	4.4	
	5	6	30	117	3.3	3.1	
161	1	9	30	112	7.6	4.7	8.5
	2	9	30	144	9.7	6.0	
	3	9	30	142	17.9	11.1	
	4	9	30	137	27.6	17.2	
	5	9	30	168	5.8	3.6	

For concrete, decreasing the combined volume applied during the four-consecutive spraying/sampling combination sequences did not show any real trend. The average recovery was 36% collected for a net volume sprayed of 160 mL per coupon, 42% for a volume of 220 mL, and 33% for 500 mL (from Task 2). Contact seems to be the main driver for liquid sample recovery from this type of material.

Table 5-11. Liquid Volume Recovery from Concrete Coupon

Volume Applied	Coupon	Spray Time (sec)				Collection Time (sec)				Liquid Recovery per Test		Average Liquid Recovery	Contact Time
mL	ID	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	mL	%	%	Sec
160	1	3	2	2	2	29	34	40	34	50	31	35[2]	35
	2	2	2	2	2	33	38	33	37	57	40		
	3	2	2	2	2	36	46	42	41	67	47		
	4	2	2	2	2	29	19	-	31	30	NA[1]		
220	1	3	3	4	3	42	38	44	34	105	45	42	39
	2	3	3	3	3	31	32	33	29	97	45		
	3	3	3	3	3	33	38	44	38	78	36		
	4	3	3	3	3	38	33	34	36	90	42		

[1] Volume recovered by the NHSRC BioLab; the collection time for Q3 was not recorded.

[2] The average liquid recovery was calculated using the recorded collected times.

The calculated volumetric fraction (%) of the collection liquid recovered from the laminated wood coupons was found to be dependent on the total volume sprayed with an average recovery of 57% for 18 mL of initial spray, 71% for 54 mL, and 80% for 100 mL (from Task 1, at an equivalent elapsed time of 30 sec). The low recoveries observed from this nonporous material at the low volume (18 mL) may be the result of the relative losses in the nozzle and tubing of the wet vacuum sampling device.

Table 5-12. Liquid Volume Recovery from Laminated Wood Coupon

Target Volume Applied	Coupon	Spray Time	Elapsed Time	Collection Time	Liquid Recovery per Test		Average Liquid Recovery
mL	ID	sec	sec	sec	mL	%	%
18	1	1	30	164	10.5	58	57
	2	1	30	199	12.9	72	
	3	1	30	167	12.7	71	
	4	1	31	183	NA	21	
	5	1	30	171	11.3	63	
54	1	3	30	183	37.5	69	71
	2	3	30	194	40.5	75	
	3	3	30	164	33	61	
	4	3	30	157	32	59	
	5	3	30	234	49.8	92	
NA = Not available; data corrupted							

5.1.3.2 Spore Recovery as a Function of Liquid Volume Sprayed

The spore recoveries as a function of total sample liquid volume sprayed onto both carpet and laminated wood materials are presented in Table 5-13. The results show that for carpet, decreasing the volume of liquid spray onto the coupons had a negative effect on the overall spore recovery. The spore recovery for a 100 and 150 mL volume spray resulted in average spore recoveries between 3.4 and 4.8%, while the recovery for a 250-mL spray was 31% (at an equivalent elapsed time of 30 sec). For the laminated wood material, the effect on the spore recovery of the volume sprayed was found to be insignificant, with an average recovery varying between 29 to 38% for volume of liquid agent sprayed between 20 and 100 mL

Table 5-13. Spore Recovery from Carpet and Laminated Wood Coupons

Spore Recovery (CFU) Summary for Various Sprayed Volumes								
Test ID	Material	Volume Sprayed	Spray Duration	Positive Control Recovery		Average CFU Recovered		
		mL	sec	Average (CFU)	Stdev (CFU)	Average (CFU)	Stdev (CFU)	%
3A	Carpet	100	6	1.56×10^6	1.65×10^5	7.49×10^4	4.01×10^4	4.8
		150	9			5.37×10^4	4.50×10^4	3.4
3C	Laminate	20	1	1.47×10^6	1.58×10^5	4.51×10^5	1.60×10^5	31
		50	3			5.60×10^5	2.15×10^5	38

For the concrete coupons, the spore recovery was not affected by the liquid volume sprayed as seen in Table 5-14. The spore recovery varied from a low average of 29% (500 mL, contact time 45 sec) as seen in Table 5-6 to a high recovery of 36% (220 mL, 39 sec) found in Table 5-14.

Table 5-14. Spore Recovery from Concrete Coupon

Spore Recovery (CFU) Summary for Various Sprayed Volumes									
Test ID	Material	Surface Partition	Spray Duration	Volume Sprayed	Positive Control Recoveries		Average CFU Recovered		
		Per Coupon	sec	mL	Average (CFU)	Standard Deviation (CFU)	Average (CFU)	Standard Deviation (CFU)	%
3B	Concrete	4	3	220	1.33×10^6	3.74×10^4	4.77×10^4	1.61×10^4	36
			2	160			4.24×10^4	2.27×10^4	32

5.1.4 Phase I: Summary

The Phase I test results showed the following suggested sampling conditions:

- DI water-Tween® liquid achieved the highest recovery among the tested liquid types.
- The spore recoveries were found to be dependent on the recovered volumetric fraction of liquid sprayed onto the coupons for carpet and concrete.
- Elapsed time between the liquid application and vacuuming of the target material had little or no effect on the wet vacuum sampling spore recovery for laminated wood and carpet materials. Concrete surface test results showed a marked effect of elapsed time on the spore recovery effectiveness. Concrete surfaces need to be vacuumed immediately after the liquid is applied.
- The total volume of liquid sprayed onto the carpet material seems to affect the spore recovery. Carpet surface requires enough volume of liquid to be applied to the surface for higher spore and liquid volume recovery. For laminated wood, the effect of the volume sprayed on the spore recovery seems to be insignificant. For concrete, the liquid volume seems to have a negligible effect within the tested conditions.

5.2 Phase II - Commercially-Available Wet Vacuum Cleaner Evaluation

The spore recovery efficiencies were estimated for four types of wet vacuum cleaners. Sampling efficiency was estimated by comparing the spore recovery from test coupons to the recovery from inoculum control coupons. Since each test coupon was inoculated four times using the same MDI, while control coupons were inoculated once, the resulting spores recovered from control coupons were multiplied by four when calculating comparative recoveries. The spore recoveries for all control coupons (stainless steel, and material-specific), then the commercial vacuum cleaner recoveries, and finally proposed sampling and extraction procedures are discussed in the next sections.

5.2.1 Control Sample Recoveries

Stainless steel inoculum control coupons were used to verify the magnitude and repeatability of spore loadings for every inoculation event. A total of 36 stainless steel coupons were inoculated with *Bg* spores, and 38 stainless steel coupons were inoculated with *Btk* spores. Both test organisms were sampled using the wipe sampling approach during all the reported testing events.

The data obtained for the inoculum controls are shown in Table 5-15 and illustrated in Figure 5-4. The repeatability of the inoculation control checks was within the data criteria set at 50% RSD for these levels of inoculation controls.

Table 5-15. Spore Recovery from Concrete Coupon

Target Organism	Replicates	Spore Recovery (CFU)		
		Average	Standard Deviation	RSD (%)
Bg	36	1.85E+08	9.31E+07	50.3
Btk	38	8.71E+07	4.15E+07	47.6

5.2.2 Wet-Vacuum Cleaner Evaluation

The sampling efficiency of the commercially-available cleaners is a measure of the spores recovered from a contaminated material surface by the vacuums as compared to the spores recovered from inoculum control coupons, sampled using wipes. The spore recovery efficiencies relative to the number of spores deposited was estimated for each of the four vacuum cleaners (portable, commercial, residential, and Shop Vac), for all three materials tested (carpet, vinyl flooring, and concrete) inoculated with *Bg* and *Btk* spores. The efficacy of each vacuum cleaner was also compared to the alternative sampling methods. Detailed data for Phase II are shown in Appendix A.

To determine if there were significant differences in the mean spore recovery among the vacuum cleaners, material types, and among the vacuum cleaners and the traditional sampling methods, a Tukey Multiple Comparison Test (Tukey, 1949) was used to compare all possible differences in spore recoveries between each pair of sampling method means (example: portable vacuum cleaner and vacuum sock for carpet material). This method is based on the one-way statistical analysis of variance or ANOVA. The Null Hypothesis used for the comparison testing is that the means at all levels are equal, and the alternative hypothesis is that the means of one or more levels are different. The confidence interval level was set at 95%.

5.2.2.1 Carpet

The sampling efficacies for each vacuum cleaner and the vacuum sock for carpet are presented in Table 5-16 and illustrated in Figure 5-4. The one-way ANOVA returns *Btk* spore recoveries, suggest that at the 0.05 significance level, the means of the wet vacuum cleaners are not significantly different (F-value = 3.30, p-value = 0.078) but are significantly different from the established vacuum sock sampling method (F-value = 6.49, p-value = 0.0076). However, for *Bg* spores, the results suggest that one commercial vacuum cleaner type outperformed all the other vacuum cleaners as well as the inoculum controls and the vacuum sock positive controls.

Table 5-16. Sampling Efficacy of the Various Sampling Methods for *Btk* and *Bg* Spore Recoveries from Carpet

***Btk* Spore Recovery (%)**				
Sampler Type	**Replicates**	**Mean**	**Standard Deviation**	**Standard Error of Mean**
Portable	3	24	5	19
Commercial	3	29	7	24
Residential	3	8	4	44
Shop Vac	3	17	14	83
Vacuum Sock	3	48	15	41
***Bg* Spore Recovery (%)**				
Portable	3	20	6	28
Commercial	3	140	43	31
Residential	3	46	21	46
Shop Vac	3	10	5	44
Vacuum Sock	3	27	13	48

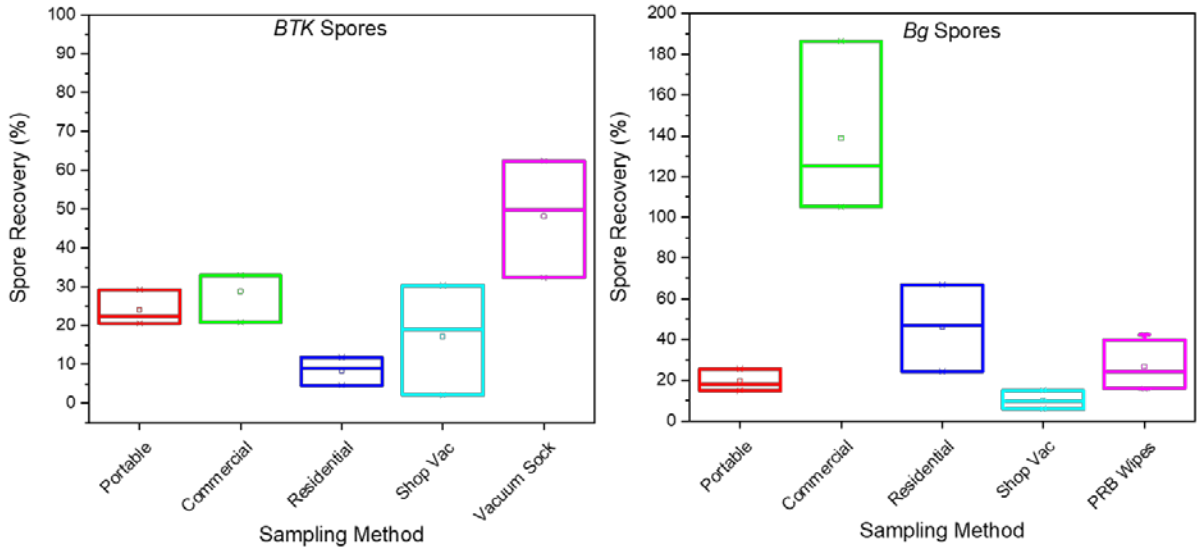

Figure 5-4. Sampling Efficacy of the Various Sampling Methods for *Btk* and *Bg* Spores Inoculated on Carpet

The one-way ANOVA performed for all vinyl results, independent of the type of surrogates, is summarized in Table 5-17. The three vacuum cleaners (portable, residential, and shop vac) showed nearly the same recoveries as the vacuum sock sampling method. The commercial wet vacuum cleaners, however, seem to be biased with the results obtained from the *Bg* spore test.

Table 5-17. Sampling Efficacy of the Various Sampling Methods for Spores (*Btk* and *Bg* data pooled) Inoculated on Vinyl Flooring

***Btk*/*Bg* Spore Recovery (Pooled Data)**				
Sampler Type	**Replicates**	**Mean**	**Standard Deviation**	**RSD**
Portable	3+3	22	5	23
Commercial	3+3	84	66	79
Residential	3+3	27	25	92
Shop Vac	3+3	14	10	73
Vacuum Sock	3+3	39	16	41

Tukey statistical tests, listed in Table 5-18, were performed with and without the results obtained with the commercial vacuum cleaners for *Bg* spores. In the absence of the commercial vacuum cleaner results, the one-way ANOVA shows that there is no statistically significant difference in the means between all four vacuum cleaner recoveries and the vacuum sock sampling technique. The one test with the commercial wet vacuum biased the comparison when it was compared to other sampling techniques.

Table 5-18. Tukey Pairwise Statistical Test Results for Various Sampling Methods on Carpet

Pairwise Comparisons	**With Commercial Wet Vacuum/*Bg* Test**		**Without the Commercial Wet Vacuum/*Bg* Test**	
	***p*-Value**	**Sig[1]**	***p*-Value**	**Sig**
Commercial/Portable	0.02337	1	0.96476	0
Residential /Portable	0.99839	0	0.97037	0
Residential/ Commercial	0.04466	1	0.99989	0
Shop Vac /Portable	0.99238	0	0.88367	0
Shop Vac /Commercial	0.00844	1	0.63004	0
Shop Vac/Residential	0.95018	0	0.54536	0
Vacuum Sock /Portable	0.88867	0	0.31414	0
Vacuum Sock/Commercial	0.15905	0	0.87182	0
Vacuum Sock/Residential	0.9694	0	0.66603	0
Vacuum Sock/ Shop Vac	0.66667	0	0.05836	0

[1]Sig equal to 1 indicates that the difference in the means is significant; when equal to 0, indicates that the difference is not significant at the 0.05 level.

5.2.2.2 Vinyl Flooring

The sampling efficacies for each vacuum cleaner and the PRB wipe method for the vinyl flooring are presented in Table 5-19 and illustrated in Figure 5-5. The one-way ANOVA returns suggest that at the 0.05 significance level, the means of the wet vacuum cleaners are not significantly different (*Btk*: F-value = 1.33, *p*-value = 0.317; *Bg*: F-value = 1.39, *p*-value = 0.314). However, the PRB wipe sampling method out-performed the wet vacuum cleaners. Confirming the results obtained for *Bg* recovery on carpet, the commercial vacuum cleaner had a better spore recovery than the other wet vacuum cleaners.

Table 5-19. Sampling Efficacy of the Various Sampling Methods for *Btk* and *Bg* Spores Recovery on Vinyl Flooring

***Btk* Spore Recovery (%)**				
Sampler Type	**Replicates**	**Mean**	**Standard Deviation**	**RSD (%)**
Portable	3	31	38	123
Commercial	3	18	11	62
Residential	3	25	4	14
Shop Vac	3	28	24	86
PRB Wipes	3	106	39	37
***Bg* Spore Recovery (%)**				
Portable	3	48	9	19
Commercial	3	87	59	68
Residential	3	41	7	17
Shop Vac	3	68	7	11
PRB Wipes	3	95	9	10

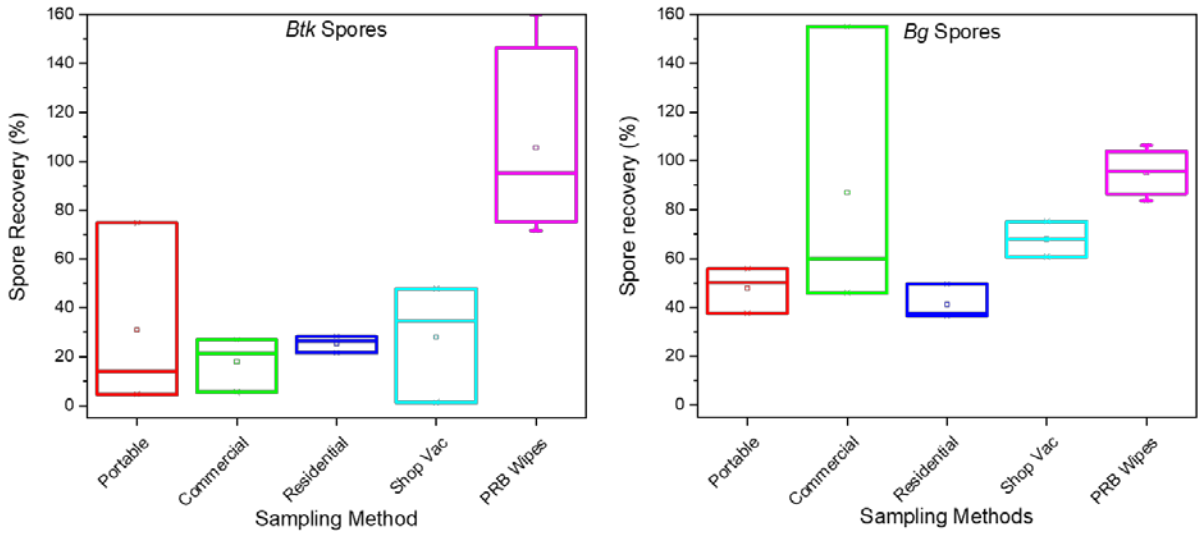

Figure 5-5. Sampling Efficacy of the Various Sampling Methods for *Btk* and *Bg* Spores Inoculated on Vinyl Flooring

The one-way ANOVA returns for all vinyl flooring results, independent of the type of surrogates, are summarized in Table 5-20. The returns, suggest that at the 0.05 significance level, the means of the wet vacuum cleaners are not significantly different (F-value = 0.397, *p*-value = 0.756). As expected, the PRB wipe sampling method, in general, out-performed the wet vacuum cleaner sampling approach.

Table 5-20. Sampling Efficacy of the Various Sampling Methods for Both *Btk* and *Bg* Spore Recovery on Vinyl Material

Btk /Bg Spore Recovery				
Sampler Type	**Replicates**	**Mean**	**Standard Deviation**	**RSD (%)**
Portable	3+3	40	26	10
Commercial	3+3	53	54	22
Residential	3+3	33	10	4
Shop Vac	3+3	48	27	11
PRB Wipes	3+3	100	27	9

5.2.2.3 Concrete

The sampling efficacies for each vacuum cleaner and the established 37-mm cassette method for concrete are presented in Table 5-21 and illustrated in Figure 5-6. The one-way ANOVA results suggest that at the 0.05 significance level, the means of the wet vacuum cleaners are not significantly different for *Btk* spores (F-value = 1.33, *p*-value = 0.317). However, for *Bg* spores, the Shop-Vac and the 37-mm cassette did not perform as well as the other wet vacuum cleaners.

Table 5-21. Sampling Efficacy of the Various Sampling Methods for *Btk* and *Bg* Spore Recovery on Concrete Material

***Btk* Spore Recovery**				
Sampler Type	**Replicates**	**Mean**	**Standard Deviation**	**RSD (%)**
Portable	3	20	13	65
Commercial	3	35	10	28
Residential	3	29	9	31
Shop Vac	3	47	12	26
37-mm Cassette	3	44	26	60
***Bg* Spore Recovery**				
Portable	3	50	19	39
Commercial	3	20	2	7.5
Residential	3	49	8	15
Shop Vac	3	6	3	51
37-mm Cassette	3	13	8	58

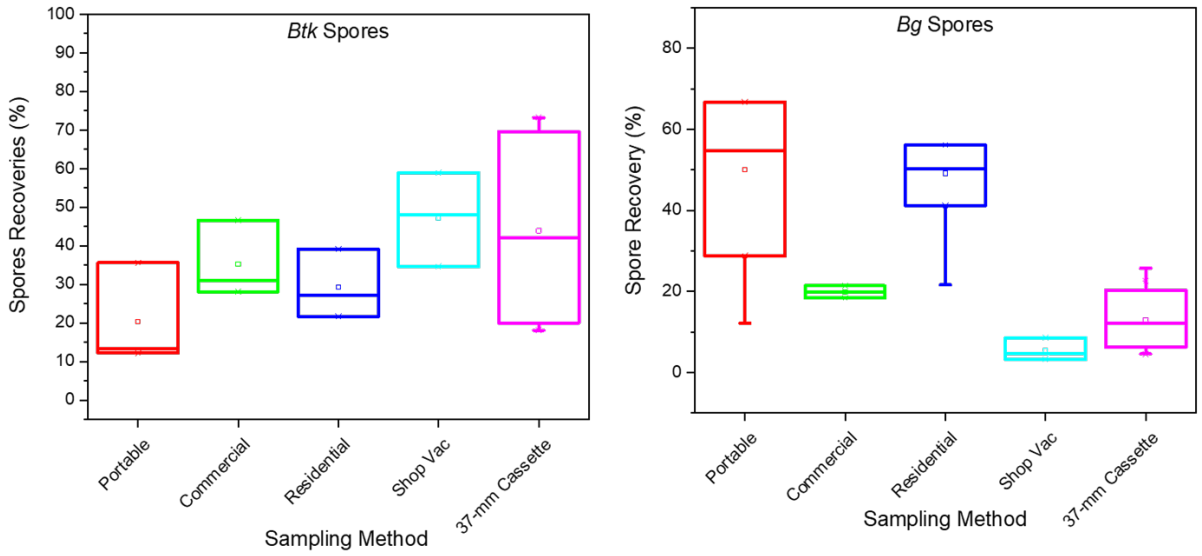

Figure 5-6. Sampling Efficacy of the Various Sampling Methods for *Btk* and *Bg* Spores Inoculated on Concrete

The one-way ANOVA results for all concrete test results, independent of the type of surrogate, are summarized in Table 5-22. The results suggest that at the 0.05 significance level, the means of the wet vacuum cleaners and the 37-mm cassette method are not significantly different (F-value = 0.474, *p*-value = 0.754).

Table 5-22. Sampling Efficacy of the Various Sampling Methods for Both *Btk* and *Bg* Spore Recovery on Vinyl Material

***Btk /Bg* Spore Recovery**				
Sampler Type	**Replicates**	**Mean**	**Standard Deviation**	**RSD (%)**
Portable	3+3	37	22	59
Commercial	3+3	28	11	38
Residential	3+3	39	13	34
Shop Vac	3+3	26	24	92
PRB Wipes	3+3	28	24	86

5.2.3 Phase II: Summary

All the wet vacuum cleaner spore recoveries were comparable to the spore recoveries of the alternate sampling methods. A two-way ANOVA (Material type/Sampling method) performed with the wet vacuum cleaners (72 samples) is shown in Table 5-23. The results demonstrated that the effect of material type on mean recoveries was not statistically significant for all types of vacuum cleaners (F-value = 0.446, *p*-value = 0.642), while the effect of sampling methods on the mean recovery was statistically significant (F-value = 3.03). The interaction of the two factors showed no significant difference in the mean recovery (F-value = 2.06, *p*-value = 0.07) at the 0.05 level.

Table 5-23. Two-Way ANOVA on the Mean Sampling Efficacy of the Wet Vacuum Cleaners

Source of Variation	SS	df	MS	F	p-Value	F critical
Material Type	846.0533	2	423.0267	0.446071	0.642244	3.150411
Sampling method	8628.085	3	2876.028	3.032698	0.036072	2.758078
Interaction (Sampling method/Material type)	11736.86	6	1956.143	2.062702	0.071075	2.254053

The overall results for the analysis of the wet vacuum cleaners (independent of material type and surrogate type) are presented in Table 5-24 and in Figure 5-7. In terms of both usability and repeatability, the residential wet vacuum cleaner was found to be the better universal wet vacuum cleaner for wide area sampling of *Bacillus* spores. The residential wet vacuum cleaner was found to be more precise (RSD = 50%), more user-friendly (lighter than the commercial wet vacuum cleaner, and less cumbersome to use than the portable vacuum cleaner (the sampler needs to bend to sample flooring), and less prone to cross-contamination than the Shop-Vac, which needs a second device for wetting the contaminated surface.

Table 5-24. Overall Sampling Efficacy of the Various Sampling Methods

***Btk /Bg* Spore Recovery**				
Sampler Type	**Replicates**	**Mean (%)**	**Standard Deviation**	**RSD (%)**
Portable	18	32	20	63
Commercial	18	55	52	96
Residential	18	33	17	51
Shop Vac	18	25	26	100

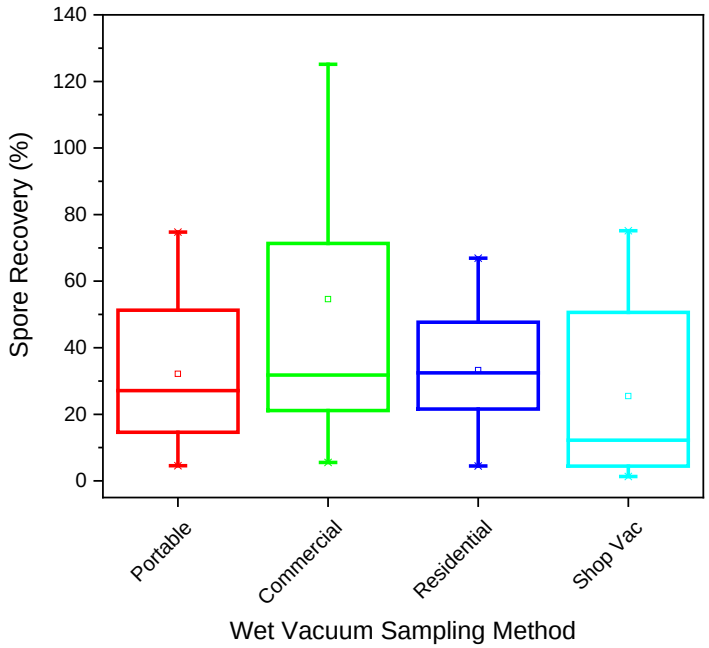

Figure 5-7. Sampling Efficacy of the Various Sampling Methods for *Bacillus* Spores Independent of Type of Material

6 Quality Assurance and Quality Control

All test activities were documented in laboratory notebooks and digital photographs. The documentation included, but was not limited to, a record for each decontamination procedure, any deviations from the quality assurance project plan, and physical impact on materials. All tests were conducted in accordance with established EPA Decontamination Technologies Research Laboratory (DTRL) and NHSRC RTP Microbiology Laboratory procedures to ensure repeatability and adherence to the data quality validation criteria set for this project.

The following sections discuss the criteria for the critical measurements and parameters, Data Quality Indicators (DQIs), and the quality assurance (QA) and quality control (QC) checks for the project

6.1 Criteria for Critical Measurements/Parameters

The Data Quality Objectives (DQOs) are used to determine the critical measurements needed to address the stated objectives and specify tolerable levels of potential error associated with simulating the prescribed decontamination environments. The following measurements were deemed to be critical to accomplish part or all project objectives:

- Volume or mass of sampling liquid,
- Spray time,
- Run time,
- Incubation temperature,
- Plated volume,
- CFU counts.

The DQIs for the critical measurements are listed in Table 6-1. DQIs were used to determine if the collected data met the quality assurance 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 be achieved routinely. The integrity of the sample 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 project used established and approved operating procedures for the maintenance and calibration of all laboratory equipment. All laboratory measuring devices such as scales and pipettors used in this project were certified as having been recently calibrated or were calibrated at the on-site EPA Metrology Laboratory at the time of use. Deficiencies, if any, were noted and the instrument replaced to meet calibration tolerances. All DQIs were within the target acceptance criteria set for this project as shown in Table 6-1.

Table 6-1. DQIs and Acceptance Criteria Validation for Critical Measurements

Measurement Parameter	Analysis Method	Acceptance Criteria	Pass or Fail Test
Mass of sampling liquid	Scale	Accuracy: 0.1 g	Pass
Volume of sampling liquid	Serological pipette – certified as calibrated	Subdivision: 0.5 mL	Pass
Time	National Institute of Standards and Technology (NIST)-calibrated stopwatch	± 1 minute	Pass
Counts of CFU per plate	Q-count	1.82×10^4 <QC Plate < 2.3×10^4	Pass
Plated volume (liquid)	Pipette	2%	Pass
Temperature	NIST-traceable thermometer (daily)	± 2 °C	Pass

6.2 Integrity of Samples and Supplies

Samples were carefully maintained and preserved to ensure their integrity. Samples were stored away from standards and other samples that could possibly cross-contaminate them. Supplies and consumables were acquired from reputable sources and were 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 that showed evidence of tampering or damage were discarded. All examinations were documented, and all supplies were appropriately labeled.

6.3 NHSRC BioLab Quality Checks

Quantitative standards do not exist for biological agents. Viable spores were counted using a QCount® colony counter. Counts generated that were either greater than 300 or less than 30 were considered outside the targeted range. If the count of colony-forming units for bacterial growth did not fall within the target range, the sample was either filtered or replated. Replates and filter plates were enumerated manually.

Before each batch of plates was enumerated on the QCount®, a QC plate was analyzed, and 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, and 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.

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

- Procedural blank coupons: Material coupons sampled in the same fashion as test coupons but not contaminated with surrogate organism prior to sampling.
- Stainless-steel and carpet positive control coupons: Coupons inoculated in tandem with the test coupons and meant to demonstrate the highest level of contamination recoverable from an inoculation event.

Additional QC checks for BioLab procedures are listed in Table 6-2. These QC checks provide assurances against cross-contamination and other biases in microbiological samples.

Table 6-2. Additional Quality Checks for Biological Measurements

Sample Type	Frequency	Acceptance Criteria	Information Provided	Corrective Action
Positive control coupon - sample from material coupon contaminated with biological agent and sampled using the existing sampling methods	Minimum of three per test	10^5 - 10^7 for *B. globigii* 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
Inoculum Control coupon – stainless steel coupon contaminated with biological agent and sampled using PRB wipes.	Minimum of three per test	10^5 - 10^7 for *B. globigii* 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
Blank tryptic soy agar 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 per plate	Used to determine the precision of the replicate plating	Re-plate sample

Most of the wet vacuum control blank (negative control), the EPA accepted sampling procedure blank (procedural blank), and the inoculum control blank (stainless-steel control blank) were non-detectable (> 93%). Some control blanks were found to be contaminated, but they had little or no effect on the final results. The source of this contamination is unknown. For negative controls, the contamination may have occurred by incomplete inactivation of spores from the materials during VHP® cycle. Procedural blanks may have become contaminated due to their presence in an area with inoculated coupons.

7 References

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APPENDIX A: PHASE II: DATA REPORT
C1: *Bg* Spores

a) Carpet Results

Material	Vacuum Type	Sample Identification	Sample Method	Recovery (CFU)	Recovery (%) Compared to Average Inoculation Control	Average Recovery (%)	Stdev Recovery (%)
Carpet	Portable - Bissell Pro-Heat*	Liquid	Procedural Blank	ND	ND		
		Negative Control	Vacuum Sock	2.42E+00	0%	0.00%	
		Positive Control	Vacuum Sock	3.35E+07	20%	32%	15%
				8.32E+07	49%		
				4.74E+07	28%		
		Inoculum Control	Gauze Wipe	1.21E+08	71%	100%	27%
				2.10E+08	123%		
				1.80E+08	106%		
		Test Coupon	Wet Vacuum	3.12E+07	18%	20%	5%
				2.52E+07	15%		
				4.34E+07	26%		
	Commercial - RugDoctor	Liquid	Procedural Blank	ND	ND		
		Negative Control	Vacuum Sock	1.06E+02	0.00005%	0.00005%	
		Positive Control	Vacuum Sock	1.15E+08	59%	42%	21%
				3.59E+07	18%		
				9.68E+07	50%		
		Inoculum Control	Gauze Wipe	1.68E+08	86%	100%	15%
				2.26E+08	116%		
				1.92E+08	98%		
		Test Coupon	Wet Vacuum	4.84E+08	248%	187%	62%
				3.64E+08	187%		
				2.44E+08	125%		
	Home Use - Hoover	Liquid	Procedural Blank	ND	ND		
		Negative Control	Vacuum Sock	ND	ND		
		Blank Inoculum Control	Wipe	ND	ND		
		Positive Control	Vacuum Sock	1.61E+07	16%	16%	6%
				2.13E+07	21%		
				1.02E+07	10%		
		Inoculum Control	Gauze Wipe	7.89E+07	78%	100%	19%
				1.16E+08	115%		
				1.07E+08	106%		
		Test Coupon	Wet Vacuum	2.46E+07	24%	46%	21%
				4.74E+07	47%		
				6.74E+07	67%		
	ShoVac	Liquid	Procedural Blank	ND	ND		
		Negative Control	Vacuum Sock	5.60E+01	0%		
		Positive Control	Vacuum Sock	4.70E+07	25%	17%	8%
				2.88E+07	15%		
				1.84E+07	10%		
		Inoculum Control	Gauze Wipe	2.02E+08	106%	100%	6%
				1.81E+08	95%		
				1.87E+08	98%		
		Test Coupon	Wet Vacuum	1.14E+07	6%	10%	4%
				1.82E+07	10%		
				2.83E+07	15%		

b) Concrete Results

Material	Vacuum Type	Sample Identification	Sample Method	Recovery (CFU)	Recovery (%) Compared to Average Inoculation Control	Average Recovery (%)	Stdev Recovery (%)
Concrete	Portable - Bissell Pro-Heat*	Liquid	Procedural Blank	ND	ND		
		Negative Control	Vacuum Sock	ND	ND		
		Cassette	Wipe	1.58E+01	0%		
		Positive Control	37 mm cassette	1.52E+07	11%	11%	4%
				1.01E+07	7%		
				2.19E+07	16%		
		Inoculum Control	Gauze Wipe	1.15E+08	83%	100%	34%
				1.93E+08	139%		
				1.08E+08	78%		
		Test Coupon	Wet Vacuum	9.25E+07	67%	50%	19%
				7.57E+07	55%		
				3.98E+07	29%		
	Commercial - RugDoctor	Liquid	Procedural Blank	ND	ND		
		Blank inoculum Control	Wipe	ND	ND		
		Positive Control	37 mm cassette	9.90E+06	6%	5%	3%
				2.19E+06	1%		
				9.90E+06	6%		
		Inoculum Control	Gauze Wipe	1.32E+08	82%	100%	23%
				2.03E+08	126%		
				1.50E+08	93%		
		Test Coupon	Wet Vacuum	3.20E+07	20%	20%	1%
				2.97E+07	18%		
				3.45E+07	21%		
	Home Use - Hoover	Liquid	Procedural Blank	ND	ND		
		Negative Control	Vacuum Sock	6.71E+02	ND		
		Blank Inoculum Control	Wipe	9.12E+01	0.00005%		
		Positive Control	37 mm cassette	1.17E+07	7%	13%	7%
				3.62E+07	21%		
				1.94E+07	11%		
		Inoculum Control	Gauze Wipe	1.71E+08	100%	100%	5%
				1.79E+08	105%		
				1.62E+08	95%		
		Test Coupon	Wet Vacuum	8.57E+07	50%	49%	8%
				7.02E+07	41%		
				9.57E+07	56%		
	ShoVac	Liquid	Procedural Blank	ND	ND		
		Negative Control	Vacuum Sock	ND	ND		
		Blank Inoculum Control	Wipe	1.01E+02			
		Positive Control	37 mm cassette	4.41E+07	27%	23%	12%
				5.15E+07	32%		
				1.45E+07	9%		
		Inoculum Control	Gauze Wipe	1.46E+08	90%	100%	10%
				1.78E+08	110%		
				1.62E+08	100%		
		Test Coupon	Wet Vacuum	7.46E+06	5%	5%	3%
				1.39E+07	9%		
				5.26E+06	3%		

c) Vinyl Results

Material	Vacuum Type	Sample Identification	Sample Method	Recovery (CFU)	Recovery (%) Compared to Average Inoculation Control	Average Recovery (%)	Stdev Recovery (%)
Vinyl	Portable - Bissell Pro-Heat*	Liquid	Procedural Blank	ND	ND		
		Negative Control	Gauze Wipe	5.60E+02	0.0001%	0.0001%	
		Positive Control	Gauze Wipe	4.28E+08	94%	96%	2%
				4.38E+08	96%		
				4.44E+08	98%		
		Inoculum Control	Gauze Wipe	4.66E+08	103%	100%	5%
				4.66E+08	103%		
				4.30E+08	95%		
		Test Coupon	Wet Vacuum	2.53E+08	56%	48%	9%
				1.71E+08	38%		
				2.27E+08	50%		
	Commercial - RugDoctor	Liquid	Procedural Blank	ND	ND		
		Blank inoculum Control	Wipe	ND	ND		
		Negative Control	Gauze Wipe	1.15E+02	0.00008%	0.00008%	
		Positive Control	Gauze Wipe	1.87E+08	131%	106%	23%
				1.23E+08	86%		
				1.46E+08	102%		
		Inoculum Control	Gauze Wipe	2.05E+08	143%	100%	38%
				1.15E+08	81%		
				1.09E+08	76%		
		Test Coupon	Wet Vacuum	8.59E+07	60%	87%	59%
				6.57E+07	46%		
				2.21E+08	155%		
	Home Use - Hoover	Liquid	Procedural Blank	4.32E+04	0.02%		
		Negative Control	Gauze Wipe	ND	ND		
		Blank Inoculum Control	Wipe	ND	ND		
		Positive Control	Gauze Wipe	1.50E+08	79%	84%	6%
				1.58E+08	83%		
				1.72E+08	90%		
		Inoculum Control	Gauze Wipe	2.94E+08	154%	100%	47%
				1.40E+08	73%		
				1.40E+08	73%		
		Test Coupon	Wet Vacuum	9.48E+07	50%	41%	7%
				7.22E+07	38%		
				7.02E+07	37%		
	ShoVac	Liquid	Procedural Blank	6.45E+04	0%		
		Negative Control	Gauze Wipe	2.66E+02	0%		
		Blank Inoculum Control	Wipe	ND	ND		
		Positive Control	Gauze Wipe	1.82E+08	129%	95%	33%
				8.80E+07	62%		
				1.33E+08	94%		
		Inoculum Control	Gauze Wipe	1.83E+08	130%	100%	38%
				8.08E+07	57%		
				1.59E+08	113%		
		Test Coupon	Wet Vacuum	1.06E+08	75%	68%	7%
				9.62E+07	68%		
				8.57E+07	61%		

C2: *Btk* Spores

a) Carpet Results

Material	Vacuum Type	Sample Identification	Sample Method	Recovery (CFU)	Recovery (%) Compared to Average Inoculation Control	Average Recovery (%)	Stdev Recovery (%)
Carpet	Portable - Bissell Pro-Heat*	Liquid	Procedural Blank	ND	ND		
		Negative Control	Vacuum Sock	ND	ND		
		Blank Inoculum Control	Wipe	ND	ND		
		Positive Control	37 mm cassette	4.40E+07	47%	62%	22%
				4.88E+07	52%		
				8.24E+07	88%		
		Inoculum Control	Gauze Wipe	9.68E+07	103%	100%	6%
				9.68E+07	103%		
				8.72E+07	93%		
		Test Coupon	Wet Vacuum	1.92E+07	21%	24%	5%
				2.74E+07	29%		
				2.10E+07	22%		
	Commercial - RugDoctor	Liquid	Procedural Blank	7.58E+05	ND		
		Blank Inoculum Control	Wipe	ND	ND		
		Negative Control	Vacuum Sock	ND	ND		
		Positive Control	Vacuum Sock	4.09E+07	39%	32%	9%
				2.70E+07	26%		
				No Data			
		Inoculum Control	Gauze Wipe	1.14E+08	109%	100%	25%
				7.55E+07	72%		
				1.25E+08	119%		
		Test Coupon	Wet Vacuum	3.43E+07	33%	29%	7%
				3.46E+07	33%		
				2.18E+07	21%		
	Home Use - Hoover	Liquid	Procedural Blank	ND	ND		
		Negative Control	Vacuum Sock	ND	ND		
		Blank Inoculum Control	Wipe	ND	ND		
		Positive Control	Vacuum Sock	3.05E+07	34%	50%	13%
				4.90E+07	55%		
				5.26E+07	60%		
		Inoculum Control	Gauze Wipe	1.17E+08	132%	100%	34%
				9.12E+07	103%		
				5.72E+07	65%		
		Test Coupon	Wet Vacuum	7.90E+06	9%	8%	4%
				3.97E+06	4%		
				1.04E+07	12%		
	ShoVac	Liquid	Procedural Blank	ND	ND		
		Negative Control	Vacuum Sock	ND	ND		
		Blank Inoculum Control	Wipe	ND	ND		
		Positive Control	Vacuum Sock	4.58E+07	45%	27%	24%
				3.87E+07	38%		
				3.51E+00	0%		
		Inoculum Control	Gauze Wipe	7.27E+07	71%	100%	25%
				1.18E+08	115%		
				1.18E+08	115%		
		Test Coupon	Wet Vacuum	2.12E+06	2%	17%	14%
				3.12E+07	30%		
				1.95E+07	19%		

b) Concrete Results

Material	Vacuum Type	Sample Identification	Sample Method	Recovery (CFU)	Recovery (%) Compared to Average Inoculation Control	Average Recovery (%)	Stdev Recovery (%)
Concrete	Portable - Bissell Pro-Heat*	Liquid	Procedural Blank	ND	ND		
		Negative Control	Vacuum Sock	ND	ND		
		Blank Inoculum Control	Wipe	ND	ND		
		Positive Control	37 mm cassette	1.20E+07	16%	18%	8%
				2.10E+07	27%		
				8.80E+06	11%		
		Inoculum Control	Gauze Wipe	5.80E+07	75%	100%	23%
				7.92E+07	103%		
				9.36E+07	122%		
		Test Coupon	Wet Vacuum	1.02E+07	13%	20%	13%
				2.74E+07	36%		
				9.35E+06	12%		
	Commercial - RugDoctor	Liquid	Procedural Blank	ND	ND		
		Negative Control	Vacuum Sock	ND	ND		
		Blank Inoculum Control	Wipe	ND	ND		
		Positive Control	37 mm cassette	1.48E+08	86%	59%	38%
				2.53E+07	15%		
				1.27E+08	74%		
		Inoculum Control	Gauze Wipe	1.59E+08	93%	100%	24%
				2.16E+08	126%		
				1.38E+08	80%		
		Test Coupon	Wet Vacuum	7.96E+07	47%	35%	10%
				5.28E+07	31%		
				4.80E+07	28%		
	Home Use - Hoover	Liquid	Procedural Blank	ND	ND		
		Negative Control	Vacuum Sock	ND	ND		
		Blank Inoculum Control	Wipe	ND	ND		
		Positive Control (Can 1)	37 mm cassette	7.15E+07	123%	73%	43%
				2.77E+07	48%		
		Positive Control (Can 3)		2.83E+07	49%		
				3.87E+07	67%		
				3.18E+07	55%		
		Inoculum Control (Can 1)	Gauze Wipe	8.48E+07	146%	100%	38%
				6.85E+07	118%		
		Inoculum Control (Can 3)		2.78E+07	48%		
				6.34E+07	109%		
				4.60E+07	79%		
		Test Coupon	Wet Vacuum	1.57E+07	27%	29%	9%
				1.26E+07	22%		
				2.27E+07	39%		
	ShoVac	Liquid	Procedural Blank	ND	ND		
		Negative Control	Vacuum Sock	ND	ND		
		Blank Inoculum Control	Wipe	ND	ND		
		Positive Control	37 mm cassette	1.00E+07	27%	26%	3%
				1.00E+07	27%		
				8.20E+06	22%		
		Inoculum Control	Gauze Wipe	2.66E+07	72%	100%	36%
				3.22E+07	88%		
				5.14E+07	140%		
		Test Coupon	Wet Vacuum	2.16E+07	59%	47%	12%
				1.76E+07	48%		
				1.27E+07	35%		

c) Vinyl Results

Material	Vacuum Type	Sample Identification	Sample Method	Recovery (CFU)	Recovery (%) Compared to Average Inoculation Control	Average Recovery (%)	Stdev Recovery (%)
Vinyl	Portable - Bissell Pro-Heat*	Liquid	Procedural Blank	ND	ND		
		Blank inoculum Control	Wipe	ND	ND		
		Negative Control	Gauze Wipe	ND	ND		
		Positive Control	Gauze Wipe	9.92E+07	129%	105%	27%
				8.40E+07	110%		
				5.85E+07	76%		
		Inoculum Control	Gauze Wipe	8.16E+07	107%	100%	12%
				8.24E+07	108%		
				6.58E+07	86%		
		Test Coupon	Wet Vacuum	5.73E+07	75%	31%	38%
				3.49E+06	5%		
				1.07E+07	14%		
	Commercial - RugDoctor	Liquid	Procedural Blank	ND	ND		
		Blank inoculum Control	Wipe	ND	ND		
		Negative Control	Gauze Wipe	ND	ND		
		Positive Control	Gauze Wipe	8.32E+07	165%	160%	6%
				7.70E+07	153%		
				8.16E+07	162%		
		Inoculum Control	Gauze Wipe	6.24E+07	124%	100%	28%
				3.52E+07	70%		
				5.34E+07	106%		
		Test Coupon	Wet Vacuum	1.36E+07	27%	18%	11%
				1.07E+07	21%		
				2.78E+06	6%		
	Home Use - Hoover	Liquid	Procedural Blank	ND	ND		
		Negative Control	Gauze Wipe	ND	ND		
		Blank Inoculum Control	Wipe	ND	ND		
		Positive Control	Gauze Wipe	5.34E+07	82%	86%	5%
				5.41E+07	83%		
				5.90E+07	91%		
		Inoculum Control	Gauze Wipe	4.51E+07	70%	100%	33%
				6.15E+07	95%		
				8.80E+07	136%		
		Test Coupon	Wet Vacuum	1.72E+07	26%	25%	3%
				1.83E+07	28%		
				1.40E+07	22%		
	ShoVac	Liquid	Procedural Blank	ND	ND		
		Negative Control	Gauze Wipe	ND	ND		
		Blank Inoculum Control	Wipe	ND	ND		
		Positive Control	Gauze Wipe	7.48E+07	53%	72%	27%
				8.32E+07	59%		
				1.43E+08	102%		
		Inoculum Control	Gauze Wipe	1.04E+08	74%	100%	24%
				1.47E+08	105%		
				1.70E+08	121%		
		Test Coupon	Wet Vacuum	1.83E+06	1%	5%	4%
				1.33E+07	9%		
				5.51E+06	4%		

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