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Evaluation of Electrostatic Sprayers for Use in a Personnel Decontamination Line Protocol for Biological Contamination Incident Response Operations

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Evaluation of Electrostatic Sprayers for Use in a Personnel Decontamination Line Protocol for Biological Contamination Incident Response Operations

Assessment and Evaluation Report

National Homeland Security Research Center
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Disclaimer

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µL	microliter(s)
µm	micrometer(s)
ADA	Aerosol deposition apparatus
Ba	*Bacillus anthracis*
Bg	*Bacillus atrophaeus* var. *globigii*
BioLab	EPA Microbiology Laboratory
CFU	Colony-forming unit(s)
CMAD	Consequence Management Advisory Division
CRZ	Contamination Reduction Zone
DB	Diluted bleach
DCMD	Decontamination and Consequence Management Division
DFU	Dry Filter Unit
DHS	U.S. Department of Homeland Security
DI	Deionized
DQI	Data quality indicator
EPA	U.S. Environmental Protection Agency
EtO	Ethylene oxide
EZ	Exclusion Zone
FAC	Free available chlorine
ft	feet
ID	Identification
in	inch(es)
H_2O_2	Hydrogen peroxide
HSRP	Homeland Security Research Program
kJ	kiloJoule
L	liter(s)
LR	Log reduction
MDI	Metered dose inhaler
min	minute
mL	milliliter(s)
mL/min	milliliter(s) per minute
N	Normal
ND	Non-detect
NHSRC	National Homeland Security Research Center
NIST	National Institute of Standards and Technology
pAB	pH-adjusted bleach
PBST	Phosphate-buffered saline with 0.05% Tween® 20
PPE	Personal protective equipment
psi	pound(s) per square inch
ppm	part(s) per million
QA	Quality assurance
QC	Quality control
RH	Relative humidity

RSD	Relative standard deviation
RTP	Research Triangle Park, North Carolina
SOP	Standard Operating Protocol
STS	Sodium thiosulfate
SZ	Support Zone
TSA	tryptic soy agar
VHP	vaporized hydrogen peroxide
VMD	Volume mean diameter

Executive Summary

This project supports the mission of the U.S. Environmental Protection Agency's (EPA's) Homeland Security Research Program (HSRP) of the Office of Research and Development's National Homeland Security Research Center (NHSRC) by providing vital scientific data that can inform decisions for EPA emergency responders. The focus of this study was to provide information relevant to the decontamination of personnel and personal protective equipment (PPE) after responding to an act of bioterrorism. To minimize worker exposure and to prevent the spread of potentially hazardous materials beyond the original areas of contamination, work zones will be established to allow workers to move between the non-contaminated Support Zone (SZ), the Contamination Reduction Zone (CRZ) where personnel decontamination takes place, and the Exclusion Zone (EZ) or area of contamination. A well-established decontamination line is essential for ensuring that potentially hazardous residues (chemical, biological or radiological) on worker PPE do not transfer into the SZ. Traditional electric backpack sprayers or handheld manual sprayers are often used to distribute a liquid decontaminant over the surfaces of worker PPE, but this process can generate a large volume of waste and may not always provide decontamination efficacy. Therefore, improved decontamination line strategies must be investigated to minimize the spread of contamination and reduce waste disposal costs.

A previous EPA study shows that compared to traditional sprayer systems, an electrostatic spray technology is more efficient, reduces waste, and delivers a more uniform distribution of liquids over uneven surfaces (USEPA 2015b). The current study explores the use of electrostatic sprayers as an alternative to the sprayers currently used in a decontamination line setting. Specifically, this study compares the performance of an electrostatic sprayer with a traditional electric backpack sprayer by evaluating the efficacy of each sprayer in removing or inactivating spores of *Bacillus atrophaeus* var. *globigii* (*Bg*), a surrogate for *Bacillus anthracis*, from different types of PPE materials.

A decontamination test chamber was used to evaluate the sprayers. The following seven PPE materials commonly found in PPE gloves, suits, boots, and related accessories were tested: nitrile, butyl, latex, Tyvek®, Tychem®, neoprene, and ChemTape®. Coupons measuring 14- by 14-inches were prepared from each PPE material and inoculated with 1×10^7 *Bg* spores. Test coupons were then placed in a vertical orientation in the decontamination test chamber and sprayed with a 10% diluted bleach (DB) decontamination solution until completely wet using either the backpack or electrostatic sprayer. Spray times for each type of sprayer were evaluated based on the flow rates as indicated in Table ES-1.

After a 5-minute contact time, the coupons were removed from the test chamber and sampled using a wipe sampling method. Wipe samples were collected in specimen cups containing a pre-determined volume of sodium thiosulfate (STS) neutralizing agent used to quench the decontamination reaction and preserve viable spores present in each sample. Wipe samples were then analyzed for the presence of viable spores. Overspray liquid runoff and air samples were also collected and analyzed for the presence of viable spores. The liquid runoff sample collection bottles also contained STS.

The sprayer decontamination efficacy was determined by comparing the mean Log10 number of colony forming units (CFU) observed for the inoculum controls (stainless-steel coupons

inoculated but not exposed to decontamination treatment) to the mean Log10 number of CFU observed for the decontaminated test samples.

Overall, both sprayers achieved a surface log reduction (LR) of greater than or equal to 6, with no statistically significant difference between the two sprayers (*p*-value = 0.49) (Table ES-1) For three of the seven test materials, no surface CFU were detected when the electrostatic sprayer was used. In contrast, there were CFU detected on coupons for all of the traditional backpack sprayer tests.

An effective personnel decontamination line spray technology will apply decontaminant solutions to the intended materials with: (1) high efficacy for the contaminant; (2) little to no cross-contamination among field personnel and equipment; (3) little or no spreading of contamination beyond the Exclusion Zone; and (4) minimal liquid waste generation. To assess the transport or migration of viable spores off the test surfaces that could lead to cross contamination, liquid runoff samples were collected and quantitatively analyzed. Each sprayer also was evaluated when deionized (DI) water was substituted for DB, and test coupons were sprayed under the decontamination spray test conditions to understand how sprayer application affects the physical removal of spores from a material surface. One runoff sample was collected per test and analyzed for the number of viable spores (CFU). All of the runoff samples collected from the backpack sprayer contained a large number of viable spores, whereas all of those collected from the electrostatic sprayer contained very few to no detectable viable spores. Runoff samples collected from the backpack sprayer ranged from 5.3×10^4 CFU to 5.0×10^6 CFU with a standard deviation of $\pm 1.6 \times 10^6$. Runoff samples from the electrostatic sprayer ranged from no CFU detected to 1 spore detected.

The field applicability of a spray technology also depends on its ability to minimize cross-contamination among field personnel and equipment, to limit the spread of contamination beyond the area of initial contamination, and to minimize additional risks to personnel. The number of spores physically removed via liquid runoff from test coupons indicates a potential cross-contamination risk that could impact the extent of contamination at the site. The application of decontamination solution using a backpack sprayer was observed to physically remove almost twice as many spores compared to the electrostatic sprayer, due to the liquid volume used and the tendency for runoff from the PPE materials. Therefore, use of the backpack sprayer, as tested in this study, physically removes biological contamination from the PPE surface and could result in environmental cross-contamination of PPE and other equipment in a biological decontamination line.

To evaluate a suitable spray technology for a decontamination line, liquid waste generation assessment is another important parameter to be considered, so quantifying and comparing the amount of potentially hazardous waste generated by each sprayer type was also an overarching project objective. Traditional electric backpack sprayers tend to have higher flow rates, resulting in the application of larger volumes of decontamination liquid, thus generating more liquid hazardous waste. Additionally, an electrostatic sprayer provides a more uniform distribution using a minimal amount of decontamination solution over the surface area sprayed, thereby significantly reducing waste streams and costs associated with liquid hazardous waste disposal. During decontamination testing, runoff liquid volumes were collected and measured gravimetrically. The quantity of liquid waste generated by the electrostatic sprayer was almost 75 times less than the amount generated by the backpack sprayer (Table ES-1).

Table ES-1. Summary of findings by sprayer type

Characteristic	Electrostatic Sprayer	Backpack Sprayer
Flow rate (actual)	62 mL/minute	996 mL/minute
Time required to cover a surface area of 14 in by 14 in (actual)	30 seconds	10 seconds
Sprayer efficacy across all seven test materials	≥ 6 LR (except latex material)	≥ 6 LR
Waste generation (average)	6 mL	450 mL

1.0 Introduction

The project was conducted to support jointly held missions of the U.S. Department of Homeland Security (DHS) and the U.S. Environmental Protection Agency (EPA). The EPA's Homeland Security Research Program (HSRP) provides credible information to protect human health and the environment from adverse impacts arising from terrorist threats and other contamination incidents. Within the EPA, the project supports the mission of EPA's HSRP by providing relevant information pertinent to the decontamination of contaminated zones resulting from a biological incident.

This report discusses a decontamination project that evaluated the decontamination efficacy and physical migration (transport) of *Bacillus* spores and operational efficiency of two types of sprayer technologies: electrostatic and traditional electric backpack sprayers. These sprayers were used to apply a decontamination solution to materials that are common constituents of emergency responder personal protective equipment (PPE) under operationally relevant exposure conditions and contact times. The following sections discuss the project background and objectives.

1.1 Background

Under Homeland Security Presidential Directive 10, the DHS is tasked with coordinating with other appropriate federal departments and agencies to develop comprehensive plans that "provide for seamless, coordinated Federal, state, local, and international responses to a biological attack." As part of these plans, the EPA, in a coordinated effort with DHS, is responsible for "developing strategies, guidelines, and plans for decontamination of persons, equipment, and facilities" to mitigate the risks of contamination after a biological weapons attack. EPA's National Homeland Security Research Center (NHSRC) provides 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. Within the NHSRC, the Decontamination and Consequence Management Division (DCMD) conducts research to provide expertise and guidance on the selection and implementation of decontamination methods that may ultimately provide the scientific basis for a significant reduction in the time and cost of decontamination events. The NHSRC DCMD decontamination research program goals are to provide: (1) expertise and guidance on the selection and implementation of decontamination methods; and (2) the scientific basis for a significant reduction in the time and cost of decontamination events. The NHSRC works with EPA's Office of Emergency Management, who have revised the biological personnel decontamination line protocol based on a previous NHSRC PPE decontamination study (USEPA 2015a, USEPA 2015c).

In previous studies, some of the most promising methods for applying decontaminants such as the electrostatic sprayer were found to be more efficient than the traditional electric backpack sprayer in uniform distribution for the decontamination of flat surfaces of large building materials (USEPA 2015b). However, these technologies have not been assessed for time-limited (a few minutes) applications such as the decontamination of personnel PPE and equipment in a biological decontamination line.

After the release of a hazardous biological substance, the impacted site is characterized and mapped into controlled work zones to mitigate the spread of further contamination and prepare for cleanup as shown in Figure 1-1 (USEPA 1992).

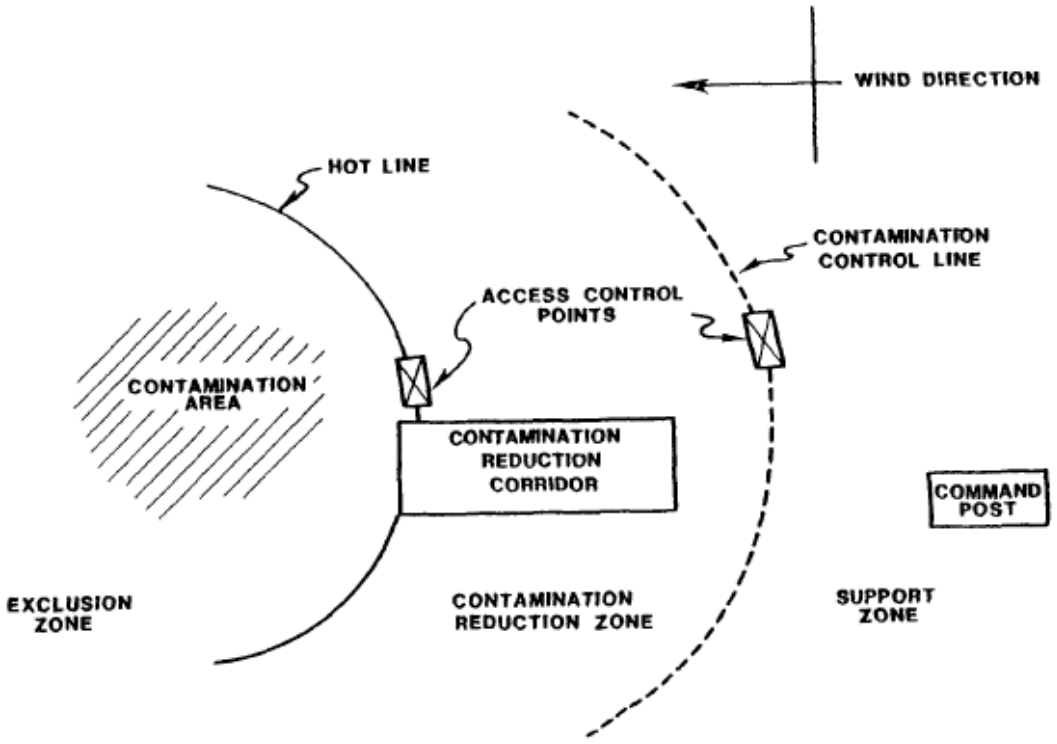


Figure 1-1. USEPA Standard Operating Safety Guides, Site Control Work Zones (USEPA 1992)

The Exclusion Zone (EZ, or Hot Zone), set up downwind of the Support Zone (SZ), is the contaminated zone and has the highest potential for exposure. The Contamination Reduction Zone (CRZ) is the transition area between the EZ and the SZ. The decontamination line is located just inside the CRZ, typically near the exit of the EZ. The purpose of the decontamination line is twofold: (1) to ensure that potentially harmful or dangerous residues on persons, samples, and equipment are confined within the CRZ; and (2) to extract personnel from their PPE safely while also protecting decontamination line personnel and minimizing liquid waste. Personnel who have been performing decontamination activities exit the EZ and move through the decontamination line in the CRZ, where traditional electric backpack sprayers or decontamination showers are often used to distribute a decontamination solution over entry personnel to decontaminate the PPE and remove potentially harmful surface residues. This process has the potential to generate a significant quantity of liquid hazardous waste. However, if an electrostatic sprayer technology could be used to achieve the same purpose but instead deliver a more uniform distribution of decontamination solution over the PPE surface while using less liquid decontaminant, decontamination efficacy may be improved and waste streams and their associated costs may be reduced.

This project addresses the direct need to evaluate alternative sprayer technologies and techniques by assessing the decontamination efficacy and consequences of using an electrostatic sprayer. The results of this study will be included as an addendum to the EPA Technical Support Working Group Task CB-CM-3499 final report, “Test Method for Standardized Evaluation of Decontamination Solutions.” The study results will also provide quantitative information relevant to technical and operational aspects of personnel decontamination, which can assist emergency responders in

mitigating health hazards to personnel operating in a chemically- or biologically-contaminated environment and in minimizing cross-contamination

1.2 Objectives

One main objective of this study was to evaluate the decontamination efficacy of electrostatic sprayer technology for use in a decontamination line. Another objective was to compare sprayer technologies currently used in decontamination lines for personnel decontamination (i.e., handheld “garden-type” sprayers) to the electrostatic sprayer technology.

To compare the two technologies, both were tested by applying a diluted bleach decontamination solution to a variety of constituents commonly found in emergency responder PPE Levels B or C. The study used operationally relevant exposure conditions and field-appropriate decontamination solution contact times to evaluate not only the surface log reduction (LR) of *Bacillus* spores but also the physical removal and migration of the spores. This study provided quantitative efficacy information relevant to sprayer decontamination methods. These results identified a useful means to: (1) assist decision makers and first responders in mitigating health hazards to personnel in the decontamination line by minimizing reaerosolization; (2) minimize the potential for contaminant migration from the incident scene; and (3) reduce liquid waste from the personnel decontamination process. Additional goals were to assess electrostatic sprayer operational efficiency and evaluate any potential safety hazards involved with its use.

2.0 Experimental Approach

The testing was conducted at EPA's Research Triangle Park (RTP) facility in North Carolina. The general experimental approach used to meet the project objectives is described below.

1. **Preparation of representative samples of test materials:** The following seven PPE materials used in suits, boots, gloves, and related accessories were selected for testing: nitrile, butyl, latex, Tyvek®, Tychem®, neoprene, and ChemTape®. Materials were categorized as plastic (Tychem®, Tyvek®, and ChemTape®) or rubber (nitrile, butyl, latex, and neoprene) for surface sampling purposes. Test coupons of each material were prepared as described in Section 3.1.1.
2. **Contamination of PPE coupons with a standardized inoculum of the target organism:** The test material coupons were contaminated using an aerosol deposition method that delivered a known quantity of spores in a repeatable fashion. Approximately 1×10^7 spores of *Bacillus atrophaeus* var. *globigii* (*Bg*), a surrogate organism for *Bacillus anthracis* (*Ba*), were deposited onto each test material coupon as discussed in Section 3.3.2.
3. **Preparation of decontamination solution:** The decontamination solution consisted of 10% diluted bleach (DB), freshly prepared on each test day as discussed in Section 3.4.2.
4. **Preparation of neutralizing agent:** STS was used as a neutralizing agent as discussed in Section 3.4.3. STS was applied to stop the decontamination activity after a prescribed exposure time. STS also was added to procedural blanks, test coupons, and runoff samples.
5. **Application of decontamination procedure on test material coupons:** Procedural blanks (non-inoculated coupon) and test coupons (inoculated) were arranged in the test chamber in a vertical position, then sprayed using either the electric backpack or the electrostatic sprayer in accordance with the pre-determined test conditions as discussed in Section 3.4. Deionized (DI) water was used for the procedural positive coupons, as a control to decouple the physical spore removal from the surface against the sporicidal activity of the decontamination solution. After the prescribed five-minute exposure time, coupons were collected and transferred to a sampling table for wipe sampling as discussed in Section 5.1.1.
6. **Coupon sampling:** Coupons were sampled using the wipe sampling method described in Section 5.1.1. Based on the material category (plastic or rubber), either three or two wipe samples were collected from each coupon. All coupon wipe samples were extracted in Phosphate Buffered Saline (135 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4) with 0.05% Tween® 20 (PBST).
7. **Collection of runoff:** Liquid runoff from the coupons was collected through the chamber drain outlet in sterile Nalgene® bottles containing pre-determined volumes of STS neutralizer.
8. **Sample extraction and analysis**: Wipe samples were extracted in PBST, and aliquots of the wipe extracts and liquid runoff samples were analyzed using an automated system for plating assays or filter plating to determine the number of colony forming units (CFU) present in each sample.
9. **Determination of decontamination efficacy:** Decontamination efficacy, as a function of the sprayer technology and material type, was measured as LR in viable spores recovered following treatment, as compared to controls. Typically, for laboratory assessments of

decontamination efficacy, an LR of 6 or greater is considered effective. Decontamination efficacy for each coupon was determined by comparing test coupon results to stainless-steel inoculum control coupon results. Quantitative assessment of residual (background) contamination was performed by sampling procedural blanks (non-inoculated coupons exposed to the same decontamination process as the test coupons). The transfer of viable organisms to decontamination liquid waste was evaluated through quantitative analysis of spraying procedure residue samples (such as liquid runoff samples). The physical removal/transfer of spores was evaluated by sampling procedural positives (sprayed with DI water instead of DB).

3.0 Experimental Materials and Methods

This section describes the test materials, test chamber, test organism and inoculation, and decontamination equipment (sprayers), solution, and neutralizer used to achieve the project objectives.

3.1 Test Materials

The representativeness and uniformity of test materials are essential in achieving adequate evaluation results. Materials are considered representative if they are typical of materials currently used in the field in terms of quality, surface characteristics, and structural integrity. For this project, representativeness was ensured by: (1) selecting test materials typically representative of PPE, and (2) obtaining these materials from appropriate suppliers. Uniformity was maintained by obtaining and preparing a quantity of material sufficient to allow the preparation of multiple test samples with presumably uniform characteristics (that is, test coupons for each test were prepared using the same batch of material).

Coupons of the following seven PPE materials were prepared on site: nitrile, butyl, latex, Tyvek®, Tychem®, neoprene, and ChemTape®. Table 3-1 summarizes the coupon materials, including their characteristics and sources.

Table 3-1. Material Specifications

Material	PPE Type	Category	Thickness (inch)	Manufacturer/Supplier Name
Stainless Steel	NA	Metal	0.02	-
Nitrile (Buna-N)	Gloves	Rubber	0.01 to 0.02	McMaster-Carr Elmhurst, IL
Butyl		Rubber	0.06 to 0.07	MSC Industrial Supply Co. Melville, NY
Latex		Rubber	0.01 to 0.02	
Tyvek® 400	Suits	Plastic	0.0059	DuPont Wilmington, DE
Tychem® QC/2000		Plastic	0.01	
Neoprene (chemical-resistant rubber)	Boots	Rubber	0.120 to 0.130	MSC Industrial Supply Co. Melville, NY
ChemTape®	Accessory	Plastic	0.0125	Kappler Guntersville, AL

Coupon fabrication and test material sterilization are discussed below.

3.1.1 Coupon Fabrication

All coupon dimensions were 14- by 14-inches (in). Material coupons were prepared on a plywood base using the PPE materials listed in Table 3-1. The following materials and equipment were used to prepare the coupons:

- 0.438-in Plywood (Plytanium 15/32 CAT PS1-09 Pine Plywood Sheathing, from Lowes, Item # 12192)
- PPE materials (Table 3-1)
- ½-in staples
- Staple gun
- Safety razor utility knife
- Table saw

- Tape measure
- Spray adhesive (Product ID 74, 3M Foam Fast Spray Adhesive Clear, Fort Worth, TX)
- Appropriate PPE (including safety glasses, cut-resistant gloves, and safety footwear)

The procedure summarized below was used to prepare all the test coupons.

1. Personnel preparing the coupons donned appropriate PPE, including safety glasses, cut-resistant gloves, and safety footwear.
2. Using a table saw, a 14- by 14-in square of Plywood was cut.

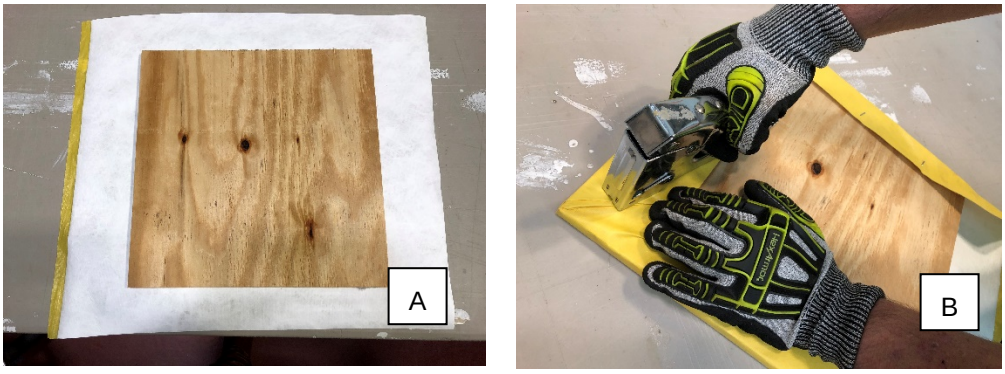


Figure 3-1. Test material, Plywood (A) and Coupon Preparation (B)

3. Using a safety razor utility knife, a 16- by 16-in square of PPE material was cut. For frail materials that tend to tear when only a single layer was wrapped around the Plywood (such as latex), a double layer of material was used to prepare the coupon.

   The material square was placed with the backing side up on a table, and the Plywood was placed over it.
4. The test material was then folded onto the Plywood and stapled in place using a staple gun (Figure 3-1 B). Thick materials such as butyl and neoprene were stuck to the Plywood using a spray adhesive. Figure 3-2 shows a finished coupon.

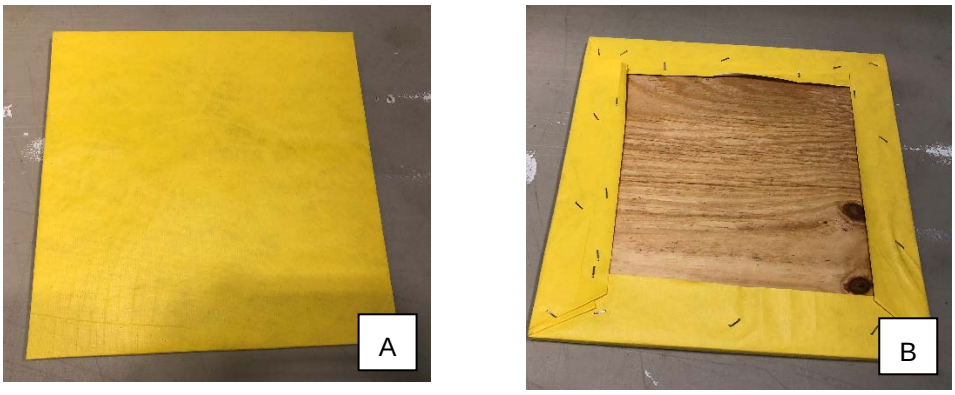


Figure 3-2. Front (A) and Back (B) of Finished Test Coupon on Plywood

5. For ChemTape®, which is 2 in wide, the tape was wrapped on the 14- by 14-in Plywood in single layers, leaving no gap between adjacent strips.

Figure 3-3. shows finished coupons of each test material.

Figure 3-3. PPE Test Coupons

3.1.2 Sterilization Process

Materials and supplies were sterilized prior to testing using a method suitable for each item. Sterilization procedures included vaporized hydrogen peroxide (VHP) sterilization, autoclaving, filter sterilization, ethylene oxide (EtO) sterilization, and pH-adjusted bleach (pAB) sterilization, as discussed in the below table (Table 3-2.)

Table 3-2. Sterilization Processes Used

Sterilization Process	Description	Materials/Supplies
Vaporized Hydrogen Peroxide® (VHP) Sterilization	Before the sterilization process, coupons and sprayers (with the lid open) were wrapped in bags, and the ADAs were placed in large plastic bins. Hydrogen peroxide vapor was produced using a STERIS VHP 1000ED generator loaded with a 35% hydrogen peroxide (H_2O_2) Vaprox® cartridge. Each sterilization cycle generated a maximum concentration of 250 parts per million (ppm) VHP and lasted four hours. Negative control coupons were used to verify coupon sterility.	Test material coupons, Aerosol deposition apparatuses (ADAs), and Sprayers
Autoclaving	Sterilized using a 30 minute gravity cycle at 121°C in a STERIS Amsco Century SV 120 Scientific Pre-Vacuum Sterilizer (STERIS Corporation, Mentor, OH). The stainless-steel coupons measured 14- by 14-in and were carefully wrapped in aluminum foil to maintain sterility when removed from the autoclave. A sterility check for the stainless-steel coupons was performed using swabs (BactiSwab® Collection and Transport System, Remel, Thermo Fisher Scientific, Waltham, MA).	Stainless-steel inoculum control coupons (0.02 inch thick), Nalgene® bottles, and carboys
Filter sterilization	Sterilized using a vacuum filter (Corning 430513, Bottle Top Vacuum Filter, 0.22 micrometer (µm) pore size, 33.2 centimeter CA membrane, Tewksbury, MA) and a sterile 1-liter (L) Pyrex bottle. Sterilized DI water was transferred into a sterile 5 L carboy. A 50-milliliter (mL) sample from each 5 L batch was sent to the NHSRC RTP Microbiology Laboratory (BioLab) for sterility analysis.	DI water

Sterilization Process	Description	Materials/Supplies
Ethylene Oxide (EtO) sterilization	Sterilized using an Andersen EtO sterilizer system (PN 333 EOGas®, Haw River, NC). The sterilization procedure is summarized below. 1. All the items to be sterilized were packed in appropriate EtO envelopes and sealed. 2. Sealed EtO envelopes were placed in appropriate sterilization bags, along with a dosimeter, humidichip, and EtO dispenser. 3. The sterilization bags were vacuum-sealed and loaded into the EtO sterilizer for an 18 hour sterilization cycle.	Sampling templates and inoculation equipment
Sterilization using pAB solution	To avoid cross contamination between tests, the interior of the test chamber was sterilized using pAB immediately before testing. This process commonly is referred to as "reset" of the test chamber. The pAB solution was prepared using DI water, 5% acetic acid, and bleach in an 8:1:1 ratio, then loaded into the pre-sterilized (with pAB) tank of a SHURflo 4 ProPack Rechargeable Electric Backpack Sprayer SRS-600 (Pentair-SHURFlo, Costa Mesa, CA). The sprayer was used to coat the interior of the test chamber with pAB. After a 10-minute (min) contact time, the chamber was rinsed with sterile DI water to remove residual pAB from the chamber. A swab (BactiSwab® Collection and Transport System, Remel, Thermo Fisher Scientific, Waltham, MA) sample of the test chamber was collected for a sterility check.	Interior of the test chamber

3.2 Test Chamber

The sprayer test chamber is located at EPA's RTP facility in North Carolina. The test chamber measures 4- by 4- by 4-feet (ft) and was designed to accommodate three 14- by 14-in coupons at a time in a horizontal or vertical position. For this project, a single PPE coupon was placed in the test chamber at a time and sprayed in a vertical position as shown in Figure 3-4.

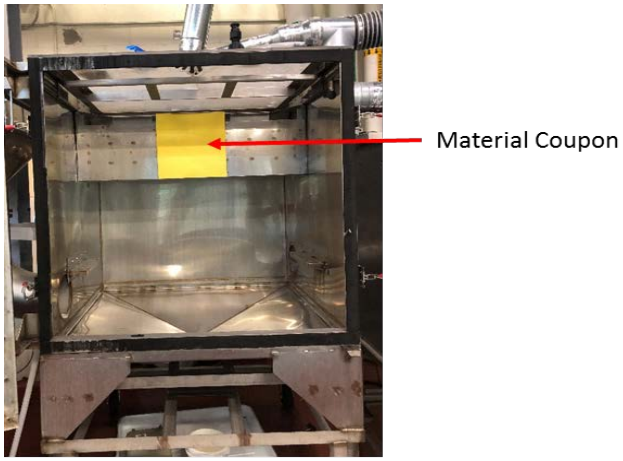


Figure 3-4. Decontamination Test Chamber with Coupon

Except for the clear acrylic front and top pieces, the test chamber is constructed of solid stainless steel. The reverse-pyramid design of the chamber bottom allows the collection of coupon runoff through a central drain with a 3-in diameter. The chamber air is exhausted to the facility's air handling system through a connection also fitted with a sampling port. The port was used to collect samples during each test so that the quantity of aerosolized spores could be estimated.

Two HOBO Relative Humidity/Temperature sensors (Model U12, Onset Computer Corporation, Bourne, MA) were placed around the spraying and inoculation areas. Temperature and humidity were measured to generate qualitative information in anticipation of helping to explain variations in project data, if any.

3.3 Test Organism and Inoculation Procedure

Details on the test organism and inoculation process are provided in the following sections.

3.3.1 Bg Surrogate for Ba

Bg, a surrogate for the spore-forming bacterial agent *Ba,* was used for this project. Like *Ba*, *Bg* is a soil-dwelling, gram-positive, aerobic microorganism but unlike *Ba, Bg* is non-pathogenic. *Bg* forms an orange-pigmented colony when grown on nutrient agar, a desirable characteristic for detecting 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).

3.3.2 Bg Spore Inoculation

The test coupons were inoculated with *Bg* spores using a metered-dose inhaler (MDI). The MDI canister contained *Bg* spores suspended in ethanol solution, HFA-134A propellant (1,1,1,2-tetrafluoroethane) gas, and Tween®. The MDI actuator is a small plastic tube in which the MDI canister is inserted (Figure 3-5(A)).

Figure 3-5. MDI Actuator (A) and Canister (B)

Each time the actuator is depressed, a repeatable number of spores are deposited on the coupon (Lee et al. 2011). MDIs selected for testing must weigh more than 10.5 grams. MDIs weighing less than 10.5 grams are retired and no longer used. Each test coupon was inoculated independently using the MDI canister and actuator. The MDIs were weighed before and after inoculation to ensure proper discharge.

For quality control (QC) purposes for the MDIs, a stainless-steel inoculation control coupon was included as the first, middle, and last coupon inoculated using a single MDI in a single test.

For the MDI inoculation procedure (Lee et al. 2011; Calfee et al. 2013), an ADA measuring 1- by 14-in was placed on the surface of the test coupon (Figure 3-6).

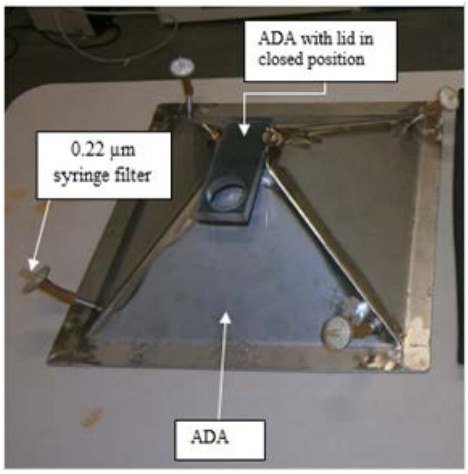


Figure 3-6. 14- by 14-in ADA with Syringe Filter

The ADA was clamped to the test coupon, and the MDI was attached to the top of the ADA. A slide below the MDI was opened, and the MDI was activated. After inoculation, the slide was closed and the MDI was removed. The assembly was kept closed while the spores were allowed to settle for 18 hours before testing. This process was repeated for each test. (Figure 3-7).

Figure 3-7. Inoculation Setup

3.4 Decontamination Equipment, Solution, and Neutralizer

This section discusses decontamination equipment (sprayers), decontamination solution, and neutralizer.

3.4.1 Sprayers

The sprayers summarized in Table 3-2 were tested.

Table 3-2. Decontamination Sprayers Tested

Sprayer Type	Description	Flow Rate
Electric backpack	SHURFlo 4 ProPack Rechargeable Electric Back Pack Sprayer SRS-600 (Pentair-SHURFlo, Costa Mesa, CA)	996 mL/minute
Electrostatic	SC-ET HD electrostatic sprayer (Electrostatic Spraying Systems ESS, Watkinsville, GA)	62 mL/minute

Each type of sprayer is discussed in more detail below.

3.4.1.1 Electric Backpack Sprayer

The SHURflo 4 SRS 600 ProPack rechargeable electric backpack sprayer used for this project measures approximately 36 in high by 24 in wide by 6 in long (Figure 3-8). This backpack sprayer has a variable speed pump, an adjustable spray cone nozzle, and the hose is made of reinforced/braided PVC. This sprayer has been used in previous EPA decontamination studies and provides a good representation of the type of handheld sprayer nozzle that is typically used in personnel decontamination lines.

Figure 3-8. Electric Backpack Sprayer

After sterilization, the 4-gallon tank of the sprayer was filled with 10% DB. The sprayer knob was tightened on each test day to ensure a consistent cone spray (several inches in diameter) on all coupons. The consistency of spray was verified by performing a spray pattern test using a construction paper. Before each test, a stop watch and a 500 mL graduated cylinder were used to verify (in triplicate) that the approximate flow rate of each sprayer was 1,020 milliliters per minute (mL/min). The liquid was collected and volume recorded based on a 10-second spray time. Readings were expected to be within 10% of the average. If they were not, the nozzle was tightened or the sprayer wand was changed, and the flow rate was re-tested until the desired flow rate was achieved.

3.4.1.2 Electrostatic Sprayer

The air-assisted SC-ET HD electrostatic sprayer shown in Figure 3-9 was used in this study.

Figure 3-9. SC-ET HD Air-Assisted Electrostatic Sprayer

This sprayer measures approximately 22 in high by 16 in wide by 10 in long and produces electrically charged spray droplets that are carried to the target in a gentle low-pressure air stream. The sprayer tank has a capacity of 4.7 L and a spray gun with hose length of 15 ft. The SC-ET HD ESS system is intended for light-duty, quick disinfection and sanitization applications and is compatible with most conventional chemicals. The sprayer also is equipped with a patented MaxCharge™ technology electrostatic spray gun that delivers droplets with a volume median diameter (VMD) of 40 µm. The electrostatic charge induced by the MaxCharge™ nozzle is strong enough to allow the droplets to move in any direction to cover surfaces homogeneously, according to the manufacturer.

Air-assisted electrostatic spray technology gives more than twice the deposition efficiency of hydraulic sprayers and non-electrostatic types of air-assisted sprayers (Kabashima et al. 1995). Prior to testing, the spray distance was set to 1 ft to cover the whole 14- by 14-in test coupon area. A stop watch and a 250-mL graduated cylinder were used to verify (in triplicate) that the approximate flow rate of the sprayer was 240 milliliters/minute (mL/min). The liquid was collected and volume recorded based on a 30-second spray time. Readings were expected to be within 10% of the average. If they were not, the spray gun was checked for bleach corrosion and re-cleaned if necessary. The flow rate was re-tested until the desired flow rate was achieved. During operation of the electrostatic backpack sprayer, personnel wore anti-static gloves (Part No. AS9674S, MCR Safety, Collierville, TN) for safety.

3.4.2 Decontamination Solution

DB (10%) was used as the decontamination agent for this study as referenced in the EPA Consequence Management Advisory Division's (CMAD's) "BioResponse Decontamination Line Standard Operating Protocol" (SOP) (USEPA 2015c). The solution was prepared in fresh 1-L batches on each test day using the procedure summarized below.

1. In a sterile container, 900 mL of DI water was added to 100 mL of Clorox® Concentrated Germicidal Bleach.
2. The solution was manually mixed for 1 min, resulting in a 10% DB solution.
3. The pH and free available chlorine (FAC) of the solution were measured before use.

3.4.3 Neutralizing Agent

Neutralizing agents are used to stop the decontamination reaction to achieve a prescribed contact time. STS has been demonstrated to be effective for bleach on both porous and nonporous surfaces (Calfee et al. 2011), so it was selected for use during this test. The volume of STS added to the sample containers (wipe and liquid runoff) was determined by measuring the FAC of the DB solution using a HACH® Hypochlorite Test Kit (Model CN-HRDT, Fisher Scientific, Waltham, MA). The HACH test kit uses an iodometric method to determine FAC and chlorite concentrations. Method development tests were conducted to ensure the effectiveness of STS before its use in this study.

A 2 normal (N) solution of STS was prepared as summarized below.

1. STS pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$, 496.4 grams) crystals were added to 1 L of DI water.
2. The solution was stirred until all the crystals dissolved completely.
3. The 2 N STS solution then was sterilized using a bottle-top filter (150 mL Corning Bottle Top Filter, 0.22 µm cellulose acetate, 33 millimeter neck, sterile, Catalog No. EK-680516, Corning, NY) and a vacuum filtration system.
4. Each batch of STS was dated, stored at 4°C, and used within six months of preparation.

4.0 Decontamination Testing

This section discusses the test matrix and approach for the decontamination coupon testing.

4.1 Test Matrix

Table 4-1 summarizes the test matrix characteristics including test material and number of coupons tested.

Table 4-1. Test Matrix

Test ID	Test Material	Category for wipe sampling	Decontamination Technology	Total No. of Material Coupons
1	Nitrile (Buna-N)	Rubber	Backpack Sprayer	12
2			Electrostatic Sprayer	12
3	Butyl	Rubber	Backpack Sprayer	12
4			Electrostatic Sprayer	12
5	Latex	Rubber	Backpack Sprayer	12
6			Electrostatic Sprayer	12
7	Tyvek®	Plastic	Backpack Sprayer	12
8			Electrostatic Sprayer	12
9	Tychem®	Plastic	Backpack Sprayer	12
10			Electrostatic Sprayer	12
11	Neoprene (chemical-resistant rubber)	Rubber	Backpack Sprayer	12
12			Electrostatic Sprayer	12
13	ChemTape®	Plastic	Backpack Sprayer	12
14			Electrostatic Sprayer	12

Each test used the coupon configuration summarized in Table 4-2.

Table 4-2. Test Coupon Configuration

Type of Coupon	No. per Test	Contaminated with 10^7 *Bg* Spores	Decontaminated
Negative control	1	No	No
Procedural blank	1	No	Yes, 10% DB
Test	3	Yes	Yes, 10% DB
Procedural positive control (blank for procedural positive coupons)	1	No	Yes, sterile DI water
Procedural positive	3	Yes	Yes, sterile DI water
Positive control	3	Yes	No
Stainless-steel inoculation control (used in calculation of decontamination efficacy, i.e., LR)	3	Yes	No

4.2 Testing Approach

The decontamination approach consisted of applying the 10% DB solution to the surface of each 14- by 14-in coupon until the coupon was completely wet (visually). This process required 10 and 30 seconds for the electric backpack and electrostatic sprayers, respectively.

The migration and physical removal of spores were evaluated as functions of the following:

- Type of sprayer (electric backpack or electrostatic)
- Type of PPE test material

The approach below was used for the testing.

1. **Test Chamber Sterilization and Cleaning**: Freshly prepared pAB was used to sterilize the test chamber as discussed in Section 3.1.2.5 before each procedural blank test. In addition, to avoid biased results in the liquid runoff samples caused by residual bleach, the test chamber also was cleaned with pAB and sterile DI water before processing the procedural positive coupons.
2. **Coupon Setup**: For testing, a single coupon was placed in a vertical orientation in the center of the test chamber (as shown in Figure 3-4). Procedural blank coupons were always tested first, followed by test coupons.
3. **Liquid Runoff**: A clean, sterile Nalgene® bottle (500 mL or 1 L) preloaded with a pre-determined volume of STS was used to collect liquid runoff by placing the bottle under the drain of the test chamber (Figure 4-1). The bottles were weighed before and after each test to determine the volume of liquid runoff generated by each type of sprayer and test material.

Figure 4-1. Liquid Runoff Collection Assembly

4. **Decontaminant application**: The 10% DB solution was applied using either the electric backpack or electrostatic sprayer as summarized below.

    a. A spray test was initiated by checking the flow rate of the sprayer as described in Section 3.4.1.1 and Section 3.4.1.2. Later in the test procedure, a spray pattern test was conducted by spraying from one foot away onto a piece of construction paper measuring 14- by 14-in mounted in the test chamber in the vertical orientation. The spray pattern was visually assessed to ensure that the spray was being discharged into the center of the paper.

    b. The coupons were sprayed using multiple side-to-side strokes (starting from the top left side of the coupon and ending at the bottom right, moving downward, in a "Z" pattern) to completely wet the coupon surface. This step was repeated as often as necessary to satisfy the required spray duration. Table A-1 in Appendix A presents the spray duration log. A contact time of five minutes, determined from CMAD's "BioResponse Decontamination Line SOP" (EPA 2015c) was allowed before sampling. Procedural blanks (coupons of each test material not contaminated with *Bg* spores) were processed first, followed by the test coupons. The physical transfer of spores using both types of sprayers was evaluated by spraying a set of coupons (Procedural positive control and material coupons) with sterile DI water. These coupons were processed using the same procedure as the test coupons.

After decontamination spraying, residual spores were recovered from the coupons using the wipe sampling technique discussed in Section 5.1.1 and assessed for viability. Liquid waste (runoff) samples were also collected and analyzed for viable spores. Together, results from these samples were used to determine the decontamination efficacy of each type of sprayer under the test conditions discussed above using 10% DB.

5.0 Sampling and Analytical Procedures

A sampling data log sheet was maintained for each sampling event (or test) that included each sample's identification (ID) number, the date, test name, sample description, and sampling start and end times. Appendix A presents a sample of that the data log. The sample ID numbers and descriptions were pre-printed on the sampling data log sheet before sampling began. Digital photographs were taken to document activities throughout the test cycle.

The following sections discuss the sample types, sample quantities, sample handling, microbiological analysis, decontamination solution characterization, and determination of efficacy.

5.1 Sample Types

The types of samples collected for this study include wipe, liquid runoff, aerosol(air), and sterility check swab samples, as discussed below.

5.1.1 Wipe Samples

The test materials were categorized as plastic (Tyvek®, Tychem®, and ChemTape®) and rubber (nitrile, butyl, latex, and neoprene). To minimize cross-contamination of decontaminated coupons, each coupon surface was being wiped completely to collect surface wipe samples, leaving no contaminated liquid residue behind. Surface wipe samples were collected using polyester-rayon blend wipes (Curity all-purpose sponges #8042, 2- by 2-in, four-ply, Covidien PLC, Dublin, Ireland). Three wipes were used on each plastic material coupon and two wipes were used on each rubber material coupon. The number of wipes required to effectively remove all liquid from the surface of each material type was determined as a part of a method development process.

The BioLab prepared the wipes for each test. Using sterile forceps, each four-ply wipe was aseptically removed from the packing and placed in an unlabeled, sterile, 120-mL specimen cup (Catalog No. 14-375-462, Fisher Scientific, Waltham, MA). Each wipe was moistened by adding 2.5 mL of sterile PBST, and the cup was capped. The wiping protocol used in this project was adopted from the protocol described by Busher et al. (2008) and Brown et al. (2007). The coupon surface was wiped by applying consistent pressure. An S-stroke motion was used both horizontally and vertically to cover the sample area as shown in Figure 5-1.

Figure 5-1. Wipe Sampling of Test Coupon

After wiping, each wipe was loosely folded and placed in a sterile specimen cup containing PBST (60 mL for plastic materials and 40 mL for rubber materials) and a pre-determined amount of STS neutralizer. Wipe start and end times were recorded using a wipe sampling log (Table A-2 in Appendix A).

5.1.2 Liquid Runoff Samples

Decontamination solutions that accumulated through the test chamber collection port (drain) were collected as liquid runoff samples. Each sample was collected in a 500 mL Nalgene® bottle pre-loaded with a pre-determined volume of STS neutralizer. Runoff collection sample volumes were determined by subtracting the weight of the collection bottle (containing only the STS neutralizer) from the weight of the bottle with the runoff sample in it. The weights were recorded using a liquid runoff collection log (Table A-3 in Appendix A).

5.1.3 Aerosol (Air) Samples

Aerosol samples were collected using Via-Cell® bioaerosol cassettes (Part No. VIA010, Bioaerosol Sampling Cassette, Zefon International, Ocala, FL) as shown in Figure 5-2.

Figure 5-2. Via-Cell® Bioaerosol Sampling Cassette

During each test, aerosol samples were collected from inside the test chamber interior and from the test chamber exhaust duct. The initial and final temperature, gas meter volume, and sample flow change in enthalpy (ΔH) was recorded for each sample using the Via-Cell® cassette log (Table A-4 in Appendix A). At the end of each sampling event, the Via-Cell® cartridge was aseptically retrieved from the pump and placed in the Via-cell® pouch. The outside of the pouch was sterilized using bleach wipes before transport to the BioLab for analysis.

5.1.4 Sterility Check Swab Samples

Pre-moistened swabs (BactiSwab® Collection and Transport System, Remel, Thermo Fisher Scientific, Waltham, MA) were used to wipe specified areas to test for the presence of spores. A single swab sample was collected for each of the following types of equipment for each test:

- ADA and ADA gasket;
- Sprayer (electric backpack or electrostatic);
- Test chamber; and
- Coupons (test material and stainless-steel coupons).

An unused sterile swab sample was used as a laboratory blank.

5.2 Sample Quantities

Table 5-1 summarizes the sample quantities and the number of samples collected during each testing event.

Table 5-1. Sample Quantities

Sample Name	Sample Description	Replicates	Samples Collected
Test coupon (2-3 wipes per coupon)	14 - by 14-in material coupon inoculated and decontaminated with DB	3 per material and sprayer type	3 specimen cups, 1 per replicate
Procedural positive coupon (2-3 wipes per coupon)	14- by 14-in material coupon inoculated and sprayed with DI water	3 per material and sprayer type	3 specimen cups, 1 per replicate
Negative control coupon (2-3 wipes per coupon)	14- by 14-in material coupon not contaminated or decontaminated	1 per material and sprayer type	1 specimen cup per test
Procedural blank coupon (2-3 wipes per coupon)	14- by 14-in material coupon not contaminated but decontaminated with DB	1 per material and sprayer type	1 specimen cup per test
Procedural positive control coupon (2-3 wipes per coupon)	14- by 14-in material coupon not contaminated but decontaminated with sterile DI water	1 per material and sprayer type	1 specimen cup per test
Positive control coupon (2-3 wipes per coupon)	14- by 14-in material coupon contaminated but not decontaminated	3 per material and sprayer type	3 specimen cups, 1 per replicate
Stainless-steel inoculation control coupon (2-3 wipes per coupon)	14- by 14-in stainless-steel coupon contaminated but not decontaminated	3 per inoculation event, inoculated immediately before each positive control coupon	3 specimen cups, 1 per replicate
Liquid runoff	Effluent from sprayed diluted bleach containing STS neutralizer	1 per sample type and material	4 per test
Via-cell® cassette	Air sample – chamber and exhaust duct	Not applicable	2 per test
Sterility check sample	Swab sample and DI water sample	Not applicable	7 swabs per test and 1 DI water sample per test

5.3 Sample Handling

After the collection of coupon surface wipe and liquid runoff samples, the samples were sealed in secondary containment and transported to the BioLab for analysis. This section discusses the sample containers, preservation, and custody.

5.3.1 Sample Containers

For each wipe sample, the primary container was an individual sterile specimen cup. Secondary and tertiary containment consisted of sterile sampling bags. Liquid runoff samples were collected in individual sterile and labeled Nalgene® bottles. A single plastic container was used to store the samples in the decontamination laboratory during sampling and for transport to the BioLab.

5.3.2 Sample Preservation

All sample specimen cups and bottles were stored in secondary containment and kept together until processing. All individual sample containers remained sealed while in the decontamination laboratory, during transport, and until processing in the BioLab. Upon arrival at the Biolab, samples were

unpackaged immediately and stored at 4 °C until processed. Hold times in the laboratory did not exceed one week.

5.3.3 Sample Custody

After sample collection for a single test was completed, all biological samples were immediately transported to the BioLab accompanied by a completed Chain of Custody form.

5.4 Microbiological Analysis

The NHSRC Bio-contaminant Laboratory analyzed all samples for presence (sterility check samples) and to quantify the CFU per sample (wipe samples, liquid samples, and filter samples). Multiple wipes used per test coupon were combined into one sample container and extracted together. Samples were processed using a variety of methods including spiral plating, spread plating, filter plating and or the high debris method, developed by the BioLab.

For all sample types, the BioLab analyzed samples to quantify the number of viable spores (CFU) per sample. For all sample types, PBST was used as the extraction buffer. Each sample was aliquoted and plated in triplicate using a spiral plater (Autoplate 5000, Advanced Instruments Inc., Norwood, MA), which deposits the extracted sample in exponentially decreasing amounts across a rotating agar plate in concentric lines to achieve three tenfold serial dilutions on each plate. Plates were incubated at 35 ± 2 °C for 16 to 19 hours. During incubation, colonies develop along the lines where the sample was deposited (see Figure 5-3). The colonies on each plate were enumerated using a QCount® colony counter (Advanced Instruments Inc., Norwood, MA).

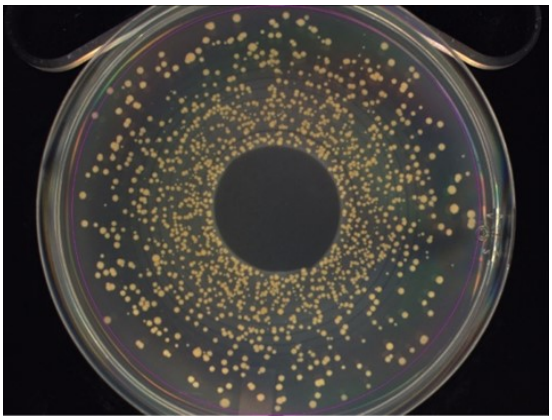


Figure 5-3. Bacterial Colonies on Spiral-plated Agar Plate

Positive control samples were diluted 100-fold (10^{-2}) in PBST before spiral plating, while 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 automatically calculates the CFU/mL in a sample based on the dilution plated and the number of colonies that develop on the plate. The QCount® records the data in an MS Excel spreadsheet.

Only plates meeting the threshold of at least 30 CFU were used for spore recovery estimates. Samples below the 30-CFU threshold were either spiral plated again using a less diluted sample aliquot, spread plated in triplicate, or filter plated. The follow-up plating method and volumes used were based on the CFU data from the initial QCount® results. All plating was performed on tryptic soy

agar (TSA) plates, and the plates were incubated at 35 ± 2 °C for 20 to 24 hours before manual enumeration. Figure 5-4 shows a filter plate with colonies of *Bg*.

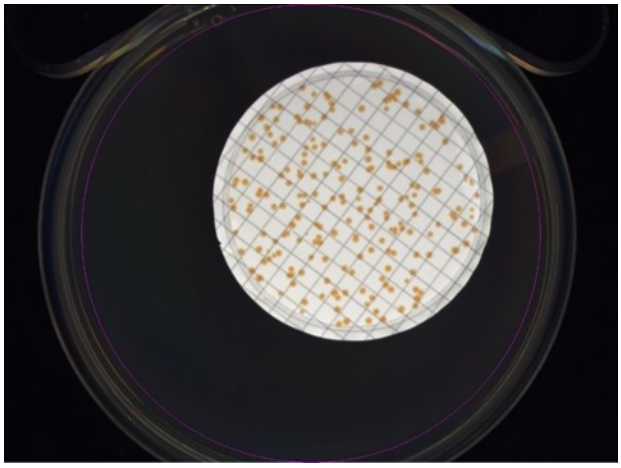


Figure 5-4. Bacterial Colonies on Filter Plate

5.5 Decontamination Solution Characterization

This section discusses the characterization of the 10% DB solution, which involved the determination of pH and temperature and FAC by titration, as discussed below.

5.5.1 pH

The pH of the decontamination solution was measured daily or after each new solution was prepared, using a calibrated pH meter (Model No. 35614-30, Oakton® pH 150, Oakton Instruments, Vernon Hills, IL). The temperature sensor included with the pH meter was factory-calibrated and checked monthly by comparison of the displayed value to a National Institute of Standards and Technology (NIST)-certified thermometer or other thermometer known to be accurate.

5.5.2 FAC by Titration

The FAC of the DB solution was measured immediately after preparation using an iodometric method that uses a HACH digital titrator (Model #16900, HACH, Loveland, CO) and a HACH reagent titration kit. The HACH digital titrator manual discusses the titration procedure and FAC concentration (https://pim-resources.coleparmer.com/instruction-manual/24908-00.pdf , accessed August 21, 2018).

5.6 Determination of Efficacy

The overall effectiveness of a decontamination technique is a measure of the ability of the technique to inactivate or remove spores from material surfaces. Data reduction was performed on measurements of the total viable spores (CFU) recovered from each sampled surface or material.

Decontamination efficacy for a particular material was calculated in terms of the LR. The number of spores (CFU) recovered from each test coupon (CFU_t) and positive-control coupon (CFU_{pc}) was transformed to its $\log_{10}$ value. Then, the mean of the $\log_{10}$ values for each test coupon (three replicates) was subtracted from the mean of the $\log_{10}$ values for each positive control (three replicates), as follows:

$$\text{Efficacy (LR)} = \boxed{(\log CFU_{pc})} - \boxed{(\log CFU_t)}$$

where CFU_{pc} is the number of CFU recovered from the inoculum positive control coupons (stainless steel coupons not decontaminated), and CFU_t is the number of CFU recovered from the test coupons. When filter plates had no CFU detected, a value of 1 CFU was input, resulting in a log value of 0. Many of the decontamination efficacy results are presented or discussed in terms of whether a 6 LR of the micro-organism population was obtained for a particular material and test condition. The 6 LR benchmark is used, since a decontaminant that achieves an LR of 6 or greater (when a 6–7 log challenge is used) for a particular material is considered an effective sporicidal decontaminant (USEPA 2007). We caution, however, that effective decontamination in the laboratory setting may not always transfer to similar efficacy in a field- or full-scale, more realistic setting. Further, a 6 LR still might not be safe for a highly contaminated area. For example, a 6 LR of spores against a spore loading of 8 or 9 log CFU would leave significant remaining viable spores and could potentially pose a health hazard.

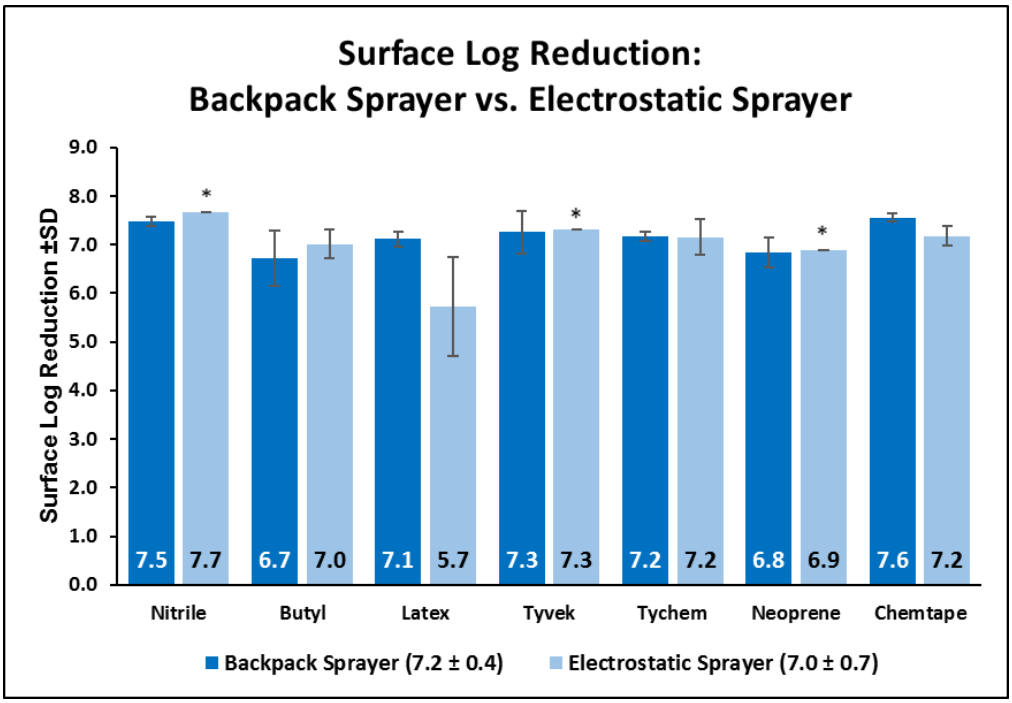
6.0 Results and Discussion

This type of laboratory study was conducted to evaluate actual PPE materials and spray technologies that may be used in a biological personnel decontamination line. The wet decontamination step may be conducted after gross decontamination procedures to ensure the biological agent is inactivated prior to doffing of PPE. This study examined the decontamination efficacy of the two types of sprayers tested, spore disposition (the transport or migration of spores to the air or as liquid runoff), and the operational efficiency of each type of sprayer tested as discussed below. A results summary is provided at the end of this section.

6.1 Decontamination Efficacy

In this section, the decontamination efficacy of the two sprayers (traditional backpack and electrostatic) is discussed. Decontamination is considered effective when there is an LR of greater than or equal to 6 or 1×10^6 CFUs (USEPA 2007).

Figure 6-1 summarizes the surface decontamination efficacies for the two sprayers on each tested material type.



*Denotes no CFU detected above detection limit

Figure 6-1. Surface Decontamination Efficacy

Overall, both sprayers achieved a surface LR ≥ 7 for at least five of the seven PPE material types, with no statistically significant difference between the two sprayers when all LR values were pooled and compared (p-value = 0.49). Spore CFU quantities for the inoculum controls were on the order of 10^7 CFU. For three of the seven test materials, no CFU were detected on the material surfaces when the electrostatic sprayer was used. In contrast, non-detects were not observed for any of the backpack sprayer tests. Because residual spores were quantified on the PPE material in many cases,

full decontamination had not occurred on these materials. The slightly lower electrostatic sprayer efficacy (LR = 5.7) observed for latex may be a result of its observed hydrophilicity but why not see same effect for other sprayer? The decontamination solution immediately ran off the latex material upon spraying with the electrostatic sprayer, perhaps preventing the contact time needed to fully inactivate the *Bg* spores. Hydrophilicity of the latex material could have resulted in a flat decontamination solution droplet formation on its surface, causing a lower contact angle as shown in Figure 6-2).

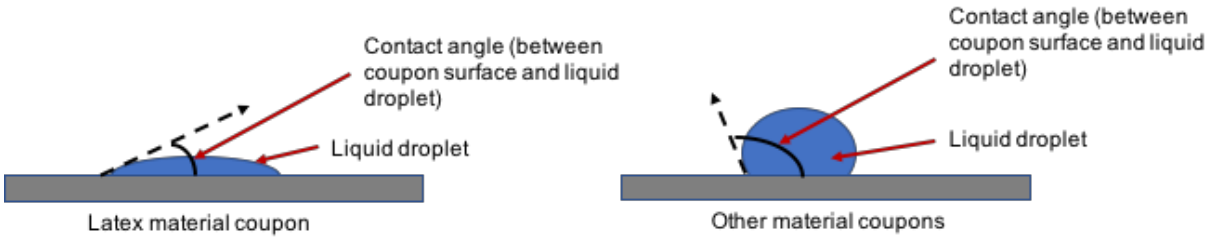


Figure 6-2. Representation of Contact Angle of Liquid Droplets on Coupon Surfaces

Hydrophilic surfaces have contact angles of less than 90º (American Chemical Society 2014.) Hydrophilic surface droplet formation would have resulted in the coalescing of droplets and subsequent immediate runoff of the decontamination solution. During testing, the electrostatic sprayer solution did not form proper droplets on the latex material. Instead, the liquid spray was observed to coalesce and run off the material immediately, preventing the contact time necessary to fully decontaminate the material. Figure 6-3 shows: A) the beading of solution typically seen on all test PPE materials except latex as well as B) the coalescence of the beads on latex for the electrostatic sprayer.

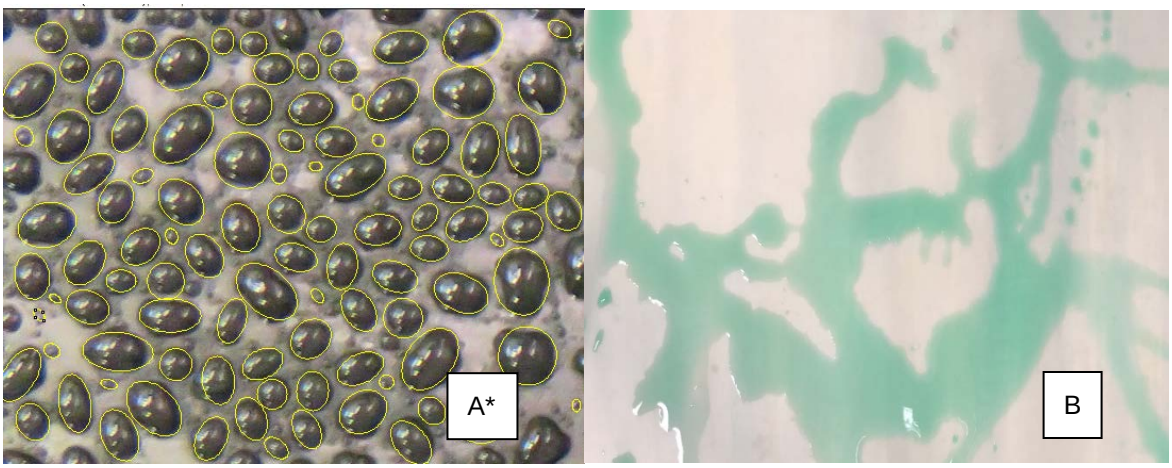


Figure 6-3. Typical Beading of droplets seen on Butyl, Neoprene, Nitrile, Chemtape®, Tychem® and Tyvek®* (A) and coalescence of droplets on Latex (B)

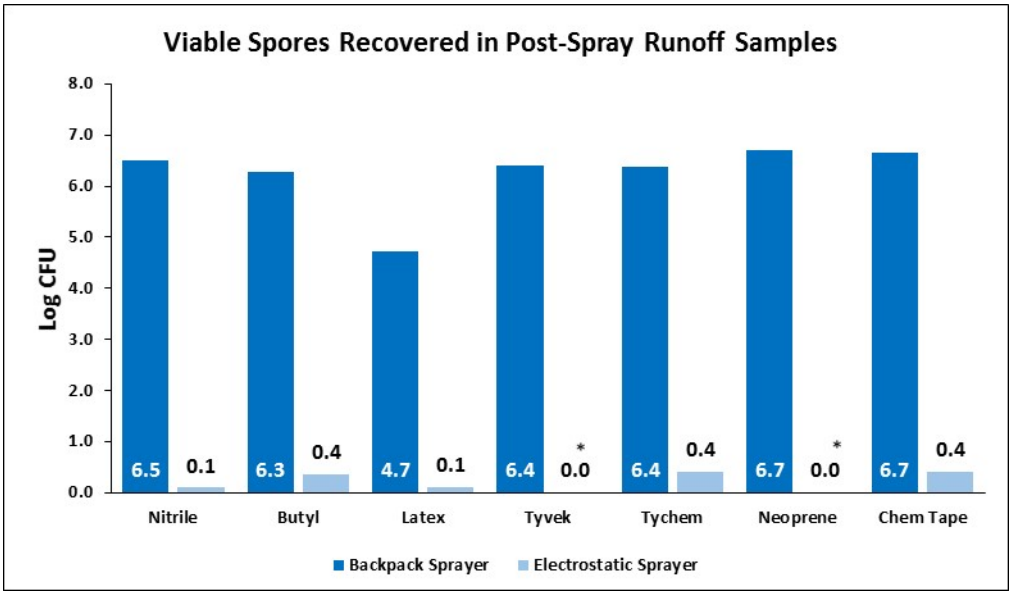
*Image created using ImageJ software

Finally, the latex material was less robust than the other materials, so the latex material was applied to the coupons in a double layer to prevent tearing. This variation in coupon preparation may have contributed to the large standard deviation observed for the electrostatic sprayer and the reduced surface LR results.

6.2 Spore Disposition (Fate and Transport of Spores)

The field applicability of a spray technology depends not only on its surface decontamination performance but also its likelihood of transferring spores from a material surface to its surrounding environment (i.e., cross-contamination). To assess the potential of viable spores to be physically washed off the test coupon surfaces, all liquids used in the decontamination process were collected and quantitatively analyzed. To provide a conservative estimate of spore fate and transport, runoff samples were neutralized immediately upon collection by pre-loading collection tubes with the STS neutralizing agent.

During each decontamination spray test, coupons of each material type were spray tested in triplicate. One combined runoff sample was collected per material test and includes runoff from triplicate coupons into one container. and analyzed for the number of viable spores. Figure 6-4 summarizes the log number of viable spores (CFU) collected in the runoff samples for each material type.



*Denotes no CFU detected exceeding detection limit

Figure 6-4. Log CFU *Bg* Spores in Liquid Runoff Samples

As the figure shows, all the runoff samples collected from the electric backpack sprayer contained a large number of viable spores, whereas those collected from the electrostatic sprayer contained very few to no detectable viable spores. This significant difference in spores collected in runoff between the two sprayers is due to the considerable less decontaminant used to cover the PPE coupon surface using the electrostatic sprayer. The application flow rate is higher for the electric backpack sprayer, which results in more runoff as compared to the electrostatic sprayer. More liquid applied leads to more physical transport of spores off the PPE material. Table B-1 in Appendix B presents the decontamination efficacy results for each material in more detail.

The field applicability for a spray technology used for personnel decontamination also depends on its potential to: (1) minimize cross-contamination among field personnel and equipment; (2) limit the spread of contamination beyond the site originally impacted; and (3) minimize additional exposure risks requiring further remediation action. Assessment of these factors requires an understanding of how a sprayer effects the physical removal of spores from a material surface. Each sprayer also was

evaluated when DI water was substituted for DB, and the test coupons were sprayed under the decontamination test conditions. The number of viable spores (CFU) physically removed from test coupons indicates a potential cross-contamination risk from migration of spores off PPE, which could be tracked outside the decontamination line area. Figure 6-5 summarizes the recovery of spores for the procedural positive coupons sprayed with DI water for each sprayer type and test material.

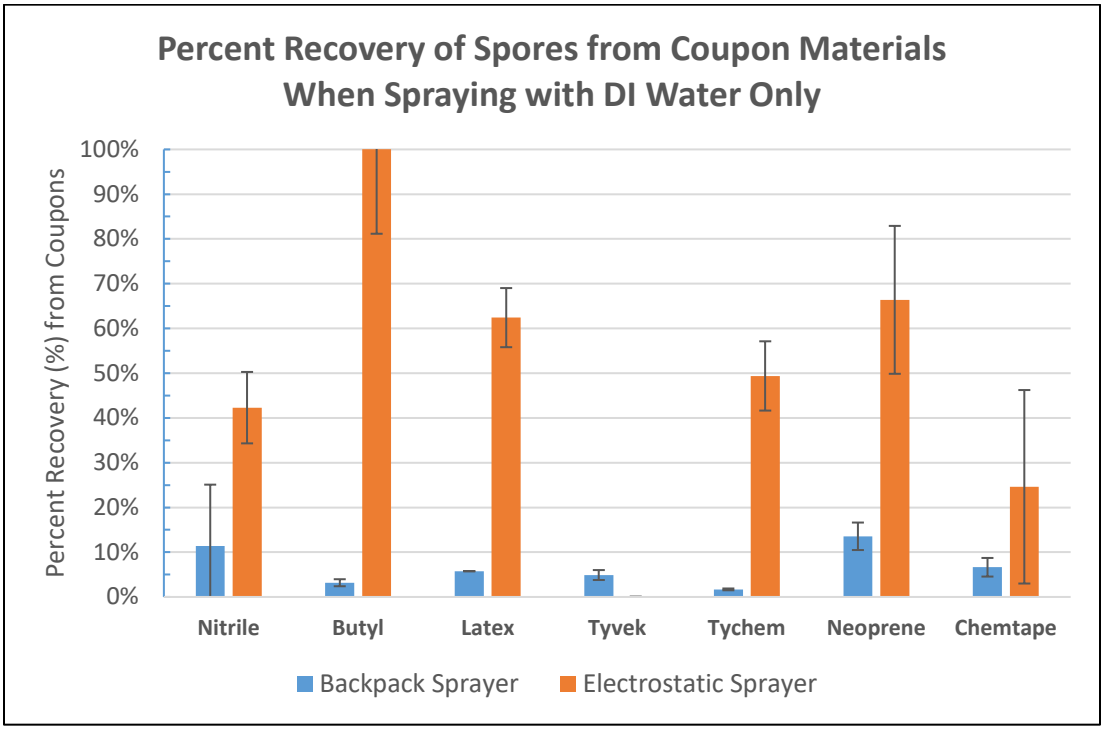


Figure 6-5. Percentage of *Bg* Spores Recovered from Procedural Positive Coupons

As implied in the above figure, the backpack sprayer physically removed more spores during the liquid application for all material types than the electrostatic sprayer, which led to lower percent recovery of spores from coupon surfaces. Percent recovery was calculated as amount recovered on procedural positive (CFU)/inoculated controls (CFU) X 100. Percent recoveries from the runoff solution are not shown in the figure but were consistently higher for the backpack sprayer as compared to the electrostatic sprayer, indicating that use of the backpack sprayer, as tested in this study, physically removes biological contamination from the PPE surface and could result in environmental cross-contamination of PPE and other equipment in a biological decontamination line. Table B-2 in Appendix B presents results for percent recovery achieved during the DI water wash-down for each material and each sprayer in detail. Much greater recovery of spores from the PPE surfaces was observed with the electrostatic sprayer, with the exception of Tyvek®. We believe that the low recovery from Tyvek® may have been due to an inoculation malfunction or residual decontaminant in the test chamber.

Via-Cell® bioaerosol cassette samples were also collected to study the fate of the spores further. Two cassettes were used to evaluate re-aerosolization during each spray test. One cassette was placed inside the test chamber, and the other cassette was connected to the exhaust duct of the test chamber. The sampling was conducted eight diameters downstream and two diameters upstream of any flow disruptions. The Via-Cell® bioaerosol cassettes were installed after sterilizing the test chamber. The cassettes were operated only during the spraying of test coupons. During most tests,

no spores were detected in the air samples. Table B-3 in Appendix B presents results for the fate of spores during aerosol sampling for each material and each sprayer in more detail.

Controlled reaerosolization experiments should be conducted during PPE decontamination spray tests using other bioaerosol sampling techniques like Dry Filter Units (DFUs) that sample a much greater volume of air, to validate the results obtained using the above method.

6.3 Liquid Waste Generation

In a previous EPA study evaluating the decontamination line protocol (USEPA 2015a), liquid waste generated during decontamination was found to be a key carrier of contamination. EPA recommends avoiding large volumes of liquid waste generation unless a completely effective decontamination technique (with immediate efficacy) is used. Otherwise, biological contaminants may be transported outside the decontamination line area. Additionally, liquid waste generated from a biological decotamination line may be costly to dispose of and will likely cause difficulty in finding a disposal facility willing to accept the liquid waste.

To evaluate decontamination line suitability for a spray technology, waste assessment must be considered, so quantifying and comparing the amount of potentially hazardous liquid waste generated by each sprayer type was a project objective. Traditional backpack sprayers have the potential to generate a significant quantity of liquid hazardous waste due to the volume sprayed and runoff from PPE. Additionally, these types of sprayers typically cause overspray (excess liquid that spreads beyond an area being sprayed) when spraying PPE surfaces, which could lead to cross-contamination outside the decontamination setup. The electrostatic sprayer could be used to achieve more uniform distribution of decontamination solution over the surface area sprayed, as well as forming a “liquid film” that adheres to the material, thereby significantly reducing waste streams and costs for liquid hazardous waste disposal. During decontamination testing, runoff liquid volumes were collected and measured gravimetrically. Figure 6-6 summarizes the average amount of liquid waste produced by each sprayer type over the range of test materials.

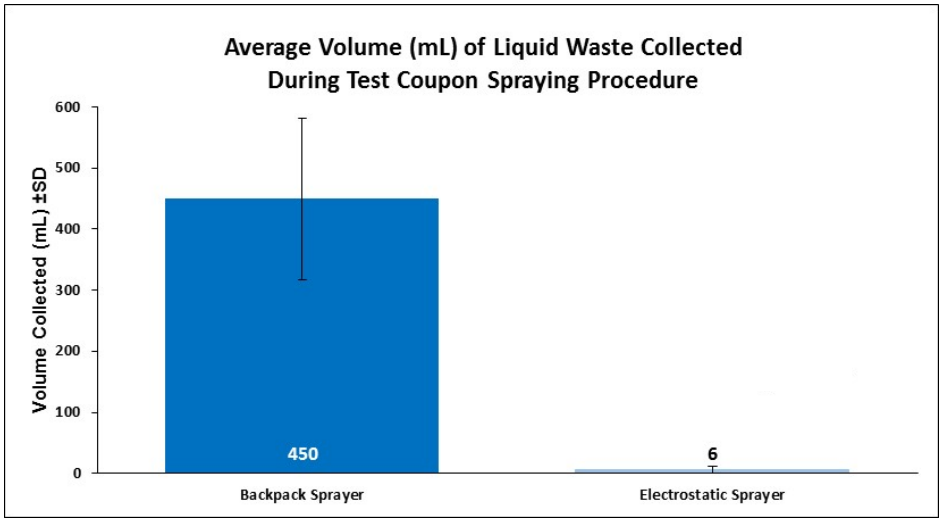


Figure 6-6. Average Volume of Liquid Waste Generated during Spraying

As the figure shows, the amount of liquid waste generated by the electrostatic sprayer is 75 times less than the amount generated by the backpack sprayer, suggesting that waste reduction and operational

cost savings can be achieved through the use of an electrostatic sprayer for personnel decontamination.

6.4 Results Summary and Discussion

Average surface decontamination results for both sprayer types indicated an LR of greater than or equal to 6 for most materials (except latex), suggesting that both sprayer types provide the same level of decontamination efficacy (*p*-value = 0.49). However, liquid runoff sample results for the regular backpack sprayer show a significant number of viable spores in the runoff, indicating that the spores were washed off the test coupons during the decontamination process. Conversely, for the electrostatic sprayer, few to no viable spores were observed in the liquid runoff samples for all material types, suggesting that the spores were not washed off the coupons and were inactivated during the five-minute contact time using the DB solution.

Overall, the electrostatic sprayer demonstrated the ability to contain spores on the coupon surfaces, which resulted in a significant reduction in the number of spores that migrated in the pre-neutralized decontamination runoff compared to the backpack sprayer. In tests using DI water only, the backpack sprayer physically removed (through migration) significantly more spores from the PPE coupons than the electrostatic sprayer, demonstrating the negative consequence of potential contamination to be transferred from the PPE to the decontamination area, which may lead to cross contamination outside the CRZ if the spores are not fully inactivated. Additionally, liquid hazardous waste disposal costs could be increased.

Table 6-1 demonstrates the overall comparison of the two sprayer technologies and highlights the pros and cons for electrostatic sprayers and traditional backpack sprayers.

Table 6-1. Sprayer Comparison

	Traditional Backpack Sprayer		Electrostatic Sprayer (ESS)	
	Pros	**Cons**	**Pros**	**Cons**
Efficacy	**X** >6 log reduction		**X** >6 log reduction	
Liquid Spray Volume		**X**	**X** 16X less	
Waste Generated		**X**	**X** 75X less	
Coupon Coverage spray time	**X** 3X less			**X**
Droplet particle size		**X**	**X** Smaller droplet size (40 µm) leads to more surface area and better coverage	
Electrostatic Attraction		**X**	**X** Wraparound effect leads to multisurface coverage	
Electric shock	**X** No risk of electrical shock		**X** Wear anti-static gloves and use bonding strap to prevent electrostatic buildup	
Cross contamination		**X** Runoff introduces potential for cross contamination	**X** Very little runoff minimizes cross contamination	
Cost	**X** 10X less than ESS			**X**

Based on the study results, use of the electrostatic sprayer technology in the decontamination line could reduce the risk for cross-contamination of personnel and equipment compared to the regular backpack sprayer. Additionally, the electrostatic sprayer generated 75 times less liquid runoff than the backpack sprayer, suggesting that the electrostatic sprayer could reduce waste volumes and associated disposal costs.

Although the spray duration of the electrostatic sprayer was three times longer than the traditional backpack sprayer, the liquid waste from the electrostatic sprayer rarely contained viable spores, and the waste stream volume was significantly reduced. Therefore, the disadvantage of increased decontamination line spraying time may be outweighed by the significant advantages in waste reduction and the decreased risk of personnel cross-contamination and spread of contamination beyond the impacted site. It is not certain how much longer it will take to fully cover a person with the

electrostatic sprayer once scaled up to a real-world scenario. Therefore, additional experiments are underway to address the difference in spray duration between the two technologies when decontaminating a mannequin outfitted with a full Level C PPE ensemble.

Additional pilot-scale studies utilizing more elaborate field-deployable decontamination systems and full Levels of B or C PPE ensembles are suggested as next steps to confirm these results and clarify the time and cost impacts of electrostatic sprayer use in a mock decontamination line setting. Specifically, the time to fully spray and decontaminate a PPE ensemble with the electrostatic sprayer needs to be evaluated as it will help determine whether the technique is operationally feasible.

7.0 Quality Assurance and Quality Control

All test activities were documented in narratives in laboratory notebooks through digital photographs. The documentation included, but was not limited to, a record for each spray test procedure, deviations from the quality assurance project plan, and physical impacts on materials and equipment. All tests were conducted in accordance with established EPA Decontamination Technologies Research Laboratory and BioLab 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 quality assurance (QA)/ QC checks for the project.

7.1 Criteria for Critical Measurements and Parameters

Data quality objectives are used to determine the critical measurements needed to address the stated project objectives and specify tolerable levels of potential errors associated with simulating the prescribed decontamination environments. Digital photographs were taken throughout the testing and sampling phases. The following measurements were deemed critical to accomplish part or all of the project objectives:

- pH of 10% DB solution;
- FAC of 10% DB solution;
- Volume of liquid needed to wet the coupon surface using sprayers;
- Backpack sprayer spray diameter at 1 foot;
- Electrostatic sprayer diameter at 1 foot;
- Flow rate of backpack sprayer;
- Flow rate of electrostatic sprayer; and
- Temperature and RH (relative humidity).

7.2 DQIs

Critical measurements were used to determine if the collected data met the QA objectives. If a measurement method or device resulted in data that did not meet the DQIs for the critical measurements, data derived from the critical measurements were rejected. Decisions to accept or reject test results were based on engineering judgment used to assess the likely impact of the failed criterion on the conclusions drawn from the data. The acceptance criteria were set at the most stringent levels that can routinely be achieved.

Table 7-1 lists the DQIs for the critical measurements. As the table shows, all the DQIs were within the target acceptance criteria set for this project.

Table 7-1. DQIs for Critical Measurements

Critical Measurement	Analysis Method	Accuracy/Precision	Acceptance Criteria
CFU per plate	Spiral plater/QCount	50% RSD amongst the triplicate plating	50% RSD amongst the triplicate plating
Incubation chamber temperature	NIST-traceable thermometer (daily)	± 2 °C	Not applicable

Critical Measurement	Analysis Method	Accuracy/Precision	Acceptance Criteria
Spray application time	NIST-calibrated stopwatch	± 1 minute/hour	± 2 minutes (2 × ± 1 min)
Spray application volume	NIST-calibrated stopwatch	± 1 second/hour	14- by 14-inch coupon surface wetted with liquid
pH	pH meter/NIST-traceable buffer solutions	± 0.01 pH unit	pH > 7
Collection of effluent at specified time	Graduated cylinder	± 1 mL	± 10% of target value
Sprayer pressure	Class B pressure gauge	± 2 psi	± 20% of target value

Notes: psi = Pounds per square inch; RSD = Relative standard deviation

7.3 QA/QC Checks

Many QA/QC checks were used during this project to ensure that the data collected met all the critical measurement requirements listed in Table 7-1. The measurement and parameter criteria were set at the most stringent level that can routinely be achieved. The integrity of each sample during collection and analysis was evaluated. Control samples and procedural blanks were included along with the test samples so that well-controlled quantitative values were obtained. Replicate coupons of all materials were included for each sprayer test.

The integrity of samples and supplies, BioLab control checks, decontamination solution verification, and QA assessments and response actions are discussed below.

7.3.1 Integrity of Samples and Supplies

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

Project personnel carefully checked supplies and consumables prior to use to verify that they met specified project quality objectives. Incubation temperature was monitored using NIST-traceable thermometers. Balances and pipettes are calibrated yearly by the EPA Metrology Laboratory.

7.3.2 NHSRC BioLab Control Checks

Quantitative standards do not exist for biological agents. Viable spores were counted using an Advanced Instruments QCount® colony counter. CFU counts greater than 300 or less than 30 were considered outside the targeted range. If the CFU count for bacterial growth did not fall within the targeted range, the sample was re-plated and then re-counted.

Before each batch of plates was enumerated, 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® colony counter was performed. Obvious count errors made by the software were corrected by adjusting the settings (such as colony size, light, and field of view) and by: (1) recounting using an edit feature of the QCount® software that allows manual

removal of erroneously identified spots or shadows on the plate; or (2) adding colonies that the QCount® software may have missed.

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

Further QC samples were collected and analyzed to check the ability of the BioLab to culture the test organism as well as to demonstrate that the test materials used did not contain pre-existing spores. The checks included the following:

- **Positive control coupons:** Coupons inoculated in tandem with the test coupons to demonstrate the highest level of contamination recoverable from a particular inoculation event.
- **Unexposed field blank (negative control):** Coupons sampled in the same fashion as test coupons but not inoculated with the surrogate organism before sampling or exposed to the decontamination process.
- **Procedural blank coupons:** Material coupons handled and sampled in the same fashion as test coupons but not inoculated with the surrogate organism before sampling.
- **Sample container sterilization:** The exterior of the wipe sample container (specimen cup) and the sterile sampling bags were decontaminated by wiping all surfaces with a bleach wipe before transport from sampling location to BioLab in a secondary container.
- **Sterility checks:** Pre-moistened swabs used to wipe specified areas to test for the presence of spores for sterility checks on coupons (PPE materials and stainless steel), the test chamber, and sprayers before use in testing as discussed in Section 5.1.4; additionally, DI water samples were collected in 50 mL conical tubes (Catalog No. 14-959-49A, Fisher Scientific, Waltham, MA) for each batch of sterilized DI water used for spray test as a sterility check.
- **Blank TSA sterility controls:** Plates incubated but not inoculated.
- **Replicate plates of diluted microbiological samples:** Replicate plates for each sample.

Table 7-2 lists the additional QC checks built into the BioLab procedures designed to provide assurances against cross-contamination and other biases in the microbiological samples.

Table 7-2. Additional QC Checks for Biological Measurements

Sample Type	Frequency	Acceptance Criteria	Information Provided	Corrective Action
Positive control coupon	Minimum of three per test	1×10^7 for *Bg*, 50% RSD between coupons in each test set	Extent of recovery of inoculum on target coupon type	If outside range, discuss in the results section of this report.
Procedural blank coupon	One per test	Non-detect	Controls for sterility of materials and methods used	Analyze extracts from procedural blank without dilution. Identify and remove source of contamination if possible.
Unexposed field blank (negative control) coupon	One per test	Non-detect	Level of contamination during sampling	Clean up environment. Sterilize sampling materials before use.
Blank TSA sterility control	Each plate	No observed growth after incubation	Controls for sterility of plates	All plates incubated before use. Contaminated plates discarded.
Replicate plating of diluted microbiological samples	Each sample	Reportable CFU count of triplicate plates within 100%; reportable CFU counts between 30 and 300 CFU per plate	Precision of replicate plating	Re-plate sample

7.3.3 Decontamination Solution Verification

Volumes of components were measured as accurately as possible using appropriate measuring equipment such as volumetric flasks, serological pipette tips, and graduated cylinders. Commercial products such as Clorox® were used as a 10% DB solution source. The concentration of each new batch of DB was evaluated. DI water was used to prepare the decontamination solution.

The following parameters of the 10% DB solution were measured prior to each use:

- pH;
- FAC (in ppm);
- Temperature;
- RH.

These readings were recorded as measured. FAC was measured using a HACH® high-range bleach test kit (Method 10100, Model CN-HRDT), and pH was measured using an Oakton Acorn® Series pH 5 meter (Oakton Instruments, Vernon Hills, IL). Two HOBO Relative Humidity/Temperature sensors (Model U12, Onset Computer Corporation, Bourne, MA) were used to measure temperature and humidity around the testing area. Appendix B includes a discussion of the characterization of the decontamination solution and Table B-4, which summarizes the measurement results.

7.3.4 QA Assessments and Response Actions

The QA assessment and corrective action procedures for this project are intended to provide rapid detection of data quality problems. Project personnel were intimately involved with the data on a daily basis so that any data quality issue became apparent soon after it occurred. Some contamination in QC procedural blank samples and negative control samples was observed in some tests. However, the contamination was very minimal and had little to no effect on the project results. Table 7-3 summarizes the QA/QC assessment of spore recoveries for the various sample types. As the table shows, blank and negative sample results were present were at or near the detection limit. Only one blank sample had a recovery above the acceptable reportable quantification limit of 30 CFU per filter. With spore recoveries on the order of logs of CFU, this contamination is inconsequential.

Table 7-3. Cross-Contamination Assessment of Blank and Negative Control Samples

Test ID	Material type	Procedural Blank Spore Recovery (CFU)		Negative Control Spore Recovery (CFU)
		Surface	Runoff	Surface
1	Nitrile (Buna-N)	ND	ND	3
2		ND	ND	ND
3	Butyl	ND	7	ND
4		ND	ND	15
5	Latex	ND	32	1
6		ND	ND	ND
7	Tyvek®	ND	ND	ND
8		ND	ND	ND
9	Tychem®	1	ND	ND
10		ND	ND	ND
11	Neoprene	ND	ND	1
12		ND	ND	ND
13	ChemTape®	ND	ND	ND
14		ND	ND	1

Note: ND = None detected

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Appendices

Appendix A: Data Logs

This appendix includes examples of data logs for the spray duration, wipe sampling, liquid runoff collection, and Via-Cell® cassettes.

Table A-1. Example of Spray Duration Log

Coupon ID	Description	Spraying Start Time	Spraying End Time	Comments
DB Spray				
91-8-K-BPS-PB-01	Procedural Blank			
91-8-K-BPS-TC-01	Test Coupon 1			
91-8-K-BPS-TC-02	Test Coupon 2			
91-8-K-BPS-TC-03	Test Coupon 3			
DI Water Spray				
91-8-K-BPS-FB-01	Field Positive Blank			
91-8-K-BPS-FP-01	Field Positive Control 1			
91-8-K-BPS-FP-02	Field Positive Control 2			
91-8-K-BPS-FP-03	Field Positive Control 3			

Table A-2. Example of Wipe Sampling Log

Coupon ID	Description	Sampling Start Time	Sampling End Time	Comments
91-1-N-BPS-X-01	Field Blank			
91-1-N-BPS-NC-01	Negative Control			
91-1-N-BPS-PB-01	Procedural Blank Wipe			
91-1-N-BPS-TC-01	Test Coupon 1			
91-1-N-BPS-TC-02	Test Coupon 2			
91-1-N-BPS-TC-03	Test Coupon 3			
91-1-N-BPS-FB-01	Field Positive Blank Wipe			
91-1-N-BPS-FP-01	Field Positive Control 1			
91-1-N-BPS-FP-02	Field Positive Control 2			
91-1-N-BPS-FP-03	Field Positive Control 3			
91-1-N-BPS-PC-01	Positive Control 1			
91-1-N-BPS-PC-02	Positive Control 2			
91-1-N-BPS-PC-03	Positive Control 3			
91-1-N-BPS-IC-01	Inoculum Control 1			
91-1-N-BPS-IC-02	Inoculum Control 2			
91-1-N-BPS-IC-03	Inoculum Control 3			

Table A-3. Example of Liquid Runoff Collection Log

Sample ID	Description	Initial Weight (grams)	Final Weight (grams)	Comments
91-8-K-BPS-PR-01	Procedural blank runoff sample			
Start Via-Cell® Cassettes				
91-8-K-BPS-RF-01	Test coupon runoff sample			After test coupon 1 spray
				After test coupon 2 spray
				After test coupon 3 spray
Stop Via-Cell® Cassettes				
91-8-K-BPS-BR-01	Field positive blank runoff sample			
91-8-K-BPS-FR-01	Test coupon runoff sample			After test coupon 1 spray
				After test coupon 2 spray
				After test coupon 3 spray

Table A-4. Example of Via-Cell® Cassette Log

Sample ID	Description	Temperature (˚C)		Gas Meter Volume (L)		Sample Flow Δ H (kJ)	
		Initial	Final	Initial	Final	Initial	Final
91-8-K-BPS-VC-01	Inside test chamber						
91-8-K-BPS-VC-02	Test chamber exhaust duct						

Notes:ΔH = Change in enthalpy; kJ = Kilojoule; L = Liter

Appendix B: Data Summary

This appendix presents the data summary tables for decontamination efficacy, log recovery achieved during the DI water wash-down, and fate of spores based on aerosol sampling, followed by a discussion of the characterization of the decontamination solution and a table that includes the pH, FAC, temperature, and RH results for each testing event.

Table B-1. Decontamination Efficacy

Sprayer Type	Stainless-Steel Inoculum Control Coupons (CFU)		Test Coupons (CFU)		Surface LR (CFU)		Spores in Runoff (Log CFU)
	Average	STD	Average	STD	Average	STD	
Nitrile (Buna-N)							
Backpack	3.44E+07	1.15E+07	1.17E+00	2.90E-01	7.48	0.10	6.50
Electrostatic	4.63E+07	8.04E+06	1.00E+00	ND	7.67	ND	0.10
Butyl							
Backpack	1.79E+07	1.53E+07	5.73E+00	6.72E+00	6.73	0.57	6.27
Electrostatic	1.67E+07	1.70E+07	1.54E+00	9.41E-01	7.08	0.24	0.37
Latex							
Backpack	2.59E+07	2.63E+06	2.09E+00	8.31E-01	7.11	0.16	4.72
Electrostatic	9.48E+06	2.20E+06	4.30E+01	4.52E+01	5.64	0.74	ND
Tyvek®							
Backpack	3.23E+07	1.74E+07	2.56E+00	2.69E+00	7.26	0.43	6.41
Electrostatic	2.07E+07	1.24E+07	1.00E+00	ND	7.32	ND	ND
Tychem®							
Backpack	1.68E+07	8.51E+06	1.15E+00	2.60E-01	7.17	0.09	6.37
Electrostatic	3.25E+07	3.15E+06	2.91E+00	2.63E+00	7.16	0.37	0.41
Neoprene							
Backpack	2.21E+07	1.45E+07	3.70E+00	2.11E+00	6.84	0.31	6.70
Electrostatic	7.71E+06	4.31E+06	1.00E+00	ND	6.89	ND	ND
ChemTape®							
Backpack	4.04E+07	4.74E+06	1.13E+00	2.24E-01	7.56	0.08	6.65
Electrostatic	2.89E+07	8.47E+06	1.77E+00	7.15E-01	7.23	0.16	0.41

Notes: CFU = Colony-forming unit; ND = None detected; STD = Standard deviation

Table B-2. Percent Recovery Achieved during DI Water Wash-down

Sprayer Type	Average Recovery (%)						
	Nitrile	Butyl	Latex	Tyvek®	Tychem®	Neoprene	ChemTape®
Backpack	11.4	3.2	5.8	4.9	1.7	13.6	6.6
Electrostatic	42.3	100.5	62.4	0.0	49.4	66.4	24.6

Table B-3. Reaerosolization of Spores Based on Air Sampling

Material type	Backpack Sprayer		Electrostatic Sprayer	
	Inside Chamber	Chamber Duct	Inside Chamber	Chamber Duct
	(CFU)			
Nitrile	ND	ND	ND	32.8
Butyl	ND	ND	ND	ND
Latex	ND	ND	ND	ND
Tyvek®	42.4	3.08	86.7	ND
Tychem®	ND	ND	ND	ND
Neoprene	9.38	ND	ND	ND
ChemTape®	1.54	ND	ND	3.08

Notes: CFU = Colony-forming unit ND = None detected

Characterization of Decontamination Solution

For this study, the decontamination solution was 10% DB at a pH ranging from 10 to 12 units and FAC concentrations ranging from 6,000 to 20,000ppm. The decontamination solution of 10% DB was chosen because it is commonly used actual decontamination lines. Additionally, two HOBO Relative Humidity/Temperature sensors (Model U12, Onset Computer Corporation, Bourne, MA) were used to measure temperature and humidity around the testing area. These sensors were launched before contamination of the coupons (inoculation) and recorded temperature and humidity data points throughout spraying and sampling events. The average temperature and RH readings around the test location throughout the testing events were 23 ˚C and 46%, respectively. Table B-4 lists the pH and FAC data for the decontamination solution prepared for each test as well as the temperature and RH results for each testing event.

Table B-4. pH, FAC, Temperature, and RH per Test

Test ID	Material	Sprayer Type	pH	FAC (ppm)	Temperature (˚C)		RH (%)	
			DB Solution		HOBO 1	HOBO 2	HOBO 1	HOBO 2
1	Nitrile (Buna-N)	Backpack	11.0	8,633	22.73	23.17	47.8	35.5
2		Electrostatic	10.8	8,253	22.97	23.20	46.4	46.6
3	Butyl	Backpack	10.6	8,193	22.69	22.81	45.7	37.8
4		Electrostatic	10.9	8,673	23.07	22.92	45.2	48.7
5	Latex	Backpack	11.3	8,133	22.45	22.72	49.7	55.0
6		Electrostatic	11.0	7,952	23.07	22.92	45.2	48.7
7	Tyvek®	Backpack	10.3	8,633	22.47	24.80	50.4	53.5
8		Electrostatic	10.8	6,390	24.67	22.73	52.8	48.2
9	Tychem®	Backpack	11.0	6,450	22.77	22.97	56.47	46.7
10		Electrostatic	11.0	7,812	22.81	26.12	45.7	40.09
11	Neoprene®	Backpack	10.8	8,954	22.83	23.76	44.2	47.6
12		Electrostatic	10.8	8,012	22.70	*No data	39.1	*No data
13	ChemTape®	Backpack	10.2	8,713	22.97	23.20	46.4	46.6
14		Electrostatic	11.0	7,752	22.70	*No data	39.1	*No data

Note : For Test#12 and Test#14, HOBO 2 was observed to record to no data.

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