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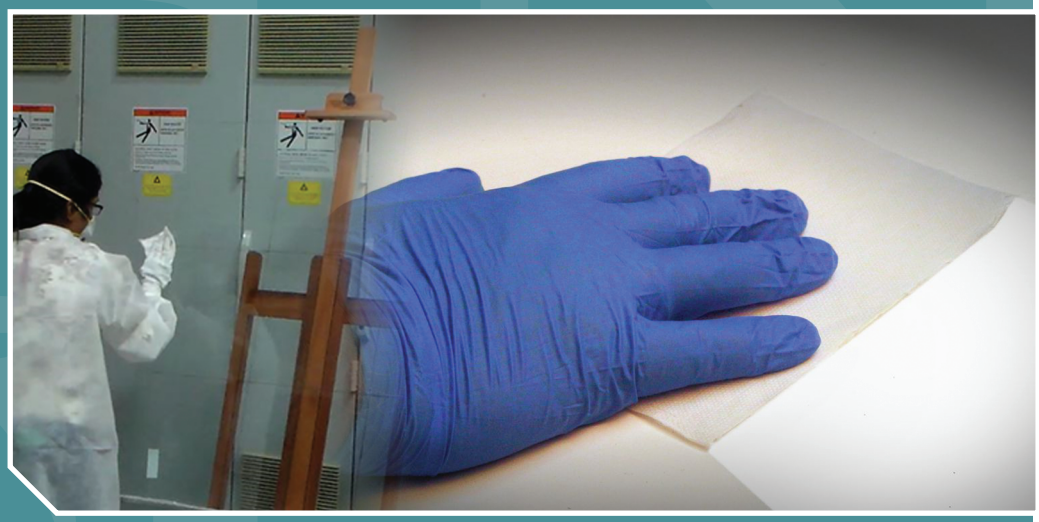
United States
Environmental Protection
Agency

Operational Testing of Sporicidal Wipes for Decontamination of Surfaces Contaminated with Bacillus anthracis Surrogate Spores

ASSESSMENT AND EVALUATION REPORT



Office of Research and Development
National Homeland Security Research Center

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ADDENDUM TO

Operational Testing of Sporicidal Wipes for Decontamination of Surfaces Contaminated with *Bacillus anthracis* Surrogate Spores

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

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Description of Addendum

This addendum to the report "Operational Testing of Sporicidal Wipes for Decontamination of Surfaces Contaminated with *Bacillus anthracis* Surrogate Spores" describes the outcome of additional research that was conducted post publication of the original report (September 2015). It considered the same objectives/goals and uses the same research approaches. Whereas the initial study focused on a medium-size 42 in. x 42 in. surface area, the study presented in this addendum focuses on a smaller 28 in. x 28 in. surface area.

Questions concerning this addendum should be addressed to the principal investigator:

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FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act

Addendum Summary

The primary objective of this research effort in its entirety was to evaluate the operational aspects of sporicidal wiping approaches as a decontamination method using commercially available sporicidal wipes on various materials. The results of the study captured in the main body of this report indicated none of the wipes demonstrated a minimum six log reduction of antimicrobial effectiveness against *B. atrophaeus* spores as required by the U.S. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) when used to inactivate spores on a medium size surface area (42 inch (in.) x 42 in.). Spatial distributions of the post decontamination spore concentration on the target coupons, as determined from discrete samples, showed that significant cross contamination occurs when wiping large area, likely due to the drying of the exposed surface area of the sporicidal wipe.

Based on these results, an additional series of tests (subject of this addendum), was conducted to evaluate the best performing decontamination wipe (Hype-Wipe® Bleach Towelette) on intermediate size coupons (28 in. x 28 in.) to avoid potential drying out of the exposed surface of the wipe before completing the decontamination process of the entire surface of the coupon.

The 2.25-fold reduction in wiped surface area using the Hype-Wipe® Bleach Towelette resulted in a 6 log reduction in viable *B. atrophaeus* spores on four (laminate, Viton™, stainless steel, and glass) out of six material surfaces. The more challenging surfaces were acrylic and painted metal with a mean log reduction in viable spores of 5.2 and 4.0, respectively.

The spatial distribution of the post-decontamination spore concentration showed that the decontamination was effective even for the more challenging hot spot located in the lower right corner of the coupon. There was trace spore migration from the inoculated quadrant to neighboring quadrants which can be attributed to human sampling errors, with the exception of acrylic and painted metal surfaces which did not produce a 6 log reduction.

Impact of this Addendum:

This additional study demonstrates that sporicidal wipes used in this study are able to achieve a 6 log reduction in viable *B. atrophaeus* spores on selected material surfaces of 28 in. x 28 in. size when a single sporicidal wipe is used, in contrast to the findings in the main body of this report that evaluated sporicidal wipes on 42 in. x 42 in. surface areas. These data will help individuals such as incident commanders and remediation personnel make informed decisions about surface decontamination after a biological contamination incident.

1 Experimental Approach

The objective of this study was to evaluate the efficacy of the Hype-Wipe® Bleach Towelette to decontaminate material coupons with small to medium sized surface areas. Various materials of intermediate size (28" x 28" = 5.44 ft^2) were assessed for decontamination effectiveness. For one material, namely, glass, a smaller 14" x 14" = 1.36 ft^2 size was included for verification of the first sporicidal wipe study by Meyer [1].

1.1 Testing Description

Test coupons were inoculated, decontaminated and sampled using the same procedure as followed during the initial testing of large surface area coupons. The variables included in this decontamination test matrix and the corresponding test codes are listed below.

1. Decontamination wipe
    a. Hype-Wipe® (H); 0.525% sodium hypochlorite; see Table 2-1 main body of report

2. Inoculation Method
    a. Hot Spot lower right corner for 28" x 28" coupons: (d)
    b. Hot Spot for 14" x 14" coupons: (0)

3. Material
    a. Laminate Countertop (L)
    b. Acrylic (A)
    c. Viton™ (V)
    d. Painted Metal (M)
    e. Stainless Steel (S)
    f. Glass 28" x 28" coupons (G1)
    g. Glass 14" x 14" coupons (G2)

4. Application variations
    a. Light Pressure application: All coupons were wiped using light pressure.

Assigned test IDs were concatenated in the order listed above. For example, decontamination test H-d-G1 indicates the light pressure application of a Hype-Wipe® (H) to decontaminate a 28" x 28" glass coupon (G1) inoculated in the lower right corner (d).

The target inoculation for "Hot Spot" testing was10^7 spores per ft^2. The upper left section (section a) of the coupon was designated as the first corner to receive the decontamination wipe and the lower right section (d) was the last section to receive the decontamination wipe. The complete decontamination matrix involved seven separate conditions as depicted in Table 1-1 by test ID.

Table 1-1: Decontamination Test Matrix for Wiping of Surfaces using Sporicidal Wipes

Test ID	Material	Inoculation Type	Surface Area	Pressure Applied	Decontamination Wipe
H-d-L	Laminate Countertop	Hot Spot d	28" x 28"	Light	Hype Wipe® Bleach Towelettes
H-d-A	Acrylic				
H-d-V	Viton™				
H-d-M	Painted Metal (Aluminum)				
H-d-S	Stainless Steel				
H-d-G1	Glass				
H-0-G2	Glass	Whole Coupon	14" x 14"		

Four individual sections of the medium 28 in. x 28 in. size surface were sampled for residual spores after a contact time of (at least) 30 min between the residual sporicidal liquid as dispensed from the decontamination wipe and the vertical surface. Recovery of spores from coupon surfaces following the decontamination technique was measured by plating the extracts from the sampling wipes. Test coupons were placed horizontally for spore inoculation and placed in a vertical position for both the wipe decontamination procedure and the subsequent wipe sampling of residual spores on the surfaces.

To ensure consistency in wipe pattern and wiping pressure applied, the same person performed this operation across all tested materials.

1.2 Definitions of Effectiveness

Surface decontamination efficacy values and the associated standard deviations were calculated as per Equations 2-1 and 2-2 in the main body of this report.

2 Material and Methods

2.1 Test Coupon Preparation and Sterilization

Six types of materials (laminate countertop, clear acrylic, Viton™, painted aluminum, stainless steel and tempered glass) were tested under this task only. These materials were selected as being typical of those commonly used in buildings and meeting industry standards or specifications for indoor use in terms of quality, surface characteristics, and structural integrity. Uniformity among the test coupons of a given material was achieved by obtaining and preparing a quantity of material sufficient to allow multiple test coupons to be prepared with presumably uniform characteristics. Coupons were re-used for the various tests after being subjected to a thorough and consistent drying and surface cleaning process following each use. The coupons were cut to the required sizes, and sterilized before use. Stainless steel coupons (14 in x 14 in, 28 in x 28 in) were prepared by using heavy duty power hydraulic shears to cut the metal from larger sheets. These stainless steel coupons were sterilized prior to use by steam autoclaving.

Coupons were sterilized with 400 parts per million (ppm) hydrogen peroxide (H_2O_2) vapor for four hours using a STERIS VHP® ED1000 generator. Prior to use, the sterilized coupons were incubated at room temperature for a minimum of 2-3 days to force off-gassing of H_2O_2 from the coupons so that biocidal activity was prevented. The following materials were included in the tests described in this addendum:

1. **Glass**. Glass coupons (3/16 in-thick tempered glass, Durham Glass, Durham, NC) were purchased pre-cut to the required sizes.

2. **Viton™** fluoroelastomer (DuPont Performance Elastomers LLC, Wilmington, DE). Coupons were cut to size from Fluor elastomer, 1/32" thick, 36" wide (Model 86075K71, McMaster-Carr, Atlanta, GA) and were glued to oriented strand board (OSB).

3. **Laminate Countertop**. Coupons were cut to size from Wilson art 48-in x 96-in Milano Amber Laminate Kitchen Countertop Sheet (Model 249780, Lowe's Home Improvement, Mooresville, NC) and were glued to oriented strand board (OSB).

4. **Acrylic**. Coupons were cut to size from LEXAN 0.093-in x 36-in x 48-in Clear Acrylic Sheet (Model 239982, Lowe's Home Improvement, Mooresville, NC).

5. **Painted Metal**. Aluminum sheets (Dillon Supply, Raleigh, NC) were cut to size, and painted with one coat of metal primer (Model 249058, Rust-Oleum, Vernon Hills, IL) followed by 2 coats of red acrylic enamel paint (Model 248647, Rust-Oleum, Vernon Hills, IL).

6. **Stainless Steel**. Stainless steel sheets (Dillion Supply, Raleigh, NC) were cut to size from larger sheets.

For the "Hot Spot" test, inoculation was performed at a target concentration of 10^7 spores/sq. ft. in the lower right corner (section d) as shown in Figure 2-1.

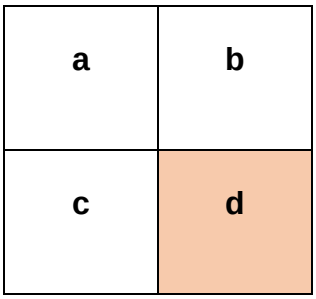


Figure 2-1: "Hot Spot" Section Inoculated (shown highlighted) on a 28" x 28" Coupons

2.2 Sample Identification (ID)

Each sample was assigned an ID based on the sample coding outlined in Table 2-1. The sampling team maintained an explicit laboratory log which included records of each unique sample number and its associated test number, contamination application, sampling method, and the date sampled. Each coupon was marked with only the material descriptor and unique code number. Once samples were transferred to the NHSRC Biocontaminant Laboratory (Biolab) for plate counting, each sample was further identified by replicate number and dilution factor. The NHSRC Biolab also included on each plate the date it was placed in the incubator.

2.3 Spore Inoculation

Information on the test organism and the inoculation of the surface is identical to the procedures used in the initial study (see Sections 3.2 and 3.3 of main body of report), except that the surface was divided into four sections (a-d) as shown in Figure 2-1.

2.4 Decontamination Procedure

The decontamination procedure followed what is described in Section 3.4 of the main body of the report.

2.5 Measurement protocol

Sterile handling of the wipes, environmental conditions, wipe sampling procedures, swab sampling, sample frequency, prevention of cross contamination, collection of representative samples and their storage and preservation were identical to those described in Section 3.6 of the main body of the report. Microbiological analysis was conducted by the NHSRC Biolab located at the EPA facility in Research Triangle Park, NC.

Table 2-1: Sample Coding

Sample Identification: 73-W-L-M-SS-N		
Category	Example Code	Description
W (Decontamination Wipe)	H	H = Hype-Wipe® Bleach Towelette
L (Inoculation Location)	d	d = lower right corner of 28" x 28" coupon 0 = entire 14" x 14" coupon
M (Material)	G	L = Laminate countertop S = Stainless Steel M = Painted Metal G2 = Glass (14" x 14") V = Viton™ A = Acrylic G1 = Glass (28" x 28")
SS (Sample Descriptor)	T(a)	XT = Procedural Blank NT = Negative Control T(a-d) = Test Coupon Quadrants D = Drip Wipe P(a-d) = Positive Coupon Quadrants SW (#) – Swab sample followed by identifier (#) of where sample was collected from: (A) = ADA (G) = Gasket (C) = Coupon (S) = Skirt (E) = Easel (B) = Blank
N (Replicate Number)	1	Sequential sample numbers
NHSRC Biolab Plate Identification: 73-W-L-M-SS-N-R-D		
73-W-L-M-SS-N	As above	As above
R (Replicate)	A	Plate replicates A-C
D (Dilution)	1	Plate Dilution 1 - 4 for 1E0 to 1E4

3 Results and Discussion

Appendix A to this addendum provides the tabulated results of the evaluation of the decontamination effectiveness of targeted wipe/material type combinations. The variables included in the test matrix included Hype-Wipe® Bleach Towelette and different material types (Glass, Laminate, Acrylic, Viton™, Painted Metal, and Stainless Steel) and inoculation method (Hot spot section d).

The results from the initial study described in the body of this report indicated that the Hype-Wipe® Bleach Towelettes were more effective than the Clorox® Health Care Wipes, Hence, Hype-Wipe® Bleach Towelettes were further tested on coupons of smaller surface area to determine if a 6 log reduction could be achieved. Coupons of 28" x 28" (71.1 x 71.1 cm) were divided into four (4) equal areas of 14" x 14" (35.6 x 35.6 cm). Each area was sampled individually, and summed to determine the recovered spore (CFU) and log CFU reduction (LR) per coupon type/wipe type combination. Furthermore, the Hype-Wipe® Bleach Towelette was also tested on glass coupons of a small surface area, 14" x 14" (35.6 x 35.6 cm), to confirm results from similar work performed by Meyer et al.[1],

The decontamination efficacy results are shown in Table 3-1. The Hype-Wipe® Bleach Towelette yielded an overall spore log reduction of 6.17 ± 0.66 across all materials with no to minimal cross-contamination.

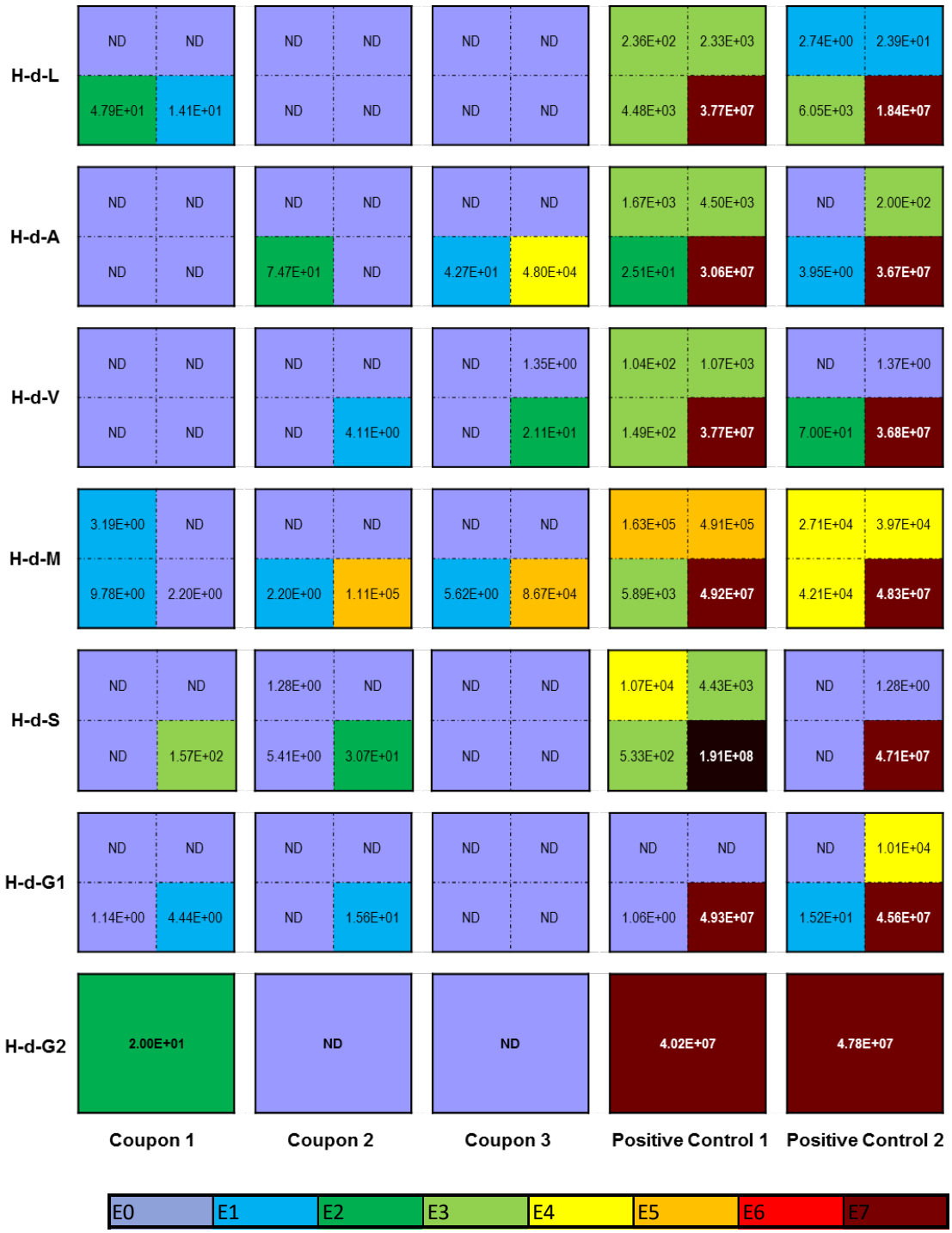
The spatial distribution of the post decontamination spore concentration for Hype-Wipe® Bleach Towelettes is illustrated in Figure 3-1 using a color-coded scheme. The results show clearly that the decontamination was effective even for the hot spot located in the lower right corner (the more challenging condition) of the coupon for smaller surface areas. There is trace spore migration from the inoculated quadrant to neighboring quadrants which can be attributed to human sampling errors, with the exception of acrylic (two out of three replicates) and all painted metal surfaces. These materials did not produce the desired 6 log reduction in viable spores. All procedural blank coupon surfaces exhibited no cross-contamination.

To confirm the decontamination approach used in this study (which was verified for sampling for a 1 ft^2 (929 cm^2) material coupon), a mini-test involving glass coupons was performed which successfully re-affirmed the findings (i.e., a better than 6-log inactivation of spores) previously claimed [1].

Residual spores collected from sterile drip Clean-Wipes™ lined with the easel tray supporting the test coupon, and stored for a minimum of 24 hours at 4^oC in sterile specimen cups, showed an overall contamination of less than 0.006% of the inoculated spore burden. This contamination may be credited to the liquid runoff during the wiping procedure and/or re-aerosolization/deposition of spores during the coupon setup/decontamination/sampling events.

Table 3-1. Hype-Wipe® Decontamination Results.

Decontamination Wipe	Test ID	Surface Area	Material	Average Recovery (CFU) (n=2 [PC] or n=3 [Test Coupons])		Log Reduction
				PC Control	Test Coupon	
Hype-Wipe®	H-d-L	28" x 28"	Laminate	$2.80 \times 10^7 \pm 1.36 \times 10^7$	$2.30 \times 10^1 \pm 3.50 \times 10^1$	6.52 ± 0.48
	H-d-A	28" x 28"	Acrylic	$3.36 \times 10^7 \pm 4.29 \times 10^6$	$1.60 \times 10^4 \pm 2.77 \times 10^4$	5.20 ± 1.25
	H-d-V	28" x 28"	Viton™	$3.72 \times 10^7 \pm 6.14 \times 10^5$	$1.09 \times 10^1 \pm 1.13 \times 10^1$	6.70 ± 0.27
	H-d-M	28" x 28"	Painted Metal	$4.91 \times 10^7 \pm 9.78 \times 10^5$	$6.58 \times 10^4 \pm 5.82 \times 10^4$	3.97 ± 1.27
	H-d-S	28" x 28"	Stainless Steel	$1.19 \times 10^8 \pm 1.02 \times 10^8$	$6.64 \times 10^1 \pm 8.18 \times 10^1$	6.58 ± 0.60
	H-d-G1	28" x 28"	Glass	$4.74 \times 10^7 \pm 2.63 \times 10^6$	$8.68 \times 10^0 \pm 7.70 \times 10^0$	6.88 ± 0.26
	H-0-G2	14" x 14"	Glass	$4.40 \times 10^7 \pm 5.38 \times 10^6$	$7.11 \times 10^0 \pm 1.12 \times 10^1$	7.33 ± 0.49



Spore loading concentration color coded scheme; ND: no viable spores detected.

Figure 3-1: Post Decontamination Spore Concentration (CFU/area) Spatial Distribution.

4 Quality Assurance and Quality Control

All test activities were documented via narratives in laboratory notebooks and the use of digital photography. The documentation included, but was not limited to, a record for each decontamination procedure, any deviations from the initial test plan and physical impacts on materials. All tests were conducted in accordance with developed Decontamination Technologies Research Laboratory (DTRL) and NHSRC Biolab MOPs to ensure repeatability and adherence to the data quality validation criteria set for this project.

4.1 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 errors associated with simulating the prescribed decontamination environments. The following measurements were deemed to be critical to accomplish part or all of the project objectives:

- Sample volume collected
- Plated volume
- Counts or CFU.

Data quality indicators (DQIs) for the critical measurements were used to determine if the collected data met the quality assurance objectives. A list of these DQIs can be found in Table 4-1. Failure to provide a measurement method or device to meet these goals resulted in a rejection of results derived from the critical measurement. For instance, if the plated volume of a sample was not known (i.e., was not 100 % complete), then that sample was declared invalid. If a collected sample was lost or did not meet the criteria for other reasons, then another sample was collected to take its place.

Table 4-1. Critical Measurement Criteria

Critical Measurement	Measurement Device	Accuracy	Precision	Detection Limit
Sample Volume	Serological pipette	Subdivision 0.5 mL	± 0.2 mL	± 0.1 mL
Plated Volume	Pipet	± 2%	± 1%	NA
CFU/plate	Counting	± 10% (between 2 counters)	± 5	1 CFU

NA = not applicable
NIST = National Institute of Standards and Technology
RSD = relative standard deviation

4.2 Quality Control Checks

Many quality assurance (QA)/quality control (QC) checks were used in this project to ensure that the data collected meet all the critical measurements listed in Table 4-1. The measurements/parameters criteria were set at the most stringent level that can routinely be achieved. The integrity of the 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. Background checks for the presence of bacterial spores were included as part of the standard protocol. Replicate coupons were included for each set of test conditions. 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 quality control checks that were performed in this project are described in the following sections.

4.2.1 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.

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 showing evidence of tampering or damage were discarded and not used. All examinations were documented and supplies were appropriately labeled. Project personnel carefully checked supplies and consumables prior to use to verify that they met specified task quality objectives and did not exceed expiration dates. All pipettes were calibrated yearly by an outside contractor (Calibrate, Inc.), incubation temperature was monitored using NIST-traceable thermometers, and balances were calibrated yearly by the EPA Metrology Laboratory.

4.2.2 NHRSC Biolab Control Checks

Quantitative standards do not exist for biological agents. Quantitative determinations of organisms in this investigation did not involve the use of analytical measurement devices. Rather, the CFU were enumerated

manually and recorded. If the CFU count for bacterial growth did not fall within the target range, the sample was either filtered or re-plated. For each set of results (per test), a second count was performed on 25 percent of the plates within the quantification range (plates with 30 - 300 CFU). All second counts were found to be within 10 percent of the original count.

4.3 QA/QC Sample Acceptance Criteria

The acceptance criteria for the critical CFU measurements were set at the most stringent level that could be achieved routinely. Positive controls and procedural blanks were included along with the test samples in the experiments so that well-controlled quantitative values were obtained. Background checks were also included as part of the standard protocol. Replicate coupons were included for each set of test conditions. Further QC samples were collected and analyzed to check the ability of the NHSRC Biolab to culture the test organism, as well as to demonstrate that materials used in this effort did not themselves contain spores. The checks included:

- Negative control coupons: sterile coupons that underwent the same sampling process;
- Field blank coupons: sterile coupons carried to the decontamination location but not decontaminated;
- Laboratory blank coupons: sterile coupons not removed from NHSRC Biolab;
- Laboratory material coupons: includes all materials, individually, used by the NHSRC Biolab in sample analysis; and
- Stainless steel positive control coupons: coupons inoculated but not fumigated.

Additional QA/QC objectives are shown in Table 4-2. These provide assurances against cross-contamination and other biases of microbiological samples.

Table 4-2. QA/QC Sample Acceptance Criteria

Sample Type	Purpose	Acceptance Criteria	Corrective Actions	Frequency
Negative Control Coupons	Determine extent of cross-contamination in test area	None	Values on test coupons of the same order of magnitude will be considered to have resulted from cross-contamination	One per test
Field Blank Coupons	Verify the presence of coupons does not introduce contamination into samples	No detectable spores	Determine source of contamination and remove	One per sample type per test
Laboratory Blank Coupons	Verify the sterility of coupons following autoclaving	No detectable spores	Determine source of contamination and remove	One per test per coupon type
Laboratory Material Coupons	Verify the sterility of materials used to analyze viable spore count	No detectable spores	Determine source of contamination and remove	Three per material per test
Blank TSA Sterility Control (plate incubated, but not inoculated)	Controls for sterility of plates	No observed growth following incubation	All plates are incubated prior to use, so any contaminated plates will be discarded	Each plate
Positive Control Coupons	Used to determine the extent of inoculation on the coupons	5×10^6 CFU, ± 0.5 log or 5×10^4 CFU, ± 0.5 log	Outside target range: discuss potential impact on results with EPA WACOR; correct loading procedure for next test and repeat depending on decided impact	Three per coupon type in Task 1. One per test in Task 2
Inoculation Control Coupons	Used to determine drift in the MDI	The CFU recovered from the first coupon must be ± 0.5 log of the last coupon	Reject results and repeat test	Two per inoculation
Replicate Plating of Diluted Microbiological Samples	Used to determine variability in CFU counts	The reportable CFU of triplicate plates must be within 100 %. Reportable CFU are between 30 and 300 CFU per plate	Re-plate sample	Each sample

WACOR = Work Assignment Contracting Officer Representative.

4.4 QA/QC Test Results Validation

The QA/QC control test results for the whole sampling campaign are shown in Table 4-3. All field blanks and inoculum blanks were found to be non-detects. All of the negative controls and procedural blanks were found to be non-detects, or in a few cases, at the detection limit.

Table 4-3. QA/QC Sample Acceptance Criteria

Test Description				Average recovery CFU			
Test ID	Surface Area	Surface	Inoculation	Field Blank	Negative Control	Procedural Blank	Inoculum Control Blank
H-d-L	28" x 28"	Laminate	Hot Spot d	ND	ND	ND	ND
H-d-A	28" x 28"	Acrylic	Hot Spot d	ND	ND	ND	ND
H-d-V	28" x 28"	Viton™	Hot Spot d	ND	3	ND	ND
H-d-M	28" x 28"	Painted Metal	Hot Spot d	ND	ND	ND	ND
H-d-S	28" x 28"	Stainless Steel	Hot Spot d	ND	7	ND	ND
H-d-G1	28" x 28"	Glass	Hot Spot d	ND	ND	7	ND
H-0-G2	14" x 14"	Glass	Whole Coupon	ND	ND	ND	ND

4.5 Instrument Calibrations

The project used established and approved operating procedures for the maintenance and calibration of all laboratory equipment. All laboratory measuring devices used in this project were certified as having been recently calibrated or were calibrated by the on-site EPA Metrology Laboratory at the time of use. Calibration of instruments was done at the frequency shown in Table 4-7. Any deficiencies were noted and the instrument adjusted and recalibrated within 24 hours to meet calibration tolerances.

Table 4-7: Instrument Calibration Frequency

Equipment	Calibration/Certification	Expected Tolerance
Thermometer	Compare to independent NIST thermometer (this is a thermometer that is recertified annually by either NIST or an International Organization for Standardization (ISO)-17025 facility value once per quarter	± 1 °C
RH Sensor	Compare to calibration salts once a week	± 5 %
Stopwatch	Compare against NIST Official U.S. time at http://nist.time.gov/timezone.cgi?Eastern/d/-5/java once every 30 days	±1 min/30 days
Clock	Compare to office U.S. Time @ time.gov every 30 days	± 1 min/30 days
Scale	Check calibration with Class 2 weights before and after each use daily	± 0.1% weight
Pipettes	Certified as calibrated at time of use/recalibrated by gravimetric evaluation of pipette performance to manufacturer's specifications every year.	± 5%

4.6 QA Assessments and Response Actions

QA assessments are an integral part of a quality system. This project was assigned an EPA QA Category III rating which merited technical system and performance audits. At regular intervals, the test team leader and the team QA officer internally evaluated QA performance and reported the audit results to EPA management and key project team individuals. Any identified deficiencies and corrective actions to be taken were reported via an interoffice memorandum submitted to the responsible project participants.

An integral part of any QA program is well-defined procedures for correcting data quality problems. The overall goals of the QA program address the following aspects of data quality:

- Problem prevention
- Problem definition
- Problem correction.

For this type of testing, data-quality problems usually require immediate, on-the-spot corrective action.

The QA assessment and action procedures followed in this project were intended to provide for 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. Corrective actions were taken as soon as practical when and if a problem was observed. The nature of the problem and corrective steps taken were noted in the project notebook of record.

4.7 Data Reduction

Data reduction for all tests performed included the total CFU recovered from each replicate coupon, the average recovered CFU and standard deviation for each group of coupons, and LRs. For each combination of test coupon material and sample type, the groups of coupons included the following:

- Positive control areas (replicates, average, standard deviation)
- Test areas (replicates, average, standard deviation)
- Procedural blank coupons.

Efficacy was defined as the extent (by LR) to which the agent extracted from the coupons after the treatment with the decontamination procedure was reduced below that extracted from positive control areas (not exposed to the decontamination procedure). The detection limit of a sample depended on the analysis method and so could vary. The detection limit of a plate was assigned a value of 1 CFU, but the fraction of the sample plated varied. For instance, the detection limit of a 0.1 mL plating of a 20 mL sample suspension was 200 CFU (1 CFU/0.1 mL * 20 mL), but if all 20 mL of the sample were filter-plated, the detection limit was 1 CFU.

The cumulative standard deviation for the LR is calculated as follows:

Let S_{Un} and S_{Tr} denote the standard deviations of the log reduction values for the untreated carriers (positive controls) and the treated carriers (post decontamination samples), respectively. Then, the cumulative standard deviation is calculated as follows:

$$S_{LR} = [(S^2_{Un} / n_{Un}) + (S^2_{TR} / n_{Tr})]^{1/2} \qquad 4\text{-}1$$

where: n_{Un} and n_{Tr} designate the number of control and post-decontamination samples, respectively.

4.8 Data Reporting

Data generated included notes recorded in a laboratory notebook (e.g., gravimetric records and assessment of decontamination solutions) and electronic files created by digital camera. Written records included observations, numerical data produced by any instrument that was not digitally recorded, and all variables specific to any experiment. Photographs were taken of each procedure and protocol conducted in general and of any unusual result. Digital files were maintained in their raw form on each of two computers in the laboratory, on desk computers used by test personnel, and on the EPA local network for backup. Processed data files were kept on desk computers and backed up on the EPA network on a biweekly basis. Two laboratory notebooks at a time were maintained for this project, one in the laboratory for notes related to the inoculation and sampling procedures, and another in the NHSRC Biolab for all notes related to biological sample analysis and coupon sterilization documentation.

5 Summary Addendum

As a follow-up to the study described in the main body of this report, one commercially-available sporicidal wipe (Hype-Wipe® Bleach Towelettes) was evaluated on the ability to decontaminate six types of materials (laminate, Viton™, acrylic, painted steel, stainless steel, and glass). The decontamination efficacy of the Hype-Wipe® Bleach Towelette was evaluated on a 28 in. x 28 in. surface area with a hot spot inoculated area).

Results indicate that the Hype-Wipe® Bleach Towelette is efficacious (better than 6 log reduction in viable spores) when used to decontaminate four out of the six materials. Lower efficacy values were obtained for the acrylic and painted metal surfaces. The lower efficacy is associated with a notable redistribution of spores for the acrylic material (but only for two out of the three replicates) and for all painted metal replicate coupons.

A direct comparison of results discussed in the main body of this report (utilizing 42 in. x 42 in. surface areas) against the results for the 28 in. x 28 in. surfaces indicate that the latter surface size should be considered as a maximum surface area that can be decontaminated efficaciously using a single sporicidal wipe. Considering the observed variability in efficacy across the tested surfaces, the actual application procedure would likely benefit from a second sporicidal wipe application for removal of redistributed spores.

6 References Addendum

1. K.M. Meyer, J.A.Tufts., M.W. Calfee, and L. Oudejans, *Efficacy of sporicidal wipes for inactivation of a Bacillus anthracis surrogate.* Journal of Applied Microbiology, 117(60, 1634-1644, 2014.

ADDENDUM APPENDIX A: Spore Recoveries

Table A-1: Individual Coupon Decontamination Test Results

Hype-Wipe® Bleach Towelette Results 28 in. x 28 in.											
Test ID	Positive Controls		Test Coupons			Drip Wipes			Decontamination Wipes		
	PC 1	PC 2	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
H-d-L	3.77E+07	1.84E+07	6.4E+01	2.77E+00	2.77E+00	2.25E00	9.46E-01	9.72E-02	ND		
H-d-A	3.06E+07	3.67E+07	2.62E+00	7.66E+01	4.80E+04	9.72E-01	5.83E+00	4.29E+02			
H-d-V	3.77E+07	3.68E+07	2.75E+00	6.13E+00	2.37E+01	2.47E+04	5.75E+00	2.59E+02			
H-d-M	4.98E+07	4.84E+07	1.57E+01	1.11E+05	8.67E+04	7.06E+03	7.47E+02	2.59E+03			
H-d-S	1.91E+08	4.71E+07	1.59E+02	3.8E+01	2.61E+00	2.10E+00	3.28E+01	6.00E+00			
H-d-G1	4.93E+07	4.56E+07	6.67E+00	1.72E+01	2.19E+00	4.35E+01	1.84E+00	8.97E-01			
Hype-Wipe® Bleach Towelette Results 14 in. x 14 in.											
H-d-G2	4.02E+07	4.78E+07	2.00E+01	6.67E+01	6.67E-01	1.24E+04	1.85E+02	3.24E+00	ND		

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