EPA/600/R-15/188 | September 2015 | www2.epa.gov/research



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



Assessment and Evaluation Report

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



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

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

The U.S. Environmental Protection Agency through its Office of Research and Development funded and managed the research described here under contract EP-C-09-027, Work Assignments 5-73 and 6-73, with ARCADIS U.S., Inc. It has been subjected to the Agency's review and has been approved for publication. Note that approval does not signify that the contents necessarily reflect the views of the Agency. Mention of trade names, products, or services does not convey official EPA approval, endorsement, or recommendation.

Questions concerning this document or its application should be addressed to the principal investigator:

Lukas Oudejans, Ph.D.

U.S. Environmental Protection Agency Office of Research and Development National Homeland Security Research Center 109 T.W. Alexander Drive (Mail Drop: E343-06) Research Triangle Park, NC 27711 Phone: (919) 541-2973 Email: Oudejans.Lukas@epa.gov

Acknowledgments

This effort was directed by the principal investigator (PI) from the Office of Research and Development's (ORD) National Homeland Research Center (NHSRC), to address critical capability or knowledge gaps identified by NHSRC's Program to Align Research and Technology with the Needs of Environmental Response (PARTNER).

Project Team:

Lukas Oudejans, Ph.D. [Principal Investigator] NHSRC, ORD, US EPA

Sang Don Lee, Ph.D. NHSRC, ORD, US EPA

M. Worth Calfee, Ph.D. NHSRC, ORD, US EPA

Leroy Mickelsen CMAD, OEM, US EPA

This effort was completed under U.S. EPA contract #EP-C-09-027 with ARCADIS-US, Inc. The support and efforts provided by ARCADIS-US, Inc. are gratefully acknowledged.

Also acknowledged are;

Ramona Sherman and Eletha Brady-Roberts (Quality Assurance) NHSRC, ORD, US EPA Cincinnati, OH 45220

Joan Bursey (Editorial Review) NHSRC, ORD, US EPA Research Triangle Park, NC 27711

The peer reviewers of this report are also acknowledged for their input to this product:

Joseph Wood, ORD/NHSRC

Shannon Serre, OEM/CMAD

Benjamin Franco, Region 3

Table	e of Co	ontents	\$	
Disc	laimer			iii
Ackr	nowled	gment	S	iv
List	of Tabl	es		viii
List	of Figu	ires		xi
Acro	nyms	and Ab	obreviations	xii
Exec	utive \$	Summa	ary	xiv
1	Intro	ductior	n	1
	1.1	Backg	round	1
	1.2	Projec	t Objectives and Process	1
2	Expe	riment	al Approach	2
	2.1	Projec	t Description	2
		2.1.1	PHASE 1: Operational Aspects of Decontamination by Sporicidal Wipes	2
		2.1.2	PHASE 2: Evaluation of Efficacy of Hypochlorite Wipes	3
	2.2	Testing	g Program Description	3
		2.2.1	PHASE 1: Operational Aspects of Decontamination by Sporicidal Wipes	3
			2.2.1.1 Task 1 – Determination of Effective Surface Coverage of Sporicidal Wipes	4
			2.2.1.2 Task 2 – Surface Retention Times of Wipe Decontaminant Liquid	5
			2.2.1.3 Task 3 - Impact of Wiping Pressure	5
		2.2.2	PHASE 2: Evaluation of Efficacy of Hypochlorite Wipes	6
	2.3	Definit	tions of Effectiveness	9
3	Mate	rial and	d Methods	11
	3.1	Test C	Coupon Preparation and Sterilization	11
	3.2	Bacillu	us Spore Preparation	11
	3.3	Coupo	on Inoculation Procedures	12
	3.4	Decon	ntamination Procedure	13
	3.5	PHASI	E 1 Measurement Protocol	14
		3.5.1	Sampling Approach	14
		3.5.2	Gravimetric Measurements	14
		3.5.3	Method Verification	15
	3.6	PHASI	E 2 Measurement Protocol	15

	3.6.1	Sterile Handling of Wipes	15
	3.6.2	Sampling Site Environmental Conditions	15
	3.6.3	Wipe Sampling Procedures	16
	3.6.4	Swab Sampling	19
	3.6.5	Phase 1 Sample Identification	19
	3.6.6	Phase 2 Sample Identification	20
	3.6.7	Sample Frequency	21
	3.6.8	Prevention of Cross Contamination of Samples during Sampling	23
	3.6.9	Collecting Representative Samples	23
	3.6.10	Sample Storage and Preservation	24
	3.6.11	Sample Holding Time	24
	3.6.12	Sample Chain of Custody	24
	3.6.13	Sample Archiving	25
	3.6.14	Microbiological Analysis	25
Res	ults and	Discussion	26
4.1	PHASE	E 1: Operational Aspects of Decontamination by Sporicidal Wipes Results	26
	4.1.1	Determination of Effective Surface Coverage of Sporicidal Wipes	26
	4.1.2	Surface Retention Times of Wipe Decontaminant Liquid Results	28
	4.1.3	Impact of Wiping Pressure	30
4.2	PHASE	E 2: Evaluation of Efficacy of Hypochlorite Wipes Results	31
Qua	lity Ass	urance and Quality Control	39
5.1	Criteria	for Critical Measurements/Parameters	39
5.2	Quality	Control Checks	40
	5.2.1	Integrity of Samples and Supplies	40
	5.2.2	NHRSC Biolab Control Checks	41
5.3	QA/QC	Sample Acceptance Criteria	41
	5.3.1	QA/QC Test Results Validation	43
	5.3.2	Sample Hold Time	44
5.4	Instrum	nent Calibrations	47
5.5	QA Ass	sessments and Response Actions	48
5.6	Data R	eduction	49
5.7	Data R	eporting	49

	5.8 QAPP Amendments and Deviations		49	
		5.8.1	Formal Amendments	49
		5.8.2	QAPP Deviations	50
6	SUM	MARY		51
7	REFE	RENCE	S	53
APPE	NDIX	A - PHA	SE 1: Operational Aspects of Decontamination by Sporicidal Wipes	54
	Test R	esults fo	r Task 1: Determination of Effective Surface Coverage of Sporicidal Wipes	54
	Test R	esults fo	r Task 2: Surface Retention Times of Wipe Decontaminant Liquid	70
	Test R	esults fo	r Task 3: Impact of Wiping Pressure	78
APPE	NDIX	B - PHA	ASE 2: Evaluation of Efficacy of Hypochlorite Wipes	86

List of Tables

Table 2-1. Sporicidal Wipe Information	3
Table 2-2. Task 1 Test Matrix Conducted for Each Type of Sporicidal Wipe	4
Table 2-3. Task 2 Test Matrix per Sporicidal Wipe Type	5
Table 2-4. Task 3 Test Matrix per Sporicidal Wipe Type	6
Table 2-5. Decontamination Test Matrix for Wiping Surfaces with Sporicidal Wipes	8
Table 3-1. Sample Coding for Phase 1	20
Table 3-2: Sample Coding for Phase 2	21
Table 3-3. Wiping Efficacy - Sample Frequency	22
Table 4-1. Decontamination Results for Glass Material Type	33
Table 4-2. Decontamination Results for Painted Drywall Material Type	34
Table 5-1. Critical Measurement Criteria	40
Table 5-2. QA/QC Sample Acceptance Criteria	42
Table 5-3. QA/QC Test Results	43
Table 5-4. Test Matrix (Test Samples)	45
Table 5-5. Test Matrix – Hold Time Control Samples	46
Table 5-6. Sample Hold Time Test Results for Decontamination Efficacy Tests	47
Table 5-7. Instrument Calibration Frequency	48
Table A-1-1-1: Task 1 Results Clorox Healthcare® Bleach Germicidal Wipe/Vinyl Flooring	54
Table A-1-1-2: Task 1 Results Clorox Healthcare [®] Bleach Germicidal Wipe/Glass	55
Table A-1-1-3: Task 1 Results Clorox Healthcare® Bleach Germicidal Wipe/Melamine	56
Table A-1-1-4: Task 1 Results Clorox Healthcare® Bleach Germicidal Wipe/Painted Drywall	57
Table A-1-2-1: Task 1 Results Hype-Wipe [®] Bleach Towelette/Vinyl Flooring	58
Table A-1-2-2: Task 1 Results Hype-Wipe [®] Bleach Towelette/Glass	59
Table A-1-2-3: Task 1 Results Hype-Wipe [®] Bleach Towelette/Melamine	60
Table A-1-2-4: Task 1 Results Hype-Wipe [®] Bleach Towelette/Painted Drywall	61
Table A-1-3-1: Task 1 Results Sani-Cloth $^{\textcircled{B}}$ Bleach Germicidal Disposable Wipe/Vinyl Flooring	62
Table A-1-3-2: Task 1 Results Sani-Cloth $^{\textcircled{B}}$ Bleach Germicidal Disposable Wipe/Glass	63
Table A-1-3-3: Task 1 Results Sani-Cloth [®] Bleach Germicidal Disposable Wipe/Melamine	64
Table A-1-3-4: Task 1 Results Sani-Cloth [®] Bleach Germicidal Disposable Wipe/Painted Drywall	65
Table A-1-4-1: Task 1 Results Dispatch® Hospital Cleaner Disinfectant Towels with Bleach/Vinyl Flooring	66

Table A-1-4-2: Task 1 Results Dispatch® Hospital Cleaner Disinfectants Towels with Bleach/Glass	67
Table A-1-4-3: Task 1 Results Dispatch [®] Hospital Cleaner Disinfectant Towels with Bleach/Melamine	68
Table A-1-4-4: Task 1 Results Dispatch® Hospital Cleaner Disinfectant Towels with Bleach/Painted Drywall	69
Table A-2-1-1: Task 2 Results Clorox Healthcare® Bleach Germicidal Wipe/Vinyl Flooring	70
Table A-2-1-2: Task 2 Results Clorox Healthcare® Bleach Germicidal Wipe/Glass	70
Table A-2-1-3: Task 2 Results Clorox Healthcare® Bleach Germicidal Wipe/Melamine	71
Table A-2-1-4: Task 2 Results Clorox Healthcare® Bleach Germicidal Wipe/Painted Drywall	71
Table A-2-2-1: Task 2 Results Hype-Wipe [®] Bleach Towelette/Vinyl Flooring	72
Table A-2-2-2: Task 2 Results Hype-Wipe [®] Bleach Towelette/Glass	72
Table A-2-2-3: Task 2 Results Hype-Wipe [®] Bleach Towelette/Melamine	73
Table A-2-2-4: Task 2 Results Hype-Wipe [®] Bleach Towelette/Painted Drywall	73
Table A-2-3-1: Task 2 Results Sani-Cloth [®] Bleach Germicidal Disposable Wipe/Vinyl Flooring	74
Table A-2-3-2: Task 2 Results Sani-Cloth [®] Bleach Germicidal Disposable Wipe/Glass	74
Table A-2-3-3: Task 2 Results Sani-Cloth [®] Bleach Germicidal Disposable Wipe/Melamine	75
Table A-2-3-4: Task 2 Results Sani-Cloth [®] Bleach Germicidal Disposable Wipe/Painted Drywall	75
Table A-2-4-1: Task 2 Results Dispatch [®] Hospital Cleaner Disinfectants Towels with Bleach/Vinyl Flooring	76
Table A-2-4-2: Task 2 Results Dispatch [®] Hospital Cleaner Disinfectant Towels with Bleach/Glass	76
Table A-2-4-3: Task 2 Results Dispatch $^{\otimes}$ Hospital Cleaner Disinfectant Towels with Bleach/Melamine	77
Table A-2-4-4: Task 2 Results Dispatch® Hospital Cleaner Disinfectant Towels with Bleach/Painted Drywall	77
Table A-3-1-1: Task 3 Results Clorox Healthcare® Bleach Germicidal Wipe/Vinyl Flooring	78
Table A-3-1-2: Task 3 Results Clorox Healthcare® Bleach Germicidal Wipe/Glass	78
Table A-3-1-3: Task 3 Results Clorox Healthcare® Bleach Germicidal Wipe/Melamine	79
Table A-3-1-4: Task 3 Results Clorox Healthcare® Bleach Germicidal Wipe/Painted Drywall	79
Table A-3-2-1: Task 3 Results Hype-Wipe [®] Bleach Towelette/Vinyl Flooring	80
Table A-3-2-2: Task 3 Results Hype-Wipe [®] Bleach Towelette/Glass	80
Table A-3-2-3: Task 3 Results Hype-Wipe [®] Bleach Towelette/Melamine	81
Table A-3-2-4: Task 3 Results Hype-Wipe [®] Bleach Towelette/Painted Drywall	81
Table A-3-3-1: Task 3 Results Sani-Cloth [®] Bleach Germicidal Disposable Wipe/Vinyl Flooring	82
Table A-3-3-2: Task 3 Results Sani-Cloth [®] Bleach Germicidal Disposable Wipe/Glass	82
Table A-3-3-3: Task 3 Results Sani-Cloth [®] Bleach Germicidal Disposable Wipe/Melamine	83
Table A-3-3-4: Task 3 Results Sani-Cloth [®] Bleach Germicidal Disposable Wipe/Painted Drywall	83
Table A-3-4-1: Task 3 Results Dispatch [®] Hospital Cleaner Disinfectant Towels with Bleach/Vinyl Flooring	84

Table A-3-4-2: Task 3 Results Dispatch [®] Hospital Cleaner Disinfectant Towels with Bleach/Glass	84
Table A-3-4-3: Task 3 Results Dispatch $^{ m e}$ Hospital Cleaner Disinfectant Towels with Bleach/Melamine	85
Table A-3-4-4: Task 3 Results Dispatch® Hospital Cleaner Disinfectant Towels with Bleach/Painted Drywall	85
Table B-1: Decontamination Test Results	86

List of Figures

Figure 2-1. Easel Used to Hold Coupons	7
Figure 3-1. Aerosol Deposition Apparatus	12
Figure 3-2. MDI and Actuator	12
Figure 3-3. "Hot Spot" Sections Inoculated (shown highlighted) on 42" x 42" Coupons	13
Figure 3-4. Clorox Healthcare [®] Bleach Germicidal Wipe Canister	14
Figure 3-5. Folding of Wipe for Wipe Sampling Step 1 (horizontal wiping pathway)	16
Figure 3-6. Horizontal Wiping Pathway	16
Figure 3-7. Folding of Wipe for Wipe Sampling Step 2 (vertical wipe sampling pathway)	17
Figure 3-8. Vertical Wiping Pathway	17
Figure 3-9. Folding of Wipe for Wipe Sampling Step 3 (diagonal wipe sampling pathway)	17
Figure 3-10. Diagonal Wiping Pathway	18
Figure 3-11. Folding of Wipe for Wipe Sampling Step 4 (perimeter wipe sampling pathway)	18
Figure 3-12. Perimeter Wiping Pathway	18
Figure 4-1. Residual Bleach Recovered from Melamine and Glass Material	26
Figure 4-2. Residual Bleach Recovered from Vinyl Flooring and Painted Drywall Material	27
Figure 4-3. Percent Liquid Dispensed on Material as a Function of Material Type, for Hype-Wipe $^{\otimes}$	27
Figure 4-4. Temporal Residual Bleach Recovered following a Decontamination Event using Clorox [®] Bleach Wipe and Hype-Wipe [®] Bleach Towelette	29
Figure 4-5. Temporal Residual Bleach Recovered Following a Decontamination Event using Sani-Cloth® Wipe and Dispatch® Towel with Bleach	29
Figure 4-6. Effect of Application Pressure on Liquid Dispensed Using Clorox [®] Bleach Wipe and Hype- Wipe [®] Bleach Towelette	30
Figure 4-7. Effect of Application Pressure on Liquid Dispensed using Sani-Cloth [®] Wipe and Dispatch [®] Towel with Bleach	31
Figure 4-8. Spatial Post-Decontamination Residual Spore Concentration Using Hype-Wipe [®] Bleach Towelette /Glass Material at Different Inoculation Locations.	35
Figure 4-9. Spatial Post-Decontamination Residual Spore Concentration using Clorox Healthcare [®] Wipe/Glass Material at Different Inoculation Locations	36
Figure 4-10. Spatial Post-Decontamination Residual Spore Concentration using Hype-Wipe [®] Bleach Towelette /Painted Drywall Material at Different Inoculation Locations	37
Figure 4-11. Spatial Post-Decontamination Residual Spore Concentration using Clorox Healthcare [®] Wipe/Painted Drywall Material at Different Inoculation Locations	38

Acronyms and Abbreviations

ADA ANOVA	aerosol deposition apparatus Analysis of Variance
ASTM ATCC	American Society for Testing and Materials, now ASTM International American Type Culture Collection
B.	Bacillus
Bg	Bacillus globigii
°C	degrees Celsius
C diff	Clostridium difficile
CFU	colony forming unit(s)
CI	confidence interval
cm	centimeter(s)
cm ²	square centimeter
CMAD	Consequence Management Advisory Division
CoC	chain of custody
DI	deionized
DPG	(U.S. Army) Dugway Proving Ground
DQI DQO	data quality indicator data quality objective
DTRL	Decontamination Technologies Research Laboratory
EPA	U. S. Environmental Protection Agency
ft	foot (feet)
ft ²	square foot (feet)
h	hour
H_2O_2	hydrogen peroxide
ID	identification
in	inch(es)
ISO	International Organization for Standardization
LR	log reduction
m² MDI	square meter(s) metered dose inhaler
mg	milligram(s)
Min	minute(s)
MOP	miscellaneous operating procedure
NHSRC	National Homeland Security Research Center
NIST	National Institute of Standards and Technology
OEM	Office of Emergency Management
OPP	Office of Pesticide Programs
ORD	Office of Research and Development
OSB	oriented strand board
pAB	pH-adjusted bleach
PARTNER	Program to Align Research and Technology with the Needs of Environmental Response
PBST PI	phosphate-buffered saline with Tween 20 principal investigator
ppm	part(s) per million
PRB	polyester rayon blend
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
RH	relative humidity
StDev	standard deviation

STS	sodium thiosulfate
TSA	tryptic soy agar
VHP	vaporous hydrogen peroxide
WACOR	work assignment contract officer representative

Executive Summary

The likelihood of a biological incident such as the 2001 U.S. Postal Service system facilities contamination through the introduction of a few letters containing *Bacillus anthracis* spores prompted the U.S. Environmental Protection Agency (EPA), in a coordinated effort with other federal agencies, to develop strategies, guidelines, and plans for decontamination of facilities and equipment to mitigate the risks of contamination following a biological weapons attack.

As a part of the 2001 remediation efforts, various surface cleaning methods were used in buildings, including those buildings that were fumigated prior to the clearance for reoccupation and normal use. These "low-tech" methods included combinations of disposal of contaminated items, vacuuming, and the use of liquid sporicides such as a pH-adjusted bleach (pAB) solution.

The use of commercially-available sporicidal wipes was presented as an effective decontamination technique for the complete removal/inactivation of *Bacillus (B.) atrophaeus* spores [1], a surrogate for *Bacillus anthracis* from a 12 inch (in) by 12 in material. This report specifically addresses the results of the operational aspects of decontamination by sporicidal wipes and evaluates the efficacy of a single wipe for the removal/inactivation of *B. atrophaeus* spores under varied conditions of application (type of sporicidal wipe, material type, and application pressure) for larger surface areas tested (one to nine square feet (ft²)) under conditions that mimic their potential realistic field use.

The operational aspects of the wiping decontamination technique were evaluated, using four commercially available sporicidal wipes, on painted drywall, vinyl tile, melamine board, and glass. All four sporicidal wipes contained sodium hypochlorite ("bleach") as the active ingredient. The use of individually packaged, single-use Hype-Wipe[®] Bleach Towelettes resulted in a higher volume of bleach dispensed, for all types of materials tested, compared to the other wipes that are sold in bulk canisters. The bulk canister wipes are also more susceptible to moisture loss because the canister lid must be opened to access individual wipes. The amount of liquid dispensed increased with the size of the surface area that was wiped. However, the amount of liquid bleach dispensed per unit area decreased when larger areas were wiped. Among the four materials tested, the least amount of liquid bleach was transferred to glass, while painted drywall had the propensity to retain the most liquid.

Another prerequisite for the high inactivation of spores would be the need to keep the surface wet for at least five minutes [1]. The residual amount of liquid remaining on the surface after a five-minute contact time was measured and compared to the amount of liquid present on the surface without the five-minute contact time. All surfaces were considered "wet" after the five-minute contact time, based on mass recovered after five minutes. Liquid solutions dispensed from Hype-Wipe® Bleach Towelettes, and to a lesser extent from Clorox Healthcare® Bleach Germicidal Wipes, were found to be least susceptible to losses due to evaporation or absorption into the material.

A higher pressure applied during wiping resulted in higher liquid solution volume dispensed compared to slight (low) pressure for all material/wipe type combinations. For these particular tests, the effect of applying higher pressure (compared to slight pressure) resulted in an increase of approximately 60% in liquid being dispensed from the Hype-Wipe[®] Bleach Towelettes to the coupon and up to a 300% increase in liquid from the Sani-Cloth[®] wipes.

The decontamination efficacy of a subset of two commercially available bleach wipes (Hype-Wipe[®] Bleach Towelette, and Clorox Healthcare[®] Wipe) was evaluated on a medium size surface area of 12.25 ft² (1.36 square meters (m²)) for different inoculation methods (hot spot versus broad area) and wipe application pressure method (slight versus high). Such a 3.5 foot (ft) × 3.5 ft surface area is considered to be representative of an area that can be wiped by a single person without physically relocating to a different position.

The results indicate that the Hype-Wipe[®] Bleach Towelette was more effective in inactivating *Bg* spores than the Clorox Healthcare[®] Wipe, both from glass and drywall surfaces. For glass, the overall log reductions in spores by decontamination with the Hype-Wipe[®] Bleach Towelette and Clorox Healthcare[®] Wipe were $4.3 \pm$ 0.6 and 3.4 ± 1.0 , respectively, while for painted drywall, the log reductions were lower, namely, 3.6 ± 0.9 and 2.5 ± 0.9 , respectively. The Hype-Wipe[®] Bleach Towelette may have been more effective as it dispenses more sporicidal liquid per decontamination wipe than the Clorox Healthcare[®] Wipe. The wipe application pressure seems to have little or no effect on the decontamination efficacy of the wipe, despite more liquid being dispensed from either decontamination wipe. The higher applied pressure may only have caused a more pronounced redistribution of spores without improvement in overall efficacy of wiping the 12.25 square foot (ft²) surface area.

Three of the twenty four tests came back with a significant number (>500) of viable spores on the procedural blank or negative control. Such contamination of blanks negates the ability to demonstrate a 6 log kill in viable spores. However, other tests clearly show the inability of either sporicidal wipe to inactivate all spores from a 3.5 ft \times 3.5 ft surface area. As such this contamination does not impact the overall conclusions of this study.

The decontamination wipes that were used during the decontamination process were stored for a minimum of 24 hours (h) at 4 degrees Celsius (°C) in sterile specimen cups. No viable *Bacillus globigii* (*Bg*) spores were detected on these used wipes after the 24 h wait period. This implies that collected spores on the wipes were inactivated

Spatial distributions of the post-decontamination spore concentration on the target coupons, as determined from discrete samples, showed that cross contamination occurs during the decontamination wiping. The likelihood of cross contamination increases as the towelette moisture content decreases. The folding of the towelette when switching wiping motions exposes a fresh sporicidal solution on the wipe. However, the same decrease in moisture content results in further redistribution of spores over areas that are already cross contaminated. The decontamination scheme approach used in this study, as optimized for sampling for a 1 ft² (929 square centimeter (cm²)) area [1], was found to be unsuitable for larger 12.25 ft² (1.36 m²) surface areas. Further laboratory testing should be conducted in which sporicidal wipes are used only as long as the exposed surface remains wet to reduce cross contamination of material and wipe surfaces.

Impact of the Study:

In contrast to the findings of a recent study that evaluated sporicidal wipes on 1 ft \times 1 ft surface areas [1], the current study demonstrates that sporicidal wipes were unable to achieve a 6 log reduction in viable *B. atrophaeus* spores on material surfaces of 3.5 ft \times 3.5 ft size when a single sporicidal wipe was used. A reduction in wiped area per sporicidal wipe or use of multiple wipes is expected to improve the overall efficacy. However, such an approach was not evaluated as part of this study.

1 Introduction

1.1 Background

This project supports the mission of the U.S. Environmental Protection Agency (EPA) Office of Research and Development's (ORD) Homeland Security Research Program (HSRP) by providing information relevant to the decontamination of equipment or areas contaminated as a result of biological contamination incident. Homeland Security Presidential Directive (HSPD)-10 tasked the U.S. Department of Homeland Security (DHS) with coordinating the 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, EPA, in conjunction with DHS and other agencies, is "developing strategies, guidelines, and plans for decontamination of persons, equipment, and facilities" to mitigate the risks of contamination following 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.

In 2001, the introduction of letters containing *Bacillus anthracis* (causative agent of anthrax) spores into the U.S. Postal Service system resulted in the contamination of several facilities. Although most of the facilities in which these letters were processed or received in 2001 were heavily contaminated [2], the facilities were successfully remediated with approaches such as fumigation with chlorine dioxide or vaporous hydrogen peroxide (VHP®). It is well agreed that available, effective and economical decontamination methods having the capacity to be employed over wide areas (outdoor and indoor) are required to increase preparedness for such a release.

Prior to the fumigation used in heavily contaminated facilities, surface cleaning methods were used in primarily contaminated facilities and secondarily contaminated areas (e.g., cross contaminated letters that came in contact with the anthrax spores contained in letters or tracked from primarily contaminated sites) or showing a minimal presence of the anthrax spores. These "low-tech" methods included combinations of disposal of contaminated items, vacuuming, and the use of liquid sporicides such as a pH-adjusted bleach (pAB) solution.

1.2 Project Objectives and Process

The primary objective of this project was to provide agencies responding to occurrences of biological contamination with operational criteria that would lead to efficacious decontamination of various materials/surfaces contaminated with *Bacillus anthracis*. This report specifically addresses the results of investigating four commercially available sporicidal wipes for potential use in the removal/inactivation of *B. anthracis* spores under varied conditions of application (type of sporicidal wipe, type of surface, application pressure, and size of area). This study was conducted in two phases: the first phase addressed the operational aspects of decontamination using sporicidal wipes by measuring the wetting ability of four different sporicidal wipe products whose efficacies had previously been evaluated by Meyer et al. [1]. The second phase of the study consisted of evaluating two of the four sporicidal wipes for their decontamination efficacy of medium-size contaminated surfaces, following inoculation of various material surfaces with *B. atrophaeus* spores, which served as a surrogate for *B. anthracis*.

2 Experimental Approach

This section describes the project, the testing program, test materials, test facilities and equipment, general decontamination approach and test conditions, and the methods that were used to evaluate the data related to the project objectives. Testing was conducted in a spray chamber located in High Bay Room H122A at EPA's facility in Research Triangle Park, North Carolina.

2.1 Project Description

2.1.1 PHASE 1: Operational Aspects of Decontamination by Sporicidal Wipes

A preliminary study was conducted to develop the operational criteria that would be most effective for decontamination of medium size (1-9 ft²) surfaces by wiping the surfaces with sporicidal (decontamination) wipes. Four different commercially-available decontamination wipes were evaluated for their ability to wet the surfaces of four common porous/nonporous materials (painted drywall, vinyl tile, melamine board, and glass) of various sizes by measuring the amount of decontamination liquid remaining on the surface following wipe application.

The amount of liquid remaining on each material coupon following wipe application was determined gravimetrically, using the weight loss of the wipe. The effects of wiping pressure on the weight loss of the wipe was also evaluated. Additionally, the gravimetric loss of liquid from the wiped coupon surface was determined over a five-minute period. This mass of liquid remaining on a coupon after five minutes (min) could be considered an indicator of the relative efficacies of the decontamination wipes since inactivation of bacterial spores is not expected to occur on a dried surface. Results of this preliminary study aided in the selection of two sporicidal wipes for use in Phase 2 of the project. Phase 2 was designed to evaluate the effectiveness of sporicidal wipes in decontaminating surfaces inoculated with bacterial spores.

The four commercially available sporicidal/antimicrobial wipes examined in this preliminary study were:

- 1. Clorox Healthcare® Bleach Germicidal Wipe
- 2. Sani-Cloth® Bleach Germicidal Disposable Wipe
- 3. Dispatch[®] Hospital Cleaner Disinfectant Towel with Bleach
- 4. Hype-Wipe[®] Bleach Towelette.

Relevant physical and chemical properties of these wipes are summarized in Table 2-1.

Table 2-1. Sporicidal Wipe Information

Product	Vendor	Size	Active Ingredients	Components	EPA Registration ^a	Minimum Contact Time (min)
Clorox Healthcare® Bleach Germicidal Wipe	Clorox [®] Professional Products Co.	6.75 in x 9 in (17.1 cm x 22.8 cm)	Sodium hypochlorite	Sodium hypochlorite 0.1-1.0%, sodium metasilicate 0.1-1.0%, sodium hydroxide 0.1-1.0%	67619-12 ^b	3c
Sani-Cloth [®] Bleach Germicidal Disposable Wipe	Professional Disposables International, Inc.	6 in x 10.5 in (15.2 cm x 26.7 cm)	Sodium hypochlorite	Trisodium phosphate dodecahydrate 1-2.5%, sodium hypochlorite <1%	9480-8 ^b	4 ^c
Dispatch [®] Hospital Cleaner Disinfectants Towels with Bleach	Clorox [®] Professional Products Co.	6.75 in x 8 in (17.1 cm x 20.3 cm)		Sodium hydroxide <0.2%, sodium metasilicate <0.6%, sodium hypochlorite <1.0%	56392-8 ^b	5°
Hype-Wipe [®] Bleach Towelette	Current Technologies Inc.	6 in x 12 in (15.2 cm x 30.4 cm)	Sodium hypochlorite	Single-use Towelettes saturated with 0.525% sodium hypochlorite (a 1:10 dilution)	70590-1 ^b	4c

^a Registration with EPA's Office of Pesticide Programs (OPP) indicates EPA/OPP has evaluated the data provided by the manufacturer to show the product is effective and has no unreasonable adverse effects on humans, the environment, and nontarget species and has issued a registration or license for use in the United States.

^b Wipe has been registered as sporicidal against *Clostridium difficile* but not specifically for use against *B. anthracis*.

^c Contact time reflects the prescribed time a surface has to be wet to kill *Clostridium difficile (C. diff)*.

2.1.2 PHASE 2: Evaluation of Efficacy of Hypochlorite Wipes

The objective of this task was to evaluate the efficacy of two commercially available hypochlorite wipes to decontaminate material coupons with medium-size surface areas (i.e., $42 \text{ in } \times 42 \text{ in } = 12.25 \text{ ft}^2$) contaminated with *B. atrophaeus* spores (1 × 10⁷ colony forming units (CFU)). Work conducted in the Phase I preliminary study measured the wetting ability of four different sporicidal wipe products with efficacies previously evaluated by Meyer et al.[1]. Two of the four hypochlorite wipes of different wetting abilities were evaluated for their decontamination efficacy on two different indoor materials, namely, glass and painted drywall. Glass represents nonporous indoor materials, while painted drywall represents a more porous indoor material. The two hypochlorite wipes selected for the decontamination efficacy experiments were:

- a. Clorox Healthcare[®] Bleach Germicidal Wipe
- b. Hype-Wipe® Bleach Towelette.

The relevant physical and chemical properties of each of these two sporicidal wipes are summarized in Table 2-1.

2.2 Testing Program Description

2.2.1 PHASE 1: Operational Aspects of Decontamination by Sporicidal Wipes

The operational aspects of the wiping decontamination technique were evaluated through three different tasks. Task 1 consisted of determining the amount of liquid remaining on each material coupon of known

surface area following wipe application. Task 2 evaluated the loss of liquid from coupon surfaces over a required five-minute contact time period following wipe application. Task 3 evaluated the influence on the results in relation to the applied pressure when wiping the surface. These three tasks, described in the following sections, were used as indicators of the relative efficacies of the decontamination wipes.

2.2.1.1 Task 1 – Determination of Effective Surface Coverage of Sporicidal Wipes

Four different decontamination wipes were evaluated to determine how much surface area could potentially be wiped with a single wipe, based on whether the surface remained wet for required time. A single wipe was used to wipe surfaces ranging in size from 1.0 to 9.0 ft² of each material type. Each surface was wiped and each wipe was refolded according to the patterns and directions described in Section 3.6.3. To ensure consistency in wipe pattern and wiping pressure applied, the same person performed this operation across all Task 1 tests. The amount of liquid dispensed by each type of wipe on a target surface area for each specific type of material was determined by measuring its weight loss following the decontamination wiping of the surface. Table 2-2 provides the Task 1 test matrix that was repeated for all four decontamination wipe types. Five replicate samples were tested for each type of wipe/material/surface area combination. The largest area wiped under Task 1 was limited to 3 ft x 3 ft. This was a smaller area than the actual decontamination testing area of 3.5 ft x 3.5 ft (Phase 2) that was established after completion of Phase 1 testing.

Test ID	Material	Coupon Area Sampled per Wipe (ft x ft)	Replicates
		1 x 1	
	Painted Drywall	1.5 x 1.5	
1-D		2 x 2	5 per coupon area
		2.5 x 2.5	
		3 x 3	
		1 x 1	
	Vinyl Tile Melamine Board	1.5 x 1.5	
1-V		2 x 2	5 per coupon area
		2.5 x 2.5	
		3 x 3	
		1 x 1	
		1.5 x 1.5	
1-M		Melamine Board 2 x 2	5 per coupon area
		2.5 x 2.5	
		3 x 3	
1.0	Class	1 x 1	E por coupon aroc
1-G	Glass	1.5 x 1.5	5 per coupon area

Table 2-2. Task 1 Test Matrix Conducted for Each Type of Sporicidal Wipe

Test ID	Material	Coupon Area Sampled per Wipe (ft x ft)	Replicates
		2 x 2	
		2.5 x 2.5	
		3 x 3	

2.2.1.2 Task 2 – Surface Retention Times of Wipe Decontaminant Liquid

Decontamination wipes have a manufacturer-recommended contact time for *C. diff.* This task was designed to determine how much decontamination liquid remained on a horizontal coupon surface for a set time period following the wiping application. Coupons of all four materials were wiped with the decontamination wipes and then allowed to dry for five minutes. Determination of the amount of liquid remaining on a coupon after the five-minute period was accomplished by sampling the surface with one or more dry (non-sporicidal) wipes and measuring the liquid recovery based on weight gain of the dry wipe(s). The amount of decontamination liquid lost from the coupon during the five-minute drying period was assessed by comparing the weight gain of these dry wipes after the five-minute contact time to the weight gain of the same dry wipes used to sample the coupons immediately after the decontamination wipe procedure. One ft² coupons of each material were used in this task. Table 2-3 shows the Task 2 test matrix that was repeated for all four decontamination wipe types.

Table 2-3.	Task 2 Test	Matrix per	Sporicidal Wipe Type	

Test ID	Material	Drying Time (minutes)	Replicates
2-D	Painted Drywall	0	5
		5	
2-V	Vinyl Tile	0	5
2-V	VIIIyi The	5	5
2.14	Malamina Doord	0	5
2-M	Melamine Board	5	
	Glass	0	5
2-G		5	

2.2.1.3 Task 3 - Impact of Wiping Pressure

The amount of decontamination solution dispensed by each type of wipe on a coupon surface, as a function of the pressure applied by the sampling person during the wiping process, was examined in this task. The four types of decontamination wipes were evaluated on the four different material surfaces to determine if application pressure affected the amount of decontamination solution dispensed onto a coupon. A single decontamination wipe was used per coupon and was applied using slight or higher pressure by the same sampling personnel. The change in weight of the decontamination wipe was measured immediately after wiping. The forces applied were not quantified, yet are referred to as "slight" and "heavy" to indicate relative

pressure. One ft² coupons of each material were used for this task. Table 2-4 shows the Task 3 test matrix that was repeated for all four decontamination wipe types.

Test ID	Material	Pressure Applied to Wipe	Replicates
		 Slight pressure 	F
3-D	Painted Drywall	 Heavy Pressure 	5
2.14	Vinyl Tile	 Slight pressure 	5
3-V		 Heavy Pressure 	
2.14	Melamine Board	 Slight pressure 	- 5
3-M		 Heavy Pressure 	
	Glass	 Slight pressure 	_
3-G		 Heavy Pressure 	5

Table 2-4. Task 3 Test Matrix per Sporicidal Wipe Type

2.2.2 PHASE 2: Evaluation of Efficacy of Hypochlorite Wipes

The testing approach for evaluation of decontamination methods involved wiping of contaminated surfaces following inoculation of various material surfaces with *B. atrophaeus* spores, which served as a non-pathogenic surrogate for *B. anthracis. B. atrophaeus* spores are also known to be valid surrogates for decontamination studies involving hypochlorite solutions [3]. Decontamination wiping was performed using hypochlorite-impregnated wipes. Individual sections of the medium 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 wipes used for sampling. Aliquots from the extract liquids were spread plated using serial dilutions onto culture media and following an 18 to 24 h incubation at 35 °C ± 2 °C, the media plates were enumerated and the CFU were quantified. Results of test samples were compared to the results of positive control samples consisting of contaminated surfaces that had not been subjected to decontamination.

Two commercially available hypochlorite wipes were evaluated to determine their decontamination efficacy on the nine 14 in x 14 in inoculated sections of the 42 in x 42 in glass and painted drywall coupon surfaces. A single decontamination wipe was used to decontaminate the 42 in by 42 in surface using the same wiping method established in the preliminary study (Section 2.2.1). While the test coupons were placed horizontally for spore inoculation, the coupons were placed vertically for both the wipe decontamination procedure and the subsequent wipe sampling of residual spores on the surfaces. Coupons were maintained in an upright position using a heavy duty easel (Lyptus Wood "Dulce" Easel, Richeson Art, Kimberly, WI), as shown in Figure 2-1. The easel was modified slightly to better accommodate both the coupons and dry Polyester Rayon Blend (PRB) wipes placed below the coupons. These wipes were added to capture potential runoff of wipe solution from the near vertical surface. The easel was sterilized using Dispatch[®] wipes (Clorox[®] Professional Products Co. Oakland, CA). Five minutes after the Dispatch sterilization, the easel was wiped again with a separate wipe soaked with 3% sodium thiosulfate (STS) to neutralize/remove any remaining

hypochlorite, and then again with an alcohol wipe (VWR[®] Pre-moistened Clean-Wipes[™] with Isopropyl Alcohol/Deionized Water, VWR, Radnor PA).



Figure 2-1. Easel Used to Hold Coupons

Variables included in this decontamination test matrix and the corresponding test codes are listed below.

- 1. Decontamination Wipe
 - a. Clorox Healthcare® (C)
 - b. Hype-Wipe[®] (H)
- 2. Inoculation Method
 - a. Hot Spot upper left corner(inoculation location a) (1)
 - b. Hot Spot center (inoculation location e) (2)
 - c. Hot Spot lower right corner (inoculation location i) (3)
 - d. Broad area, all nine sections (0)
- 3. Material
 - a. Glass (G)
 - b. Painted Drywall (D)
- 4. Application Variations
 - a. Slight Pressure application (1)
 - b. Higher Pressure application (2)

Assigned test identifications (IDs) were contaminated in the order shown in Table 2-5. For example, decontamination test C2D1 indicates the use of a Clorox Healthcare[®] wipe (C) to wipe the 42"x42" surface that was inoculated in the center (2). The material was painted drywall (D) and slight pressure (1) was applied during the wiping of the surface. The target inoculation for the Hot Spot testing was 1×10^7 spores per ft², while the target inoculation for the Broad Area testing was 1×10^6 spores per ft². Note that only the

center 12 in x 12 in of a 14 in x 14 in coupon is inoculated, as the surrounding one-inch border is covered during the inoculation process.

For both Hot Spot and Broad Area decontamination wiping, 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 (i) was the last section to receive the decontamination wipe (see Section 3.3). The complete decontamination matrix involved 24 separate conditions, as depicted in Table 2-5 by test ID.

Test Code	Material	Inoculation Method and Location	Decontamination Wipe Used	Pressure Applied
C1G1	Glass	а	Clorox Healthcare®	Slight
C1D1	Painted drywall	а	Clorox Healthcare®	Slight
C2D1	Painted drywall	е	Clorox Healthcare®	Slight
C2G1	Glass	е	Clorox Healthcare®	Slight
C3G1	Glass	i	Clorox Healthcare®	Slight
H0D1	Painted drywall	Broad Area	Hype-Wipe [®]	Slight
H0D2	Painted drywall	Broad Area	Hype-Wipe [®]	Heavy
H1D1	Painted drywall	а	Hype-Wipe [®]	Slight
H1G1	Glass	а	Hype-Wipe [®]	Slight
H2G1	Glass	е	Hype-Wipe [®]	Slight
H3D1	Painted drywall	i	Hype-Wipe [®]	Slight
H3G1	Glass	i	Hype-Wipe [®]	Slight
H3D2	Painted drywall	i	Hype-Wipe [®]	Heavy
H3G2	Glass	i	Hype-Wipe [®]	Heavy
C3G2	Glass	i	Clorox Healthcare®	Heavy
H2D1	Painted drywall	е	Hype-Wipe [®]	Slight
C3D1	Painted drywall	i	Clorox Healthcare®	Slight
C0G1	Glass	Broad Area	Clorox Healthcare®	Slight
C0G2	Glass	Broad Area	Clorox Healthcare®	Heavy
C3D2	Painted drywall	I	Clorox Healthcare®	Heavy
H0G1	Glass	Broad Area	Hype-Wipe [®]	Slight
H0G2	Glass	Broad Area	Hype-Wipe [®]	Heavy
C0D1	Painted drywall	Broad Area	Clorox Healthcare®	Slight
C0D2	Painted drywall	Broad Area	Clorox Healthcare®	Heavy

Table 2-5. Decontamination Test Matrix for Wiping Surfaces with Sporicidal Wipes

2.3 Definitions of Effectiveness

The surface decontamination efficacy for each decontamination technique and surface material combination was evaluated by measuring the difference in the logarithm of the measured CFU before decontamination (determined from sampling of the positive control coupons) and after decontamination (determined from sampling of the test coupons) for that material. This value is reported as a log reduction (LR) on the specific material surface as defined in Equation 2-1.

$$\eta_{i} = \frac{\sum_{k=1}^{N_{c}} \log(CFU_{c,k})}{N_{c}} - \frac{\sum_{k=1}^{N_{i}} \log(CFU_{s,k})}{N_{s}}$$
(2-1)

where:

$$\begin{split} \eta_i &= \begin{array}{ll} & \text{Surface decontamination effectiveness; the average log} \\ \eta_i &= \begin{array}{ll} & \text{Surface decontamination effectiveness; the average log} \\ & \text{reduction (LR) of spores on a specific material surface (surface material designated by i)} \\ \\ & \\ & \\ \hline \frac{\sum_{k=1}^{N_c} \log(CFU_{c,k})}{N_c} \end{array} &= \begin{array}{ll} & \text{The average of the logarithm (or geometric mean) of the} \\ & \text{number of viable spores (determined by CFU) recovered on the} \\ & \text{control coupons (C indicates control and N_c is the number of control coupons)} \\ \\ & \\ \hline \frac{\sum_{k=1}^{N_c} \log(CFU_{s,k})}{N_s} \end{array} &= \begin{array}{ll} & \text{The average of the logarithm (or geometric mean) of the} \\ & \text{number of viable spores (determined by CFU) remaining on the} \\ & \text{surface of a decontaminated coupon (S indicates a decontaminated coupon and N_s is the number of coupons tested).} \end{array}$$

When no viable spores were detected, a value of 0.5 CFU was assigned for $CFU_{S,k}$, and the efficacy was reported as greater than or equal to the value calculated by Equation 2-1.

The standard deviation of the average LR of spores on a specific material (η_i) is calculated by Equation 2-2:

$$SD_{\eta_{i}} = \sqrt{\frac{\sum_{k=1}^{N_{s}} (x_{k} - \eta_{i})^{2}}{N_{s} - 1}}$$
(2-2)

where:

- SD_{η_i} = Standard deviation of η_i , the average LR of spores on a specific material surface
 - η_i = The average LR of spores on a specific material surface (surface material designated by *i*)
 - $x_k = \frac{\text{The average of the LR from the surface of a decontaminated}}{\text{coupon (Equation 2-3)}}$
 - Ns = Number of test coupons of a material surface type.

$$x_{k} = \frac{\sum_{k=1}^{N_{s}} ((\overline{\log(CFU_{c})} - \log(CFU_{s,k})))}{N_{s}}$$
(2-3)

Where:

$$\overline{\log(CFU_C)} = \frac{\sum_{k=1}^{N_C} \log(CFU_{C,k})}{N_C}$$

=

Represents the "mean of the logs" (geometric mean), the average of the logarithm-transformed number of viable spores (determined by CFU) recovered on the control coupons (C = control coupons, N_c = number of control coupons, k = test coupon number and N_s is the number of test coupons)

 $CFU_{s,k} =$ Number of CFU on the surface of the kth decontaminated coupon

$$N_s = {{Total number (1,k) of decontaminated coupons of a} \atop {material type.}}$$

In this report, decontamination efficacy is generally reported in terms of LR for a particular material. Results may include whether the average LR for a particular test is \geq 6.0, since a decontaminant that achieves \geq 6 LR (against a 6-7 log challenge) is generally considered effective during laboratory efficacy tests. It is important to note that demonstrated effectiveness in the laboratory can help predict the performance of a decontamination procedure/product in the field, but the 6 LR benchmark should not be considered in direct connection with achieving specific clearance goals.

3 Material and Methods

3.1 Test Coupon Preparation and Sterilization

Four types of materials (painted dry wall, vinyl tile, melamine, and glass) were used for Phase 1 testing, while only painted dry wall and glass were used for Phase 2. These four types of materials were selected as being typical of materials 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 contamination control coupons (14 in x 14 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.

For Phase 1, all coupons were cleaned with an alcohol wipe and air-dried immediately prior to use to remove any incidental dust and grime that may have accumulated throughout the repeated use of the same materials. Coupons for wiping efficacy tests (Phase 2) were sterilized with 400 parts per million (ppm) hydrogen peroxide (H₂O₂) vapor for four h using a STERIS VHP® ED1000 (STERIS Life Sciences, Mentor, OH) generator. Prior to use, the coupons treated with VHP® were kept at room temperature for a minimum of 2-3 days to force off-gassing of residual H₂O₂ from the coupons so that biocidal activity was prevented.

- Painted Drywall. Drywall coupons were cut to size from a 4 ft x 8 ft SHEETROCK Brand 1/2 inthick Drywall Panel (National Gypsum Company, Charlotte, NC) and painted with two coats of white latex paint (Behr Premium Plus Interior Flat White Latex Paint, Home Depot, Durham, NC). Test ID code D.
- 2. **Vinyl Tile**. Coupons were cut to size from Armstrong 12 ft-wide River pattern Staggered Slate Brown Multi Vinyl Sheet (Model G4820, Home Depot, Durham, NC) and were glued to oriented strand board (OSB). Test ID code **V**.
- 3. **Melamine Boards**. Coupons were cut to size from a 4 ft x 8 ft sheet of $\frac{3}{4}$ in Melamine (Model 461877, Home Depot, Durham, NC). Test ID code **M**.
- 4. **Glass**. Glass coupons (3/16 in-thick tempered glass, Durham Glass, Durham, NC) were purchased pre-cut to the required sizes. Test ID code **G**.

3.2 Bacillus Spore Preparation

The test organism for this work was a powdered spore preparation consisting of a mixture of *B. atrophaeus* (formerly known as *Bg*) spores (American Type Culture Collection (ATCC) 9372) and silicon dioxide particles. The preparation was obtained from the U.S. Army Dugway Proving Ground (DPG) Life Science Division. The preparation procedure is fully reported in Brown et al. [4] and can be summarized as follows: After 80-90 percent sporulation, the suspension is centrifuged to generate a preparation of approximately 20 percent solids. The final product in the form of a powdered matrix containing approximately 1 x 10¹¹ viable spores per

gram is prepared by dry blending and jet milling the dried spores with fumed silica particles (Degussa, Frankfurt am Main, Germany).

The powdered preparation as received was loaded into metered dose inhalers (MDIs) at the EPA test site according to a proprietary protocol. Control checks for each MDI were included in each batch of coupons contaminated with a single MDI.

3.3 Coupon Inoculation Procedures

Coupons (test and positive controls) were inoculated independently with spores of *B. atrophaeus* by being placed into a separate dosing chamber, called an Aerosol Deposition Apparatus (ADA), designed to fit one 14 in x 14 in coupon of any thickness [5]. The ADA consisted of a stainless steel hood sized to cover the area of a square test coupon exactly. In the center at the top of the hood was an opening to which an MDI and an MDI actuator were attached. Photographs of an ADA, an MDI, and an MDI actuator used in this project are shown in Figures 3-1 and 3-2.



Figure 3-1. Aerosol Deposition Apparatus



Figure 3-2. MDI and Actuator

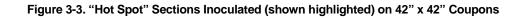
Prior to the start of any experiment, all components needed for the aerosol deposition of spores onto material coupon surfaces using the ADA deposition approach must be sterilized and stored in a sterile environment until use. ADAs and coupons were sterilized with a 250-ppm, 4-h VHP sterilization cycle, while the MDI actuator, with attached MDI adaptor, was wiped with pAB, then rinsed with deionized (DI) water. Sterilization was not necessary for binder clips, MDIs, vortex, or the aerosol trap.

The MDI was discharged a single time into the ADA. The MDIs are claimed to provide 200 discharges per MDI. The number of discharges per MDI was tracked so that use did not exceed this value. Additionally, the weight of each MDI was determined after completion of the contamination of each coupon. The contamination control coupons (14 in x 14 in stainless steel coupons) were inoculated as the first, middle, and last coupons within a single group of coupons inoculated by any one MDI within a single test. Puffed spores were allowed to settle onto the coupon surfaces for a minimum period of 18 h.

For both Hot Spot and Broad Area inoculations, *B. atrophaeus* spores were inoculated onto 14 in x 14 in sections of 42 in x 42 in coupons. Dosing was conducted according to established National Homeland Security Research Center (NHSRC) laboratory aseptic procedures described previously [6, 7].

For the Hot Spot tests, inoculation was performed at a target concentration of 1×10^7 spores/ft² in one of the nine 14 in x 14 in sections: the upper left corner (a), the center (e), and the lower right corner (i), as shown in Figure 3-3.

a	b	С
d	e	f
g	h	i



For the Broad Area testing, all nine (14 in x 14 in) sections were inoculated at a target concentration of 1 x 10^6 spores/ft² for each section.

3.4 Decontamination Procedure

The decontamination procedure for all four decontamination wipes is described below:

- Three of the four wipes were supplied in canisters. The Clorox Healthcare[®] Bleach Germicidal Wipe is shown in Figure 3-4. Each canister was gently rolled and/or inverted 3-4 times to distribute liquid in advance of removing Towelettes. Sterile gloves were used to remove the first 2-3 wipes from the container and these wipes were discarded based on American Society for Testing and Materials, now ASTM International (ASTM) 2896-12. Gloves were changed as necessary to maintain sterility. Hype-Wipe[®] Bleach Towelettes were individually packaged and did not require this step.
- 2. The same wiping procedure utilized for sampling the coupon surfaces (as described in Section 3.6.3) was used for the decontamination procedure.
- 3. Each decontamination wipe was stored in a separate sterile conical tube for at least 24 h after wipe testing. After 24 h, the decontamination wipe was neutralized by adding 35 mL of phosphatebuffered saline with Tween 20 (PBST) with 2.5% STS to the conical tube.

The steps to neutralize these specific decontamination wipes are based on the study by Meyer et al. [1].



Figure 3-4. Clorox Healthcare® Bleach Germicidal Wipe Canister

3.5 PHASE 1 Measurement Protocol

3.5.1 Sampling Approach

The sampling approaches used for the Phase 1 operational aspect testing and Phase 2 wipe decontamination testing were identical (detailed in Section 3.6.3). During Phase 1, coupons from the same material were chosen randomly for sampling to avoid a systematic bias that could have been introduced due to personnel fatigue or changes in room environment. Each of the 25 coupons per material (five replicates, five sizes) was assigned a random unique integer between 1 and 25. Wiping occurred in numerical order. A Microsoft Excel 2013 random function coupled with the index and rank function was used to create a random coupon number generator.

3.5.2 Gravimetric Measurements

The amount of liquid retained on the coupons following the decontamination wiping procedure was determined by measuring the weights of the wipes before and after use. The following gravimetric procedure for Tasks 1 and 3 was used:

- Using a recently calibrated analytical balance, accurate to ±1 milligram (mg), tare the balance using a new weigh boat and measure the weight of the wipe to the nearest 1 mg immediately following removal from the bulk canister or individual package (Hype-Wipe[®] Bleach Towelettes only).
- 2. Wearing gloves, use the wipe for decontamination of the coupon according to the procedure detailed in Section 3.6.3.
- 3. Immediately place the used wipe in the same weigh boat used in Step 1 and determine the weight of the used wipe to the nearest 1 mg.
- 4. The difference between weights in Step 1 and Step 3 is reported as the weight loss of the wipe which is equated to the amount of liquid transferred to the coupon.

For Task 2, the following procedure was used.

- 1. Tare the balance using a new weigh boat, and measure the combined weight of two dry wipes to the nearest 1 mg immediately following removal from the bulk container (canister).
- 2. Remove one wipe from the boat and record the weight of the second wipe alone.
- 3. Use a two-wipe variation of the procedure outlined in Section 3.6.3 to sample the entire surface. Start with the first wipe, using horizontal and vertical strokes, flipping the wipe over between horizontal and vertical strokes.
- 4. Remove the second wipe from the weigh boat, place first wipe in the weigh boat, and record the weight of the first wipe alone after use.
- 5. Use the second wipe to sample the entire surface with diagonal and perimeter strokes.
- 6. Immediately place the second wipe in the same weigh boat and determine the weight of both used wipes together.
- 7. The difference between weights in Step 6 and Step 1 is reported as the weight gain and is equated to the amount of decontamination liquid remaining on the coupon following the drying period.

Steps 2 and 4 were included initially to determine individual wipe weight increases as needed.

3.5.3 Method Verification

A procedural blank was obtained as a means of verifying the methods used for Tasks 1 and 3. A procedural blank was acquired by handling a decontamination wipe as per the procedure but without applying the wipe to a surface. The weight loss of the wipe determined in this manner was taken to be indicative of the amount of decontamination liquid that remains on the glove.

The dry wipe sampling method was verified by applying 10 mL of a 5000 ppm hypochlorite solution in 1 mL drops to the surface of the nonporous coupon materials and using the dry wipe method to measure recovery. For verification, the recovery had to be within a range of 75% to 125% of the amount applied, with an expected variance not exceeding 30%.

3.6 PHASE 2 Measurement Protocol

3.6.1 Sterile Handling of Wipes

New containers of decontaminant wipes were opened for each test day. The first three wipes were not considered to be representative of the package and were discarded based on ASTM 2896-12 for small scale Petri dish experiments, which stated, in part: "For multi-count containers only, use sterile gloves to remove 2-3 wipes from the container and discard. For canisters, gently roll and/or invert 3-4 times to distribute liquid in advance of removing towelettes. If possible, have another person hold the towelette container to avoid contamination of the gloves when removing the wipes. Change gloves as necessary to maintain sterility."

3.6.2 Sampling Site Environmental Conditions

Ambient environmental conditions such as temperature, relative humidity (RH), and barometric pressure can affect the evaporation rate of liquids from surfaces. Taking these influences into account was especially important for Phase 1 testing. All tests were conducted at room temperature, ambient RH and ambient barometric pressure. RH and temperature were monitored during testing, and tests were conducted on days with environmental conditions within 35-60% RH and 20-25 °C. All coupons were conditioned under ambient conditions for one week before use.

3.6.3 Wipe Sampling Procedures

Within a single test, surface sampling of the materials was completed for all procedural blank coupons before sampling of any test material was performed. Surface sampling was done by wipe sampling in accordance with the protocols documented below. Prior to the sampling event, all materials needed for sampling were prepared using aseptic techniques. The materials specific to the wipe sampling protocol are included in the relevant sections below. The general sampling supplies were sterile or sterilized/disinfected for each sampling event.

- a. Don a fresh pair of latex or nitrile gloves for the wipe sampling procedure.
- b. With gloved hand, grasp the wetted decontamination wipe. Using the other hand, gently fold the wipe (Figure 3-5). Do not squeeze the wipe to avoid losses of the wetting solvent.

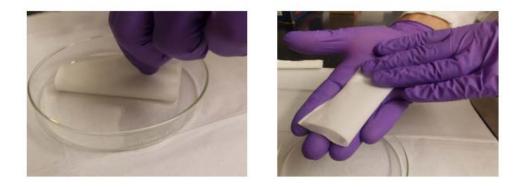


Figure 3-5. Folding of Wipe for Wipe Sampling Step 1 (horizontal wiping pathway)

c. Starting in the top left corner, wipe the surface **horizontally**, working downward, to cover the surface completely. The horizontal wipe sampling pathway is shown in Figure 3-6.

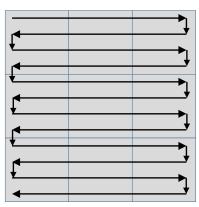


Figure 3-6. Horizontal Wiping Pathway

d. Using both hands, gently refold the wipe so that that surface used for the horizontal wipe sampling is now on the inside (Figure 3-7).

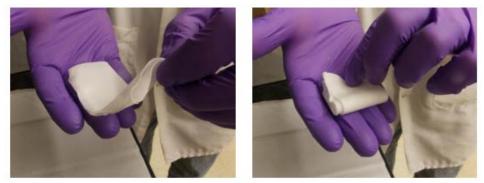


Figure 3-7. Folding of Wipe for Wipe Sampling Step 2 (vertical wipe sampling pathway)

e. Starting in the bottom left corner, wipe the surface vertically, working toward the right, to cover the surface completely. The vertical wipe sampling pathway is shown in Figure 3-8.

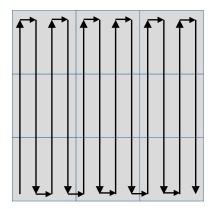


Figure 3-8. Vertical Wiping Pathway

f. Using both hands, gently refold the wipe diagonally, so that that surface used for the vertical wipe sampling is now on the inside (Figure 3-9).

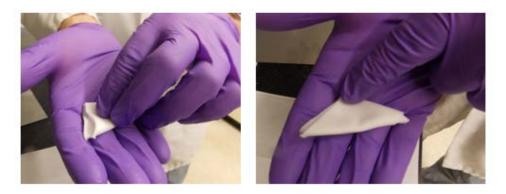


Figure 3-9. Folding of Wipe for Wipe Sampling Step 3 (diagonal wipe sampling pathway)

g. Starting in the top left corner, wipe the surface **diagonally**, working toward the bottom right corner, to cover the surface completely. The diagonal wipe sampling pathway is shown in Figure 3-10.

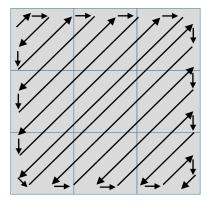


Figure 3-10. Diagonal Wiping Pathway

h. Using both hands, gently refold the wipe so that that surface used for the diagonal wipe sampling is now on the inside (Figure 3-11).



Figure 3-11. Folding of Wipe for Wipe Sampling Step 4 (perimeter wipe sampling pathway)

i. Starting in any corner, wipe the **perimeter** of the coupon. The perimeter wipe sampling pathway is shown in Figure 3-12.

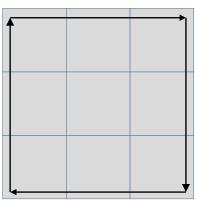


Figure 3-12. Perimeter Wiping Pathway

Following decontamination of the spore-inoculated coupons, each of the nine 14 in x 14 in sections (including both inoculated and non-inoculated sections in the Hot Spot tests) of the 42 in x 42 in test coupon and the nine 14 in x 14 in sections in a positive control coupon were sampled individually using PRB wipes in accordance with the wipe sampling procedure described above. Sampling was performed after a coupon was visually dry and no sooner than 30 minutes after decontamination wiping was complete.

Because the coupons were placed vertically on the easel, additional dry PRB wipes were placed under the bottom edge of the coupon to capture any drips that might migrate downward and off the coupon during wiping. These dry wipes were also considered to be samples for determination of viable spores. Decontamination wipe samples were placed in 35 mL of PBST with 3% STS immediately following use.

LR was determined using the sum of viable spores collected on individual wipes from all sections of each coupon and the wipes below the coupons as compared to the sum of viable spores collected on individual wipes from positive control coupons not subjected to decontamination.

This standard wiping method was used to ensure high reproducibility of laboratory results as it ensured that the whole surface area was wiped in a consistent manner. This test method may not reflect the performance when used by responders. However, this approach reflects the best possible case when utilizing these sporicidal wipes.

3.6.4 Swab Sampling

The general approach for swab sampling was to use a moistened swab (BactiSwab[®] Collection and Transport System, Remel, Thermo Fisher Scientific, Waltham, MA) to wipe a specified area to recover bacterial spores. Swab samples were collected from all decontamination procedure equipment before use to serve as sterility checks.

3.6.5 Phase 1 Sample Identification

Each sample was assigned an ID based on the sample coding outlined in Table 3-1. This ID was used in written records in laboratory notebooks documenting the surface area, surface type, pressure applied, etc.

Table 3-1. Sample Coding for Phase 1

Coupon Identification: WA73-T-M-D-SS-N				
Category	Example Code	Description		
T (Task)	(1,2,3)	Task under which the sample was collected		
M (Material)	V G M D	Vinyl Tile Glass Melamine Board Painted Drywall		
D (Decontamination Wipe)	1 2 3 4	Clorox Healthcare [®] Bleach Germicidal Wipe Sani-Cloth [®] Bleach Germicidal Disposable Wipe Dispatch [®] Hospital Cleaner Disinfectant Towel with Bleach Hype-Wipe [®] Bleach Towelette		
	W#	Decontamination wipe For Task 1, # will be the number of coupons wiped		
SS (Sample Descriptor)	D#	Dry wipe For Task 2, # will be the time in minutes after which the sample is collected		
	P(X)#	For Task 3, $\#$ will the 1 – slight pressure, , or 3 – higher pressure PA for person A, PB for person B		
N (Replicate Number)	N	Sequential numbers		

3.6.6 Phase 2 Sample Identification

Each sample was assigned a unique ID based on the sample coding outlined in Table 3-2. 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.

Table 3-2: Sample Coding for Phase 2

	Coupon Identification: 73-TID-L-M-D-SS-N				
Category	Example Code	Description			
TID (Test ID)	1C	For Task 1*: T=1, ID = C (Clorox [®] wipe) or H (Hype-Wipe [®])			
L (Inoculation Location)	а	a = the upper left corner, e = the center, i = the lower right corner, 0 = broad area; see Figure 3-2			
M (Material)	G	G = Glass D = Painted Drywall			
D Decontamination Application	2	For Task 1: 1 = Slight Pressure 2 = Higher Pressure			
	T(#) – Wipe samples from test coupons	XT for blank coupon (negative) # represents section the test sample was obtained from (a-i)			
	D	Drip sample			
SS (Comple descriptor)	(X)M	MDI control sample from stainless steel coupons; XM for Blank Sample			
(Sample descriptor)	SW (#)	Swab sample- followed by identifier (#) of where sample was collected (A) - ADA (C) - Coupon			
	P(#) – Wipe samples from Positive Control coupons	# represents section the test sample was obtained from (a-i)			
N (Replicate Number) 1 Se		Sequential test numbers			
	NHSRC Biolab Plate Iden	tification: 73-TID-L-M-D-SS-N -R-D			
73-TID-L-M-D-SS-N	As above				
R (Replicate)	R	A – C			
D (Dilution)	1	0 to 4, for 10E0 to 10E4			

* Original study included Task 2 related to use of robots for decontamination. Only outcome from Task 1 is reported here.

3.6.7 Sample Frequency

Table 3-3 lists the sample frequency for decontamination tests using the wiping approach.

Table 3-3. Wiping Efficacy - Sample Frequency

Sample Type	Quantity	Frequency	Location/Condition	Purpose
Test Coupon Samples	Three coupon replicates per test; nine wipe samples per coupon for a total of 27 samples	One set per material	Decontaminated	To determine the number of viable spores after decontamination
Procedural Blank	One coupon of equal size as test coupon, not inoculated	One per material	Decontaminated	To determine extent of cross contamination during testing
Negative Control Coupon	One 14 in x 14 in coupon	One per material	Not decontaminated	To determine extent of cross contamination and/or the sterility of coupons
Task 1 Positive Control Coupon	Two coupons per material, inoculated as the first and last coupon during the inoculation procedure; nine wipe samples per coupon for a total of 18 wipe samples	One set per material	Not decontaminated	To determine the number of viable spores recoverable from the coupons
MDI Control Coupons (stainless steel)	Three per inoculation event, as the first and last inoculations. Also includes the middle inoculation if more than 25 inoculations are performed	Three per inoculation	Not decontaminated	To determine the number of viable spores deposited onto the coupons and to assess the stability of the MDI
Laboratory Material Blanks	Three per material	Once per use of material	NA	To demonstrate sterility of extraction and plating materials
ADAs Sterility Check (swab samples)	One per sterilized batch of ADAs	Once before testing	NA	To demonstrate sterility of ADAs
Drip Wipe	Three per coupon	Three composite samples per test	Underneath the coupon	Determine the transport of spores off decontaminated surface through drips
Decontamination Wipe	One per coupon	Three per test	The decontamination wipe itself	Determine the survival rates of the spores on the wipe and the physical removal of viable spores after 24-h hold time

3.6.8 Prevention of Cross Contamination of Samples during Sampling

Sampling posed a potentially significant opportunity for cross contamination of samples. To minimize the possibility of cross contamination, several management controls were implemented:

- In accordance with aseptic techniques, a three-person sampling team was utilized, including a "lead sampler," an "assistant sampler," and a "coupon handler."
- The "coupon handler" was designated as the only person to operate the ADA and handle the test coupons during the sampling event.
- The "lead sampler" handled only the sampling media (wipes and liquids) and performed the surface sampling of the test coupons.
- The "assistant sampler" was responsible for and handled only the containers for receiving the sample collection media (e.g., a PRB wipe). The "lead sampler" deposited the collection media into the sample containers that were opened, held, and sealed by the "assistant sampler." Sealed samples were handled only by the "assistant sampler" who was solely designated to perform the following actions using aseptic technique:
 - The sealed bag with the sample was placed into another sterile plastic bag that was then sealed; the exterior of that bag was then decontaminated using a bleach wipe.
 - The double-bagged sample was placed into a sample container for transport.
 - The exterior of the transport container was decontaminated by wiping all surfaces with a bleach wipe or a towelette moistened with a solution of hypochlorite prior to transport from the sampling location to the on-site NHSRC Biolab.
- After the sample was placed into the container for transport, the "coupon handler" placed the sampled test coupons in pAB for decontamination prior to reuse or disposal.
- At the completion of each sampling event, each member of the sampling team changed their gloves in preparation for working with the next sample.

As a further precaution to avoid cross contamination of samples, the order of coupon sampling was from coupons least contaminated with *B. atrophaeus* to those most contaminated; i.e., (1) all blank coupons, (2) all decontaminated coupons, and (3) positive control coupons.

Strict adherence to aseptic laboratory technique was followed by the NHSRC Biolab to recover, plate, culture, and analyze samples. The order of analysis was the same as the order of sampling; i.e., (1) all blank coupons, (2) all decontaminated coupons, and (3) all positive control coupons.

3.6.9 Collecting Representative Samples

The representativeness and uniformity of test coupon materials was considered essential in achieving defensible evaluation results. Material representativeness was achieved by using materials that were typical of those currently used in buildings in terms of quality, surface characteristics, and structural integrity and which conformed to industry standards or specifications for indoor use. Material uniformity was achieved by obtaining and preparing a quantity of material sufficient to allow multiple test samples to be prepared with presumably uniform characteristics.

3.6.10 Sample Storage and Preservation

After sample collection, sample integrity was accomplished by triple containment of samples: (1) sample collection container, (2) sterile bag with exterior sterilized during the sample packaging process, and (3) clean container holding all samples from a test. All individual sample containers remained sealed while in the coupon decontamination laboratory and while in transport to the NHSRC Biolab. The sampling person did not handle any samples after they were relinquished to the support person during placement into the primary sample container.

All samples received were stored in a refrigerator at 4 °C \pm 2 °C until they were analyzed. All samples were allowed to stabilize at room temperature for one h prior to analysis.

3.6.11 Sample Holding Time

After sample collection for a single test was completed, all biological samples taken for that test were transported to the NHSRC Biolab immediately along with the appropriate chain of custody (CoC) form(s). The quality assurance project plan (QAPP) for this project stated that samples were to be stored no longer than five days before commencing the primary analysis. However, given the volume of samples generated over a short period of time, the samples were stored in the refrigerator for longer than five days before commencing the primary analysis. A method development test was conducted to determine if the holding times had a negative effect on the samples. No significant change was observed in the samples and bacterial colony counts. These results are explained in detail in Section 5.3.2.

3.6.12 Sample Chain of Custody

Test schedules were coordinated with the NHSRC Biolab so that samples were collected, transferred, and analyzed within the established timeframe, with the exception of hold times as discussed above. To ensure the integrity of samples and to maintain a timely and traceable transfer of samples, an established and proven CoC approach was used. The primary objective of the CoC was to create an accurate written record that could be used to trace the possession of a sample from the moment of its creation through the reporting of the results. A sample was said to be in custody if it was in any one of the following states:

- In actual physical possession
- In view, after being in physical possession
- In physical possession and locked up so that no one could tamper with it
- In a secured area, restricted except to authorized personnel
- In transit.

Test team members received copies of the test plans prior to each test. Pre-test briefings were then held to apprise all participants of the objectives, test protocols, and CoC procedures to be followed.

In the transfer of custody, each custodian signed, recorded, and dated the transfer. Sample transfer was either on a sample-by-sample basis or on a bulk basis. A CoC record listing the samples by their IDs always accompanied the samples at each stage of their journey from initial collection through analysis and final disposition. When turning over possession of samples, the transferor and recipient both signed, dated, and

noted the time on the CoC record sheet. The recipient of the samples was declared to be the custodian and was responsible for the samples until a subsequent transfer or disposition was made.

3.6.13 Sample Archiving

All samples and diluted samples were archived for at least two weeks following completion of analysis. This archival period allowed for review of the data to determine if any re-plating of selected samples was required. Samples were archived by maintaining the primary extract at 4 °C \pm 2 °C in a sealed extraction tube.

3.6.14 Microbiological Analysis

The NHSRC Biolab located at the EPA facility in Research Triangle Park, NC, analyzed samples either qualitatively for spore presence (quality control, swab samples) or quantitatively for the number of viable spores recovered per sample (CFU). Details of the analysis procedures are provided below. A laboratory notebook was used to document the details of each sampling event (or test).

Spores were extracted from the PRB wipes by adding 20 mL PBST to each tube, then agitating the tubes using a vortex mixer (set to maximum rotation) for two minutes in 10-second intervals. For all sample types, liquid extracts were serially diluted tenfold (in PBST) and 0.1 mL spread-plated in triplicate onto tryptic soy agar (TSA) plates. Plates were incubated at 35 °C \pm 2 °C for 18 to 24 h, and CFU were enumerated visually. Only plates containing between 30 and 300 CFU were utilized for recovery estimates. Extracts were diluted and replated if none of the tenfold dilutions resulted in all three plates containing colony counts within the acceptable range. All extracts were stored at 4 °C \pm 2 °C. Total spore recovery was calculated by multiplying the mean CFU counts from triplicate plates by the inverse of the volume plated, by the dilution factor, and finally by the total volume of the extract. Any samples below countable criteria (30-300 CFU) on the primary dilution plates were subsequently filter plated through 0.2 µm pore-size filters (Nalgene, Rochester, NY), with the filters placed onto TSA plates followed by incubation at 35 °C \pm 2°C for 18-24 h. The CFU counts from these plates were used to calculate recovery in these circumstances.

Prior to erecting a coupon on the easel, the easel tray was lined with sterile drip wipes (Fisherbrand[™] Dry Clean-Wipes[™], catalog no. 06-664-14, Pittsburgh, PA). The drip wipes collected any runoff/spores that were displaced from the coupons during the decontamination wiping process. After the coupons were sampled, the drip wipes were aseptically transferred to a sterile specimen cup and stored in the refrigerator at 4 °C for a minimum of 24 h, after which they were neutralized by adding 70 mL of PBST with 2.5% STS.

The decontamination wipes were also stored in sterile specimen cups following the decontamination procedure. They were refrigerated at 4 °C for a minimum of 24 h after which they were neutralized by adding 35 mL of PBST with 2.5% STS. The drip wipes as well as the decontamination wipes followed the same extraction and microbial analysis procedure as the PRB sampling wipes.

4 Results and Discussion

4.1 PHASE 1: Operational Aspects of Decontamination by Sporicidal Wipes Results

Appendix A provides the tabulated results of Tasks 1, 2, and 3 of the preliminary study designed to establish the wiping techniques and to aid in the selection of the decontamination wipes to be used in Phase 2 to evaluate the effectiveness of decontamination methods. The conclusions from these test results are explained in detail in the following sections.

4.1.1 Determination of Effective Surface Coverage of Sporicidal Wipes

The objective of this task was to determine how much liquid was dispensed from a single wipe. In this task, the weight loss of a wet wipe following the prescribed wiping procedure was taken to be equal to the amount of bleach deposited on a coupon. The individually packaged, single-use Hype-Wipe[®] Bleach Towelettes resulted in higher amount of bleach dispensed, for all types of materials tested, compared to the other wipes that are susceptible to drying out faster once their canister lid is opened. The weight losses were the highest for the largest areas wiped. As expected, the amount of dispensed liquid bleach per unit area decreased with increasing areas wiped as illustrated in Figures 4-1 and 4-2 for all material/wipe combinations tested. Among the four materials tested, the least amount of liquid bleach was transferred to glass, while painted drywall retained the most liquid (Figure 4-3). Since a surface area of 9 ft² resulted in 40 to 50% of the bleach being dispensed regardless of material/wipe type tested, glass (nonporous) and painted drywall (porous) were selected as the materials to be tested during the Phase 2 decontamination testing.

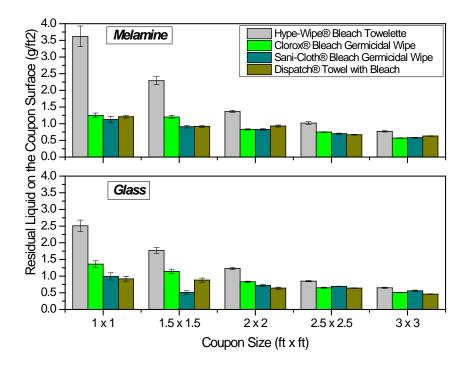


Figure 4-1. Residual Bleach Recovered from Melamine and Glass Material

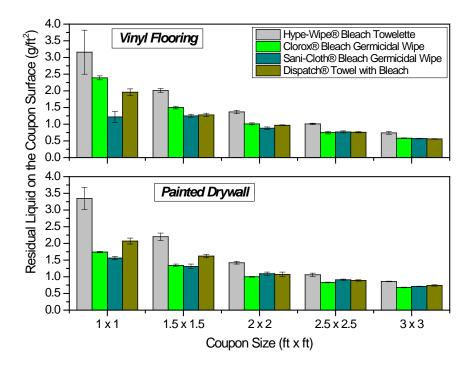


Figure 4-2. Residual Bleach Recovered from Vinyl Flooring and Painted Drywall Material

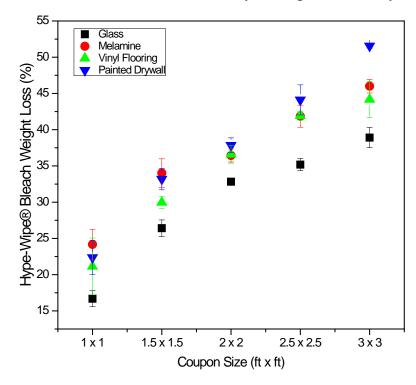


Figure 4-3. Percent Liquid Dispensed on Material as a Function of Material Type, for Hype-Wipe®

4.1.2 Surface Retention Times of Wipe Decontaminant Liquid Results

This task was designed to assess the propensity of each material to retain the liquid bleach from each wipe type on its surface over time and to assess the temporal liquid loss as a function of the material/wipe combination. Two sets of five replicate 1 ft x 1 ft coupons for each material were used for this task. The amount of decontamination liquid dispensed was obtained for each different type of wipe by the same method used in Task 1. The amount of liquid remaining on a coupon immediately after the decontamination event (time zero) and after a five-minute drying time was determined by dry wiping of the surface. The liquid remaining on the surface was equated with the increase in weight of the dry wipes at the two time points (0, and five (5) minutes). The time point of five minutes was selected as the longest minimum contact time, across the four different sporicidal wipes, that the decontamination liquid was supposed to have with the material to achieve the efficacy criteria as demonstrated on a 1 ft x 1 ft coupon by Meyer et al. [1]. The results for the temporal recovery of the bleach solution for each type of material/wipe type combination are presented in Figure 4-4 for Clorox[®] Bleach Wipe and Hype-Wipe[®] Bleach and in Figure 4-5 for Sani-Cloth[®] Bleach Germicidal Disposable Wipe and Dispatch[®] Hospital Cleaner Disinfectant Towel with Bleach, respectively.

The residual liquid solution from the Hype-Wipe[®] Bleach Towelettes, and to a lesser extent the liquid solution from the Clorox Healthcare[®] Bleach Germicidal Wipe, was found to adhere better to the materials tested and was less susceptible to loss through evaporation or absorption through the material surface over time. The residual liquid solution from the Sani-Cloth[®] Bleach Germicidal Disposable Wipe was found to disappear the most through evaporation or absorption through the materials tested, the vinyl flooring material was associated with the lowest loss in liquid over time, while painted drywall was the most affected. Although, all the tested wipes were saturated with 0.525% sodium hypochlorite (bleach), the contact time between the bleach and a specific surface may be affected by the secondary active ingredients such as alcohols, surfactants, and stabilizers.

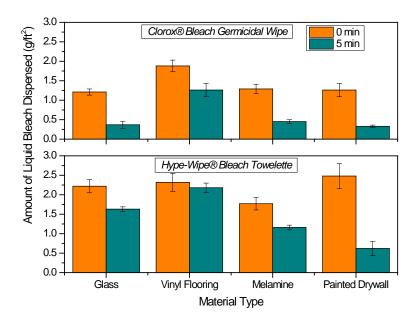


Figure 4-4. Temporal Residual Bleach Recovered following a Decontamination Event using Clorox[®] Bleach Wipe and Hype-Wipe[®] Bleach Towelette

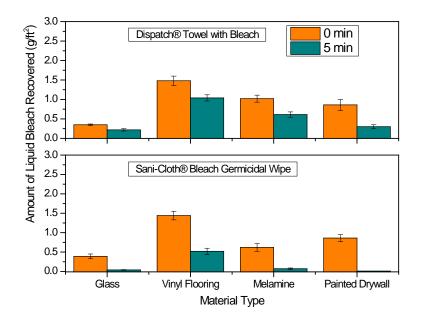


Figure 4-5. Temporal Residual Bleach Recovered Following a Decontamination Event using Sani-Cloth[®] Wipe and Dispatch[®] Towel with Bleach

4.1.3 Impact of Wiping Pressure

This task was to determine the effect of the application pressure on the decontamination wipe while wiping the coupons. Coupons (1 ft x 1 ft) of each material were used for this task, and results are shown in Figures 4-6 and 4-7. Higher pressure application resulted in a higher volume of liquid solution dispensed compared to the slight (low) pressure application for all material/wipe type combinations. For these particular tests, the effect of applying higher pressure, compared to slight pressure, resulted in an increase of approximately 60% of the liquid dispensed from the Hype-Wipe[®] Bleach Towelettes to the coupon and up to 300% from Sani-Cloth[®] wipes. The decontamination efficiency of a particular wipe type may be affected by the operational aspect of the decontamination procedure such as the application pressure.

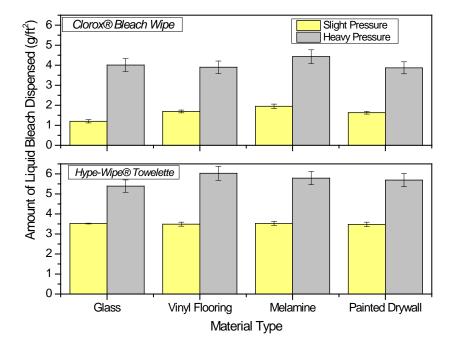


Figure 4-6. Effect of Application Pressure on Liquid Dispensed Using Clorox[®] Bleach Wipe and Hype-Wipe[®] Bleach Towelette

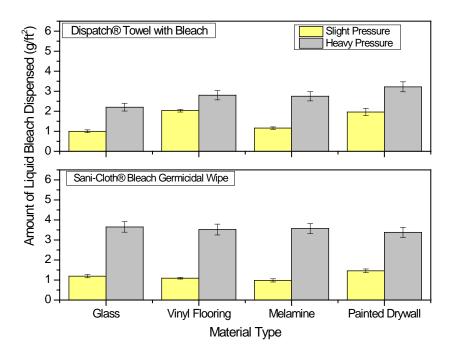


Figure 4-7. Effect of Application Pressure on Liquid Dispensed using Sani-Cloth[®] Wipe and Dispatch[®] Towel with Bleach

4.2 PHASE 2: Evaluation of Efficacy of Hypochlorite Wipes Results

Appendix B provides the tabulated recoveries of the Phase 2 testing designed to evaluate the decontamination effectiveness of targeted wipe/material type combinations to decontaminate a 12.25 ft² surface. The variables evaluated in the complex test matrix included two types of decontamination wipes (Hype-Wipe[®] Bleach Towelette, and Clorox Healthcare[®] Wipe), two material types (glass and painted drywall), two inoculation spatial distributions (Hot Spot versus Broad Area), and the effect of wipe application pressure (slight and higher pressure). In addition, the fate of the spores is presented for different inoculation as shown in Figure 3-3), and for the broad area inoculation.

The two different hypochlorite wipes were tested on 3.5 ft x 3.5 ft (107 cm x 107 cm) coupons, consisting of 3 x 3 sections of 14 in x 14 in (35.6 cm x 35.6 cm) sampling areas. Each 14 in x 14 in area was sampled individually after a 30-min contact time following the wiping of the whole surface. Surfaces were wet with locally dryer areas due to, e.g., the gravitational pull of the residual dispensed liquid toward the bottom of the panel. Recovered spores (CFU) for each section were summed to determine the CFU and log CFU reduction per coupon type/wipe type combination across the whole 42 in x 42 in area. The decontamination efficacy results are shown in Tables 4-1 and 4-2 for glass and painted drywall, respectively. Some (3 out of 24) of the procedural blanks had more than 500 spores. For those three test conditions, a 6 log reduction would be impossible to demonstrate. It should be noted that the average recovered number of spores from test

coupons were significantly higher than those from the procedural blank suggesting a less than 6 log reduction. See Section 5.3.1 for a more detailed discussion.

One-way analysis of variance (one-way ANOVA), revealed that, at the 95% confidence interval (CI), the mean decontamination efficiencies of the types of wipes used in this study are statistically different (p value = 0.031). Hype-Wipe[®] Bleach Towelettes with an overall average spore LR of 3.8 ± 0.8 were, in general, more effective than the Clorox Healthcare[®] Wipe with an overall average spore LR of 3.0 ± 1.0 , independent of the inoculation location. Similar work performed by Meyer et al. [1] showed that these selected wipes completely inactivated *Bacillus* spores (LR ≥ 6.0) on smaller 14 in x 14 in (35.6 cm x 35.6 cm) coupons for similar wipe type/material combination decontamination approaches. Mean decontamination efficacies were not statistically different across the two materials (p values = 0.436 and 0.294 for Clorox Healthcare[®] Wipe and Hype-Wipe[®] Bleach Towelettes, respectively). The wipe application pressure seems to have little or no effect on the decontamination efficacy of the wipe. No detailed statistical analysis was performed on this small subset of test samples.

To interpret cross contamination of the wipes during the decontamination process, the spatial distribution of the post-decontamination spore concentration for both the Hype-Wipe® Bleach Towelette and Clorox Healthcare® Wipe on glass and painted drywall material is illustrated in Figures 4-8 to 4-11 using a colorcoded scheme for the spore loading concentration. The results show clearly that the wipe decontamination was most effective when the Hot Spot inoculation occurred in the upper left corner, which is the same location where the (horizontal motion) decontamination wiping started, and worst for the Hot Spot located in the lower right corner of the coupon (where the decontamination wiping ended). For the lower right corner inoculation test, the first surface area of the hypochlorite wipe used to decontaminate the coupon using horizontal motion (working downward to completely cover the surface) may have dried before reaching this Hot Spot. The dried wipe may have then become contaminated without inactivation of spores. The cross contamination between the wipe surfaces (spent dry wipe surface folded inwards and newly saturated wipe surface) may also have occurred during the folding of the wipe, and spores were subsequently distributed throughout the other areas of the coupon during the vertical wipe decontamination pathway. The third (diagonal wiping) and the fourth (perimeter wiping) pathways may have exacerbated the cross contamination, due to faster drying of the exposed surface, since only one fourth and one eighth of the original surface area were used during these two last steps.

Residual spores collected from sterile drip Clean-Wipes[™] that lined the easel tray supporting the test coupon and stored for a minimum of 24 h at 4 °C in sterile specimen cups showed an overall contamination of less than 0.03% of the inoculated spore burden. This contamination may be credited to liquid runoff during the decontamination wiping procedure and/or re-aerosolization/deposition of spores during the coupon setup/decontamination/sampling events. Enumeration of CFU on the decontamination wipes that were used during the decontamination process and stored for a minimum of 24 h at 4 °C in sterile specimen cups resulted in no spores being detected. This indicates that spores collected on the sporicidal wipe became inactivated.

Decontamination	Inoculation	Applied	Average Rec	Log	
Wipe Type	Location	Sampling Pressure	Positive Control	Test Coupons	Reduction
	Upper left corner (a)	Slight	3.17 x 10 ⁷ ± 2.19 x 10 ⁷	2.60 x 10 ² ± 1.10 x 10 ²	5.12 ± 0.88
	Center (e)	Slight ¹	4.43 x 10 ⁷ ± 8.50 x 10 ⁶	$2.26 \text{ x } 10^4 \pm 1.20 \text{ x } 10^4$	3.86 ± 0.91
Hype-Wipe [®] Bleach	Lower right	Slight	4.35 x 10 ⁷ ± 9.66 x 10 ⁶	1.72 x 10 ⁷ ± 1.14 x 10 ⁴	3.60 ± 0.56
Towelette	corner (i)	Heavy	3.90 x 10 ⁷ ± 2.83 x 10 ⁶	1.38 x 10 ³ ± 9.94 x 10 ²	4.63 ± 0.91
	Broad Area	Slight	6.08 x 10 ⁶ ± 1.24 x 10 ⁶	$4.33 \text{ x } 10^2 \pm 5.53 \text{ x } 10^2$	4.52 ± 1.42
		Heavy	7.91 x 10 ⁶ ± 1.74 x 10 ⁶	$3.49 \text{ x } 10^3 \pm 4.58 \text{ x } 10^3$	3.88 ± 1.26
	Upper left corner (a)	Slight	$3.06 \text{ x } 10^7 \pm 9.68 \text{ x } 10^6$	3.32 x 10 ³ ± 4.21 x 10 ³	4.32 ± 0.94
	Center (e)	Slight	5.04 x 10 ⁷ ± 1.27 x 10 ⁷	2.84 x 10 ⁶ ± 1.41 x 10 ⁶	1.91 ± 0.42
Clorox Healthcare®	Lower right	Slight	2.14 x 10 ⁷ ± 1.31 x 10 ⁷	1.61 x 10 ³ ± 2.12 x 10 ³	4.50 ± 1.20
Bleach Germicidal Wipe	corner (i)	Heavy	4.83 x 10 ⁷ ± 8.74 x 10 ⁶	1.70 x 10 ⁴ ± 9.18 x 10 ⁴	3.50 ± 0.21
		Slight	7.11 x 10 ⁶ ± 1.44 x 10 ⁶	1.21 x 10 ⁴ ± 6.21 x 10 ³	2.86 ± 0.27
	Broad Area	Heavy	$6.55 \times 10^6 \pm 9.02 \times 10^5$	$3.34 \times 10^4 \pm 1.08 \times 10^3$	3.31 ± 0.14

Table 4-1. Decontamination Results for Glass Material Type

¹Procedural blank showed relatively high contamination for these tests (>5 x 10² spores)

Decontamination	Inoculation	Applied	Average Reco		
Wipe Type Location		Sampling Pressure	Positive Control	Test Coupons	LR
	Upper left corner (a)	Slight	4.54 x 10 ⁷ ± 1.24 x 10 ⁷	3.25 x 10 ³ ± 4.36 x 10 ³	4.63 ± 1.33
	Center (e)	Slight	6.11 x 10 ⁷ ± 1.45 x 10 ⁷	$5.54 \text{ x } 10^4 \pm 2.85 \text{ x } 10^4$	3.47 ± 0.79
Hype-Wipe®	lower right	Slight	5.03 x 10 ⁷ ± 3.11 x 10 ⁷	$2.04 \text{ x } 10^4 \pm 1.53 \text{ x } 10^4$	3.77 ± 0.86
Bleach Towelette	corner (i)	Heavy ¹	3.76 x 10 ⁷ ± 1.27 x 10 ⁶	$4.75 \text{ x } 10^5 \pm 2.14 \text{ x } 10^5$	2.20 ± 0.26
	Broad Area	Slight ¹	7.22 x 10 ⁶ ± 1.70 x 10 ⁶	7.77 x $10^3 \pm 4.27$ x 10^3	3.02 ± 0.23
		Heavy	$1.06 \text{ x } 10^7 \pm 6.65 \text{ x } 10^5$	$8.37 \text{ x } 10^3 \pm 5.07 \text{ x } 10^3$	3.22 ± 0.39
	Upper left corner (a)	Slight	5.38 x 10 ⁷ ± 6.46 x 10 ⁶	$1.00 \text{ x } 10^4 \pm 1.35 \text{ x } 10^4$	4.24 ± 1.08
	Center (e)	Slight	7.52 x 10 ⁷ ± 1.97 x 10 ⁷	1.77 x 10 ⁵ ± 5.53 x 10 ⁴	2.73 ± 0.23
Clorox Healthcare®	lower right corner (i)	Slight	4.43 x 10 ⁷ ± 9.52 x 10 ⁶	1.23 x 10 ⁶ ± 5.31 x 10 ⁵	1.64 ± 0.11
Bleach Germicidal Wipe		Heavy	3.67 x 10 ⁷ ± 7.35 x 10 ⁶	$2.78 \text{ x } 10^5 \pm 3.44 \text{ x } 10^5$	2.46 ± 0.32
		Slight	9.87 x 10 ⁶ ± 5.66 x 10 ⁵	8.13 x 10 ⁴ ± 2.79 x 10 ⁴	2.20 ± 0.18
	Broad Area	Heavy	9.39 x 10 ⁶ ± 2.60 x 10 ⁶	$1.09 \text{ x } 10^5 \pm 2.8 \text{ x } 10^4$	1.95 ± 0.08

Table 4-2. Decontamination Results for Painted Drywall Material Type

¹Procedural blanks show relatively high contamination for these tests (>5 x 10² spores)

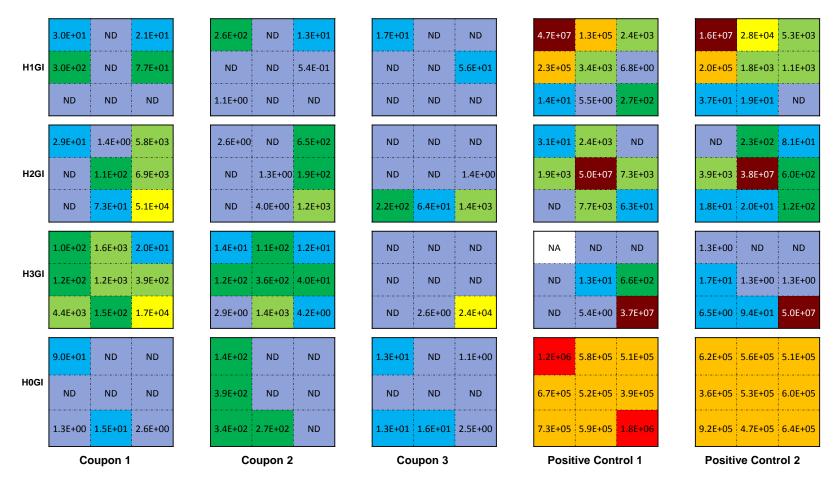


Figure 4-8. Spatial Post-Decontamination Residual Spore Concentration Using Hype-Wipe[®] Bleach Towelette /Glass Material at Different Inoculation Locations.

EO	E1	E2	E3	E4	E5	E6	E7
----	----	----	----	----	----	----	----

Spore loading concentration color coded scheme; ND: no viable spores detected; NA: Not Available due to sample loss.

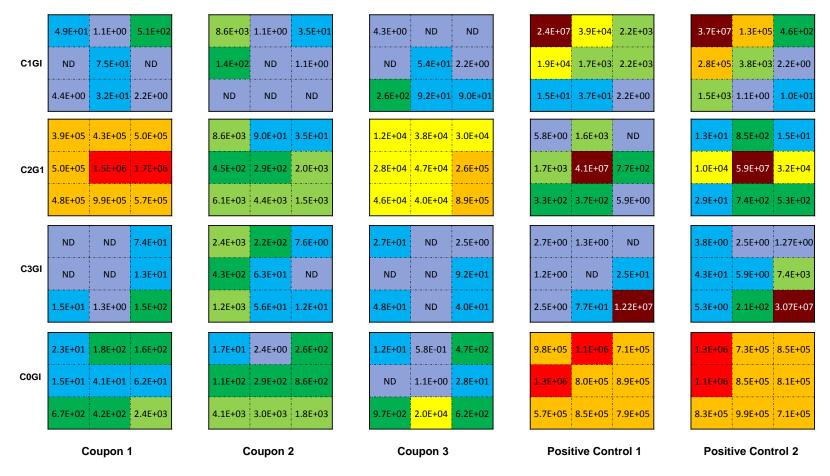


Figure 4-9. Spatial Post-Decontamination Residual Spore Concentration using Clorox Healthcare® Wipe/Glass Material at Different Inoculation Locations

E0 E1 E2 E3 E4 E5 E6 E7 E	
---------------------------	--

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

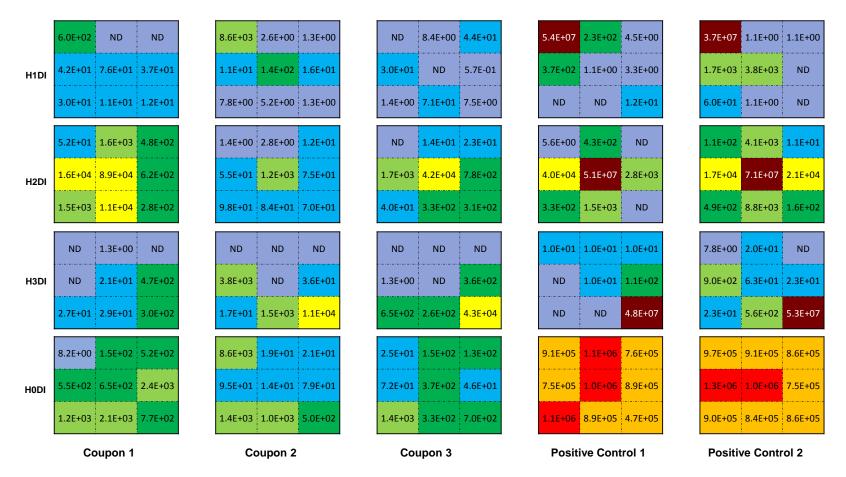


Figure 4-10. Spatial Post-Decontamination Residual Spore Concentration using Hype-Wipe[®] Bleach Towelette /Painted Drywall Material at Different Inoculation Locations

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

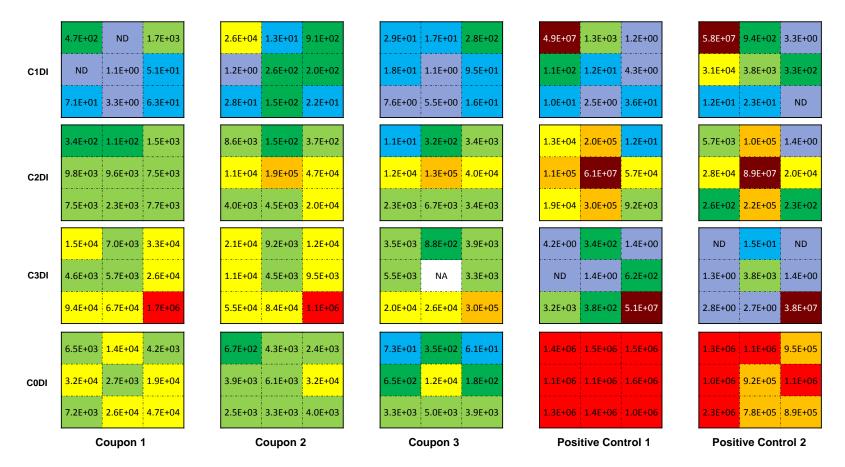


Figure 4-11. Spatial Post-Decontamination Residual Spore Concentration using Clorox Healthcare® Wipe/Painted Drywall Material at Different Inoculation Locations

Spore loading concentration color coded scheme; ND: no viable spores detected; NA: Not Available due to sample loss.

5 Quality Assurance and Quality Control

This project was performed under the approved Category III QAPPs entitled "Decontamination Solution Methods for Bacillus anthracis Surrogates Sporicidal Wipes Product Assessment Part I", July, 2014, and "Evaluation of Sporicidal Wipes and Liquid Agents for Decontamination of Anthrax-Contaminated Surfaces by Hand and Robotic Cleaners", November, 2014.

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 QAPP, and physical impacts on materials. All tests were conducted in accordance with developed Decontamination Technologies Research Laboratory (DTRL) and NHSRC Biolab Miscellaneous Operating Procedures (MOPs) to ensure repeatability and adherence to the data quality validation criteria set for this project.

5.1 Criteria for Critical Measurements/Parameters

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

- Weight of the decontamination and dry wipes
- Sample volume collected
- Plated volume
- Counts of 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 5-1. Failure to provide a measurement 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.

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 two counters)	± 5	1 CFU
Weight	Scale	0.004 g	0.0001 g	NA

NA = not applicable

5.2 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 5-1. 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.

5.2.1 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 National Institute of Standards and Technology (NIST)-traceable whenever possible. Supplies and consumables were examined for evidence of tampering or damage upon receipt and prior to use, as appropriate. Supplies and consumables showing evidence of tampering or damage were discarded 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.

5.2.2 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.

5.3 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 blanks: transfer of sterile wipes from the sample tube to an empty conical tube at the decontamination location;
- 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 5-2. These QA/QC objectives provide assurances against cross contamination and other biases in the microbiological samples.

Table 5-2. 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 Blanks	Verify the sampling wipes do 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 ones will be discarded	Each plate
Positive Control Coupons	Used to determine the extent of inoculation on the coupons	1 x 10 ⁶ CFU, \pm 0.5 log (Broad Area Inoculation) or 1 x 10 ⁷ CFU, \pm 0.5 log (Hot Spot Inoculation)	Outside target range: discuss potential impact on results with EPA WACOR; correct loading procedure for next test and repeat depending on decided impact	Two per coupon type.
Procedural Blank Coupons	Determine cross contamination during the testing procedures	No detectable spores	Determine source of contamination and remove	One for each test condition
Inoculation Control Coupons	Used to determine drift in the MDI	The CFU recovered from the first coupon must be \pm 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.

5.3.1 QA/QC Test Results Validation

The QA/QC control test results for the whole sampling campaign are shown in Table 5-3. All field blanks and inoculum blanks were found to be non-detects. However, some of the negative control blanks (2 out of 24) and some of the procedural blanks (3 out of 24) were found to have approximately 5 x 10² CFU per coupon. The source of this contamination is unknown. For negative controls, the contamination may have occurred by incomplete inactivation of spores from the materials by the VHP cycle and the subsequent reuse of such a coupon in the next test. Procedural blanks may have become contaminated due to their presence in an area with inoculated coupons present. Due to the high levels of spores in the negative control and procedural blanks, this study cannot demonstrate a minimum of 6 LR antimicrobial efficacy for every test condition. For the test conditions where cross contamination occurred, the number of spores recovered from especially the hot spot inoculated test coupons were significantly higher than those from the associated procedural blank still suggesting that the sporicidal wipes were unable to deactivate all spores. The vast majority of sporicidal wipe tests with associated non-detects in procedural blank or negative control support the general observation that a 6 log efficacy was not reached when wiping a 3.5 ft x 3.5 ft surface area.

Decontamination Wipe Type					Glass		Painted Drywall					
	Inoculation Location	Applied Sampling Pressure	Average Recovery (CFU)					Average Recovery (CFU)				
			Field Blank	Negative Control	Procedural Blank	Inoculum Control Blank	Field Blank	Negative Control	Procedural Blank	Inoculum Control Blank		
	Upper left corner (a)	Slight	ND	ND	103	ND	ND	30	ND	ND		
	Center (e)	Slight	ND	ND	3680	ND	ND	ND	ND	ND		
Hype-Wipe [®]	Lower right corner (i)	Slight	ND	ND	270	ND	ND	ND	ND	ND		
Bleach Towelette		Heavy	ND	ND	450	ND	ND	1980	1580	ND		
	Broad Area	Slight	ND	ND	ND	ND	ND	3870	99	ND		
		Heavy	ND	4	ND	ND	ND	ND	ND	ND		
	Upper left corner (a)	Slight	ND	500	ND	ND	ND	6	ND	ND		
	Center (e)	Slight	ND	ND	<200*	ND	ND	6	293	ND		
Clorox Healthcare®	Lower right	Slight	ND	ND	30	ND	ND	ND	ND	ND		
Bleach Germicidal Wipe	corner (i)	Heavy	ND	ND	ND	ND	ND	ND	ND	ND		
	Dread Area	Slight	ND	524	200	ND	ND	ND	ND	ND		
	Broad Area	Heavy	ND	4	ND	ND	ND	ND	2	ND		

Table 5-3. QA/QC Test Results

ND: Non Detect

*: No spores detected during dilution plating; filter plating results considered outlier. Upper limit based on enumeration detection limit of 1 spore in 0.1 mL dilution extract.

5.3.2 Sample Hold Time

Due to the need for extended holding times of the samples before primary analysis (as discussed in Section 3.6.11), a testing sequence was conducted to evaluate the potential reduction of bacterial spores in the wipe sample containers as a function of time.

The hypochlorite sporicidal wipes listed below were tested on medium size coupon surfaces to estimate the occurrence and potential reduction of bacterial spores as a function of remediation activities. The surface tested was glass (which represented nonporous indoor building materials, and coupons were 12.25 ft² in area (3.5 ft x 3.5 ft)).

- a. Clorox Healthcare® Bleach Germicidal Wipe
- b. Hype-Wipe® Bleach Towelette
- c. pÅB wipe prepared in house by soaking Fisherbrand[™] dry wipes in 4 mL pAB.

The testing approach consisted of the following sequentially conducted steps:

- a. The test coupons were first subjected to a specific decontamination wipe; note that the coupons were not inoculated prior to decontamination.
- b. After an exposure time of 30 minutes, the coupons were wipe sampled as follows:
 - areas a, b, and c were sampled together and labeled as the Day 1 sample;
 - areas d, e, and f were sampled and labeled as the Day 4 sample, and
 - Lastly, areas g, h, and i were labeled as the Day 7 sample.
- c. Each of the sampled wipe sample containers was inoculated on Day 1 (one day following sampling) with 0.1 mL of 2 x 10⁸ CFU/mL microbial suspension (test organism solution) to result in a final suspension that contains approximately 2 x 10⁷ CFU of the microorganism.
- d. Analysis of the samples occurred by adding 20 mL of PBST to each container on the scheduled day (Day 1, Day 4 or Day 7), followed immediately by extracting and plating, as shown in Table 5-4.

Table 5-4. Test Matrix (Test Samples)

Test ID	Decontaminant	Coupon Section	Analyzed and Plated (Day)	Number of Wipe Samples
73-(C/H/P)-0-G-1-Ta-1		а		C Test Samples (3)
73-(C/H/P)-0-G-1-Tb-1		b	Day 1	H Test Samples (3)
73-(C/H/P)-0-G-1-Tc-1	C = Clorox Healthcare® Bleach Germicidal Wipe H = Hype-Wipe® Bleach Towelette	C		P Test Samples (3)
73-(C/H/P)-1-G-1-Td-1		d		C Test Samples (3)
73-(C/H/P)-1-G-1-Te-1		е	Day 4	H Test Samples (3)
73-(C/H/P)-1-G-1-Tf-1	P = pAB Wipe	f		P Test Samples (3)
73-(C/H/P)-7-G-1-Tg-1		g		C Test Samples (3)
73-(C/H/P)-7-G-1-Th-1		h	Day 7	H Test Samples (3)
73-(C/H/P)-7-G-1-Ti-1		i		P Test Samples (3)

The positive control and negative control coupons were not subjected to the decontamination wipe. The negative control coupon and each of the positive control coupon sections were directly wipe-sampled with a PRB wipe. While the positive coupons were inoculated with spores, the negative control coupons were not. The procedural blank coupon was decontaminated with the hypochlorite wipe and then sampled, but did not undergo inoculation. The control sample test matrix for this hold time study is shown in Table 5-5.

Table 5-5. Test Matrix – Hold Time Control Samples

Test ID	Туре	Decontaminant	Coupon Section	Analyzed and Plated (Day)	Number of Wipe Samples
73-(C/H/P)-0-G-1-Pa-1			а		C Test Samples (3)
73-(C/H/P)-0-G-1-Pb-1			b	Day 1	H Test Samples (3)
73-(C/H/P)-0-G-1-Pc-1			С		P Test Samples (3)
73-(C/H/P)-1-G-1-Pd-1		C = Clorox	d		C Test Samples (3)
73-(C/H/P)-1-G-1-Pe-1	Positive Control	Healthcare [®] Bleach Germicidal Wipe	e	Day 4	H Test Samples (3)
73-(C/H/P)-1-G-1-Pf-1		H = Hype-Wipe [®] Bleach Towelette	f		P Test Samples (3)
73-(C/H/P)-7-G-1-Pg-1			g		C Test Samples (3)
73-(C/H/P)-7-G-1-Ph-1		P = pH-Amended Bleach Wipe	h	Day 7	H Test Samples (3)
73-(C/H/P)-7-G-1-Pi-1			i		P Test Samples (3)
73-(C/H/P)-(0/1/7)-G-1-XT-1	Procedural Blank	•	NA	0 = Day 1	1 per decontamination wipe per day (9)
73-(C/H/P)-(0/1/7)-G-1-NT-1	Negative Control		NA	1 = Day 4 7 = Day 7	1 per decontamination wipe per day (9)

The results from the hold tests, summarized in Table 5-6, indicate that the samples were not affected by the longer hold time prior to analysis. There was no reduction of bacterial spores in the sample containers as a function of time.

Decontamination Wipe			Average Recovery FU)	Test Sa Average Rec	LR		
	Day	Average	StDev	Average	StDev	Average	StDev
	1	1.42 x 10 ⁷	9.33 x 10⁵	1.34 x 10 ⁷	9.34 x 10⁵	0.02	0.03
Clorox Healthcare®	4	1.40 x 10 ⁷	1.04 x 10 ⁶	1.35 x 10 ⁷	1.83 x 10 ⁶	0.08	0.06
	7	1.26 x 10 ⁷	4.82 x 10⁵	1.55 x 10 ⁷	1.08 x 10 ⁶	-0.04	0.03
	1	1.37 x 10 ⁷	1.48 x 10 ⁶	1.46 x 10 ⁷	7.57 x 10 ⁵	-0.03	0.02
Hype-Wipe [®]	4	1.44 x 10 ⁷	5.18 x 10 ⁵	1.40 x 10 ⁷	4.54 x 10 ⁵	-0.01	0.01
	7	1.21 x 10 ⁷	4.73 x 10⁵	1.36 x 10 ⁷	3.85 x 10⁵	0.00	0.01
	1	1.42 x 10 ⁷	1.34 x 10 ⁶	1.47 x 10 ⁷	1.77 x 10 ⁶	-0.01	0.05
pAB Wipe	4	1.35 x 10 ⁷	1.03 x 10 ⁶	1.38 x 10 ⁷	7.49 x 10 ⁵	0.01	0.02
	7	1.29 x 10 ⁷	9.05 x 10⁵	1.27 x 10 ⁷	4.23 x 10 ⁵	0.05	0.01

Table 5-6. Sample Hold Time Test Results for Decontamination Efficacy Tests

StDev = Standard deviation.

5.4 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 5-7. Any deficiencies were noted and the instrument adjusted and recalibrated within 24 h to meet calibration tolerances. All other equipment (e.g., incubators, biological safety cabinets, refrigerators) used at the time of evaluation were verified as being certified, calibrated, or validated.

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	± 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%
pH meter	Perform a two-point calibration with standard buffers that bracket the target pH before each use.	± 0.1 pH unit
HACH High Range Bleach Test Kit	Titrate standard solution of 1000 ppm NaClO2 before each use	± 10%

Table 5-7. Instrument Calibration Frequency

NIST: National Institute of Standards and Technology

ISO: International Organization for Standardization

5.5 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 that 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.

5.6 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 could therefore 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.

5.7 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.

5.8 **QAPP** Amendments and Deviations

5.8.1 Formal Amendments

During the course of this research effort, two amendments were added to the initial QAPP entitled "Decontamination Solution Methods for Bacillus anthracis Surrogates. Sporicidal Wipes Product Assessment Part I.", August 11, 2014. This QAPP described the Phase I testing. A first amendment described the Phase Il evaluation of the sporicidal efficacy of wipes and was approved on December 3, 2014. A second amendment described the holding tests (see Section 5.3.2). The second amendment was provided without a formal approval requirement.

5.8.2 QAPP Deviations

One deviation was documented. The hold time test (Section 5.3.2; Amendment II to the QAPP) included pAB wipes. The QAPP amendment text suggested that these pAB wipes were also evaluated as part of this study. This was not the case; only four sporicidal products were evaluated in Phase I and two sporicidal products in Phase II. There is no impact to the conclusions of this study.

6 SUMMARY

Four commercially-available sporicidal wipes (Clorox Healthcare[®] Bleach Germicidal Wipe, Sani-Cloth[®] Bleach Germicidal Disposable Wipe, Dispatch[®] Hospital Cleaner Disinfectant Towel with Bleach, and Hype-Wipe[®] Bleach Towelettes) were evaluated on their use to decontaminate four types of materials (painted drywall, vinyl tile, melamine board, and glass).

A first assessment involved the measurement of the amount of liquid dispensed to a surface. The individually packaged, single-use, Hype-Wipe[®] Bleach Towelettes resulted in the highest volume of sporicidal solution (bleach) dispensed to the surface of all types of materials tested. This result is not surprising as the initial amount of liquid held by these wipes is also the highest of the four wipe materials tested. Other wipes that are kept in bulk in their canister dispensed less liquid. Of those, the Clorox Healthcare[®] Wipe dispensed the highest amount of liquid. Among the four materials tested, the least amount of liquid bleach was transferred to glass, while painted drywall had the propensity to retain the most.

The liquid solutions dispensed from Hype-Wipe[®] Bleach Towelettes, and to a lesser extent from Clorox Healthcare[®] Bleach Germicidal Wipes, were less susceptible to loss through evaporation from or adsorption into the material surface over time. All decontamination wipes dispensed enough liquid (with bleach as the active ingredient) to keep the tested surfaces "wet" based on visual observation over the five-min contact time.

A higher pressure application resulted in higher liquid solution volume dispensed compared to slight (low) pressure application for all material/wipe type combinations. For these particular tests, the effect of applying higher pressure resulted in an increase of approximately 60% in liquid being dispensed from the Hype-Wipe[®] Bleach Towelettes to the coupon and up to a 300% increase in liquid from the Sani-Cloth[®] wipes.

Two sporicidal wipes were selected for further evaluation of their efficacy to decontaminate medium size surfaces. The decontamination efficacies of the Hype-Wipe[®] Bleach Towelette and Clorox Healthcare[®] Wipe were evaluated on medium-size surface areas (12.25 ft², 1.37 m²) for different inoculation methods (Hot Spot versus Broad Area) and wipe application pressure method (slight versus high pressure).

Results indicate that the Hype-Wipe[®] Bleach Towelette was slightly more effective in inactivating *Bg* spores than the Clorox Healthcare[®] Wipe. This difference may be attributed to the higher amount of liquid that was dispensed from the Hype-Wipe[®] Bleach Towelette when compared to the Clorox Healthcare[®] Wipe. The overall log reduction in viable spores, including all inoculation conditions and both materials, by the Hype-Wipe[®] Bleach Towelette and Clorox Healthcare[®] Wipe was 3.8 ± 0.8 and 3.0 ± 1.0 , respectively. The wipe application pressure seems to have little or no effect on the decontamination efficacy of the wipe. Three sporicidal wipe tests may have been biased by significant number of spores (>500) on procedural blank or negative control coupons. Excluding results from those tests does not change the average log reduction in viable spores across all inoculation conditions for either sporicidal wipe.

The decontamination wipes that were used during the decontamination process were stored for a minimum of 24 h at 4 °C in sterile specimen cups. No viable *Bg* spores were detected on these wipes after use.

Post-decontamination sampling of coupons showed that cross contamination occurs, especially when the exposed surface area of the bleach decontamination wipe is drying out due to the wiping of dry surface

areas. This cross contamination seems to have started with the contamination of the spent surface of the wipe, when it dried, resulting in contamination of other areas of the coupons during subsequent wiping.

Neither sporicidal wipe meets the 6 log reduction in viable spores when wiping a larger 12.25 ft² (1.37 m²) surface area. The presence of a significant number of viable spores on some of the procedural blanks or negative controls may bias this statement negatively. However, the overall impact of this cross contamination should be considered minimal as the vast majority of tests resulted in a less than 6 log reduction in viable spores. These results are in contrast to the previously reported results where \geq 6 log reductions were achieved when wiping a 1 ft² surface area [1]. Further testing should be considered to find the maximum area these wipes can be used by conducting efficacy tests to verify a 6 LR.

7 REFERENCES

- 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, 2014. **117**(6): 1634-44.
- 2 C.P. Weis, A.J. Intrepido, A.K. Miller, P.G. Cowin, M.A. Durno, J.S. Gebhart, and R. Bull, *Secondary aerosolization of viable Bacillus anthracis spores in a contaminated U.S. Senate office*, Journal of the American Medical Association, 2002. 288(22): 2853-8.
- U.S. EPA, Office of Research and Development, Systematic Investigation of Liquid and Fumigant Decontamination Efficacy against Biological Agents Deposited on Test Coupons of Common Indoor Materials. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-11/076, 2011.
- G.S. Brown, R.G. Betty, J.E. Brockmann, D.A. Lucero, C.A. Souza, K.S. Walsh, R.M. Boucher, M. Tezak, M.C. Wilson, and T. Rudolph, *Evaluation of a wipe surface sample method for collection of Bacillus spores from nonporous surfaces*. Applied and Environmental Microbiology, 2007. **73**(3): 706-710.
- 5. M.W. Calfee, S.D. Lee, and S.P. Ryan, *A rapid and repeatable method to deposit bioaerosols on material surfaces.* Journal of Microbiological Methods, 2013. **92**(3): 375-380.
- 6. S.D. Lee, S.P. Ryan, and E.G. Snyder, *Development of an aerosol surface inoculation method for Bacillus spores.* Applied and Environmental Microbiology, 2011. **77**(5): 1638-1645.
- M.W. Calfee, S.P. Ryan, J.P. Wood, L. Mickelsen, C. Kempter, L. Miller, M. Colby, A. Touati, M. Clayton, N. Griffin-Gatchalian, S. McDonald and R. Delafield, *Laboratory evaluation of large-scale decontamination approaches*. Journal of Applied Microbiology, 2012. **112**(5): 874-82.

APPENDIX A - PHASE 1: Operational Aspects of Decontamination by Sporicidal Wipes

Test Results for Task 1: Determination of Effective Surface Coverage of Sporicidal Wipes

Wipe	ci		Random	Weight	of Wet	Net	Net Wipe	Net Weight	Average	StDev
Туре	Size	ID	Number Assignment	Initial	Final	Weight Loss (g)	Time (seconds)	Loss (g/ft ²)	Weight Loss (g/ft²)	Weight Loss (g/ft ²)
		WA73-1-V-1-W1-1	7	13.58	11.25	2.33	19.00	2.33		
		WA73-1-V-1-W1-2	24	12.91	10.54	2.37	18.00	2.37	2.39	
	1 x 1	WA73-1-V-1-W1-3	5	13.23	10.88	2.36	19.00	2.36		0.06
		WA73-1-V-1-W1-4	19	12.46	9.99	2.47	17.00	2.47		
		WA73-1-V-1-W1-5	10	12.55	10.13	2.42	18.00	2.42		
		WA73-1-V-1-W2-1	4	12.44	8.98	3.47	25.00	1.54		
ring		WA73-1-V-1-W2-2	12	12.34	8.94	3.40	24.00	1.51	1.50	0.04
Clorox Healthcare® Bleach Germicidal Wipe/Vinyl Flooring	1.5 x 1.5	WA73-1-V-1-W2-3	23	13.04	9.65	3.39	24.00	1.51		
inyl I		WA73-1-V-1-W2-4	11	11.98	8.74	3.24	28.00	1.44		
ipe/V		WA73-1-V-1-W2-5	9	12.57	9.15	3.42	24.00	1.52		
al W		WA73-1-V-1-W3-1	1	12.91	8.89	4.03	34.00	1.01	1.01	0.03
nicid	2 x 2	WA73-1-V-1-W3-2	21	12.28	8.21	4.06	34.00	1.02		
Gerr		WA73-1-V-1-W3-3	22	12.98	8.73	4.24	36.00	1.06		
each		WA73-1-V-1-W3-4	8	12.54	8.62	3.92	33.00	0.98		
B		WA73-1-V-1-W3-5	15	13.11	9.07	4.05	34.00	1.01		
Icare		WA73-1-V-1-W4-1	6	14.34	9.55	4.79	42.00	0.77		0.04
lealt		WA73-1-V-1-W4-2	13	12.55	8.07	4.48	44.00	0.72		
H XO	2.5 x 2.5	WA73-1-V-1-W4-3	14	12.06	7.65	4.42	43.00	0.71	0.75	
Cloi		WA73-1-V-1-W4-4	25	13.56	8.82	4.74	43.00	0.76		
		WA73-1-V-1-W4-5	3	12.66	7.58	5.07	42.00	0.81		
		WA73-1-V-1-W5-1	18	12.59	7.33	5.26	51.00	0.58		
		WA73-1-V-1-W5-2	16	13.05	7.82	5.23	53.00	0.58		
	3 x 3	WA73-1-V-1-W5-3	20	12.53	7.26	5.27	54.00	0.59	0.58	0.01
		WA73-1-V-1-W5-4	2	13.23	7.87	5.36	52.00	0.60		
		WA73-1-V-1-W5-5	17	12.27	7.09	5.18	50.00	0.58		

Table A-1-1-1: Task 1 Results Clorox He	althcare [®] Bleach Germicidal Wipe/Vinyl Flooring

Wipe Type	Size	ID	Random Number Assignment	U	of Wet es (g) Final	Net Weight Loss (a)	Net Wipe Time (seconds)	Net Weight Loss	Average Weight Loss (g/ft ²)	StDev Weight Loss (g/ft ²)
		WA73-1-G-1-W1-1	13	12.17	10.72	1.45	21.00	(a/ft²) 1.45		
		WA73-1-G-1-W1-2	11	12.08	10.61	1.46	20.00	1.46	1.36	0.10
	1 x 1	WA73-1-G-1-W1-3	6	11.96	10.68	1.29	21.00	1.29		
		WA73-1-G-1-W1-4	10	12.43	11.06	1.37	18.00	1.37		
		WA73-1-G-1-W1-5	5	12.89	11.67	1.22	19.00	1.22		
		WA73-1-G-1-W2-1	20	12.33	9.71	2.62	22.00	1.16		
		WA73-1-G-1-W2-2	21	12.63	9.99	2.64	24.00	1.17		0.07
ass	1.5 x 1.5	WA73-1-G-1-W2-3	18	11.49	9.20	2.29	23.00	1.02	1.14	
Clorox Healthcare [®] Bleach Germicidal Wipe/Glass		WA73-1-G-1-W2-4	14	12.56	9.98	2.57	26.00	1.14		
II Wig		WA73-1-G-1-W2-5	3	12.51	9.81	2.70	25.00	1.20		
nicida	2 x 2	WA73-1-G-1-W3-1	15	12.13	8.90	3.22	28.00	0.81	0.83	0.02
Germ		WA73-1-G-1-W3-2	22	12.25	9.02	3.23	26.00	0.81		
each		WA73-1-G-1-W3-3	16	12.57	9.12	3.45	29.00	0.86		
Ble		WA73-1-G-1-W3-4	23	12.22	8.86	3.36	31.00	0.84		
Icare		WA73-1-G-1-W3-5	4	12.10	8.84	3.26	32.00	0.82		
lealth		WA73-1-G-1-W4-1	19	12.36	8.43	3.92	40.00	0.63		0.02
OX H		WA73-1-G-1-W4-2	7	12.30	8.17	4.13	39.00	0.66		
Cloi	2.5 x 2.5	WA73-1-G-1-W4-3	25	12.28	8.25	4.03	37.00	0.64	0.65	
		WA73-1-G-1-W4-4	2	12.50	8.33	4.17	38.00	0.67		
		WA73-1-G-1-W4-5	24	12.42	8.27	4.15	35.00	0.66		
		WA73-1-G-1-W5-1	17	12.19	7.56	4.63	56.00	0.51		
		WA73-1-G-1-W5-2	1	13.09	8.61	4.48	55.00	0.50		
	3 x 3	WA73-1-G-1-W5-3	9	11.82	7.34	4.47	51.00	0.50	0.52	0.03
		WA73-1-G-1-W5-4	8	12.63	7.99	4.64	53.00	0.52		
		WA73-1-G-1-W5-5	17	12.27	7.09	5.18	54.00	0.58		

Table A-1-1-2: Task 1 Results Clorox Healthcare® Bleach Germicidal Wipe/Glass

Wipe Туре	Size	ID	Random Number Assignment	Weight Wipe	of Wet es (g)	Net Weight Loss (g)	Net Wipe Time (seconds)	Net Weight Loss (g/ft²)	Average Weight Loss (g/ft ²)	StDev Weight Loss (g/ft ²)
				Initial	Final	-		(g/it ²)	(9/11-)	
		WA73-1-M-1-W1-1	3	12.10	10.88	1.22	18.00	1.22		
		WA73-1-M-1-W1-2	25	11.92	10.55	1.38	19.00	1.38		
	1 x 1	WA73-1-M-1-W1-3	23	11.46	10.23	1.22	20.00	1.22	1.25	0.07
		WA73-1-M-1-W1-4	21	11.78	10.59	1.20	18.00	1.20		
		WA73-1-M-1-W1-5	7	11.63	10.39	1.24	20.00	1.24		
		WA73-1-M-1-W2-1	6	12.65	9.84	2.81	25.00	1.25		
e		WA73-1-M-1-W2-2	2	12.19	9.37	2.82	27.00	1.26		
amin	1.5 x 1.5	WA73-1-M-1-W2-3	24	11.62	9.04	2.58	24.00	1.15	1.20	0.05
/Mela		WA73-1-M-1-W2-4	15	12.28	9.59	2.68	26.00	1.19		
Wipe		WA73-1-M-1-W2-5	12	12.73	10.11	2.63	27.00	1.17		
idal		WA73-1-M-1-W3-1	9	11.60	8.34	3.26	36.00	0.82		
rmic		WA73-1-M-1-W3-2	4	12.07	8.80	3.27	37.00	0.82	-	
Clorox Healthcare [®] Bleach Germicidal Wipe/Melamine	2 x 2	WA73-1-M-1-W3-3	8	12.12	8.81	3.32	35.00	0.83	0.83	0.02
Bleac		WA73-1-M-1-W3-4	20	13.09	9.84	3.25	35.00	0.81		
re® E		WA73-1-M-1-W3-5	16	12.20	8.73	3.47	34.00	0.87		
Ithca		WA73-1-M-1-W4-1	18	12.82	8.16	4.66	44.00	0.75		
Hea		WA73-1-M-1-W4-2	14	11.25	6.48	4.77	45.00	0.76		
orox	2.5 x 2.5	WA73-1-M-1-W4-3	5	11.88	7.29	4.58	48.00	0.73	0.75	0.01
C		WA73-1-M-1-W4-4	19	13.00	8.33	4.68	51.00	0.75		
		WA73-1-M-1-W4-5	11	11.99	7.36	4.63	48.00	0.74		
	3 x 3	WA73-1-M-1-W5-1	10	12.16	6.98	5.18	56.00	0.58		
		WA73-1-M-1-W5-2	17	12.25	7.23	5.02	56.00	0.56	1	
		WA73-1-M-1-W5-3	22	13.25	8.00	5.26	53.00	0.58	0.57	0.01
		WA73-1-M-1-W5-4	13	12.03	6.89	5.14	52.00	0.57	1	
		WA73-1-M-1-W5-5	17	12.27	7.09	5.18	55.00	0.58	1	

Table A-1-1-3: Task 1 Results Clorox Healthcare® Bleach Germicidal Wipe/Melamine

Wipe Type	Size	ID	Random Number Assignment	Weight Wipe Initial	of Wet es (g) Final	Net Weight Loss (g)	Net Wipe Time (seconds)	Net Weight Loss g/(ft²)	Average Weight Loss (g/ft ²)	StDev Weight Loss (g/ft ²)
		WA73-1-D-1-W1-1	16	12.21	10.44	1.77	27.00	1.77		
		WA73-1-D-1-W1-2	9	11.07	9.36	1.71	25.00	1.71		
	1 x 1	WA73-1-D-1-W1-3	24*	11.48	9.75	1.73	21.00	1.73	1.74	0.02
		WA73-1-D-1-W1-4	2	12.15	10.40	1.75	24.00	1.75		
		WA73-1-D-1-W1-5	17	11.52	9.80	1.72	23.00	1.72		
=		WA73-1-D-1-W2-1	4	11.44	8.37	3.07	34.00	1.37		
rywa		WA73-1-D-1-W2-2	7	12.00	9.01	2.99	33.00	1.33		
ed D	1.5 x 1.5	WA73-1-D-1-W2-3	12	11.48	8.58	2.89	35.00	1.29	1.34	0.04
ainte		WA73-1-D-1-W2-4	21	12.11	9.10	3.01	33.00	1.34		
pe/P		WA73-1-D-1-W2-5	14	12.27	9.13	3.14	34.00	1.39		
Clorox Healthcare [®] Bleach Germicidal Wipe/Painted Drywall		WA73-1-D-1-W3-1	15	12.09	8.11	3.98	41.00	0.99		
icida		WA73-1-D-1-W3-2	13	12.12	8.07	4.05	46.00	1.01		
Germ	2 x 2	WA73-1-D-1-W3-3	1	12.10	8.03	4.07	44.00	1.02	1.00	0.01
ich (WA73-1-D-1-W3-4	22	12.15	8.18	3.97	41.00	0.99		
Blea		WA73-1-D-1-W3-5	19	12.33	8.37	3.96	46.00	0.99		
are®		WA73-1-D-1-W4-1	25*	12.25	7.11	5.14	48.00	0.82		
lthc		WA73-1-D-1-W4-2	6	12.10	6.82	5.29	51.00	0.85		
Hea	2.5 x 2.5	WA73-1-D-1-W4-3	8	12.18	7.08	5.10	54.00	0.82	0.83	0.01
orox		WA73-1-D-1-W4-4	3	11.81	6.68	5.13	52.00	0.82		
C		WA73-1-D-1-W4-5	10	12.21	6.99	5.22	51.00	0.83		
		WA73-1-D-1-W5-1	5	12.22	6.10	6.11	62.00	0.68		
		WA73-1-D-1-W5-2	20	11.50	5.49	6.01	62.00	0.67		
	3 x 3	WA73-1-D-1-W5-3	18	12.21	5.93	6.28	67.00	0.70	0.66	0.05
		WA73-1-D-1-W5-4	23	11.52	5.42	6.11	61.00	0.68		0.00
		WA73-1-D-1-W5-5	17	12.27	7.09	5.18	66.00	0.58		

Table A-1-1-4: Task 1 Results Clorox Healthcare[®] Bleach Germicidal Wipe/Painted Drywall

* 25 completed before 24

Wipe Type	Size	ID	Random Number Assignment	Weight Wipe Initial	of Wet es (g) Final	Net Weight Loss (g)	Net Wipe Time (seconds)	Net Weight Loss g/(ft²)	Average Weight Loss (g/ft ²)	StDev Weight Loss (g/ft ²)
		WA73-1-V-4-W1-1	6	15.02	11.83	3.19	28.00	3.19		
		WA73-1-V-4-W1-2	22	14.07	12.01	2.05	33.00	2.05		
	1 x 1	WA73-1-V-4-W1-3	13	15.20	11.51	3.69	23.00	3.69	3.16	0.66
		WA73-1-V-4-W1-4	20	15.11	11.86	3.25	24.00	3.25		
		WA73-1-V-4-W1-5	12	15.11	11.46	3.64	24.00	3.64		
		WA73-1-V-4-W2-1	14	15.14	10.63	4.51	38.00	2.00		
		WA73-1-V-4-W2-2	23	15.06	10.70	4.36	35.00	1.94		
bu	1.5 x 1.5	WA73-1-V-4-W2-3	8	15.07	10.60	4.46	41.00	1.98	2.01	0.06
loori		WA73-1-V-4-W2-4	2	15.08	10.37	4.71	45.00	2.09		
Hype-Wipe® Bleach Towelette/Vinyl Flooring		WA73-1-V-4-W2-5	4	15.01	10.47	4.54	49.00	2.02		
e/Vir		WA73-1-V-4-W3-1	16	15.09	9.32	5.77	44.00	1.44		
elett		WA73-1-V-4-W3-2	1	15.01	9.46	5.55	53.00	1.39		
Tow	2 x 2	WA73-1-V-4-W3-3	25	15.00	9.55	5.45	47.00	1.36	1.37	0.05
ach		WA73-1-V-4-W3-4	3	14.93	9.73	5.20	55.00	1.30		
Ble		WA73-1-V-4-W3-5	24	15.01	9.51	5.50	50.00	1.37		
Vipe		WA73-1-V-4-W4-1	15	15.13	8.85	6.28	62.00	1.01		
pe-V		WA73-1-V-4-W4-2	11	15.00	8.65	6.35	57.00	1.02		
Н	2.5 x 2.5	WA73-1-V-4-W4-3	18	15.24	8.74	6.50	57.00	1.04	1.01	0.02
		WA73-1-V-4-W4-4	10	15.09	8.76	6.34	57.00	1.01		
		WA73-1-V-4-W4-5	9	15.03	8.84	6.19	58.00	0.99		
		WA73-1-V-4-W5-1	7	15.05	8.85	6.20	75.00	0.69		
		WA73-1-V-4-W5-2	21	15.07	8.39	6.68	72.00	0.74		
	3 x 3	WA73-1-V-4-W5-3	19	15.02	8.03	7.00	76.00	0.78	0.71	0.09
		WA73-1-V-4-W5-4	17	15.08	8.04	7.04	73.00	0.78		
		WA73-1-V-4-W5-5	17	12.27	7.09	5.18	78.00	0.58		

Table A-1-2-1: Task 1 Results Hype-Wipe® Bleach Towelette/Vinyl Flooring

Wipe Type	Size	ID	Random Number Assignment	Weight Wipe Initial		Net Weight Loss (g)	Net Wipe Time (seconds)	Net Weight Loss (g/ft ²)	Average Weight Loss (g/ft ²)	StDev Weight Loss (g/ft ²)
		WA73-1-G-4-W1-1	19	14.97	12.55	2.42	24.00	2.42		
		WA73-1-G-4-W1-2	20	14.99	12.67	2.32	22.00	2.32		
	1 x 1	WA73-1-G-4-W1-3	4	15.38	12.87	2.51	28.00	2.51	2.51	0.17
		WA73-1-G-4-W1-4	16	14.97	12.42	2.55	27.00	2.55		
		WA73-1-G-4-W1-5	1	15.01	12.25	2.76	25.00	2.76		
		WA73-1-G-4-W2-1	7	15.06	11.28	3.78	31.00	1.68		
		WA73-1-G-4-W2-2	21	15.03	11.09	3.94	33.00	1.75		
	1.5 x 1.5	WA73-1-G-4-W2-3	13	15.18	10.98	4.21	34.00	1.87	1.77	0.09
ass		WA73-1-G-4-W2-4	9	15.04	11.20	3.84	32.00	1.71		
te/GI		WA73-1-G-4-W2-5	25	15.25	11.06	4.19	32.00	1.86		
Hype-Wipe® Bleach Towelette/Glass		WA73-1-G-4-W3-1	6	15.28	10.16	5.13	41.00	1.28		
Tow		WA73-1-G-4-W3-2	8	15.15	10.22	4.94	40.00	1.23		
ach	2 x 2	WA73-1-G-4-W3-3	22	14.95	10.08	4.86	38.00	1.22	1.23	0.03
Ble		WA73-1-G-4-W3-4	23	15.24	10.29	4.95	38.00	1.24		
'ipe®		WA73-1-G-4-W3-5	15	14.56	9.76	4.80	39.00	1.20		
N-90		WA73-1-G-4-W4-1	10	15.15	9.72	5.43	49.00	0.87		
Hyp		WA73-1-G-4-W4-2	2	15.00	9.61	5.39	57.00	0.86		
	2.5 x 2.5	WA73-1-G-4-W4-3	3	15.06	9.85	5.22	50.00	0.83	0.85	0.02
		WA73-1-G-4-W4-4	5	15.09	9.73	5.36	52.00	0.86		
		WA73-1-G-4-W4-5	18	15.11	9.98	5.14	49.00	0.82		
		WA73-1-G-4-W5-1	14	14.94	8.95	5.99	66.00	0.67		
		WA73-1-G-4-W5-2	11	15.00	9.47	5.53	63.00	0.61		
	3 x 3	WA73-1-G-4-W5-3	12	15.09	9.31	5.79	64.00	0.64	0.63	0.04
		WA73-1-G-4-W5-4	24	15.16	9.25	5.91	63.00	0.66		
		WA73-1-G-4-W5-5	17	12.27	7.09	5.18	62.00	0.58		

Table A-1-2-2: Task 1 Results Hype-Wipe® Bleach Towelette/Glass

Wipe Type	Size	ID	Random Number Assignment	Weight Wip∈	of Wet es (g)	Net Weight Loss (g)	Net Wipe Time (seconds)	Net Weight Loss	Average Weight Loss	StDev Weight Loss (g/ft²)
			5	Initial	Final			(g/ft²)	(g/ft²)	
		WA73-1-M-4-W1-1	17	15.02	11.55	3.47	24.00	3.47		
		WA73-1-M-4-W1-2	23	14.94	11.57	3.37	27.00	3.37		
	1 x 1	WA73-1-M-4-W1-3	12	14.97	11.51	3.46	31.00	3.46	3.62	0.31
		WA73-1-M-4-W1-4	5	14.99	11.31	3.68	28.00	3.68		
		WA73-1-M-4-W1-5	11	15.00	10.87	4.14	30.00	4.14		
		WA73-1-M-4-W2-1	3	15.01	9.77	5.25	40.00	2.33		
		WA73-1-M-4-W2-2	2	15.09	9.69	5.40	48.00	2.40		
	1.5 x 1.5	WA73-1-M-4-W2-3	10	15.18	10.44	4.74	42.00	2.11	2.29	0.12
mine		WA73-1-M-4-W2-4	4	15.10	9.76	5.34	46.00	2.37	_	
Vela		WA73-1-M-4-W2-5	15	15.22	10.24	4.98	47.00	2.21		
Hype-Wipe [®] Bleach Towelette/Melamine		WA73-1-M-4-W3-1	1	15.07	9.70	5.37	48.00	1.34		
wele		WA73-1-M-4-W3-2	22	14.98	9.52	5.45	62.00	1.36		
h To	2 x 2	WA73-1-M-4-W3-3	9	15.17	9.78	5.39	56.00	1.35	1.37	0.03
leac		WA73-1-M-4-W3-4	19	14.86	9.27	5.59	60.00	1.40		
e® E		WA73-1-M-4-W3-5	20	15.07	9.48	5.58	63.00	1.40		
-Wip		WA73-1-M-4-W4-1	16	15.25	9.06	6.20	63.00	0.99		
lype		WA73-1-M-4-W4-2	24	15.17	9.13	6.04	62.00	0.97		
	2.5 x 2.5	WA73-1-M-4-W4-3	7	15.22	8.67	6.55	66.00	1.05	1.02	0.04
		WA73-1-M-4-W4-4	18	15.06	8.60	6.46	68.00	1.03		
		WA73-1-M-4-W4-5	6	15.13	8.64	6.49	65.00	1.04		
		WA73-1-M-4-W5-1	14	14.95	8.09	6.87	86.00	0.76		
		WA73-1-M-4-W5-2	8	15.13	8.04	7.09	81.00	0.79		
	3 x 3	WA73-1-M-4-W5-3	21	15.08	7.99	7.09	83.00	0.79 0	0.73	0.09
	3 × 3	WA73-1-M-4-W5-4	25	14.97	8.17	6.80	81.00	0.76]	0.07
		WA73-1-M-4-W5-5	17	12.27	7.09	5.18	82.00	0.58		

Table A-1-2-3: Task 1 Results Hype-Wipe® Bleach Towelette/Melamine

Wipe Type	Size	ID	Random Number Assignment		t of Wet es (g) Final	Net Weight Loss (g)	Net Wipe Time (seconds)	Net Weight Loss (g/ft ²)	Average Weight Loss (g/ft ²)	StDev Weight Loss (g/ft ²)
		WA73-1-D-4-W1-1	11	15.03	11.83	3.20	27.00	3.20		
		WA73-1-D-4-W1-2	18	15.01	11.73	3.28	30.00	3.28		
	1 x 1	WA73-1-D-4-W1-3	12	14.93	11.20	3.73	32.00	3.73	3.35	0.33
		WA73-1-D-4-W1-4	2	15.12	12.20	2.92	28.00	2.92		
		WA73-1-D-4-W1-5	15	14.89	11.25	3.64	28.00	3.64		
		WA73-1-D-4-W2-1	21	14.91	10.10	4.81	37.00	2.14		
		WA73-1-D-4-W2-2	20	14.92	9.67	5.25	68.00	2.34		
wall	1.5 x 1.5	WA73-1-D-4-W2-3	25	15.02	10.10	4.92	39.00	2.19	2.20	0.11
Hype-Wipe® Bleach Towelette/Painted Drywall		WA73-1-D-4-W2-4	17	14.79	10.13	4.67	37.00	2.08		
nted		WA73-1-D-4-W2-5	5	15.07	9.93	5.14	42.00	2.28		
e/Pai		WA73-1-D-4-W3-1	16	14.96	9.51	5.45	54.00	1.36		
elette		WA73-1-D-4-W3-2	7	15.04	9.17	5.87	57.00	1.47		
Towe	2 x 2	WA73-1-D-4-W3-3	14	14.88	9.19	5.70	44.00	1.42	1.42	0.04
ach ⁻		WA73-1-D-4-W3-4	24	15.11	9.33	5.78	53.00	1.45		
Blea		WA73-1-D-4-W3-5	6	15.07	9.45	5.62	60.00	1.40		
'ipe®		WA73-1-D-4-W4-1	22	15.17	8.40	6.77	58.00	1.08		
N-əc		WA73-1-D-4-W4-2	10	14.95	8.75	6.20	63.00	0.99		
Hyp	2.5 x 2.5	WA73-1-D-4-W4-3	19	15.00	8.63	6.37	57.00	1.02	1.06	0.05
		WA73-1-D-4-W4-4	3	15.09	8.11	6.99	65.00	1.12		
		WA73-1-D-4-W4-5	9	15.00	8.14	6.86	55.00	1.10) 7 5 6 7 7	
		WA73-1-D-4-W5-1	13	15.11	7.27	7.84	89.00	0.87		
		WA73-1-D-4-W5-2	23	14.99	7.30	7.69	83.00	0.85		
	3 x 3	WA73-1-D-4-W5-3	8	14.92	7.23	7.69	79.00	0.85		0.13
		WA73-1-D-4-W5-4	1	15.04	7.24	7.80	78.00	0.87		
		WA73-1-D-4-W5-5	17	12.27	7.09	5.18	79.00	0.58		

Table A-1-2-4: Task 1 Results Hype-Wipe® Bleach Towelette/Painted Drywall

Wipe Type	Size	ID	Random Number Assignment		of Wet es (g)	Net Weight Loss	Net Wipe Time (seconds)	Net Weight Loss	Average Weight Loss	StDev Weight Loss
			5	Initial	Final	(g)	· · ·	(g/ft ²)	(g/ft²)	(g/ft ²)
		WA73-1-V-2-W1-1	14	11.03	9.94	1.10	17.00	1.10		
		WA73-1-V-2-W1-2	15	11.10	9.92	1.18	17.00	1.18		
	1 x 1	WA73-1-V-2-W1-3	5	10.28	9.15	1.13	20.00	1.13	1.22	0.16
		WA73-1-V-2-W1-4	24	10.85	9.64	1.20	21.00	1.20		
		WA73-1-V-2-W1-5	22	11.01	9.52	1.49	21.00	1.49		
		WA73-1-V-2-W2-1	2	10.59	7.68	2.92	28.00	1.30		
-		WA73-1-V-2-W2-2	13	10.38	7.67	2.71	28.00	1.20		
ooring	1.5 x 1.5	WA73-1-V-2-W2-3	19	10.25	7.31	2.94	29.00	1.31	1.25	0.05
nyl FI		WA73-1-V-2-W2-4	10	10.77	8.08	2.69	26.00	1.20		
ipe/Vi		WA73-1-V-2-W2-5	20	11.20	8.40	2.79	26.00	1.24		
ble W		WA73-1-V-2-W3-1	12	11.10	7.76	3.35	37.00	0.84		
sposal		WA73-1-V-2-W3-2	18	11.30	7.61	3.69	39.00	0.92		
Sani-Cloth [®] Bleach Germicidal Disposable Wipe/Vinyl Flooring	2 x 2	WA73-1-V-2-W3-3	21	11.42	7.90	3.52	33.00	0.88	0.88	0.04
rmicio		WA73-1-V-2-W3-4	6	10.47	7.02	3.45	36.00	0.86		
ih Gei		WA73-1-V-2-W3-5	25	11.08	7.44	3.64	32.00	0.91		
Bleac		WA73-1-V-2-W4-1	17	10.26	5.39	4.87	66.00	0.78		
loth®		WA73-1-V-2-W4-2	7	10.59	5.53	5.06	48.00	0.81		
ani-C	2.5 x 2.5	WA73-1-V-2-W4-3	8	10.22	5.64	4.58	45.00	0.73	0.77	0.03
S		WA73-1-V-2-W4-4	1	10.39	5.70	4.69	45.00	0.75		
		WA73-1-V-2-W4-5	4	10.60	5.61	5.00	43.00	0.80	_	
		WA73-1-V-2-W5-1	9	10.83	5.78	5.05	57.00	0.56	6 7 9 0.57	
		WA73-1-V-2-W5-2	11	10.41	5.25	5.16	52.00	0.57		
	3 x 3	WA73-1-V-2-W5-3	23	10.71	5.44	5.27	56.00	0.59		0.01
		WA73-1-V-2-W5-4	16	10.21	5.20	5.01	56.00	0.56		
		WA73-1-V-2-W5-5	17	12.27	7.09	5.18	66.00	0.58		

Table A-1-3-1: Task 1 Results Sani-Cloth[®] Bleach Germicidal Disposable Wipe/Vinyl Flooring

Wipe Type	Size	ID	Random Number Assignment	Weight Wipe Initial	of Wet es (g) Final	Net Weight Loss (g)	Net Wipe Time (seconds)	Net Weight Loss (g/ft ²)	Average Weight Loss (g/ft ²)	StDev Weight Loss (g/ft ²)
		WA73-1-G-2-W1-1	10	11.12	10.20	0.92	18.00	0.92		
		WA73-1-G-2-W1-1	10	11.06	9.88	1.18	17.00	1.18		
	1 x 1	WA73-1-G-2-W1-2	7	10.64	9.00	0.92	19.00	0.92	0.99	0.11
	1.4.1	WA73-1-G-2-W1-3	8	10.04	9.72	0.92	17.00	0.92	0.77	0.11
		WA73-1-G-2-W1-4	0 19	10.74	9.79	0.95	19.00	0.95		
		WA73-1-G-2-W2-1	15	11.07	9.99	1.07	23.00	0.48		
ass	15 15	WA73-1-G-2-W2-2	23	11.03	9.77	1.26	24.00	0.56	0.51	0.05
e/Gl	1.5 x 1.5	WA73-1-G-2-W2-3	3	10.82	9.81	1.02	23.00	0.45	0.51	0.05
Wip		WA73-1-G-2-W2-4	18	10.81	9.58	1.23	25.00	0.55		
sable		WA73-1-G-2-W2-5	13	10.98	9.79	1.19	25.00	0.53		
sods		WA73-1-G-2-W3-1	2	10.94	8.07	2.86	32.00	0.72		
al Di		WA73-1-G-2-W3-2	9	11.14	8.14	3.00	33.00	0.75		
nicid	2 x 2	WA73-1-G-2-W3-3	16	11.06	8.34	2.71	34.00	0.68	0.72	0.03
Gern		WA73-1-G-2-W3-4	5	11.05	8.08	2.96	33.00	0.74		
ach (WA73-1-G-2-W3-5	24	10.69	7.84	2.86	33.00	0.71		
Sani-Cloth [®] Bleach Germicidal Disposable Wipe/Glass		WA73-1-G-2-W4-1	1	11.13	6.96	4.17	40.00	0.67		
loth		WA73-1-G-2-W4-2	11	11.18	6.85	4.33	39.00	0.69		
ani-C	2.5 x 2.5	WA73-1-G-2-W4-3	22	11.16	6.77	4.40	40.00	0.70	0.69	0.01
Š		WA73-1-G-2-W4-4	21	10.83	6.48	4.35	42.00	0.70		
		WA73-1-G-2-W4-5	25	11.01	6.70	4.31	39.00	0.69		
		WA73-1-G-2-W5-1	12	10.92	6.09	4.82	51.00	0.54		
		WA73-1-G-2-W5-2	14	10.77	5.57	5.20	54.00	0.58		
	3 x 3	WA73-1-G-2-W5-3	20	10.92	5.64	5.28	59.00	0.59	0.57	0.02
		WA73-1-G-2-W5-4	4	11.01	6.06	4.95	55.00	0.55		0.02
		WA73-1-G-2-W5-5	17	12.27	7.09	5.18	55.00	0.58		

Table A-1-3-2: Task 1 Results Sani-Cloth[®] Bleach Germicidal Disposable Wipe/Glass

Wipe Type	Size	ID	Random Number Assignment	Weig Wet V (g	Vipes	Net Weight Loss (g)	Net Wipe Time (seconds)	Net Weight Loss (g/ft²)	Average Weight Loss (g/ft ²)	StDev Weight Loss (g/ft ²)
		WA73-1-M-2-W1-1	22	10.70	9.65	1.05	18.00	1.05		
		WA73-1-M-2-W1-2	2	11.00	9.82	1.18	19.00	1.18		
	1 x 1	WA73-1-M-2-W1-3	10	11.05	9.96	1.09	20.00	1.09	1.13	0.09
		WA73-1-M-2-W1-4	3	10.81	9.75	1.06	19.00	1.06		
		WA73-1-M-2-W1-5	21	10.70	9.44	1.25	19.00	1.25		
		WA73-1-M-2-W2-1	6	11.02	8.85	2.17	29.00	0.97		
(D		WA73-1-M-2-W2-2	11	10.83	8.70	2.13	25.00	0.94		
Sani-Cloth [®] Bleach Germicidal Disposable Wipe/Melamine	1.5 x 1.5	WA73-1-M-2-W2-3	5	10.39	8.39	2.00	30.00	0.89	0.91	0.04
e/Mel		WA73-1-M-2-W2-4	24	10.60	8.66	1.94	27.00	0.86		
Wipe		WA73-1-M-2-W2-5	18	10.96	8.93	2.03	29.00	0.90		
sable		WA73-1-M-2-W3-1	15	10.61	7.31	3.30	30.00	0.82		
Dispo		WA73-1-M-2-W3-2	25	10.58	7.45	3.13	31.00	0.78		
cidal [2 x 2	WA73-1-M-2-W3-3	8	11.06	7.60	3.46	35.00	0.87	0.83	0.03
ermic		WA73-1-M-2-W3-4	20	11.00	7.65	3.34	31.00	0.84		
ich G		WA73-1-M-2-W3-5	9	11.01	7.72	3.29	34.00	0.82		
Blea		WA73-1-M-2-W4-1	23	11.30	7.05	4.25	34.00	0.68		
Cloth		WA73-1-M-2-W4-2	16	10.77	6.50	4.28	45.00	0.68		
ani-C	2.5 x 2.5	WA73-1-M-2-W4-3	17	11.06	6.54	4.52	47.00	0.72	0.70	0.02
0)		WA73-1-M-2-W4-4	14	11.10	6.57	4.53	43.00	0.72		
		WA73-1-M-2-W4-5	1	10.68	6.36	4.32	43.00	0.69		
		WA73-1-M-2-W5-1	7	10.41	5.26	5.16	58.00	0.57		
		WA73-1-M-2-W5-2	13	10.65	5.46	5.18	50.00	0.58		
	3 x 3	WA73-1-M-2-W5-3	19	10.65	5.50	5.14	49.00	0.57	0.58	0.01
		WA73-1-M-2-W5-4	12	10.93	5.61	5.32	51.00	0.59		0.01
		WA73-1-M-2-W5-5	17	12.27	7.09	5.18	52.00	0.58		

Table A-1-3-3: Task 1 Results Sani-Cloth® Bleach Germicidal Disposable Wipe/Melamine

Wipe Type	Size	ID	Random Number Assignment	Weig Wet V (c	Vipes	Net Weight Loss (g)	Net Wipe Time (seconds)	Net Weight Loss	Average Weight Loss (g/ft ²)	StDev Weight Loss (g/ft ²)
				Initial	Final			(g/ft ²)	(y/11²)	
		WA73-1-D-2-W1-1	24	11.02	9.42	1.61	25.00	1.61		
		WA73-1-D-2-W1-2	23	11.27	9.66	1.61	26.00	1.61		
	1 x 1	WA73-1-D-2-W1-3	17	10.84	9.35	1.49	22.00	1.49	1.56	0.05
		WA73-1-D-2-W1-4	2	10.56	9.05	1.52	22.00	1.52		
		WA73-1-D-2-W1-5	4	10.63	9.09	1.55	22.00	1.55		
_		WA73-1-D-2-W2-1	1	10.64	7.92	2.71	32.00	1.21		
rywal		WA73-1-D-2-W2-2	21	10.92	7.86	3.06	32.00	1.36		
ed Di	1.5 x 1.5	WA73-1-D-2-W2-3	18	11.16	8.07	3.10	35.00	1.38	1.31	0.07
Daint		WA73-1-D-2-W2-4	22	11.01	7.99	3.03	34.00	1.34	4	
/ipe/ł		WA73-1-D-2-W2-5	5	10.95	8.09	2.86	35.00	1.27		
Sani-Cloth [®] Bleach Germicidal Disposable Wipe/Painted Drywall		WA73-1-D-2-W3-1	3	10.53	6.27	4.26	42.00	1.06		
posa		WA73-1-D-2-W3-2	14	11.20	7.06	4.14	NA	1.04		
I Dis	2 x 2	WA73-1-D-2-W3-3	20	11.19	6.67	4.52	43.00	1.13	1.09	0.05
icida		WA73-1-D-2-W3-4	6	10.69	6.32	4.36	45.00	1.09		
Germ		WA73-1-D-2-W3-5	11	11.22	6.64	4.58	46.00	1.15		
ach (WA73-1-D-2-W4-1	9	11.16	5.52	5.65	52.00	0.90		
Ble		WA73-1-D-2-W4-2	13	10.90	5.15	5.75	53.00	0.92		
Cloth	2.5 x 2.5	WA73-1-D-2-W4-3	19	11.24	5.36	5.88	86.00	0.94	0.91	0.02
àni-(WA73-1-D-2-W4-4	7	10.94	5.30	5.64	53.00	0.90		
, ,		WA73-1-D-2-W4-5	16	10.78	5.29	5.49	51.00	0.88		
		WA73-1-D-2-W5-1	8	11.10	4.66	6.44	71.00	0.72)	
		WA73-1-D-2-W5-2	10	11.21	4.94	6.27	78.00	0.70		
	3 x 3	WA73-1-D-2-W5-3	15	11.22	4.87	6.35	73.00	0.71		0.06
		WA73-1-D-2-W5-4	12	11.29	4.74	6.56	75.00	0.73		
		WA73-1-D-2-W5-5	17	12.27	7.09	5.18	73.00	0.58		

Table A-1-3-4: Task 1 Results Sani-Cloth[®] Bleach Germicidal Disposable Wipe/Painted Drywall

NA: Not Available

Wipe Туре	Size	ID	Random Number Assignment		of Wet es (g) Final	Net Weight Loss (g)	Net Wipe Time (seconds)	Net Weight Loss (g/ft²)	Average Weight Loss (g/ft ²)	StDev Weight Loss (g/ft²)
		WA73-1-V-3-W1-1	20	13.35	11.39	1.96	24.00	1.96		
		WA73-1-V-3-W1-2	16	13.10	11.27	1.83	21.00	1.83		
	1 x 1	WA73-1-V-3-W1-3	25	12.93	10.86	2.08	18.00	2.08	1.96	0.10
		WA73-1-V-3-W1-4	1	12.47	10.42	2.04	18.00	2.04		
		WA73-1-V-3-W1-5	10	12.98	11.11	1.88	19.00	1.88		
orinç		WA73-1-V-3-W2-1	13	12.82	9.91	2.91	33.00	1.29		
yl Flo		WA73-1-V-3-W2-2	5	12.67	9.63	3.04	29.00	1.35		
Vin	1.5 x 1.5	WA73-1-V-3-W2-3	19	12.87	10.06	2.81	28.00	1.25	1.28	0.05
leach		WA73-1-V-3-W2-4	22	12.99	10.26	2.73	22.00	1.21	_	
ith B		WA73-1-V-3-W2-5	6	12.99	10.04	2.95	25.00	1.31		
els w		WA73-1-V-3-W3-1	4	12.64	8.73	3.92	34.00	0.98		
Tow		WA73-1-V-3-W3-2	11	12.98	9.09	3.89	31.00	0.97		
ctant	2 x 2	WA73-1-V-3-W3-3	7	12.84	8.95	3.89	35.00	0.97	0.97	0.01
sinfe		WA73-1-V-3-W3-4	12	12.95	9.14	3.81	37.00	0.95		
er Dis		WA73-1-V-3-W3-5	23	13.19	9.36	3.84	30.00	0.96		
lean		WA73-1-V-3-W4-1	14	13.56	8.88	4.68	36.00	0.75		
ital C		WA73-1-V-3-W4-2	24	13.00	8.32	4.68	36.00	0.75		
losp	2.5 x 2.5	WA73-1-V-3-W4-3	2	13.09	8.43	4.65	38.00	0.74	0.76	0.02
ch [®]		WA73-1-V-3-W4-4	21	13.18	8.32	4.87	54.00	0.78		
Dispatch $^{\circ}$ Hospital Cleaner Disinfectant Towels with Bleach/Vinyl Flooring		WA73-1-V-3-W4-5	8	13.46	8.51	4.95	50.00	0.79		
		WA73-1-V-3-W5-1	17	13.50	8.56	4.94	56.00	0.55		
		WA73-1-V-3-W5-2	3	13.06	7.99	5.08	57.00	0.56		
	3 x 3	WA73-1-V-3-W5-3	18	12.93	8.04	4.89	54.00	0.54	0.56	0.01
		WA73-1-V-3-W5-4	15	13.37	8.24	5.13	58.00	0.57		
		WA73-1-V-3-W5-5	17	12.27	7.09	5.18	57.00	0.58		

Table A-1-4-1: Task 1 Results Dispatch® Hospital Cleaner Disinfectant Towels with Bleach/Vinyl Flooring

Wipe Type	Size	ID	Random Number Assignment		of Wet es (g)	Net Weight Loss (g)	Net Wipe Time (seconds)	Net Weight Loss (g/ft²)	Average Weight Loss (g/ft ²)	StDev Weight Loss (g/ft ²)
				Initial	Final			(g/it-)	(g/it-)	(g/it-)
		WA73-1-G-3-W1-1	10	10.55	9.69	0.86	21.00	0.86		
		WA73-1-G-3-W1-2	17	11.12	10.27	0.85	20.00	0.85		
	1 x 1	WA73-1-G-3-W1-3	7	10.09	9.15	0.94	21.00	0.94	0.92	0.07
		WA73-1-G-3-W1-4	5	11.01	10.04	0.98	19.00	0.98		
		WA73-1-G-3-W1-5	19	11.64	10.66	0.99	18.00	0.99		
ass		WA73-1-G-3-W2-1	15	10.44	8.43	2.01	24.00	0.89		
th/GI		WA73-1-G-3-W2-2	13	11.68	9.50	2.18	24.00	0.97		
Bleac	1.5 x 1.5	WA73-1-G-3-W2-3	3	10.53	8.62	1.92	26.00	0.85	0.88	0.06
vith I		WA73-1-G-3-W2-4	18	11.76	9.86	1.90	24.00	0.84		
els v		WA73-1-G-3-W2-5	12	10.93	9.06	1.87	27.00	0.83		
Dispatch [®] Hospital Cleaner Disinfectants Towels with Bleach/Glass		WA73-1-G-3-W3-1	23	10.95	8.38	2.56	32.00	0.64		
tants		WA73-1-G-3-W3-2	9	10.59	8.11	2.48	33.00	0.62		
nfect	2 x 2	WA73-1-G-3-W3-3	16	10.86	8.45	2.41	40.00	0.60	0.64	0.03
Disi		WA73-1-G-3-W3-4	4	12.11	9.34	2.77	31.00	0.69		
aner		WA73-1-G-3-W3-5	24	12.25	9.64	2.61	34.00	0.65		
al Cle		WA73-1-G-3-W4-1	1	11.06	7.12	3.94	43.00	0.63		
spita		WA73-1-G-3-W4-2	11	11.91	7.85	4.06	45.00	0.65		
Hc	2.5 x 2.5	WA73-1-G-3-W4-3	22	12.77	8.76	4.00	40.00	0.64	0.64	0.01
batch		WA73-1-G-3-W4-4	21	12.04	8.02	4.02	41.00	0.64		
Dis	рисіл 1	WA73-1-G-3-W4-5	25	13.00	8.91	4.10	42.00	0.66		
		WA73-1-G-3-W5-1	2	11.81	7.65	4.16	60.00	0.46		
		WA73-1-G-3-W5-2	20	11.89	7.67	4.22	58.00	0.47		
	3 x 3	WA73-1-G-3-W5-3	14	10.88	6.79	6.79 4.09 59.00 0.45 0.4 6.36 4.09 59.00 0.45 0.45	59.00	0.45	0.48	0.05
		WA73-1-G-3-W5-4	8	10.44	6.36			5.00		
		WA73-1-G-3-W5-5	17	12.27	7.09	5.18	61.00	0.58		

Table A-1-4-2: Task 1 Results Dispatch® Hospital Cleaner Disinfectants Towels with Bleach/Glass

Wipe Type	Size	ID	Random Number Assignment		of Wet es (g)	Net Weight Loss (g)	Net Wipe Time (seconds)	Net Weight Loss (g/ft²)	Average Weight Loss (g/ft ²)	StDev Weight Loss (g/ft ²)
				Initial	Final			(9/11-)	(9/11-)	
		WA73-1-M-3-W1-1	24	12.51	11.29	1.21	19.00	1.21		
		WA73-1-M-3-W1-2	13	10.55	9.38	1.17	21.00	1.17		
	1 x 1	WA73-1-M-3-W1-3	5	13.94	12.76	1.19	19.00	1.19	1.21	0.04
		WA73-1-M-3-W1-4	25*	13.58	12.39	1.20	20.00	1.20		
		WA73-1-M-3-W1-5	9	14.10	12.82	1.27	23.00	1.27		
ine		WA73-1-M-3-W2-1	4	12.92	10.90	2.02	30.00	0.90		
lelan		WA73-1-M-3-W2-2	10	14.59	12.61	1.98	28.00	0.88		
ach/N	1.5 x 1.5	WA73-1-M-3-W2-3	14	10.90	8.85	2.05	32.00	0.91	0.92	0.03
Blea		WA73-1-M-3-W2-4	8	14.80	12.71	2.09	29.00	0.93		
with		WA73-1-M-3-W2-5	12	14.81	12.64	2.17	28.00	0.96		
Dispatch® Hospital Cleaner Disinfectant Towels with Bleach/Melamine		WA73-1-M-3-W3-1	3	13.90	10.38	3.52	32.00	0.88		
nt Tc		WA73-1-M-3-W3-2	18	12.97	9.28	3.69	35.00	0.92		
fecta	2 x 2	WA73-1-M-3-W3-3	22	13.09	9.20	3.88	59.00	0.97	0.93	0.03
Disin		WA73-1-M-3-W3-4	15	13.25	9.52	3.73	37.00	0.93		
aner		WA73-1-M-3-W3-5	16	13.67	9.99	3.68	34.00	0.92		
l Clea		WA73-1-M-3-W4-1	23	13.54	9.49	4.05	40.00	0.65		
spital		WA73-1-M-3-W4-2	17	12.46	8.33	4.13	39.00	0.66		
[®] Ho:	2.5 x 2.5	WA73-1-M-3-W4-3	11	14.02	9.77	4.26	43.00	0.68	0.67	0.01
atch		WA73-1-M-3-W4-4	1	12.80	8.56	4.24	42.00	0.68		
Disp		WA73-1-M-3-W4-5	21*	12.69	8.46	4.23	44.00	0.68		
		WA73-1-M-3-W5-1	20	13.05	7.52	5.53	56.00	0.61		
		WA73-1-M-3-W5-2	19	13.90	8.21	5.69	54.00	0.63		
	3 x 3	WA73-1-M-3-W5-3	7	14.35	8.53	5.82	54.00	0.65	0.62	0.03
		WA73-1-M-3-W5-4	2	13.29	7.57	5.72	61.00	0.64		
		WA73-1-M-3-W5-5	17	12.27	7.09	5.18	59.00	0.58		

Table A-1-4-3: Task 1 Results Dispatch® Hospital Cleaner Disinfectant Towels with Bleach/Melamine

Wipe Type	Size	ID	Random Number Assignment	Weight Wipe		Net Weight Loss (g)	Net Wipe Time (seconds)	Net Weight Loss	Average Weight Loss	StDev Weight Loss (g/ft ²)
			<u> </u>	Initial	Final			(g/ft ²)	(g/ft²)	
		WA73-1-D-3-W1-1	17	13.90	11.96	1.94	22.00	1.94		
		WA73-1-D-3-W1-2	11	13.90	11.86	2.04	25.00	2.04		
	1 x 1	WA73-1-D-3-W1-3	13	13.56	11.44	2.12	28.00	2.12	2.07	0.09
		WA73-1-D-3-W1-4	2	14.01	11.88	2.13	26.00	2.13		
=		WA73-1-D-3-W1-5	15	14.04	11.90	2.15	25.00	2.15		
Jrywa		WA73-1-D-3-W2-1	22	14.14	10.63	3.51	30.00	1.56		
ted D		WA73-1-D-3-W2-2	8	13.80	10.20	3.61	31.00	1.60		
/Pain	1.5 x 1.5	WA73-1-D-3-W2-3	3	13.92	10.12	3.80	29.00	1.69	1.62	0.05
each		WA73-1-D-3-W2-4	7	14.27	10.69	3.57	31.00	1.59		
th Bi		WA73-1-D-3-W2-5	12	14.09	10.39	3.70	31.00	1.64		
Dispatch® Hospital Cleaner Disinfectant Towels with Bleach/Painted Drywall		WA73-1-D-3-W3-1	19	14.10	10.04	4.06	39.00	1.02		
Towe		WA73-1-D-3-W3-2	25	13.96	9.93	4.03	44.00	1.01		
tant	2 x 2	WA73-1-D-3-W3-3	23	13.87	9.69	4.18	41.00	1.04	1.07	0.07
infec		WA73-1-D-3-W3-4	16	14.07	9.48	4.59	46.00	1.15		
r Disi		WA73-1-D-3-W3-5	5	14.03	9.51	4.52	43.00	1.13		
eane		WA73-1-D-3-W4-1	6	14.11	8.54	5.57	62.00	0.89		
al CI		WA73-1-D-3-W4-2	14	13.75	8.27	5.48	57.00	0.88		
ospi	2.5 x 2.5	WA73-1-D-3-W4-3	9	13.83	8.10	5.73	55.00	0.92	0.89	0.02
h®H		WA73-1-D-3-W4-4	4	13.79	8.21	5.58	59.00	0.89		
spato		WA73-1-D-3-W4-5	24	14.10	8.71	5.39	59.00	0.86		
Di		WA73-1-D-3-W5-1	21	13.80	7.22	6.58	60.00	0.73		
		WA73-1-D-3-W5-2	18	14.21	7.63	6.57	61.00	0.73		
	3 x 3	WA73-1-D-3-W5-3	20	14.37	7.54	6.83	63.00	0.76	0.70	0.07
		WA73-1-D-3-W5-4	10	13.64	7.21	6.43	74.00	0.71	1	
		WA73-1-D-3-W5-5	17	12.27	7.09	5.18	62.00	0.58		

Table A-1-4-4: Task 1 Results Dispatch[®] Hospital Cleaner Disinfectant Towels with Bleach/Painted Drywall

Test Results for Task 2: Surface Retention Times of Wipe Decontaminant Liquid

Wipe	Wait		Weight Wipe		Net	Weight Wipe	t of Dry es (g)	Net Weight	Average Amount of	StDev of the Average
Туре	time (min)	ID	Initial	Final	Weight Loss (g)	Initial	Final	Gain (g)	Liquid Recovered g/ft ²	Liquid Recovered g/ft ²
al		WA73-2-V-1-D0-1	12.241	9.194	3.047	4.581	6.65	2.069		
Germicidal J		WA73-2-V-1-D0-2	12.003	9.438	2.565	4.802	6.566	1.764		
Gern	0	WA73-2-V-1-D0-3	12.304	9.499	2.805	4.762	6.498	1.736	1.88	0.15
Bleach G Flooring		WA73-2-V-1-D0-4	12.019	9.394	2.625	4.592	6.594	2.002		
		WA73-2-V-1-D0-5	12.184	9.123	3.061	4.617	6.455	1.838		
Healthcare® Wipe/Vinyl		WA73-2-V-1-D5-1	11.973	9.223	2.75	4.668	5.899	1.231		
alth Nipe		WA73-2-V-1-D5-2	12.37	8.968	3.402	4.563	6.018	1.455		
× He	5	WA73-2-V-1-D5-3	12.041	9.107	2.934	4.845	5.88	1.035	1.26	0.17
Clorox		WA73-2-V-1-D5-4	11.949	8.846	3.103	4.574	5.749	1.175		
C		WA73-2-V-1-D5-5	12.176	9.275	2.901	4.7	6.104	1.404		

Table A-2-1-1: Task 2 Results Clorox Healthcare® Bleach Germicidal Wipe/Vinyl Flooring

Table A-2-1-2: Task 2 Results Clorox Healthcare® Bleach Germicidal Wipe/Glass

Wipe Type	Wait time (min)	ID		of Wet es (g)	Net Weight Loss (g)		t of Dry es (g)	Net Weight Gain (g)	Average Amount of Liquid Recovered	StDev of the Average Liquid
			Initial	Final		Initial	Final		g/ft ²	Recovered g/ft ²
al		WA73-2-G-1-D0-1	12.817	11.195	1.622	4.576	5.684	1.108		
nicid		WA73-2-G-1-D0-2	11.382	9.398	1.984	4.806	6.123	1.317		
Bleach Germicidal lass	0	WA73-2-G-1-D0-3	11.978	9.957	2.021	4.539	5.774	1.235	1.21	0.08
each s		WA73-2-G-1-D0-4	12.169	10.378	1.791	4.672	5.813	1.141		
		WA73-2-G-1-D0-5	11.54	9.641	1.899	4.769	5.998	1.229		
Healthcare [®] Wipe/G		WA73-2-G-1-D5-1	11.75	10.23	1.52	4.555	4.93	0.375		
alth		WA73-2-G-1-D5-2	11.94	9.9	2.04	4.545	5.061	0.516		
k He	5	WA73-2-G-1-D5-3	12.144	10.626	1.518	4.602	4.979	0.377	0.37	0.09
Clorox		WA73-2-G-1-D5-4	12.234	10.218	2.016	4.658	4.945	0.287		
Ö		WA73-2-G-1-D5-5	11.657	9.602	2.055	4.717	5.009	0.292		

Wipe	Wait			of Wet es (g)	Net		t of Dry es (g)	Net Weight	Average Amount of	StDev of the Average
Туре	time (min)	ID	Initial	Final	Weight Loss (g)	Initial	Final	Gain (g)	Liquid Recovered g/ft ²	Liquid Recovered g/ft ²
dal		WA73-2-M-1-D0-1	11.446	9.553	1.893	4.594	5.811	1.217		
Germicidal		WA73-2-M-1-D0-2	12.851	10.68	2.171	4.582	6.038	1.456		
Gerr	0	WA73-2-M-1-D0-3	12.227	10.465	1.762	4.609	5.942	1.333	1.29	0.12
		WA73-2-M-1-D0-4	12.018	10.014	2.004	4.578	5.896	1.318		
thcare [®] Bleach Wipe/Melamine		WA73-2-M-1-D0-5	11.764	9.753	2.011	4.599	5.747	1.148		
Healthcare [®] Wipe/Me		WA73-2-M-1-D5-1	11.773	9.832	1.941	4.585	5.082	0.497		
Wip		WA73-2-M-1-D5-2	12.259	10.383	1.876	4.574	5.03	0.456		
Неа	5	WA73-2-M-1-D5-3	11.816	9.782	2.034	4.572	4.957	0.385	0.45	0.05
Clorox		WA73-2-M-1-D5-4	12.422	10.633	1.789	4.601	5.115	0.514		
Cic		WA73-2-M-1-D5-5	12.3	10.156	2.144	4.587	5.005	0.418		

Table A-2-1-3: Task 2 Results Clorox Healthcare® Bleach Germicidal Wipe/Melamine

Table A-2-1-4: Task 2 Results Clorox Healthcare® Bleach Germicidal Wipe/Painted Drywall

Wipe	Wait		Weight of V (g		Net		t of Dry es (g)	Net Weight	Average Amount of	StDev of the Average
Туре	time (min)	ID	Initial	Final	Weight Loss (g)	Initial	Final	Gain (g)	Liquid Recovered g/ft ²	Liquid Recovered g/ft ²
dal		WA73-2-D-1-D0-1	12.729	10.198	2.531	4.599	5.958	1.359		
nicio		WA73-2-D-1-D0-2	12.278	10.152	2.126	4.592	5.668	1.076		
Bleach Germicidal ed Drywall	0	WA73-2-D-1-D0-3	11.836	9.808	2.028	4.498	5.637	1.139	1.26	0.17
lealthcare [®] Bleach Ge Wipe/Painted Drywall		WA73-2-D-1-D0-4	12.076	9.686	2.39	4.514	6.013	1.499		
		WA73-2-D-1-D0-5	11.668	9.421	2.247	4.531	5.742	1.211		
Healthcare [®] Wipe/Paint		WA73-2-D-1-D5-1	12.24	10.249	1.991	4.624	4.971	0.347		
lithc pe/F		WA73-2-D-1-D5-2	11.131	9.55	1.581	4.594	4.916	0.322		
Τ.	5	WA73-2-D-1-D5-3	12.212	10.137	2.075	4.612	4.909	0.297	0.33	0.03
Clorox		WA73-2-D-1-D5-4	11.74	9.562	2.178	4.587	4.881	0.294		
CIC		WA73-2-D-1-D5-5	11.964	9.81	2.154	4.579	4.944	0.365		

Wipe	Wait		Weight of V (g		Net		t of Dry es (g)	Net Weight	Average Amount of	StDev of the Average
Туре	time (min)	ID	Initial	Final	Weight Loss (g)	Initial	Final	Gain (g)	Liquid Recovered g/ft ²	Liquid Recovered g/ft ²
١٨		WA73-2-V-4-D0-1	16.952	13.454	3.498	4.688	7.341	2.653		
i/م		WA73-2-V-4-D0-2	17.301	14.118	3.183	4.728	6.95	2.222		
Towelette/Vinyl ng	0	WA73-2-V-4-D0-3	17.113	13.871	3.242	4.721	7.098	2.377	2.32	0.23
owel		WA73-2-V-4-D0-4	17.261	14.165	3.096	4.684	6.976	2.292		
leach To Flooring		WA73-2-V-4-D0-5	17.008	13.433	3.575	4.712	6.748	2.036		
Bleach Floori		WA73-2-V-4-D5-1	17.056	13.721	3.335	4.729	6.847	2.118		
В		WA73-2-V-4-D5-2	17.034	13.913	3.121	4.734	6.945	2.211		
Vipe	5	WA73-2-V-4-D5-3	17.097	13.847	3.25	4.724	6.766	2.042	2.18	0.12
Hype-Wipe®		WA73-2-V-4-D5-4	16.966	13.611	3.355	4.709	6.897	2.188		
Н		WA73-2-V-4-D5-5	17.233	14.084	3.149	4.718	7.073	2.355		

Table A-2-2-1: Task 2 Results Hype-Wipe® Bleach Towelette/Vinyl Flooring

Table A-2-2-2: Task 2 Results Hype-Wipe® Bleach Towelette/Glass

Wipe Type	Wait time (min)	ID	Weight of V (g)		Net Weight Loss (g)	Weigh Wipe	t of Dry es (g)	Net Weight Gain (g)	Average Amount of Liquid Recovered	StDev of the Average Liquid Recovered
			Initial	Final		Initial	Final		g/ft ²	g/ft ²
ass		WA73-2-G-4-D0-1	17.266	13.874	3.392	4.725	6.798	2.073		
15/C		WA73-2-G-4-D0-2	17.233	13.922	3.311	4.742	6.951	2.209		
ette	0	WA73-2-G-4-D0-3	17.354	14.109	3.245	4.684	6.72	2.036	2.22	0.17
Towelette/Glass		WA73-2-G-4-D0-4	17.245	13.492	3.753	4.724	7.05	2.326		
		WA73-2-G-4-D0-5	17.339	13.641	3.698	4.712	7.143	2.431		
Bleach		WA73-2-G-4-D5-1	17.272	13.694	3.578	4.699	6.314	1.615		
8		WA73-2-G-4-D5-2	17.297	13.753	3.544	4.739	6.295	1.556		
Vipe	5	WA73-2-G-4-D5-3	17.324	13.819	3.505	4.676	6.283	1.607	1.63	0.06
Hype-Wipe		WA73-2-G-4-D5-4	17.347	13.685	3.662	4.717	6.359	1.642		
Нуі		WA73-2-G-4-D5-5	17.368	13.73	3.638	4.729	6.45	1.721		

Wipe	Wait time	ID	Weight of W (g)	et Wipes	Net Weight		t of Dry es (g)	Net Weight	Average Amount of Liguid	StDev of the Average
Туре	(min)	טו	Initial	Final	Loss (g)	Initial	Final	Gain (g)	Recovered g/ft ²	Liquid Recovered g/ft ²
ē		WA73-2-M-4-D0-1	17.095	13.889	3.206	4.707	6.458	1.751		
amin		WA73-2-M-4-D0-2	17.117	14.212	2.905	4.677	6.593	1.916		
Bleach Towelette/Melamine	0	WA73-2-M-4-D0-3	16.296	13.541	2.755	4.709	6.275	1.566	1.77	0.16
elette		WA73-2-M-4-D0-4	17.084	13.967	3.117	4.731	6.68	1.949		
Towe		WA73-2-M-4-D0-5	17.173	14.306	2.867	4.714	6.388	1.674		
ach		WA73-2-M-4-D5-1	17.313	14.235	3.078	4.67	5.838	1.168		
		WA73-2-M-4-D5-2	17.449	14.165	3.284	4.708	5.93	1.222		
Vipe	5	WA73-2-M-4-D5-3	17.244	14.44	2.804	4.719	5.785	1.066	1.16	0.06
Hype-Wipe®		WA73-2-M-4-D5-4	17.266	14.372	2.894	4.723	5.881	1.158		
É,		WA73-2-M-4-D5-5	17.195	14.108	3.087	4.707	5.913	1.206		

Table A-2-2-3: Task 2 Results Hype-Wipe® Bleach Towelette/Melamine

Table A-2-2-4: Task 2 Results Hype-Wipe® Bleach Towelette/Painted Drywall

Wipe	Wait time	ID	Weight of V	•	Net Weight	Weight Wipe	t of Dry es (g)	Net Weight	Average Amount of Liquid	StDev of the Average
Туре	(min)	טו	Initial	Final	Loss (g)	Initial	Final	Gain (g)	Recovered g/ft ²	Liquid Recovered g/ft ²
-		WA73-2-D-4-D0-1	17.091	13.441	3.65	4.734	6.933	2.199		
intec		WA73-2-D-4-D0-2	17.186	13.379	3.807	4.736	6.982	2.246		
ie/Pa	0	WA73-2-D-4-D0-3	17.142	13.518	3.624	4.737	7.117	2.38	2.48	0.32
/elett		WA73-2-D-4-D0-4	17.29	13.578	3.712	4.738	7.314	2.576		
each Tow Drywall		WA73-2-D-4-D0-5	17.092	13.166	3.926	4.736	7.716	2.98		
each Dry		WA73-2-D-4-D5-1	17.15	13.866	3.284	4.738	5.256	0.518		
e [®] Bl		WA73-2-D-4-D5-2	17.222	13.672	3.55	4.718	5.551	0.833		
-Wipe	5	WA73-2-D-4-D5-3	17.167	13.41	3.757	4.729	5.242	0.513	0.62	0.18
Hype-Wipe [®] Bleach Towelette/Painted Drywall		WA73-2-D-4-D5-4	17.154	13.371	3.783	4.731	5.163	0.432		
		WA73-2-D-4-D5-5	17.209	13.573	3.636	4.716	5.514	0.798		

Wipe	Wait			Wet Wipes g)	Net	Weight Wipe	t of Dry es (g)	Net Weight	Average Amount of	StDev of the Average
Туре	time (min)	ID	Initial	Final	Weight Loss (g)	Initial	Final	Gain (g)	Liquid Recovered g/ft ²	Liquid Recovered g/ft ²
ble		WA73-2-V-2-D0-1	13.543	11.385	2.158	4.698	6.236	1.538		
osal		WA73-2-V-2-D0-2	13.339	11.694	1.645	4.727	5.991	1.264		
Disp	0	WA73-2-V-2-D0-3	13.468	11.758	1.71	4.699	6.228	1.529	1.44	0.11
rmicidal I Flooring		WA73-2-V-2-D0-4	14.214	12.336	1.878	4.634	6.074	1.44		
ermi I Flo		WA73-2-V-2-D0-5	12.939	11.053	1.886	4.725	6.159	1.434		
Bleach Ge Wipe/Vinyl		WA73-2-V-2-D5-1	13.625	11.89	1.735	4.755	5.221	0.466		
Blea Vipe/		WA73-2-V-2-D5-2	14.465	12.349	2.116	4.688	5.304	0.616		
oth®	5	WA73-2-V-2-D5-3	12.967	11.264	1.703	4.629	5.217	0.588	0.52	0.08
Sani-Cloth [®] Bleach Germicidal Disposable Wipe/Vinyl Flooring		WA73-2-V-2-D5-4	14.139	12.259	1.88	4.704	5.123	0.419		
Saı		WA73-2-V-2-D5-5	13.623	11.722	1.901	4.741	5.235	0.494		

Table A-2-3-1: Task 2 Results Sani-Cloth[®] Bleach Germicidal Disposable Wipe/Vinyl Flooring

 Table A-2-3-2: Task 2 Results Sani-Cloth[®] Bleach Germicidal Disposable Wipe/Glass

Wipe	Wait time	ID	3	Wet Wipes g)	Net Weight		t of Dry bes	Net Weight	Average Amount of Liquid	StDev of the Average Liquid
Туре	(min)		Initial	Final	Loss	Initial	Final	Gain	Recovered g/ft ²	Liquid Recovered g/ft ²
ble		WA73-2-G-2-D0-1	13.267	12.065	1.202	4.63	4.981	0.351		
oo sa		WA73-2-G-2-D0-2	13.536	12.274	1.262	4.637	5.084	0.447		
Disp	0	WA73-2-G-2-D0-3	13.262	11.952	1.31	4.702	5.045	0.343	0.39	0.06
Bleach Germicidal Disposable Wipe/Glass		WA73-2-G-2-D0-4	14.075	12.708	1.367	4.708	5.166	0.458		
ach Germic Wipe/Glass		WA73-2-G-2-D0-5	13.434	12.226	1.208	4.691	5.046	0.355		
ch G /ipe/		WA73-2-G-2-D5-1	14.05	12.85	1.2	4.665	4.704	0.039		
Blea		WA73-2-G-2-D5-2	13.66	12.49	1.17	4.635	4.68	0.045		
oth®	5	WA73-2-G-2-D5-3	13.117	11.89	1.227	4.687	4.722	0.035	0.04	0.01
Sani-Cloth®		WA73-2-G-2-D5-4	13.621	12.437	1.184	4.805	4.839	0.034		
Sar		WA73-2-G-2-D5-5	13.846	12.614	1.232	6.753	6.78	0.027		

Wipe	Wait			Wet Wipes g)	Net		t of Dry pes	Net	Average Amount of	StDev of the Average Liquid Recovered g/ft ²
Туре	time (min)	ID	Initial	Final	Weight Loss	Initial	Final	Weight Gain	Liquid Recovered g/ft ²	
ble		WA73-2-M-2-D0-1	13.076	11.806	1.27	4.605	5.204	0.599		
osal		WA73-2-M-2-D0-2	13.085	11.796	1.289	4.652	5.426	0.774		
Bleach Germicidal Disposable Wipe/Melamine	0	WA73-2-M-2-D0-3	13.124	11.873	1.251	4.692	5.217	0.525	0.62	0.10
cidal ine		WA73-2-M-2-D0-4	13.113	11.865	1.248	4.712	5.279	0.567	_	
leach Germicid. Wipe/Melamine		WA73-2-M-2-D0-5	13.261	11.745	1.516	4.581	5.234	0.653		
ch G be/M		WA73-2-M-2-D5-1	13.065	11.805	1.26	4.745	4.79	0.045		
Blea		WA73-2-M-2-D5-2	13.367	12.156	1.211	4.551	4.637	0.086		
oth®	5	WA73-2-M-2-D5-3	12.966	11.519	1.447	4.639	4.724	0.085	0.07	0.02
Sani-Cloth®		WA73-2-M-2-D5-4	13.079	11.664	1.415	4.65	4.728	0.078		
Sai		WA73-2-M-2-D5-5	13.002	11.709	1.293	4.617	4.671	0.054		

Table A-2-3-3: Task 2 Results Sani-Cloth® Bleach Germicidal Disposable Wipe/Melamine

Table A-2-3-4: Task 2 Results Sani-Cloth® Bleach Germicidal Disposable Wipe/Painted Drywall

Wipe	Wait		0	Wet Wipes (g)	Net		t of Dry pes	Net	Average Amount of	StDev of the Average
Туре	time (min)	ID	Initial	Final	Weight Loss	Initial	Final	Weight Gain	Liquid Recovered g/ft ²	Liquid Recovered g/ft ²
ble		WA73-2-D-2-D0-1	13.517	11.965	1.552	4.61	5.523	0.913		
oosa		WA73-2-D-2-D0-2	13.194	11.857	1.337	4.588	5.353	0.765		
Disp	0	WA73-2-D-2-D0-3	12.892	11.591	1.301	4.616	5.607	0.991	0.86	0.09
nicidal D Drywall		WA73-2-D-2-D0-4	12.769	11.493	1.276	4.637	5.486	0.849	_	
ermi ed D		WA73-2-D-2-D0-5	13.427	11.836	1.591	4.663	5.469	0.806		
[®] Bleach Gern Wipe/Painted		WA73-2-D-2-D5-1	13.66	12.179	1.481	4.622	4.633	0.011		
Blea /ipe/I		WA73-2-D-2-D5-2	13.139	11.81	1.329	4.598	4.605	0.007		
oth® V	5	WA73-2-D-2-D5-3	13.718	12.184	1.534	4.551	4.563	0.012	0.01	0.00
Sani-Cloth [®] Bleach Germicidal Disposable Wipe/Painted Drywall		WA73-2-D-2-D5-4	13.32	11.819	1.501	4.614	4.622	0.008		
Sal		WA73-2-D-2-D5-5	13.336	11.793	1.543	4.582	4.594	0.012		

Wipe	Wait time	ID	Weight of Wet Wipes (g)		Net Weight	Weight Wipe	t of Dry es (g)	Net Weight	Average Amount of Liguid	StDev of the Average
Туре	(min)		Initial	Final	Loss (g)	Initial	Final	Gain (g)	Recovered g/ft ²	Liquid Recovered g/ft ²
ts		WA73-2-V-3-D0-1	13.909	11.911	1.998	4.567	6.084	1.517		
Hospital Cleaner Disinfectants with Bleach/Vinyl Flooring	0	WA73-2-V-3-D0-2	13.447	11.478	1.969	4.548	5.934	1.386	1.48	0.12
sinfe -loor		WA73-2-V-3-D0-3	12.172	10.542	1.63	4.57	5.928	1.358		
er Di inyl F		WA73-2-V-3-D0-4	12.915	11.211	1.704	4.607	6.099	1.492		
ch/Vi		WA73-2-V-3-D0-5	13.244	11.408	1.836	4.523	6.188	1.665		
ital C Blea		WA73-2-V-3-D5-1	12.301	10.496	1.805	4.54	5.679	1.139		
losp with		WA73-2-V-3-D5-2	12.713	11.063	1.65	4.591	5.682	1.091		
	5	WA73-2-V-3-D5-3	13.117	11.135	1.982	4.581	5.547	0.966	1.04	0.08
Dispatch [®] Towels		WA73-2-V-3-D5-4	13.093	11.102	1.991	4.568	5.513	0.945		
Di		WA73-2-V-3-D5-5	12.924	11.05	1.874	4.567	5.615	1.048		

Table A-2-4-1: Task 2 Results Dispatch® Hospital Cleaner Disinfectants Towels with Bleach/Vinyl Flooring

Table A-2-4-2: Task 2 Results Dispatch® Hospital Cleaner Disinfectant Towels with Bleach/Glass

Wipe Type	Wait time (min)	ID	Weight of Wet Wipes (g)		Net Weight Loss	Weight Wipe		Net Weight Gain (g)	Average Amount of Liquid Recovered	StDev of the Average Liquid
	()		Initial	Final	(g)	Initial	Final	Guill (g)	g/ft ²	Recovered g/ft ²
t		WA73-2-G-3-D0-1	12.791	11.935	0.856	4.566	4.903	0.337		
ectai		WA73-2-G-3-D0-2	12.225	11.382	0.843	4.573	4.897	0.324		
isinf ass	0	WA73-2-G-3-D0-3	12.481	11.553	0.928	4.587	4.935	0.348	0.35	0.02
her D ch/GI		WA73-2-G-3-D0-4	12.771	11.884	0.887	4.493	4.861	0.368		
Cleaner Disinfectant Bleach/Glass		WA73-2-G-3-D0-5	13.046	12.082	0.964	4.601	4.982	0.381		
		WA73-2-G-3-D5-1	12.939	11.876	1.063	4.512	4.694	0.182		
:h® Hospital Towels with		WA73-2-G-3-D5-2	13.008	12.086	0.922	4.497	4.759	0.262		
ch [®] F Tow	5	WA73-2-G-3-D5-3	12.443	11.364	1.079	4.571	4.774	0.203	0.22	0.03
Dispatch [®] Hospital Towels with		WA73-2-G-3-D5-4	12.3	11.491	0.809	4.562	4.803	0.241		
D		WA73-2-G-3-D5-5	12.541	11.519	1.022	4.505	4.707	0.202		

Wipe	Wait			f Wet Wipes (g)	Net Weight	Weight Wipes		Net	Average Amount of	StDev of the Average
Туре	time (min)	ID	Initial	Final	Loss (g)	Initial	Final	Weight Gain (g)	Liquid Recovered g/ft ²	Liquid Recovered g/ft ²
Ŧ		WA73-2-M-3-D0-1	13.678	11.775	1.903	4.832	5.763	0.931		
ectar	0	WA73-2-M-3-D0-2	13.012	10.936	2.076	4.764	5.686	0.922	1.02	
tch [®] Hospital Cleaner Disinfe Towels with Bleach/Melamine		WA73-2-M-3-D0-3	12.765	10.866	1.899	4.798	5.822	1.024		0.09
her D /Melå		WA73-2-M-3-D0-4	13.101	11.428	1.673	4.589	5.711	1.122		
Clear each		WA73-2-M-3-D0-5	12.627	11.099	1.528	4.825	5.934	1.109		
ital (th Blo		WA73-2-M-3-D5-1	12.953	11.143	1.81	4.803	5.314	0.511		
Hosp Is wit		WA73-2-M-3-D5-2	13.041	11.456	1.585	4.73	5.416	0.686		
ch® I owel	5	WA73-2-M-3-D5-3	13.42	11.367	2.053	4.686	5.365	0.679	0.61	0.07
Dispatch® Hospital Cleaner Disinfectant Towels with Bleach/Melamine		WA73-2-M-3-D5-4	13.228	11.551	1.677	4.712	5.31	0.598		
ā		WA73-2-M-3-D5-5	13.416	11.892	1.524	4.809	5.401	0.592		

Table A-2-4-3: Task 2 Results Dispatch® Hospital Cleaner Disinfectant Towels with Bleach/Melamine

Table A-2-4-4: Task 2 Results Dispatch[®] Hospital Cleaner Disinfectant Towels with Bleach/Painted Drywall

Wipe	Wait time	ID	Weight of	Wet Wipes (g)	Net Weight		t of Wet es (g)	Net	Average Amount of	StDev of the Average Liquid Recovered g/ft ²
Туре	(min)		Initial	Final	Loss (g)	Initial	Final	Weight Gain (g)	Liquid Recovered g/ft ²	
Ŧ		WA73-2-D-3-D0-1	12.719	10.93	1.789	4.581	5.427	0.846		
Disinfectant ed Drywall		WA73-2-D-3-D0-2	13.156	11.473	1.683	4.535	5.313	0.778		
Hospital Cleaner Disinfecta with Bleach/Painted Drywall	0	WA73-2-D-3-D0-3	12.942	11.394	1.548	4.618	5.647	1.029	0.86	0.14
		WA73-2-D-3-D0-4	12.222	10.81	1.412	4.573	5.534	0.961		
Clear ch/Pa		WA73-2-D-3-D0-5	13.721	12.132	1.589	4.564	5.258	0.694		
oital (3leac		WA73-2-D-3-D5-1	12.554	10.856	1.698	4.58	4.869	0.289		
Hosp vith E		WA73-2-D-3-D5-2	13.209	11.692	1.517	4.594	4.937	0.343		
ch [®] l els v	5	WA73-2-D-3-D5-3	12.678	11.127	1.551	4.631	4.852	0.221	0.30	0.05
Dispatch [®] Hospital Cleaner Towels with Bleach/Paint		WA73-2-D-3-D5-4	12.511	11.025	1.486	4.609	4.928	0.319		
Ω		WA73-2-D-3-D5-5	12.398	10.599	1.799	4.57	4.902	0.332		

Test Results for Task 3: Impact of Wiping Pressure

Wipe	Pressure Applied	ID	Weight of W	'et Wipes (g)	Net Weight	Average Weight Loss	StDev Weight Loss	
Туре	Applied		Initial	Final	– Loss (g/ft ²)	(g/ft ²)	(g/ft ²)	
al	Slight	WA73-3-V-1-P1-1	13.637	12.052	1.585			
nicida		WA73-3-V-1-P1-2	12.481	10.815	1.666			
Bleach Germicidal Flooring		WA73-3-V-1-P1-3	13.132	11.447	1.685	1.69	0.07	
Bleach G Flooring	110000010	WA73-3-V-1-P1-4	12.598	10.835	1.763			
		WA73-3-V-1-P1-5	13.076	11.342	1.734			
ealthcare® Wipe/Vinyl		WA73-3-V-1-P3-1	12.142	8.453	3.689			
althc Vipe/		WA73-3-V-1-P3-2	12.524	8.396	4.128			
k He	Higher Pressure	WA73-3-V-1-P3-3	12.357	8.418	3.939	3.90	0.31	
Clorox Healthcare® Wipe/Vinyl		WA73-3-V-1-P3-4	12.817	8.839	3.978			
Ö		WA73-3-V-1-P3-5	12.886	9.134	3.752			

Table A-3-1-1: Task 3 Results Clorox Healthcare® Bleach Germicidal Wipe/Vinyl Flooring

Table A-3-1-2: Task 3 Results Clorox Healthcare® Bleach Germicidal Wipe/Glass

Wipe Type	Pressure Applied	ID		Weight of Wet Wipes (g)		Average Weight Loss	StDev Weight Loss	
туре	Applieu		Initial	Final	Loss (g/ft ²)	(g/ft ²)	(g/ft²)	
dal		WA73-3-G-1-P1-1	13.253	12.168	1.085			
nicio		WA73-3-G-1-P1-2	11.702	10.404	1.298			
Germicidal		WA73-3-G-1-P1-3	12.806	11.655	1.151	1.20	0.08	
Ч.		WA73-3-G-1-P1-4	11.519	10.312	1.207			
care [®] Bleac Wipe/Glass		WA73-3-G-1-P1-5	13.087	11.849	1.238			
are [®] ipe/(WA73-3-G-1-P3-1	12.355	8.421	3.934			
lthc: V		WA73-3-G-1-P3-2	12.726	8.634	4.092			
Hea	Higher Pressure	WA73-3-G-1-P3-3	12.821	8.835	3.986	4.01	0.32	
Clorox Healthcare [®] Wipe/	Tressure	WA73-3-G-1-P3-4	13.015	9.156	3.859			
Clo		WA73-3-G-1-P3-5	12.624	8.439	4.185			

Wipe	Pressure Applied	ID	Weight of V		Net Weight Loss (g/ft ²)	Average Weight Loss	StDev Weight Loss	
Туре	Applieu		Initial	Final	LUSS (g/II ⁻)	(g/ft²)	(g/ft²)	
lal		WA73-3-M-1-P1-1	11.91	10.063	1.847			
Germicidal		WA73-3-M-1-P1-2	12.8	10.892	1.908			
Gern	Slight Pressure	WA73-3-M-1-P1-3	12.939	10.857	2.082	1.95	0.11	
		WA73-3-M-1-P1-4	12.145	10.096	2.049			
Blea		WA73-3-M-1-P1-5	12.873	10.991	1.882			
thcare [®] Bleach Wipe/Melamine		WA73-3-M-1-P3-1	12.927	8.395	4.532			
Wip		WA73-3-M-1-P3-2	13.166	8.865	4.301			
Неа	Higher Pressure	WA73-3-M-1-P3-3	13.197	8.784	4.413	4.43	0.35	
Clorox Healthcare® Wipe/M		WA73-3-M-1-P3-4	12.628	8.298	4.33			
C	Clo	WA73-3-M-1-P3-5	12.835	8.265	4.57			

Table A-3-1-3: Task 3 Results Clorox Healthcare® Bleach Germicidal Wipe/Melamine

Table A-3-1-4: Task 3 Results Clorox Healthcare® Bleach Germicidal Wipe/Painted Drywall

Wipe	Pressure	ID	Weight of (Wet Wipes g)	Net Weight	Average Weight Loss	StDev Weight Loss	
Туре	Applied		Initial	Final	Loss (g/ft ²)	(g/ft ²)	(g/ft ²)	
lal		WA73-3-D-1-P1-1	12.442	10.826	1.616			
nicio		WA73-3-D-1-P1-2	12.265	10.740	1.525			
Germicidal 'all	Slight Pressure	WA73-3-D-1-P1-3	11.874	10.214	1.66	1.63	0.07	
Bleach Ge ed Drywall	11000010	WA73-3-D-1-P1-4	13.246	11.519	1.727			
		WA73-3-D-1-P1-5	12.718	11.085	1.633		l	
ealthcare [®] Bli Wipe/Painted		WA73-3-D-1-P3-1	13.022	9.396	3.626			
Ithc: pe/P		WA73-3-D-1-P3-2	13.103	9.163	3.940			
Hea Wij	Higher Pressure	WA73-3-D-1-P3-3	11.824	8.078	3.746	3.87	0.31	
Clorox Healthcare® Wipe/Paint	11035010	WA73-3-D-1-P3-4	13.109	9.113	3.996			
Cle		WA73-3-D-1-P3-5	13.005	8.951	4.054			

Wipe	Pressure	in a	Weight of (Wet Wipes g)	Net Weight	Average	StDev	
Туре	Applied	ID	Initial	Final	Loss (g/ft ²)	Weight Loss (g/ft ²)	Weight Loss (g/ft ²)	
ring		WA73-3-V-4-P1-1	17.302	13.902	3.4			
Floo	Bleach Towelette/Vinyl Flooring Pressure	WA73-3-V-4-P1-2	17.215	13.722	3.493			
inyl		WA73-3-V-4-P1-3	17.327	13.923	3.404	3.49	0.10	
tte/V		WA73-3-V-4-P1-4	17.376	13.735	3.641			
wele		WA73-3-V-4-P1-5	17.297	13.807	3.49			
:h To		WA73-3-V-4-P3-1	17.451	11.297	6.154			
3leac		WA73-3-V-4-P3-2	17.281	11.458	5.823			
pe® E	Higher ad Pressure ad Pressure	WA73-3-V-4-P3-3	17.466	11.324	6.142	6.03	0.35	
e-Wi		WA73-3-V-4-P3-4	17.364	11.369	5.995			
Hyp		WA73-3-V-4-P3-5	17.292	11.277	6.015			

Table A-3-2-1: Task 3 Results Hype-Wipe® Bleach Towelette/Vinyl Flooring

Table A-3-2-2: Task 3 Results Hype-Wipe® Bleach Towelette/Glass

Wipe Pressure Type Applied		ID	Weight of Wet Wipes (g)		Net Weight Loss (g/ft²)	Average Weight Loss	StDev Weight Loss
Type Applied		Initial	Final	2033 (g/it)	(g/ft²)	(g/ft ²)	
		WA73-3-G-4-P1-1	17.388	13.834	3.554		
lass	Bleach Towelette/Class Pressure	WA73-3-G-4-P1-2	17.297	13.805	3.492		
tte/G		WA73-3-G-4-P1-3	17.402	13.896	3.506	3.52	0.02
welet	11000010	WA73-3-G-4-P1-4	17.258	13.743	3.515		
h To		WA73-3-G-4-P1-5	17.373	13.838	3.535		
leac		WA73-3-G-4-P3-1	17.275	11.987	5.288		
		WA73-3-G-4-P3-2	17.354	11.862	5.492		
-Wip	®a diM a diM a diA H	WA73-3-G-4-P3-3	17.369	11.917	5.452	5.39	0.31
Hype		WA73-3-G-4-P3-4	17.28	11.879	5.401		
		WA73-3-G-4-P3-5	17.304	12.012	5.292		

Wipe	Pressure	ID	Weight of (Wet Wipes g)	Net Weight	Average Weight Loss	StDev Weight Loss
Туре	Applied		Initial	Final	Loss (g/ft ²)	(g/ft ²)	(g/ft ²)
e		WA73-3-M-4-P1-1	17.468	13.898	3.57		
Bleach Towelette/Melamine	0.11.1	WA73-3-M-4-P1-2	17.369	13.925	3.444	3.53	0.10
e/Mel	Slight Pressure	WA73-3-M-4-P1-3	17.242	13.674	3.568		
elette		WA73-3-M-4-P1-4	17.227	13.824	3.403		
Towe		WA73-3-M-4-P1-5	17.368	13.712	3.656		
ach		WA73-3-M-4-P3-1	17.312	11.36	5.952	5.79	0.33
		WA73-3-M-4-P3-2	17.359	11.565	5.794		
Wipe	Higher Pressure	WA73-3-M-4-P3-3	17.111	11.485	5.626		
Hype-Wipe®		WA73-3-M-4-P3-4	17.573	11.563	6.01		
Ť		WA73-3-M-4-P3-5	17.286	11.714	5.572		

Table A-3-2-3: Task 3 Results Hype-Wipe® Bleach Towelette/Melamine

Table A-3-2-4: Task 3 Results Hype-Wipe® Bleach Towelette/Painted Drywall

Wipe		ID	Weight of We	t Wipes (g)	Net Weight	Average Weight Loss	StDev Weight Loss (g/ft ²)
Туре	Applied	עו	Initial	Final	Loss (g/ft ²)	(g/ft ²)	
wall		WA73-3-D-4-P1-1	17.295	13.928	3.367		
d Dry	0.11	WA73-3-D-4-P1-2	17.266	13.892	3.374	3.48	
ainte	Slight Pressure	WA73-3-D-4-P1-3	17.487	13.98	3.507		0.11
te/Pa		WA73-3-D-4-P1-4	17.346	13.758	3.588		
velet		WA73-3-D-4-P1-5	17.41	13.833	3.577		
Bleach Towelette/Painted Drywall		WA73-3-D-4-P3-1	17.381	11.603	5.778	5.69	
leac		WA73-3-D-4-P3-2	17.392	11.622	5.770		0.33
oe® B	Higher Pressure	WA73-3-D-4-P3-3	17.524	12.05	5.474		
Hype-Wipe [®]		WA73-3-D-4-P3-4	17.317	11.489	5.828		
Hype		WA73-3-D-4-P3-5	17.561	11.984	5.577		

Wipe	Pressure	ID	Weight of We	Weight of Wet Wipes (g)		Average Weight Loss	StDev Weight Loss
Туре	Applied	Initial Final Loss (g)	(g/ft ²)	(g/ft ²)			
ble		WA73-3-V-2-P1-1	13.255	12.111	1.144		
Disposable		WA73-3-V-2-P1-2	12.916	11.852	1.064	1.09	
Disp	Slight Pressure	WA73-3-V-2-P1-3	13.01	11.908	1.102		0.04
rmicidal I Flooring		WA73-3-V-2-P1-4	13.144	12.113	1.031		
		WA73-3-V-2-P1-5	12.83	11.743	1.087		
Bleach Gei Wipe/Vinyl		WA73-3-V-2-P3-1	12.876	9.497	3.379	3.52	
Blea Nipe,		WA73-3-V-2-P3-2	12.943	9.332	3.611		
oth® \	Higher Pressure	WA73-3-V-2-P3-3	13.086	9.437	3.649		0.27
Sani-Cloth [®] Bleach Wipe/Vi		WA73-3-V-2-P3-4	12.715	9.27	3.445		
Sa		WA73-3-V-2-P3-5	12.833	9.318	3.515		

Table A-3-3-1: Task 3 Results Sani-Cloth[®] Bleach Germicidal Disposable Wipe/Vinyl Flooring

Table A-3-3-2: Task 3 Results Sani-Cloth® Bleach Germicidal Disposable Wipe/Glass

Wipe	Pressure	ID	Weight of We	et Wipes (g)	Net Weight	Average StDev Weight Loss (g/ft ²) (g/ft ²)	StDev Weight Loss
Туре	Applied		Initial	Final	– Loss (g)		(g/ft ²)
ble		WA73-3-G-2-P1-1	13.941	12.789	1.152		0.08
Disposable		WA73-3-G-2-P1-2	13.697	12.564	1.133	1.19	
Disp	Slight Pressure	WA73-3-G-2-P1-3	13.258	11.951	1.307		
cidal S		WA73-3-G-2-P1-4	14.076	12.853	1.223		
ach Germicidal Wipe/Glass		WA73-3-G-2-P1-5	13.359	12.245	1.114		
		WA73-3-G-2-P3-1	14.047	10.287	3.76		0.26
Bleach Wip		WA73-3-G-2-P3-2	13.603	10.182	3.421	3.65	
	Higher Pressure	WA73-3-G-2-P3-3	14.401	10.799	3.602		
Sani-Cloth [®]		WA73-3-G-2-P3-4	13.693	10.02	3.673		
Sar		WA73-3-G-2-P3-5	13.931	10.149	3.782		

Wipe	Pressure	D	Weight of We	Weight of Wet Wipes (g)		Average Weight Loss	StDev Weight Loss
Туре	Applied	ID	Initial	Final	Loss (g)	Weight Loss (g/ft ²)	Weight Loss (g/ft ²)
ele		WA73-3-M-2-P1-1	13.671	12.622	1.049		
Disposable		WA73-3-M-2-P1-2	13.789	12.887	0.902		
	Slight Pressure	WA73-3-M-2-P1-3	13.562	12.59	0.972	0.98	0.08
cidal ine		WA73-3-M-2-P1-4	14.118	13.217	0.901		
Germicidal Melamine		WA73-3-M-2-P1-5	14.085	13.011	1.074		
		WA73-3-M-2-P3-1	14.338	10.911	3.427	3.57	
8		WA73-3-M-2-P3-2	14.234	10.533	3.701		
loth®	Higher Pressure	WA73-3-M-2-P3-3	14.067	10.297	3.77		0.25
Sani-Cloth®		WA73-3-M-2-P3-4	13.934	10.481	3.453		
Ś		WA73-3-M-2-P3-5	14.129	10.608	3.521		

Table A-3-3-3: Task 3 Results Sani-Cloth® Bleach Germicidal Disposable Wipe/Melamine

Table A-3-3-4: Task 3 Results Sani-Cloth[®] Bleach Germicidal Disposable Wipe/Painted Drywall

Wipe Pressur	Pressure	ID	Weight of Wet	Wipes (g)	Net Weight Loss	Average Weight Loss (g/ft ²)	StDev Weight Loss (g/ft ²)
Туре	Applied	עו	Initial	Final	(g)		
e		WA73-3-D-2-P1-1	13.215	11.886	1.329		
Germicidal Disposable nted Drywall		WA73-3-D-2-P1-2	13.806	12.359	1.447		
Dispo	Slight Pressure	WA73-3-D-2-P1-3	14.107	12.544	1.563	1.46	0.09
nicidal D Drywall		WA73-3-D-2-P1-4	13.582	12.067	1.515		
ermic ed D		WA73-3-D-2-P1-5	13.327	11.869	1.458		
<u> </u>		WA73-3-D-2-P3-1	14.05	10.764	3.286		
' Blea Vipe/		WA73-3-D-2-P3-2	13.569	10.231	3.338		
loth [®] V	Higher Pressure	WA73-3-D-2-P3-3	13.181	9.62	3.561	3.38	0.25
Sani-Cloth [®] Bleach Wipe/Pa	11000010	WA73-3-D-2-P3-4	13.538	10.397	3.141		
S		WA73-3-D-2-P3-5	13.296	9.713	3.583		

Wipe		ID	Weight of Wet	Wipes (g)	Net Weight Loss	Average Weight Loss (g/ft2)	StDev Weight Loss (g/ft2)
Туре	Applied	עו	Initial	Final	(g)		
wels		WA73-3-V-3-P1-1	12.119	10.083	2.036		
Disinfectant Towels Flooring		WA73-3-V-3-P1-2	12.307	10.194	2.113		
ectar ing	Slight Pressure	WA73-3-V-3-P1-3	12.185	10.243	1.942	2.03	0.07
Disinfecta Flooring		WA73-3-V-3-P1-4	12.466	10.388	2.078	-	
		WA73-3-V-3-P1-5	12.353	10.395	1.958		
iital Cleaner Bleach/Vinyl		WA73-3-V-3-P3-1	12.107	9.24	2.867		
Hospital with Ble		WA73-3-V-3-P3-2	12.408	9.607	2.801		
	Higher Pressure	WA73-3-V-3-P3-3	12.432	9.638	2.794	2.80	0.23
Dispatch®		WA73-3-V-3-P3-4	12.448	9.719	2.729		
Disp		WA73-3-V-3-P3-5	12.124	9.302	2.822		

Table A-3-4-1: Task 3 Results Dispatch® Hospital Cleaner Disinfectant Towels with Bleach/Vinyl Flooring

Wipe Type	Pressure Applied	ID	Weight of Wet	Wipes (g)	Net Weight Loss	Average StDev Weight Weight	Weight
туре	Applieu	neu	Initial	Final	(g)	Loss (g/ft ²)	Loss (g/ft ²)
Ħ		WA73-3-G-3-P1-1	11.585	10.545	1.04		
ectai		WA73-3-G-3-P1-2	11.566	10.644	0.922	1.00	0.07
Disinfectant Slass	Slight Pressure	WA73-3-G-3-P1-3	11.683	10.648	1.035		
her D		WA73-3-G-3-P1-4	11.952	11.031	0.921		
Cleaner Disin Bleach/Glass		WA73-3-G-3-P1-5	10.874	9.813	1.061		
		WA73-3-G-3-P3-1	11.437	9.429	2.008		
® Hosp owels v		WA73-3-G-3-P3-2	11.761	9.443	2.318		0.19
ch [®] F Tow	Higher Pressure	WA73-3-G-3-P3-3	12.038	9.684	2.354	2.20	
Dispatch [®] Hospital Towels with	11035010	WA73-3-G-3-P3-4	11.639	9.506	2.133		
D		WA73-3-G-3-P3-5	11.371	9.16	2.211		

Wipe Pressure	ID	Weight of We	et Wipes (g)	Net Weight	Average Weight Loss	StDev Weight	
Туре	Applied	U	Initial	Final	Loss (g)	(g/ft ²)	Loss (g/ft ²)
t t		WA73-3-M-3-P1-1	11.548	10.334	1.214		
Disinfectant		WA73-3-M-3-P1-2	11.674	10.486	1.188	1.16	
isinf	Slight Pressure	WA73-3-M-3-P1-3	12.415	11.281	1.134		0.06
ω		WA73-3-M-3-P1-4	11.971	10.893	1.078		
Cleaner leach/M		WA73-3-M-3-P1-5	12.104	10.897	1.207		
oital (th Bl		WA73-3-M-3-P3-1	11.332	8.589	2.743		
Hospital Is with B		WA73-3-M-3-P3-2	12.541	9.637	2.904	1	
	Higher Pressure	WA73-3-M-3-P3-3	12.31	9.784	2.526	2.75	0.23
Dispatch [®] Towe		WA73-3-M-3-P3-4	11.856	9.178	2.678		
Ω		WA73-3-M-3-P3-5	12.493	9.606	2.887		

Table A-3-4-3: Task 3 Results Dispatch® Hospital Cleaner Disinfectant Towels with Bleach/Melamine

Wipe	Pressure	ID.	Weight of We	Weight of Wet Wipes (g)		Average	StDev
Туре	Applied	ID	Initial	Final	Loss (g)	Weight Loss (g/ft2)	Weight Loss (g/ft2)
nt		WA73-3-D-3-P1-1	12.611	10.721	1.89		
Disinfectant d Drywall		WA73-3-D-3-P1-2	12.337	10.208	2.129	1.96	
isinfecta Drywall	Slight Pressure	WA73-3-D-3-P1-3	13.083	10.932	2.151		0.18
0		WA73-3-D-3-P1-4	12.479	10.593	1.886		
Cleaner ch/Painte		WA73-3-D-3-P1-5	13.102	11.374	1.728		
		WA73-3-D-3-P3-1	12.424	9.161	3.263		
Hospital with Blea		WA73-3-D-3-P3-2	12.612	9.502	3.110	3.22	
Dispatch [®] I Towels v	Higher Pressure	WA73-3-D-3-P3-3	13.111	9.81	3.301		
		WA73-3-D-3-P3-4	12.957	9.769	3.188		
D		WA73-3-D-3-P3-5	12.866	9.637	3.229		

APPENDIX B - PHASE 2: Evaluation of Efficacy of Hypochlorite Wipes

Clorox Healthcare [®] Wipe Results											
Test ID	Positive Controls		Test Coupons			Drip Wipes			Decon Wipes*		
	1	2	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
5-73 C0D1	9.47E+06	1.03E+07	1.58E+05	5.95E+04	2.59E+04	3.50E+02	1.52E+02	1.19E+02	ND		
5-73 C0D2	7.55E+06	1.12E+07	6.72E+04	1.04E+05	1.55E+05	3.38E+02	4.41E+02	9.24E+02			
5-73 C0G1	6.09E+06	8.13E+06	3.99E+03	1.04E+04	2.19E+04	5.93E+01	6.48E+02	1.45E+02			
5-73 C0G2	7.19E+06	5.91E+06	3.03E+03	4.56E+03	2.41E+03	2.03E+02	1.44E+02	9.71E+01			
5-73 C1D1	4.93E+07	5.84E+07	2.40E+03	2.72E+04	4.66E+02	6.30E+03	2.65E+02	6.16E+03			
5-73 C1G1	2.38E+07	3.75E+07	6.75E+02	8.78E+03	5.04E+02	5.52E+01	2.73E+02	4.48E+02			
5-73 C2D1	6.13E+07	8.91E+07	4.62E+04	2.83E+05	2.02E+05	5.09E+04	1.57E+04	3.01E+03			
5-73 C2G1	4.14E+07	5.93E+07	7.12E+06	2.34E+04	1.39E+06	1.53E+04	NA	NA			
5-73 C3D1	5.10E+07	3.75E+07	2.00E+06	1.33E+06	3.66E+05	3.34E+04	3.36E+04	5.83E+04			
5-73 C3G1	1.22E+07	3.07E+07	2.51E+02	4.37E+03	2.13E+02	4.71E+04	1.60E+04	2.66E+03			
5-73 C3D2	4.19E+07	3.15E+07	5.59E+04	7.27E+05	5.06E+04	4.31E+03	2.87E+02	5.88E+03			
5-73 C3G2	4.21E+07	5.44E+07	1.06E+04	1.12E+04	2.92E+04	1.05E+04	5.18E+03	3.62E+04			
Hype-Wipe [®] Bleach Towelette results											
5-73 H0D1	6.02E+06	8.43E+06	8.34E+03	1.18E+04	3.22E+03	4.25E+01	3.99E+01	1.53E+02			
5-73 H0D2	1.01E+07	1.11E+07	1.13E+04	1.19E+04	1.88E+03	1.64E+02	1.16E+02	2.15E+02			
5-73 H0G1	6.96E+06	5.20E+06	1.12E+02	1.14E+03	4.86E+01	4.90E+02	2.40E+02	1.12E+02	- ND		
5-73 H0G2	6.68E+06	9.14E+06	1.20E+03	9.17E+03	1.02E+02	5.60E+01	5.94E+01	2.60E+02			
5-73 H1D1	5.42E+07	3.66E+07	8.10E+02	8.77E+03	1.64E+02	3.89E+02	2.40E+02	1.41E+02			
5-73 H1G1	4.72E+07	1.62E+07	4.30E+02	2.73E+02	7.70E+01	1.75E+01	6.30E+00	8.69E+00			
5-73 H2D1	5.09E+07	7.14E+07	1.20E+05	1.60E+03	4.48E+04	1.54E+03	2.45E+02	1.26E+02			
5-73 H2G1	5.04E+07	3.83E+07	6.42E+04	2.01E+03	1.67E+03	2.04E+04	4.34E+02	1.25E+03			
5-73 H3D1	4.81E+07	5.25E+07	8.51E+02	1.60E+04	4.44E+04	3.62E+02	6.77E+04	8.40E+03			
5-73 H3G1	3.67E+07	5.03E+07	2.51E+04	2.06E+03	2.44E+04	2.11E+04	1.36E+04	4.11E+04			
5-73 H3D2	3.67E+07	3.85E+07	1.21E+06	1.43E+05	7.53E+04	2.24E+03	4.62E+02	3.02E+02			
5-73 H3G2	3.70E+07	4.10E+07	1.78E+03	2.17E+03	1.96E+02	3.50E+04	2.43E+05	6.74E+04			

Table B-1: Decontamination Test Results

*: After a minimum of 24 h at 4 °C NA: Not Available

ND: No Viable Spores Detected



PRESORTED STANDARD POSTAGE & FEES PAID EPA PERMIT NO. G-35

Office of Research and Development (8101R) Washington, DC 20460

Official Business Penalty for Private Use \$300