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Assessment of *Bacillus* spore inactivation on indoor surfaces using commercially-available cleaning products

Office of Research and Development
Homeland Security Research Program

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Disclaimer

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List of Acronyms and Abbreviations

ADA	Aerosol Deposition Apparatus
APIC	Association for Professionals in Infection Control and Epidemiology
ATCC	American Type Culture Collection
B.	*Bacillus*
CDC	Centers for Disease Control and Prevention
CFU	Colony Forming Unit(s)
COC	Chain of Custody
COTS	Commercial Off the Shelf
DCMD	Decontamination and Consequence Management Division
DHS	Department of Homeland Security
DI	Deionized
DNA	Deoxyribonucleic Acid
DPG	Dugway Proving Ground
DQI	Data Quality Indicator
DQO	Data Quality Objective(s)
DTRL	Decontamination Technologies Research Laboratory
EPA	U. S. Environmental Protection Agency
fl. oz	Fluid Ounce
ft	Feet or Foot
ft^2	Square Feet or Foot
FAC	Free Available Chlorine
HSRP	Homeland Security Research Program
HVAC	Heating Ventilation and Air Conditioning
HVLP	High Volume Low Pressure
In	Inch(es)
L	Liter
LD50	Lethal Dose, 50%
LR	Log Reduction
MDI	Metered Dose Inhaler
Ml	Milliliter
Min	Minute
MOP	Miscellaneous Operating Procedure
NIST	National Institute of Standards and Technology
OPP	Office of Pesticide Programs
OSHA	Occupational Safety and Health Administration
OSWER	Office of Solid Waste and Emergency Response
oz	Ounce
pAB	pH-Adjusted Bleach
PEG	Polyethylene Glycol
PPE	Personal Protective Equipment
ppm	Part(s) Per Million
RH	Relative Humidity
RSD	Relative Standard Deviation
RTU	Ready to Use
QAPP	Quality Assurance Project Plan
QUATS	Quaternary Ammonium Compounds

RH	Relative Humidity
RTP	Research Triangle Park
RTU	Ready To Use
RNA	Ribonucleic Acid
SD	Standard Deviation
SNL	Sandia National Laboratories
STEL	Short-Term Exposure Limit
STS	Sodium Thiosulfate
TSA	Tryptic Soy Agar
UV	Ultraviolet (light)
VHP	Vaporous Hydrogen Peroxide
WA	Work Assignment
WACOR	Work Assignment Contract Officer's Representative

Executive Summary

This project supports the mission of the U.S. Environmental Protection Agency's Office of Research and Development's Homeland Security Research Program (HSRP) by providing information relevant to the decontamination of areas contaminated as a result of an act of terrorism or other incident.

This project evaluated "low tech"/"self-help" expedient bioagent decontamination options of porous and nonporous building materials using bleach solutions. In Phase I, the sporicidal effectiveness of various dilutions of germicidal bleach and diluted bleach with surfactant was investigated. In Phase II, bleach-based ready-to-use (RTU)-commercial-off-the-shelf (COTS) cleaning and disinfecting products were used.

The efficacy of diluted germicide and bleach with surfactants was evaluated on common building materials contaminated with aerosolized *Bacillus atrophaeus* spores (surrogate for *Bacillus anthracis*). Evaluation exercised two types of operational procedures: Phase I procedures- application of in-house prepared decontaminant (diluted bleach) via rag or sponge -10 minutes processing - water rinse using clean rag or sponge, and Phase II procedure - application of ready-to-use/commercial-off-the-shelf (RTU-COTS) decontaminant via spray - 10 minutes processing - water rinse using clean rag. Estimates of the number of spores re-aerosolized and rinsed off from the decontaminated material surface were also performed.

For the Phase I procedure, the average log reduction (LR) values ranged from 3.6 to 7.8. The decontamination effectiveness was dependent on bleach dilution, with dilution at 1:5 determined to provide a satisfactory sporicidal action. The superior mechanical removal of spores associated with the more abrasive rag application provided better sporicidal efficacy for nonporous materials, and the vertical penetration offered by use of a sponge (into the sponge) seemed to improve the decontamination efficacy for porous materials.

Addition of surfactants improved spore removal efficacy - the target decontamination rate (average LR ≥ 6) was achieved for 100% of the materials decontaminated with the rag procedure using bleach with surfactant-based liquid sporicide. Similarly, all tests in Phase II performed with RTU-COTS products containing bleach, various surfactants, and additives resulted in the full decontamination of all materials tested (average LR values from 6.7 to 8.0).

These data suggest that "low-tech"/"self-help" decontamination technology can provide greater than 6 log reductions of viable spores on the common building materials tested. The use of RTU-COTS materials removes additive-bleach compatibility issues and *a priori* eliminates any potential errors in preparation of diluted bleach and surfactant-amended bleach formulation.

The estimates of the fate of spores suggest that the low-tech liquid sporicide-based decontamination process leads to physical removal of spores from decontaminated surfaces, with a consequent transport of viable spores to the post-decontamination liquid and solid waste. Spores can also be released to the air, and can therefore be a significant source of cross-contamination during a remediation and can pose health risks to persons performing decontamination. The final "self-help" method tutorial/ instructional document resulting from this project should therefore emphasize the importance of using the required personal protective equipment (PPE). Users of this guidance should follow PPE recommendations on the product label when using these products. Mixing of these products with other chemicals is also not recommended.

1. Introduction

The Department of Homeland Security (DHS) and other appropriate Federal departments and agencies have been tasked to develop comprehensive plans which "provide for seamless, coordinated Federal, state, local, and international responses to a biological attack." As part of these plans, the U.S. Environmental Protection Agency (EPA), in a coordinated effort with DHS, is responsible for "developing strategies, guidelines, and plans for decontamination of persons, equipment, and facilities" to mitigate the risks of contamination following a biological weapons attack. EPA's Homeland Security Research Program (HSRP) 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.

Decontamination can be defined as the process of inactivating or reducing a contaminant in or on humans, animals, plants, food, water, soil, air, areas, or items through physical, chemical, or other methods to meet a cleanup goal. In terms of the surface of a material, decontamination can be accomplished by physical removal of the contaminant or via inactivation of the contaminant with antimicrobial chemicals, heat, ultraviolet (UV) light, etc. Physical removal could be accomplished via *in situ* removal of the contaminant from the material or by physical removal of the material itself (i.e., disposal). Similarly, inactivation of the contaminant can be conducted *in situ* or after removal of the material for ultimate disposal. Following the 2001 anthrax incidents, a combination of removal and *in situ* decontamination was used [1-5].The balance between the two procedures were facility-dependent and factored in many issues (e.g., physical state of the facility) [1-5]. One factor was that such remediation was unprecedented for the United States Government, and no technologies had been proven for such use at the time. The cost of disposal proved to be significant and was complicated by the nature of the waste (e.g., finding an ultimate disposal site). Since 2001, a primary focus for facility remediation has been improving the effectiveness and practical application of *in situ* decontamination methods and evaluating waste treatment options to be able to provide information necessary to optimize the decontamination/disposal paradigm. This optimization has a significant impact on reducing the cost of and time for the remediation effort.

Quick, effective and economical decontamination methods that have the capacity to be employed over wide areas (outdoor and indoor) required to increase emergency preparedness are the focus of the HSRP's research, which supports the Office of Solid Waste and Emergency Response (OSWER) and the Office of Pesticide Programs (OPP). Decontamination methods being tested by the HSRP include various "high-tech" technologies like fumigations and "low-tech" approaches like combined mechanical and chemical procedures (vacuum, scrub/wash and bleach). If proven effective, "lower-tech" approaches involving washing and cleaning with readily available equipment, washes and off-the-shelf sporicides would significantly increase the Nation's readiness to respond to a wide-area contaminant release.

Low tech decontamination approaches employing liquid and physical cleaning methods have been used following the intentional or accidental release of *Bacillus (B.) anthracis* spores in secondarily contaminated areas, (i.e., areas contaminated with a biological agent tracked from primary contaminated sites, often via materials contaminated/cross-contaminated with anthrax; e.g. letters), or in primary contaminated facilities (i.e., directly exposed to intentional or accidental release of anthrax spores) showing a minimal presence of the *B. anthracis* spores [1-5]. These methods included combinations of disposal of contaminated items,

vacuuming, and the use of liquid sporicides (e.g., pH-amended bleach solution) or a combination of mechanical and chemical procedures (vacuum, scrub/wash, and bleach) [1-5].

Bleach, (5.3%-8.3% NaOCl by weight for regular and concentrated off-the-shelf products) is the most popular chlorinating agent in use today. When added to water, bleach hydrolyzes as hypochlorous acid, sodium ion and hydroxide:

$NaOCl + H_2O \rightarrow HOCl + Na^+ + OH^-$

Additionally, some of the HOCl dissociates into hydrogen ion and hypochlorite ion:

$HOCl \rightarrow H^+ + OCl^-$

Both HOCl and OCl^- are oxidants and effective germicides. HOCl is the stronger and more effective of the two species [6-7].

Bleach is effective against a broad range of microorganisms at low contact times (5-10 minutes). The Centers for Disease Control and Prevention (CDC), Occupational Safety and Health Administration (OSHA), and Association for Professionals in Infection Control and Epidemiology (APIC) guidelines recommend bleach as a broad spectrum germicide to disinfect hard surfaces contaminated by blood spills and tough-to-kill pathogens such as *Clostridium difficile* spores and norovirus, both of which are resistant to disinfection by quaternary ammonium compounds (QACs) [6]. For resistant organisms and surfaces that are highly soiled, the CDC recommends a 1:10 dilution of 5.25% – 6.15% bleach (5250 parts per million (ppm) – 6150 ppm sodium hypochlorite solution). As a strong oxidizer, bleach reacts with nucleic acids (DNA/RNA), lipids and fatty acids associated with the cell membrane and destroys the cellular activity of structural and functional proteins [6]. Chlorine may affect a variety of metabolic processes in bacteria including inhibition of metabolic enzymes and inhibition of membrane-mediated active transport processes and respiratory activity [7]. There is no evidence of bacteria or viruses developing resistance to the powerful oxidizing action of bleach when used at recommended dilutions [6].

Addition of simple surfactants to liquid sporicides may improve decontamination efficacy because some surfactants can prevent the formation of biofilms due to surface tension that pulls apart microorganisms upon contact. A new generation of antibacterial/antiviral products known as "nanoemulsion chemicals" are formulations made from detergents and oil in water [8-9]. These antimicrobial nanoemulsions fuse with lipid-containing organisms. This fusion is enhanced by the electrostatic attraction between the cationic charge of the emulsion and the anionic charge on the pathogen, which ultimately destabilizes the pathogen lipid membrane, resulting in cell lysis and death [9].

Optimized low tech decontamination approaches can be especially useful for self-help decontamination purposes of secondary contaminated areas, like residential dwellings. However, additional information is needed to predict the decontamination efficacy of off-the-shelf sporicidal agents deployed over complex and challenging material types and developing safe and efficient concepts of operation that are both easily accessible and understandable to the general public.

1.1.Objectives

The primary objective of this study was to develop a simple “self-help” decontamination approach for building surfaces contaminated with *Bacillus atrophaeus* spores (i.e., surrogates of *Bacillus anthracis*). “Self-help” decontamination was defined as *in situ* surface-disinfection method not requiring specialized materials or equipment (i.e., cleaning supplies/products available at a local hardware store would be used). The optimized method must be easily understandable to the general public/responders without specialized training.

Several procedures using bleach-based decontamination solutions were evaluated, using basic tools expected to be easily accessible at residential dwellings (i.e., cleaning rags and sponges). The decontamination agents tested were diluted germicidal concentrated bleach and bleach with surfactants solutions and four commercial RTU-COTS bleach-based disinfecting/cleaning products (details of decontaminants are given in Section 2.4). The effectiveness of decontaminants/proposed self-help procedures was tested on 14 x14 inch coupons of the porous and nonporous common building materials (details given in Section 2.2). The operational parameters of decontamination (material compatibility of decontaminants with test materials, effectiveness of decontamination solution delivery as a function of the type of cleaning medium and medium-specific loading volume) were considered important to understand the sporicidal activity of a bleach-based decontamination process and were characterized in addition to sporicidal effectiveness. The fate of the viable spores during decontamination (transfer from decontaminated surface to air) and transfer to post-decontamination solid and liquid waste were also determined. The fate or partitioning of the spores was studied to determine whether the loss of spores from the surface were a result of inactivation or physical removal from the surface.

2. Experimental Approach

This section describes the 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 at EPA's Research Triangle Park facility (RTP, NC, USA). Sampling and analytical procedures are described in Section 3.

The general experimental approach was:

- Preparation of representative samples of nonporous and porous building materials; the 35.6 cm by 35.6 cm (14x14 in) coupon area was selected as a suitable surface for decontamination and sampling.

- Selection of bleach-based formulations and commercial COTS products for decontamination; all products used in this study are obtainable from national retailers. The products used in these studies were titrated to verify the hypochlorite concentration as this concentration will change as the product sits on the shelf.

- Contamination of model building surfaces (coupon) with standardized inocula of *B. atrophaeus* spores using an aerosol deposition method, followed by quantitative assessment of pre-decontamination spore loading by sampling positive control (non-decontaminated) coupons (n = 3 per test)

- Application of the decontamination procedure on test coupons (n = 3 to 5 coupons per each decontamination procedure tested); test coupons were decontaminated with various solutions of bleach and bleach with additives using different decontamination procedures, followed by quantitative determination of viable spores. Quantitative assessment of residual (background) contamination was performed by sampling of procedural blanks. The transfer of viable spores to post-decontamination waste and air was done by qualitative analysis of decontamination procedure residues, i.e., decontamination solution run-off, rinse water waste, expended decontamination tools (rags and sponges) and in aerosol samples.

- Determination of decontamination effectiveness as a function of the procedure/decontaminant and material type. Decontamination effectiveness was measured as log reduction (LR), defined as the amount of reduction in viable spores required to move the decimal one place, or reduce the exponent in scientific notation by one. The target LR for decontamination studies is usually LR≥6 (≥99.9999% reduction). A 6 LR target is consistent with sporicidal efficacy tests used to register sporicides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Recovery of no viable spores following treatment was considered highly effective.

This project was conducted in two phases:

1) Phase I - determining the effectiveness and operational parameters for a "low-tech" or "self-help" approach for decontamination of *B. anthracis* surrogate from selected building surfaces using a

simple liquid sporicide (diluted germicidal concentrated bleach) deployed via an uncomplicated wipe-wait-rinse technique. The experimental design assumed bleach dilution at ratios starting at 10:1 and working down to a 1:1 ratio if necessary. The candidate processing times were 10 and 30 minutes. Addition of surfactant to the bleach solution was also tested for the combination of bleach dilution and processing time that was determined to provide > 6 log spore inactivation/removal for bleach without additives. Lastly, effect of grime/organic matter presence on the selected building surface (laminate flooring) surface was also tested for the procedure that provided the highest sporicidal activity for all materials (1:5 bleach with surfactant decontamination solution, applied via rag with processing time of 10 minutes).

2) Phase II - determining the effectiveness of a "low-tech" or “self-help” approach for decontamination of *B. anthracis* surrogate from selected building surfaces using bleach-based RTU-COTS products deployed via spray-wait-rinse technique, using wiping media and processing time optimized in Phase I.

2.1. Materials

The representativeness and uniformity of test materials are essential in achieving defensible evaluation results. Material representativeness means that these materials are typical of those currently used in buildings in terms of quality, surface characteristics, and structural integrity. In this effort, representativeness will be assured by selecting test materials that are typical of those found in residential dwellings, and that meet industry standards or specifications for indoor use, and by obtaining those materials from appropriate suppliers. Material uniformity means that all these material coupons are equivalent for purposes related to testing. Uniformity will be maintained by obtaining and preparing a quantity of material sufficient to allow multiple test samples to be prepared with presumably uniform characteristics (i.e., test coupons will be cut from the interior rather than the edge of a large piece of material). Documented procedures were established for coupon preparation to ensure material uniformity.

Coupons (14 x14 inch) were prepared from selected nonporous and porous building materials (glass, finished wood flooring, grouted ceramic tile, and painted wallboard (Table 2-1, Figure 2-1) that are typical of the materials found in residential dwellings that meet industry standards or specifications for indoor use.

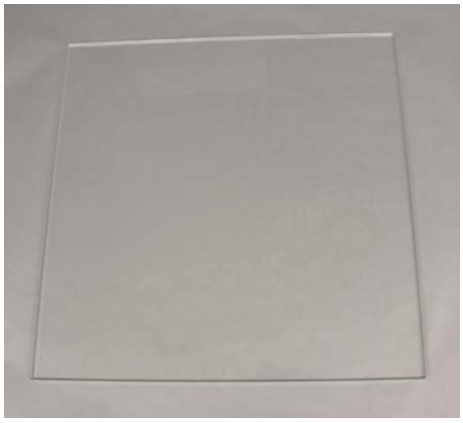
Table 2-1. Description of Building Materials for Decontamination Testing

Material	Description	Manufacturer/ Supplier Name	Coupon Surface Size, L x W (inches)	Material Preparation
Glass	Borosilicate glass, thickness 1/4 inch	McMaster-Carr	14 x 14	- Clean with water and detergent - Rinse with water and wipe dry - Sterilize (autoclave)
Finished wood flooring	7-5/8 x 50-3/4 inch laminated wood locking flooring; smooth surface	Project Source; color – /Lowe's /Winchester Oak	14 x 14	- Remove particles by wiping clean with water and wipe dry - Sterilize (VHP)*
Grouted ceramic tile	12" x 12" Ceramic mosaic tile grouted with cementitious sanded premixed grout	Lowe's /American Olean and TEC Invision	14 x 14	- Remove particles by wiping clean with water and wipe dry - Sterilize (VHP)
Painted wallboard	1/2-in x 4-ft x 8-ft drywall panel painted with premium latex interior flat paint in white	Lowe's /National Gypsum Company and Behr	14 x 14	- Remove particles by wiping clean with water and wipe dry - Sterilize (VHP)

*Vaporous hydrogen peroxide.

a. Finished wood flooring

b. Glass



c. Painted wallboard

d. Grouted mosaic tile

Figure 2-1. Test Coupons

The glass coupons were sterilized prior to use by steam autoclave utilizing a gravity cycle program. The remaining materials (painted wallboard, finished wood flooring and grouted tile) were sterilized using Vaporized Hydrogen Peroxide® (VHP). The hydrogen peroxide vapor in this study was generated using a STERIS VHP 1000ED generator (referred to as Vaporized Hydrogen Peroxide®, or VHP) loaded with a 35% H_2O_2 Vaprox® cartridge. The sterility of the coupons was verified through the use of negative control samples.

For tests with grime (test 25, Table C-1, Appendix C), coupons were loaded with grime using a high volume, low pressure (HVLP) sprayer per Miscellaneous Operating Procedure (MOP) 3163 (Appendix B). Grime consisting of 94% fine dust, 3% soot and 3% biological materials was prepared in-house using the procedure developed by Sandia National Laboratories (SNL) (recipe was adopted from “Evaluation of Surface Sampling Method Performance for *Bacillus* spores on Clean and Dirty Outdoor Surfaces” developed by Sandia National Laboratories [10]). The detailed recipe for grime is shown in Table 2-2.

Table 2-2. Grime Recipe

Vendor	Part#	Component Name	Relative composition of individual component [%]	Relative composition of classes component [%]
Powder Technology Inc., PTI	PP2G4 A2 fine	Arizona fine dust	94.00%	94% Fine dust
Powder Technology Inc., PTI	Raven 410	Carbon black	2.50%	3% Soot
National Institute of Standards and Technology, NIST*	SRM 1650b	Diesel particulate	0.25%	
Auto Parts	Off the shelf	Motor oil	0.13%	
Fisher Scientific	AC16436-0050	α-Pinene,97%	0.13%	
Fisher Scientific	S755301	Lycopodium	1.00%	3% Biological materials
Polysciences Inc.	7673	Ragweed pollen	1.00%	
Polysciences Inc.	7670	Paper Mulberry pollen	1.00%	

*National Institute of Standards and Technology

2.2. Inoculation of Coupons

Inoculation of test and positive control coupons with spores of *B. atrophaeus* was performed via aerosol deposition using a metered dose inhaler (MDI).

The test organism for this work was a powdered spore preparation of *B. atrophaeus* (American Type Culture Collection (ATCC) 9372) and silicon dioxide particles. This bacterial species was formerly known as *B. subtilis* var. *niger* and subsequently *B. globigii*. The preparation was obtained from the U.S. Army Dugway Proving Ground (DPG) Life Science Division. Briefly, after 80 – 90 percent sporulation, the suspension was centrifuged to generate a preparation of approximately 20 percent solids. A preparation resulting in a powdered matrix containing approximately $1x10^{11}$ viable spores per gram was prepared by dry blending and jet milling the dried spores with fumed silica particles (Deguss, Frankfurt am Main, Germany). The powdered preparation was loaded into metered dose inhalers (MDIs). Control checks for each MDI were included in the batches of coupons contaminated with a single MDI.

Coupons (test and positive controls) were inoculated with spores of *B. atrophaeus* from an MDI confirmed to deliver E+07 spores per discharge. Results of inoculation of positive control coupons are given in Section 5.3.1). Each coupon was contaminated independently by being placed into a separate dosing chamber into a separate dosing chamber (aerosol deposition apparatus or ADA) designed to fit one 35.6 cm by 35.6 cm (14 inch (in) by 14 in) coupon of any thickness. The MDI was discharged a single time into the dosing chamber. The number of discharges per MDI was tracked so that use did not exceed 75% of the calculated capacity. Additionally, the weight of each MDI was determined after completion of the contamination of each coupon. The inoculation control coupons (14 in by 14 in sterile 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. Prior to decontamination testing, spores were allowed to settle onto the coupon surfaces for a minimum period of 18 hours (overnight).

2.3. Preparation of Decontamination Solution

Two types of sporicides were tested: solutions prepared in-house using Clorox® Concentrated Germicidal Bleach (The Clorox® Company, USA) and Clorox® Splash-less Concentrated Bleach (The Clorox® Company, USA). RTU-COTS recipes used for preparation of in-house diluted bleach solutions are given in Table A-1 (Appendix A). RTU-COTS products were used as is. More detailed information on each sporicide can be found in Sections 2.4.1 through 2.4.5.

2.3.1 Clorox® Concentrated Germicidal Bleach

This Clorox® Concentrated Germicidal Bleach is a newer product of the Clorox® Company that replaces Regular Germicidal Bleach (Figure 2-2). Per the Clorox® Concentrated Germicidal Bleach label sodium hypochlorite concentration in this product is 8.25% (compared to 6.15% in Regular Germicidal Bleach). Per manufacturer's instructions, 1/2 cup of concentrated product shall be diluted in 1 gallon of water (1:32 dilution ratio). For disinfection, the recommended surface contact time should be at least five minutes. The dilution ratios used in this study were lower; from 1:10 to 1:5, with a contact time of 10 minutes.

> *NOTE: The concentrated product that is being sold in a smaller and more ergonomic bottle with a yellow band (Figure 2-2 a) is easily distinguishable from its non-concentrated predecessor (Figure 2-2b). However, a clear recommendation should be made in the instructional document resulting from this project to always use the new generation (concentrated) product for preparation of liquid decontaminant solution.*

Figure 2-2. Packaging of Clorox® Concentrated Germicidal Bleach (a) and Clorox® Germicidal Bleach (b)

Clorox® Splash-less Concentrated Bleach (The Clorox® Company, USA; Figure 2-3) was used to test the effect of surfactant addition on sporicidal effectiveness of diluted bleach. The concentration of sodium hypochlorite in this product is 3.985% (compared to 8.25% in Regular Concentrated Germicidal Bleach). The diluted solution of bleach with surfactant to be used in decontamination testing had the same working concentration of sodium hypochlorite as in diluted bleach without surfactant liquid decontaminant (see characterization of bleach and bleach with surfactant solutions in Section 2.5). Note that COTS bleach should be used before the expiration date as sodium hypochlorite concentrations will decrease with age.

Clorox® Splash-Less formulation has multiple surfactants in addition to sodium hypochlorite:

- Cetyl betaine: surfactant – zwitterionic (amphoteric; cationic/anionic) surfactant – cleaning agent, used as thickener and foam stabilizer.

- Cocamidopropyl betaine (coco betaine): zwitterionic (amphoteric; cationic/anionic) surfactant – high-foaming agent/foam booster, also used as thickener and foam stabilizer and viscosity enhancer; has anti-static properties; moderate emulsifier.

- Sodium xylene sulfonate: anionic surfactant – hydrotrope; solubilizes hydrophobic compounds in aqueous solutions; is generally used to stabilize other ingredients in a cleaning product to maximize effectiveness of the formula. It is also useful as a co-thickener (in combination with other ingredients) in cleaning products.

Figure 2-3. Packaging of Splash-less Clorox® Concentrated Germicidal Bleach

Utilization of RTU-COTS bleach with surfactant product was preferred over addition of other commercially available cleaning product/surfactant into bleach-based liquid sporicide formulations. Using a commercial product is a "mistake-proof approach" and removes compatibility issues from preparation of a surfactant-amended bleach formulation. Use of a COTS product is especially important if the surfactant-amended bleach is eventually to be prepared by a person without specialized training for self-help decontamination purposes.

Surfactants used in Clorox® Splash-less Concentrated Bleach (sodium xylenesulfonate, cocoamidopropyl betaine, cetyl betaine) are commonly used in a variety of other cleaning products designed for efficient removal of tough dirt and grease, e.g., Spic and Span®, which would be possible candidates for sporicidal additions to bleach.

Recipes for preparation of bleach and bleach with surfactant solutions used in this study are given in Table A-1 (Appendix A).

2.3.2 Lysol® Mold & Mildew Blaster

Lysol® Mold and Mildew Blaster with bleach is recommended for cleaning and disinfection purposes. The ingredients are water, sodium hypochlorite (2%), sodium hydroxide, sodium chloride, laurylamine oxide, and fragrance.

Lysol® Mold and Mildew Blaster is sold in 28 ounce (oz.) and 32 oz. bottles and is available from major national retailers at a price of approximately $0.10/fluid (fl.) oz. The current packaging of this product is shown in Figure 2-4.

Figure 2-4. Packaging of Lysol® Mold & Mildew Blaster

Per manufacturer's information, this product is bactericidal, fungicidal, and virucidal.

2.3.3 Tilex® Mold & Mildew Remover

Tilex® Mold & Mildew Remover is intended to disinfect and kill mold and mildew on hard nonporous surfaces. The ingredients are water, sodium hypochlorite (2.4%), sodium hydroxide, dimethicone/silica/ polyethylene glycol distearate antifoam, fragrance, lauramine oxide, and sodium silicate.

Tilex® Mold and Mildew Remover is sold in 16 oz. and 32 oz. bottles and is available from major national retailers at a price of approximately $0.12/fl. oz. The current packaging of this product is shown in Figure 2-5.

Figure 2-5. Packaging of Tilex® Mold & Mildew Remover

2.3.4 Clorox® Clean-Up Cleaner + Bleach

Clorox® Clean-Up Cleaner + Bleach is a multipurpose cleaner for cleaning, disinfection, and deodorization of most hard nonporous household surfaces. The ingredients are water, sodium hypochlorite (1.84%), sodium hydroxide, dimethicone/silica/polyethylene glycol (PEG) distearate antifoam, fragrance, lauramine oxide, and sodium silicate.

Clorox® Clean-up Cleaner + Bleach is sold in 32 oz. bottles and is available from major national retailers at a price of approximately $0.09/fl. oz. It is also available in 64 oz. and 180 oz. refill bottles ($0.07-0.10/fl. oz.). The current packaging of this product is shown in Figure 2-6.

Figure 2-6. Packaging of Clorox® Clean-Up Cleaner + Bleach

2.3.5 Clorox® Disinfecting Bleach Foamer

Clorox® Disinfecting Bleach Foamer is used as a cleaning agent as well as an antimicrobial agent designed specifically for bathroom surfaces. The ingredients are water, sodium hypochlorite (2.4%), sodium hydroxide, dimethicone/silica/PEG distearate antifoam, fragrance, lauramine oxide, sodium silicate, and alkyl dimethyl benzyl ammonium chloride (quaternary ammonium compounds or QUATS). Clorox® Disinfecting Bleach Foamer is sold in 30 oz. bottles and is available from major national retailers at a price of approximately $0.11/fl. oz. The current packaging of this product is shown in Figure 2-7.

Figure 2-7. Packaging of Clorox® Disinfecting Bleach Foamer

2.4. Characterization of Decontamination Solutions

The baseline values for pH, chlorine concentration (% sodium hypochlorite and free available chlorine (FAC)) and temperature were established experimentally prior to testing via triplicate measurements for each liquid decontaminant to be tested, including RTU products. The importance of these parameters for characterization of liquid sporicides is explained in Section 2.5.1 through 2.5.3.

The averages from these measurements were then used as the target threshold of FAC and pH for each decontamination solution prepared in-house and used in ongoing assessments of quality assurance (QA) objectives in Phase I (Section 6.2). Time of preparation of each batch of diluted bleach was also noted. For Phase II, RTU-COTS products were used as is, after the initial testing of FAC, pH and temperature parameters (Table 2-3).

Baseline characteristics (% sodium hypochlorite and pH as per Safety Data Sheet (SDS), product label and values delivered experimentally) of decontaminants used in Phase I and Phase II are listed in Table 2-3.

Table 2-3. Concentration of Sodium Hypochlorite (%) and pH of Decontamination Solutions

Sporicide	% Sodium Hypochlorite			pH	
	SDS	Label	Tested Value***	SDS	Tested Value
Prepared in-house (Phase I testing)					
Clorox® Concentrated Germicidal Bleach 1:10 dilution	0.50-1.5*	0.83*	0.67	>11*	11.4
Clorox® Concentrated Germicidal Bleach 1:5 dilution	1.0-3.0*	1.65*	1.37	>11*	11.6
Clorox® Splashless Concentrated Bleach 1:5 dilution equivalent	0.40-2.1**	1.65**	1.55	>11**	11.8
COTS (Phase II testing)					
Lysol® Mold and Mildew Blaster	1.0 - 2.5	2.00	2.52	12.3 - 12.7	12.2
Tilex® Mold and Mildew Remover	1.0 – 5.0	2.40	2.49	12.4 - 12.8	12.6
Clorox® Clean-Up Cleaner + Bleach	1.0 – 5.0	1.84	2.11	12.4 - 12.8	12.6
Clorox® Disinfecting Bleach Foamer	1.0 – 5.0	2.40	2.15	~12.2	12.6

** Full strength* Clorox® *Concentrated Germicidal Bleach concentration per SDS: 5-15%, per label: 8.3%; pH per SDS ~ 11.9*

*** Full strength* Clorox® *Concentrated Splashless Bleach concentration per SDS: 1-5%, per label: 3.985%; pH per SDS ~ 12.5*

**** As determined by iodometric titration (n = 3)*

Detailed results from characterization of decontamination solutions are given in Section 5-1 and Tables A-2 and A-3 (Appendix A).

2.4.1 Concentration of Sodium Hypochlorite (NaOCl) versus Free Available Chlorine and pH

Sodium hypochlorite (NaOCl) is the active ingredient (oxidizing agent) in bleach. For consumer products, the sodium hypochlorite strength is most commonly expressed as weight percent [wt%] of sodium hypochlorite (the weight of the sodium hypochlorite per 100 parts of solution).

The definition most commonly used to describe the oxidizing power of bleach-based products used in the decontamination research is grams per liter [g/L] of free available chlorine (FAC). FAC is any residual chlorine that is available to react with sources of bacteria or other contaminants. FAC is the sum of all of the chemical species that contain a chlorine atom in the 0 or +1 oxidation state and are not combined with ammonia or other organic nitrogen. Some species of FAC that might be present in aqueous solutions are:

- Molecular chlorine: Cl_2
- Hypochlorous acid: HOCl
- Hypochlorite: OCl^-
- Trichoride: Cl_3 - a complex formed by molecular chlorine and the chloride ions (Cl^-) [11].

In addition to free available chlorine concentration, pH significantly changes relative effectiveness of bleach as a disinfectant, since different species of chlorine ions are more prevalent at different pH levels. In the pH range 6–9, HOCl and OCl^- are the main chlorine species [11]. Depending on pH level, the ratio of these two free chlorine species changes (Figure 2-8 adapted from reference 11).

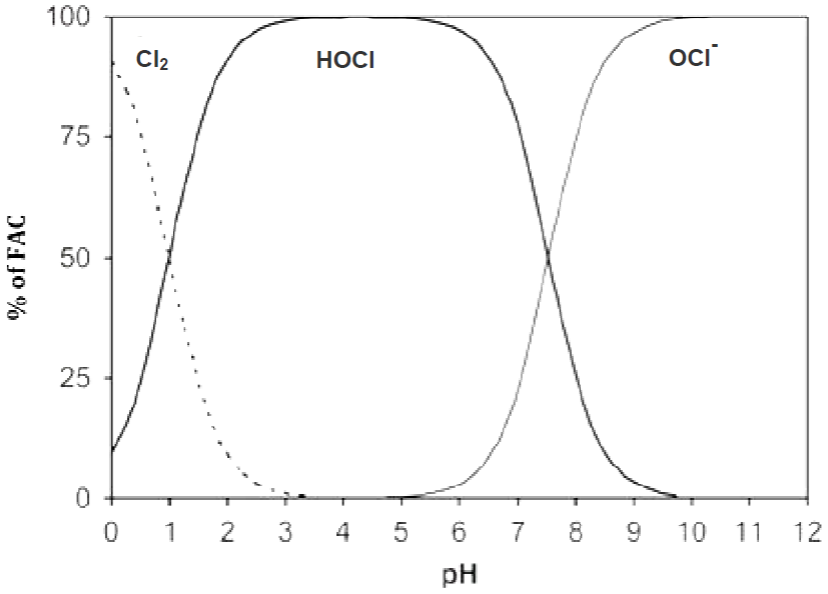


Figure 2-8. Distribution of Free Chlorine Species in Aqueous Solutions (from reference 11)

Figure 2-8 shows that chlorine hydrolysis into HOCl is almost complete at pH ≤ 4. Dissociation of HOCl into OCl^- begins at approximately 5.5 pH and increases dramatically thereafter. The formation of HOCL is important because HOCl and OCl^- do not have the same effectiveness as disinfectants. HOCl can be 80-100% more effective as a disinfectant than OCl^-. Optimum disinfection occurs at pH 5 to 6.5 where HOCl is the prevailing species of free chlorine present. As pH rises above that level, the ratio shifts towards being primarily OCl^-. At pH 7.5, the ratio is about even. When the pH value rises to 8 or higher, OCl^- is the dominant species. Therefore, assuming the concentration of the Cl_2 species is constant, the higher the pH of the solution rises above 5.5, the lower the oxidation capability and disinfecting power of the FAC [11]. In conclusion, knowing the FAC and pH of bleach solution is important to have a more complete picture of its disinfecting power.

Results for test-specific pH and FAC measurements of decontamination solutions are given in Section 5.1 and in Table A-2 and A-3 (Appendix A).

2.4.2 Temperature

Sodium hypochlorite solutions have limited storage stability. Decomposition of sodium hypochlorite solutions will occur due to the following two reactions [7]:

- Transformation into chlorate: $3\ NaOCl \rightarrow 2\ NaCl + NaClO_3$

- Release of oxygen: $2\ NaOCl \rightarrow 2\ NaCl + O_2$

In a good quality sodium hypochlorite solution, the chlorate decomposition pathway accounts for approximately 90% of the total decomposition. Increasing temperature, hypochlorite concentration and ionic strength (salt content) will increase the reaction rate of both reactions to about the same extent. UV light (sunlight) also catalyzes both decomposition reactions. The decomposition of sodium hypochlorite is minimized in the range of pH 12 to 13 (between 0.025 and 0.35% excess sodium hydroxide). Below pH 11, the rate of formation of chlorate will increase dramatically. In the pH range of 13 to 13.5, there is only a minor increase in the decomposition rate [7].

Bleach decomposition is dependent on temperature. For any given temperature, the higher the solution strengths, the faster the bleach decomposes. Briefly, for every 10 °C increase in storage temperature, the sodium hypochlorite will decompose at an increased rate factor of approximately 3.5 [7]. The rate constants (k2) of sodium hypochlorite decompositions with respect to strength and temperature are given in Table 2-4, below [7].

Table 2-4. Rate Constants (k2) of NaOCl Decompositions with Respect to Strength and Temperature*

Temperature, °C	Sodium Hypochlorite				
	15.89%	13.46%	10.82%	7.93%	4.74%
55	250	189	138	98.2	65.5
45	80.7	58.7	43.9	30.2	19.3
35	23.1	17.0	12.2	8.43	5.45
25	6.33.	4.68	3.22	2.19	1.58
15	1.65	1.15	0.80	0.53	0.30

**from reference [7]*

In this study, the temperature of the decontamination solutions was monitored carefully to determine pot-life of decontamination solution batches. A temperature increase greater than 5 °C was a threshold designating that preparation of a new batch of the decontamination solution is needed (see Section 6.2 for details). Results of temperature monitoring of diluted bleach solutions are given in Section 5.1.

2.5. Decontamination Procedure

For each decontamination test, sets of building material coupons were inserted into the test coupon holders of the small spray test chamber (Figure 2-9) in a vertical position. Chamber dimensions are 4 ft high by 4 ft wide by 4 ft deep, and it is designed to accommodate three 14 in x 14 in coupons at a time in horizontal or vertical orientation. In this study, only the vertical orientation assembly was utilized. The chamber was constructed of stainless steel with the exception of the front face and top that were clear acrylic plastic. The acrylic door was fitted with three ports, one per coupon, to allow the insertion of the backpack delivery

system in the direct middle of each vertical coupon. In this study, the decontamination solution was applied by hand, and the door was open during application to imitate real-life low-tech decontamination (Figure 2-9). After application of decontaminant, the chamber door was closed. The project personnel were wearing personal chlorine gas monitors at all times. Chlorine emissions during applications of decontamination agents were below the OSHA short-term exposure limit (STEL).

The reverse-pyramid design of the chamber bottom allowed for collection of runoff from the coupons during the decontamination procedure through a central (3 inch in diameter) drain. The bottom of the chamber has a 189 liter (L) (50 gallon) collection capacity.

Figure 2-9. Decontamination Chamber (Left) and Application of Diluted Bleach Using Rag

The chamber exhaust exits via a readily accessible connection to the facility's air handling system. Aerosol samples were collected from the chamber exhaust duct using Via-Cell® BioAerosol Cassettes (Zefon International, Inc., Ocala, FL, USA). The sampling point was eight diameters downstream and two diameters upstream of any flow disruptions.

Decontamination procedural steps were as follows:

1. Inoculation of coupons

   - Three to five (3-5) test coupons in Phase I and Phase II work, respectively, and three (3) positive control coupons were inoculated with spores of *B. atrophaeus* via aerosol deposition using a metered dose inhaler (MDI) and Aerosol Deposition Apparatus (ADA); details of the inoculation procedure are described in Section 2.3.
   - After aerosol deposition, spores were allowed to settle overnight

2. Preparation of test chamber

- The test chamber was sterilized using pH-amended bleach solution; the pH-adjusted bleach (pAB) solution used for cleaning surfaces in equipment in both the decontamination and microbiology laboratories is prepared as a 1:10 dilution of bleach in deionized (DI) water, pH-adjusted to ~6.8 using glacial acetic acid.
- Using the back sprayer, the interior surfaces of the test chamber were sprayed with pAB, and kept wet with solution for 10 minutes, and then rinsed with DI water.
- After rinsate drained from the chamber, the chamber valve was closed and the interior of the chamber was wiped down with isopropyl alcohol or ethanol.

3. Verification of critical operational parameters of decontamination solutions

   - Measurements of pH, FAC and temperature of bleach-based decontamination solution, or
   - Measurements of pH and temperature for RTU products
     - Results were recorded in a laboratory notebook.

4. Installation of aerosol sampling device in exhaust duct

   - A Zefon Via-Cell® Bioaerosol Sampling Cassette (p/n VIA010) coupled with EPA Method 5 Dry Gas meter box was used to collect air samples using the procedure described in Section 3.6

5. Installation of coupons in test chamber

   - Coupons were aseptically installed in the test chamber in the vertical orientation.
   - The position of each coupon was recorded.

6. Application of a prescribed decontamination sequence

   - In Phase I, bleach-based liquid decontaminant was applied using a sterilized rag or sponge wetted in the bucket of diluted bleach solution. The rag used was a 12 in by 12 in cotton cloth (Walmart, Durham, NC). The excess decontaminant was removed by gentle squeezing (wiping medium remained well wetted; this step was only to avoid dripping-wet wiping media), then decontamination solution was applied onto the coupon surface using a zigzag stroke technique. The coupon cleaning (wiping) action started at the upper right corner of the coupon. Using a zigzag stroke, the rag or sponge was firmly moved down and up the entire coupon surface. After application of decontaminant, the wiping medium was double-bagged in sterile bags for microbiological analysis.
   - In Phase II, RTU-COTS decontaminants were sprayed using the product-specific original spray bottle as provided by manufacturer (i.e., RTU products were not transferred to any secondary spray bottles or sprayers); each RTU-COTS solution will be applied onto the coupon surface until the surface is visibly wet, starting at the upper right corner of the coupon. Spraying then continued

from right to left, then moved slightly downward and continued from left-to-right, then right-to-left, until the entire coupon surface was covered with decontamination product.

- The time required for application of decontamination solution on each coupon was recorded. Loading volumes of decontaminant are given in Section 5.2.
- The runoff of decontaminant was collected in a clean sterile carboy loaded with neutralizer (sodium thidosulfate) that was placed under the drain of the chamber. The volume of neutralizer was determined in a series of preliminary experiments for single set (three coupons) of porous and nonporous material/wiping medium and/or decontamination solution combination.
- After a 10-minute processing time, the coupon surface was wiped using a new sterilized rag or sponge wetted in sterile water; the coupon surface was wiped with a wetted wiping medium using a zigzag stroke technique. The coupon water rinse (wiping) action always started at the upper right corner of the coupon. Using a zigzag stroke, the cloth or sponge was firmly moved down and up the entire coupon surface.
- The runoff of rinse water was collected into a separate clean sterile carboy loaded with neutralizer that was placed under drain of the chamber. The volume of neutralizer was determined in series of preliminary experiments using a single set (three coupons) of porous and nonporous material/wiping medium used for water rinse of decontaminant combination.

7. Post-decontamination procedures

   - Immediately after the rinse step was completed, the collected runoff and rinsates were characterized (volume, FAC, pH, temperature) and were taken for microbiological analyses. Coupons were moved to designated storage cabinets and allowed to dry overnight.
   - Visual inspection of the decontaminated building surfaces was then performed. Changes in color, reflectivity, and roughness were assessed qualitatively and observations were made and documented in the laboratory notebook and by digital photographs when surface change was observable.
   - After completion of the entire decontamination procedure, coupons were then moved to designated Coupon Storage Cabinets (non-contaminated control coupons were transferred to the Blank Coupon Cabinet, non-decontaminated coupons were transferred to the Positive Coupon Cabinet, decontaminated coupons were moved to Test Coupon Cabinet) and allowed to dry overnight. Coupons positions were recorded in the Coupon Tracker (Appendix).

8. Sampling and transfer of samples to the HSRP RTP Biocontaminant Laboratory for microbiological analysis

   - On the following day, coupons were sampled using sampling techniques described in Section 3.2.1 and transferred to the laboratory for microbiological analysis. Samples were transferred in sterile primary independent packaging within sterile secondary containment containing logical groups of samples for analysis. All samples were accompanied by a completed chain of custody (COC) form.

9. Determination of decontamination efficacy

   - After the HSRP RTP Biocontaminant Laboratory has performed a quantitative assessment of viable spores for each type of sample, the determination of surface decontamination efficacy will

be performed (comparison of viable spore concentrations from positive controls and test coupons; see Section 4 for details).

2.5.1. Test Matrix

Table 2-5 and Table 2-6 show the test matrix for Phase I and Phase II, respectively. The Phase I test matrix was developed as the tests progressed, based on the results (decontamination efficacy, LR obtained; see Figure 5-4 and 5-6 for details). The Phase II test matrix used processing time and rinse wiping medium selected after obtaining Phase I results (i.e., 10 minutes and cotton rag for rinse water application).

Table 2-5. Phase I Test Matrix

Test ID	Material Type	Decontamination Agent	Application Mode of Decontaminant	Application Mode of Rinse Water	Processing time
1	Finished wood flooring	Bleach 1:10	Rag	Rag	10 minutes
2	Glass	Bleach 1:10	Rag	Rag	10 minutes
3	Painted wallboard	Bleach 1:10	Rag	Rag	10 minutes
4	Grouted ceramic tile	Bleach 1:10	Rag	Rag	10 minutes
5	Finished wood flooring	Bleach 1:5	Rag	Rag	10 minutes
6	Glass	Bleach 1:5	Rag	Rag	10 minutes
7	Painted wallboard	Bleach 1:5	Rag	Rag	10 minutes
8	Grouted ceramic tile	Bleach 1:5	Rag	Rag	10 minutes
17	Finished wood flooring	Bleach 1:5	Sponge	Sponge	10 minutes
18	Glass	Bleach 1:5	Sponge	Sponge	10 minutes
19	Painted wallboard	Bleach 1:5	Sponge	Sponge	10 minutes
20	Grouted ceramic tile	Bleach 1:5	Sponge	Sponge	10 minutes
21	Finished wood flooring	Bleach with surfactant 1:5	Rag	Rag	10 minutes
22	Glass	Bleach with surfactant 1:5	Rag	Rag	10 minutes
23	Painted wallboard	Bleach with surfactant 1:5	Rag	Rag	10 minutes
24	Grouted ceramic tile	Bleach with surfactant 1:5	Rag	Rag	10 minutes
25	Finished wood flooring + grime	Bleach with surfactant 1:5	Rag	Rag	10 minutes

Table 2-6. Phase II Test Matrix

Test ID	Material Type	Decontamination Agent	Application Mode of Decontaminant	Application Mode of Rinse Water	Processing time
1	Finished wood flooring	Lysol® Mold and Mildew Blaster	Spray	Rag	10 minutes
2	Glass	Lysol® Mold and Mildew Blaster	Spray	Rag	10 minutes
3	Painted wallboard	Lysol® Mold and Mildew Blaster	Spray	Rag	10 minutes
4	Grouted ceramic tile	Lysol® Mold and Mildew Blaster	Spray	Rag	10 minutes
5	Finished wood flooring	Tilex® Mold and Mildew Remover	Spray	Rag	10 minutes
6	Glass	Tilex® Mold and Mildew Remover	Spray	Rag	10 minutes
7	Painted wallboard	Tilex® Mold and Mildew Remover	Spray	Rag	10 minutes
8	Grouted ceramic tile	Tilex® Mold and Mildew Remover	Spray	Rag	10 minutes
9	Finished wood flooring	Clorox® Clean-Up Cleaner + Bleach	Spray	Rag	10 minutes
10	Glass	Clorox® Clean-Up Cleaner + Bleach	Spray	Rag	10 minutes
11	Painted wallboard	Clorox® Clean-Up Cleaner + Bleach	Spray	Rag	10 minutes
12	Grouted ceramic tile	Clorox® Clean-Up Cleaner + Bleach	Spray	Rag	10 minutes
13	Finished wood flooring	Clorox® Disinfecting Bleach Foamer	Spray	Rag	10 minutes
14	Glass	Clorox® Disinfecting Bleach Foamer	Spray	Rag	10 minutes
15	Painted wallboard	Clorox® Disinfecting Bleach Foamer	Spray	Rag	10 minutes
16	Grouted ceramic tile	Clorox® Disinfecting Bleach Foamer	Spray	Rag	10 minutes

3. Sampling and Analytical Procedures

3.1. Types of Samples

Four major classes of operational samples were collected:

- Surface samples (see Section 3.2.1 for sampling procedures). Each test listed in Tables 2-5 and 2-6 included surface samples from each material collected in triplicate (Phase I) or quintuplicate (Phase II), positive control surface sampling in triplicate (Phase I and Phase II), and procedural blank surface samples (one per each Phase I and Phase II test).

- Liquid effluent samples (see Section 3.2.2 for sampling procedures). These samples were collected to assess the potential for viable spores to be washed off the surfaces. Composite samples of liquid waste (runoff of bleach solution and water rinsate) were collected and analyzed quantitatively for each test.

- Solid waste samples (see Section 3.2.3 for sampling procedures). Post-decontamination solid waste (rags and sponges) were important for understanding the fate of the spores and the transfer process as well as for determination of safe disposal of post-decontamination waste.

- Aerosol samples (see Section 3.2.4). Via-Cell® BioAerosol Cassette samples were collected during each decontamination and procedural blank test. These samples were used to estimate the occurrence and magnitude of fugitive emissions of viable *B. atrophaeus* spores during the decontamination process.

Additional QA/QC samples (see Section 3.3) included:

- Swab (sterility check) samples

- Material samples/field samples

- Decontamination solution samples.

3.2. Sampling Procedures

Within a single test, surface sampling of the materials was completed for all procedural blank coupons first 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 each protocol are included in the relevant sections below. The general sampling supplies were sterile or sterilized/disinfected for each sampling event. To ensure the integrity of samples and to maintain a timely and traceable transfer of samples, an established and proven chain of custody was strictly adhered to for each test.

3.2.1 Wipe Sampling

Wipe sampling is typically used for small sample areas and is effective on nonporous smooth surfaces such as ceramics, vinyl, metals, painted surfaces, and plastics. The general approach is that a moistened sterile non-cotton pad is used to wipe a specified area to recover bacteria, viruses, and biological toxins.

Sampling kits for wipes were prepared as specified in MOP 6568 (see Appendix B). All laboratory surfaces intended for use during sampling were wiped with Dispatch® bleach wipes (Clorox, Oakland, CA). Precut 50.8 cm by 50.8 cm (20 in by 20 in) sheets of absorbent bench liner were used to cover all work surfaces, replaced after each phase of a test (e.g., coupon contamination is considered one phase, decontamination another, and surface sampling a third). Sampling was conducted on only one coupon at a time. One coupon was moved from the Decontaminated Coupon Cabinet (test coupons), Test Coupon Cabinet (positive controls), or Procedural Blank Coupon Cabinet (procedural blanks) to the sampling space located immediately outside (to the front) of each cabinet. All coupons were placed horizontally for sampling, regardless of their orientation during the decontamination.

Within a single test, surface sampling of the coupons was performed starting with coupons from the lowest level of contamination and ending with the highest level of contamination (i.e., all procedural blank coupons first, followed by all test coupons, and then all positive control coupons).

Surface sampling was performed by wipe sampling in accordance with the protocols described in MOP 3144 (Appendix B). The surface area for all samples was 1175.8 cm^2 (1.3 ft^2). A template was used to cover the exterior 0.635 cm (0.25 in) of each coupon, leaving a square (34.29 cm by 34.29 cm) exposed for sampling for all coupons. The outer 0.635 cm of each coupon was not sampled to avoid edge effects.

A sampling material bin was stocked with all appropriate items for each sampling event. The bin contained enough wipe sampling kits to accommodate all required samples for the specific test. An additional kit was also included for backup. Enough gloves and bleach wipes needed to complete the test were available. Templates (35.6 cm by 35.6 cm (14 in by 14 in)) with an interior opening of 34.3 cm by 34.3 cm (13.5 in by 13.5 in) were wrapped in aluminum foil and packaged in sterile autoclave-safe bags (autoclave-sterilized using a one hour gravity cycle, 10 templates per bag) and transported with the original sterile coupons (concrete and stainless steel procedural blanks). These bags of templates were also included with the sampling kits. A sample collection bin was used to transport samples back to the Microbiology Laboratory. The exterior of the transport container was decontaminated by wiping all surfaces with a Dispatch® bleach wipe prior to transport from the sampling location to the HSRP RTP Biocontaminant Laboratory for analysis. The samples were stored at 4 ± 2 °C until processed.

3.2.2 Liquid Effluent Sampling

Liquid effluents from the decontamination process were collected using the central drain of the test chamber. Liquid effluents were collected into pre-weighted sterilized containers (amber glass bottles) preloaded with neutralizer - 2N STS (sodium thiosulfate) solution. Since neutralizer was added to the collection vessel before collection of liquid samples, the active (sporicidal) ingredient was neutralized as sample was being collected (not after collection). The runoffs and rinsates were collected as one composite sample for each test set. For each test, the total mass of liquid effluents collected was recorded for comparison of the collection vessel final weight versus the initial weight value. After collection, triplicate 100 mL aliquots were taken via aseptic technique using a new 100 mL sterile serological pipette and sterile 4 oz. container. The liquid effluent aliquots were then double-contained in sterile bags and transported to the HSRP RTP Biocontaminant Laboratory for analysis. The samples were stored at 4 ± 2 °C until processed.

3.2.3 Solid Waste Sampling

Once the application of bleach solution in Phase I was finished, the wiping medium (rag or sponge) was aseptically collected to 10” x 15” Twirl Em bag (Fisher cat no. 01-002-53) preloaded with neutralizer. This primary contained sample was then weighed, placed in a secondary sterile 10” x 15” bag and transported to the HSRP RTP Biocontaminant Laboratory for microbiological analysis. Samples were refrigerated at 4 ± 2 °C until processed.

3.2.4 Aerosol Sampling

Aerosol samples were collected from the chamber exhaust duct (Figure 3-1) using Via-Cell® BioAerosol Cassettes®. The sampling point was eight diameters downstream of and two diameters upstream of any flow disruptions.

Figure 3-1. Spray Chamber Exhaust Duct (white arrow shows the sampling point location)

A 4-in diameter galvanized duct that is 44 in long was attached to the chamber to allow for precise flow measurements and sampling. The duct was attached to the chamber using a coupling and a 90-degree

elbow. The sampling port was located 32 in (8 diameters) downstream from the 90-degree elbow that is connected to the chamber and 12 in (3 diameters) from the bend in the flexible duct that connects to the main exhaust (the sampling point location is indicated with a white arrow symbol on Figure 3-1). The exhaust air was sampled using a Via-Cell® and dry meter box operated according to MOP 3155. After sampling, the Via-Cell® cassette was placed into the special foil bag (provided by the manufacturer) and zip-closed. The red safety seal label was applied over the top of the foil bag opening to ensure sample integrity until analysis. The primary bag containing the cassette was then placed inside a pre-labeled 5.5 in x 15 in sterile bag for secondary containment. For each test day, there was a field blank (plain, unused Via-Cell® cassette). The samples were stored at 4 ± 2 °C until processed.

3.3. QA/QC Samples

3.3.1 Swab Samples

MOP 3135 (Appendix B) was used for collecting swab samples. The general approach was to use a moistened swab to wipe a specified area to recover bacterial spores. Swab samples were collected from all decontamination procedure equipment before and after use. Swab samples were also collected from materials before use as sterility checks.

3.3.2 Material Samples

Material samples provided information on the level of contamination possibly present during sampling due to contaminated materials, e.g., unused wipe kits and unused Via-Cell® cassettes were transferred from/to or to the HSRP RTP Biocontaminant Laboratory for microbiological analysis. The samples were referred to as unexposed field blank samples. There were also blank plating of microbiological supplies to check for sterility of supplies used in dilution plating.

3.3.3 Decontamination Solution Samples

Samples of decontamination solutions prepared in-house (Phase I) were evaluated for critical parameters (pH, FAC and temperature) prior to each test. The RTU-COTS products were experimentally evaluated for pH, FAC and temperature prior to start of Phase II testing, then pH and temperature were recorded prior to each decontamination test.

4. Determination of Sporicidal Effectiveness

The sporicidal effectiveness (efficacy) of a decontamination technique is a measure of the ability of the method to inactivate and/or remove the spores from a contaminated material surface (i.e., represented by coupons in this study). It is evaluated by measuring the difference in the logarithm of the measured colony forming units (CFU) before decontamination (determined from sampling the positive control coupons) and after decontamination (determined from sampling the test coupons) for the same type of material. The number of viable spores was measured as CFU and reported as a log reduction on the specific material surface as defined in Equation 3-1.

$$\eta_i = \frac{\sum_{k=1}^{N_C} \log(CFU_{C,k})}{N_C} - \frac{\sum_{k=1}^{N_t} \log(CFU_{S,k})}{N_s} \qquad (3\text{-}1)$$

where:

η_i = Surface decontamination effectiveness; the average log reduction of spores on a specific material surface (surface material designated by *i*)

$\frac{\sum_{k=1}^{N_C} \log(CFU_{C,k})}{N_C}$ = The average of the logarithm (or geometric mean) of the number of viable spores (determined by CFU) recovered on the control coupons (C indicates control and N_C is the number of control coupons)

$\frac{\sum_{k=1}^{N_t} \log(CFU_{S,k})}{N_s}$ = The average of the logarithm (or geometric mean) of the number of viable spores (determined by CFU) remaining on the surface of a decontaminated coupon (S indicates a decontaminated coupon and N_s is the number of coupons tested).

When no viable spores were detected, a value of 0.5 CFU was assigned to the maximum plated volume to determine the detection limit for $CFU_{S,k}$, and the efficacy was reported as greater than or equal to the value calculated by Eqn.3 -1.

The standard deviation of the average log reduction of spores on a specific material (η_i) is calculated by Eqn. 3-2:

$$SD_{\eta_i} = \sqrt{\frac{\sum_{k=1}^{N_s}(x_k - \eta_i)^2}{N_S - 1}} \quad (3\text{-}2)$$

where:

SD_{η_i} = Standard deviation of η_i, the average log reduction of spores on a specific material surface

η_i = The average log reduction of spores on a specific material surface (surface material designated by *i*)

x_k = The average of the log reduction from the surface of a decontaminated coupon

N_S = Number of test coupons of a material surface type.

and,

$$x_k = \frac{\sum_{k=1}^{N_s}((\overline{\log(CFU_c)} - \log(CFU_{s,k}))}{N_s} \quad (3\text{-}3)$$

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 k^{th} decontaminated coupon

N_s = Total number (1,k) of decontaminated coupons of a material type.

The surface log reduction, as calculated in accordance with Equation 3-1, represents the effectiveness of the decontamination in mitigating the contamination on selected building materials. The overall decontamination is a cumulative effect of inactivation of the spores resulting from oxidative stress after

application of a bleach-based sporicide or due to physical removal of spores from the material via wiping action. For physical removal, viable spores may either remain in the liquid waste or be re-aerosolized. Understanding the ultimate fate of the spores, not just the surface log reduction, is critical to recognizing the utility or appropriate implementation of the decontamination process. Process parameters (as well as the general nature of microbiological sampling) will not allow exact accounting of the fate of spores, or closing of the microbiological loading mass balance. However, the quantitative measurements of spores in post-decontamination waste and exhaust air are good indications of ultimate fate of viable spores. For the liquid and solid samples, the results are reported as total CFU per sample. The test-specific re-aerosolization rates are reported as CFU per volume of exhaust air sampled.

5. Results and Discussion

The primary objective of this study was to evaluate the efficacy of simple "self-help" methods for decontamination of building material surfaces using bleach-based decontamination solutions (prepared in house and commercially available products) basic tools expected to be easily accessible at residential dwellings (i.e., cleaning rags and sponges).

In addition to reduction of contamination from material surfaces, the fate of the spores was also considered important information for selection of the most effective decontamination procedure. The method that offers a satisfactory decontamination efficacy (> 6 LR), can be then translated to an optimized method (method manual, method tutorial) that is easily understandable to the general public/responders without specialized training. Any users of the products described in this report shall follow PPE recommendations listed on the label of the respective products.

This section discusses the results of characterization of decontamination solutions (Section 5.1), operational differences between individual decontamination procedures tested (Section 5.2) and the result of the determination of decontamination efficacy for each method/material combination (Section 5.3). The procedure-specific ultimate fate of the spores is discussed in Section 5.4.

5.1. Characterization of Decontamination Solutions

5.1.1 FAC Concentration

Concentration of FAC in the working decontamination solution was measured via by sodium thiosulfate/ potassium iodide titration using a commercial digital titrator (Hypochlorite (Bleach) HACH Test Kit, Model CN-HRDT; Cat No. 26871-00, Hach Company, Loveland, CO, USA). In Phase I, samples were taken from the solution container In Phase II samples were taken from the RTU-COTS product spray-bottles.

The concentration of free available chlorine in freshly prepared batches of diluted bleach solution in Phase I experiments was 6,600 ± 180 ppm for 1:10 bleach solution (n = 7), 14,000 ± 280 ppm for 1:5 bleach solution (n = 11), and 15,600 ± 670 ppm for 1:5 bleach with surfactant solution (Figure 5-1). Test-specific data for in-house prepared decontamination solutions are given in Table A-2 (Appendix A). The FAC, pH, and temperature were measured prior to testing and these are referred to as the initial assessments in Figures 5-1 through 5-3. These parameters were also measured prior to the decontamination tests and these are shown as ongoing assessments in Figures 5-1 through 5-3.

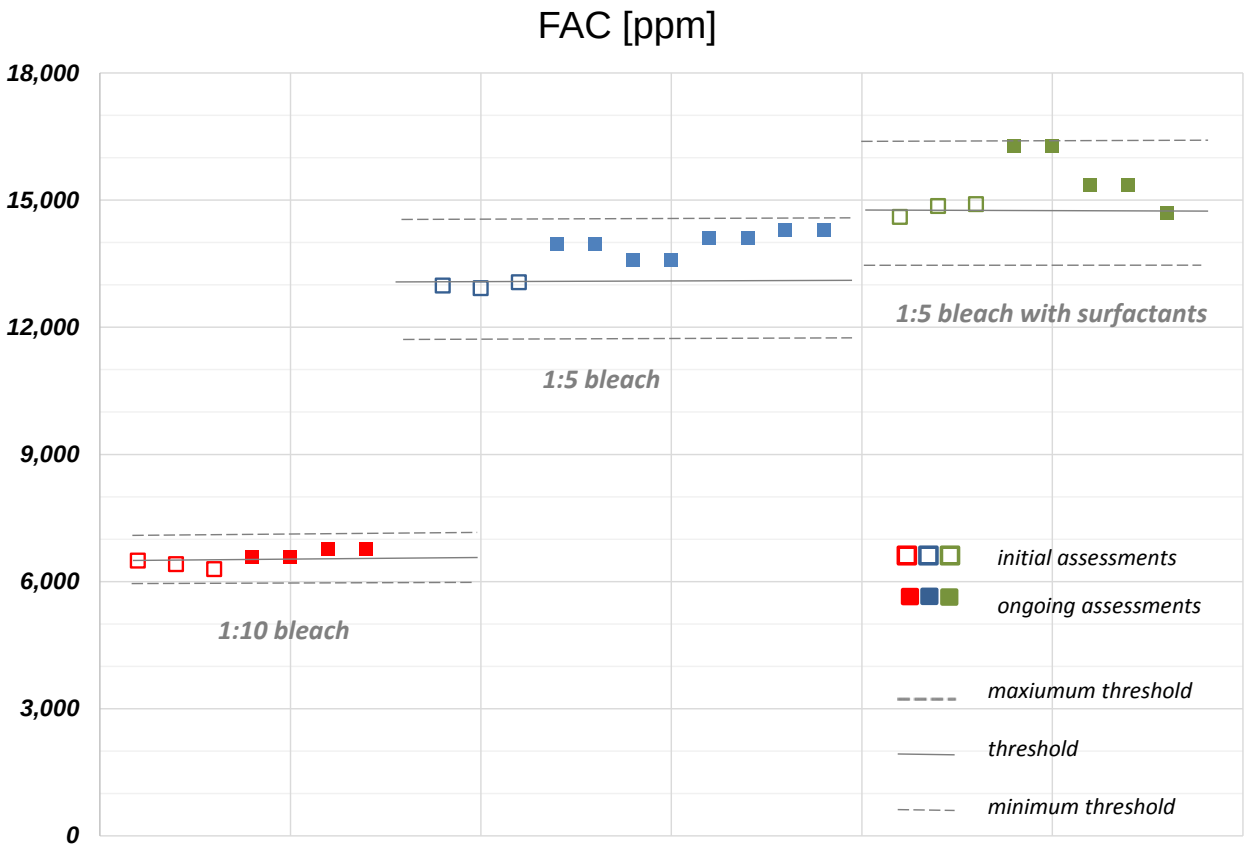


Figure 5-1. FAC of Diluted Bleach Solutions Prepared In-House (Phase I)

The FAC concentration in RTU-COTS products was 22,100 ± 1950 ppm. Product-specific FAC concentrations for decontaminants used in Phase II experiments are given in Table A-3 (Appendix A).

5.1.2 pH and Temperature

The pH and temperature measurements of decontamination solutions were performed using an Oakton pH meter equipped with a pH probe and a thermocouple probe (Oakton pH 5+, OAKTON Instruments, Vernon Hills, IL, USA).

The pH of freshly prepared batches of diluted bleach solution, as measured with the Oakton pH meter, in Phase I experiments was 11.4 ± 0.01 for 1:10 bleach solution (n = 7), 11.4 ± 0.17 for 1:5 bleach solution (n = 11), and 12.1 ± 0.01 for 1:5 bleach with surfactant solution (Figure 5-2). Test-specific data for in-house prepared decontamination solutions are given in Table A-2 (Appendix A).

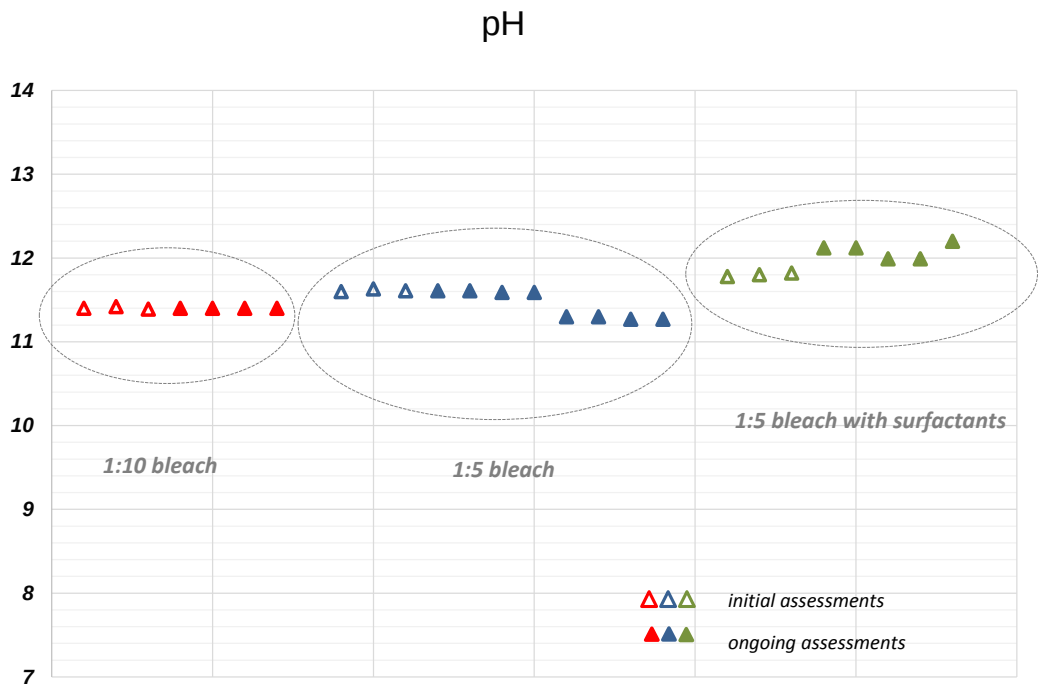


Figure 5-2. pH of Diluted Bleach Solutions Prepared In-House (Phase I)

Average temperature of freshly prepared batches of diluted bleach in Phase I of this study was 22.7 °C ± 1.2 °C. Test-specific data are shown in Figure 5-3 and in Table A-2 (Appendix A). Even though all experiments in this study were performed in the environment-controlled laboratory, the temperature variations of bleach solutions shown some minimal variation between batches prepared during colder and warmer weather conditions – batched prepared during the February-May timeframe (initial assessment tests, 1:10 dilution tests and majority of 1:5 dilution tests) had a post-mixing temperature of < 23 °C, batches prepared in June-August (latest four tests for 1:5 dilution bleach and all tests for 1:5 bleach with surfactants), had post-mixing temperature of > 23 °C (Figure 5-3). For RTU-COTS solutions used in this study temperature of decontaminants was 24.8 °C ± 1.2 °C (Table A-3, Appendix A).

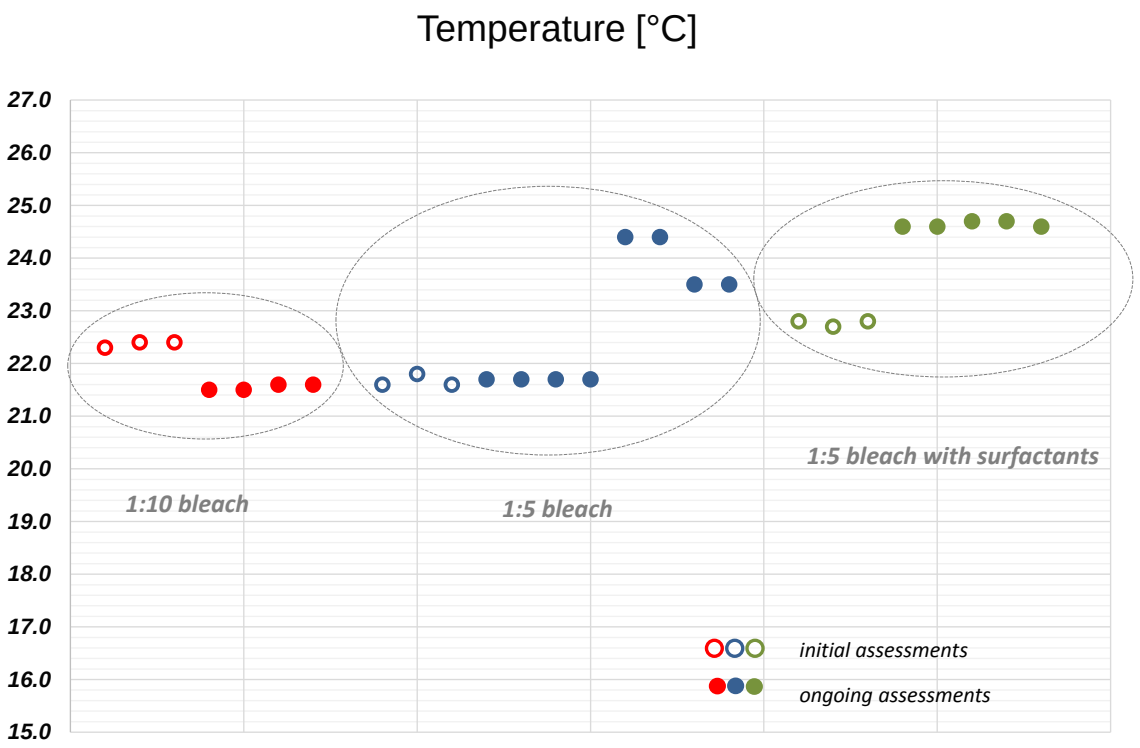


Figure 5-3. Temperature of Diluted Bleach Solutions Prepared In-House (Phase I)

5.2. Application Characterization

The critical procedural steps of decontamination - average surface loading volume of decontaminant (in milliliters (mL) per coupon, [mL/coupon]), average application time per coupon (seconds per coupon [sec/coupon]) and average processing time (in minutes per test [min/set]) were controlled and documented for each test.

5.2.1. Average application time and surface loading volumes and

Loading volumes were determined gravimetrically by weighing the wetted and expended (post-decontamination) wiping medium. The determined decontaminant loading rates varied significantly (average 34 mL per coupon, standard deviation (SD) = 17.3 mL, relative standard deviation (RSD) = 51%) between Phase I tests when solution was applied using a rag or a sponge (Table A-2). The reason for this variability is probably the variation in strength when the wiping medium was pressed against the material surface.

There was no obvious correlation between the surface loading (volume of decontaminant applied per coupon) and decontamination efficacy within the variation of the loading used in this study – samples with the highest average log reduction values (LR>7, e.g. test 21 and 22) were decontaminated on days with low to average surface loadings (27.1 ± 9.4 mL and 42 ± 4.1 mL per coupon, respectively; see Table A-2 and C-1 in Appendix A and C). Tests that had the lowest average log reduction values (LR<6) for generally

efficacious 1:5 bleach concentration (test 18 and 19) had above-average or high surface loadings of decontaminant (85.3 ± 42.2 mL and 34.7 ± 23.5 mL per coupon, respectively; Table A-2 and C-1 in Appendix A and C). A more systematic study is needed to investigate how the manual application pressure (and consequent mechanical removal of spores) versus amount of decontamination solution applied to the surface can affect the decontamination efficacy.

The Phase II replicate applications were very consistent between each type of RTU-COTS decontamination products: 20 ± 3.8 mL for Lysol® Mold & Mildew Blaster, 17.4 ± 0.86 mL for Tilex® Mold & Mildew Remover, 19.7 ± 4.4 mL for Clorox® Clean-up Cleaner + Bleach, 41.6 ± 1.83 mL Clorox® Disinfecting Bleach Foamer) (Table A-3, Appendix A). The higher loading observed for Clorox® Disinfecting Bleach Foamer is probably due to the foaming characteristics of this product (more solution had to be sprayed for the surface to appear to be "visibly wet" – surface looks wet after the foam collapses). There was no correlation between amount of RTU-COTS sprayed onto surface and decontamination efficacy (Table A-3 and C-2 in Appendix A and C) - Phase II tests all had decontamination efficacies greater than 6 logs.

The average time need for application of the decontamination solution either via wiping medium (Phase I) or via spraying (Phase II) was approximately 8-20 seconds per 14x14 inch coupon (Table A-2 and A-3, Appendix A), and varied very slightly (RSD<10%) between decontamination product and material type combinations, suggesting that the proposed decontamination methods are quickly and reproducibly deployable, and that obtaining a visible coverage with decontaminant (wet surface) is a good indicator of the successful application of the decontaminant.

5.3. Decontamination Results

5.3.1. Inoculation Results (Positive Controls)

In this study, test and positive control coupons of porous and nonporous building materials have been inoculated with aerosol-deposited *Bacillus* spores at 1 E7. Test specific results for positive controls (CFU/sample) are given in Table C-1 and C-2 (Appendix C). The average CFU recovered from positive control coupons in Phase I was 2.3 E7, with an average coefficient of variance of 25% (Table C-1, Appendix C). In Phase II, the average surface loading was 4.2 E7, with an average coefficient of variance of 29% (Table C-2, Appendix C). The average CFU recovery values for the positive controls indicate that the initial spore loading allowed decontamination experiments with a target sporicidal effectiveness ≥ 6 log reduction (LR). The average variability suggests a reproducible surface-sampling of spores. Only three tests had a variation in positive control CFU higher than 50% percent for some tests, probably because of natural variations in the coupon surface roughness. Presumably, test coupon sampling was similarly affected, hence the variability in the initial loading/CFU recovery of positive controls was not considered a source of methodical bias in calculations of the log reduction values for tests with higher (>50%) variability in CFU recovery.

5.3.2. Log Reduction Results

Tables C-1 and C-2 in Appendix C and Figure 5-4 and 5-5 summarize decontamination product-specific log reduction values. These data suggest that bleach-based liquid sporicide provides greater than 6 log reduction of spore loading on most of the common building materials tested, at concentrations of

hypochlorite of approximately 1.5% or greater, using either in-house bleach solutions applied via cotton rag processed for 10 minutes and wiped with water using clean cotton rag, or RTU-COTS applied via spraying, processed for 10 minutes and wiped with water using a clean cotton rag. The sponge application tested in Phase I seemed to be less efficacious than a cotton rag application, perhaps due to less scrubbing action offered by the soft sponge.

Material-specific results (Figures 5-6 and 5-7) suggest that efficient decontaminants (bleach dilution 1:5, bleach with surfactant dilution 1:5, all bleach-based RTU-COTS tested) performed similarly, independent of the type of material treated. The average lower LR observed for glass and bleach dilution 1:10 applied by sponge is due to the presence of an outlier that has surface decontamination efficacy almost 2 logs below an average LR for this test (LR=4.1 compared to LR of 7.7 and 5.3 for the remaining two samples in a subset).

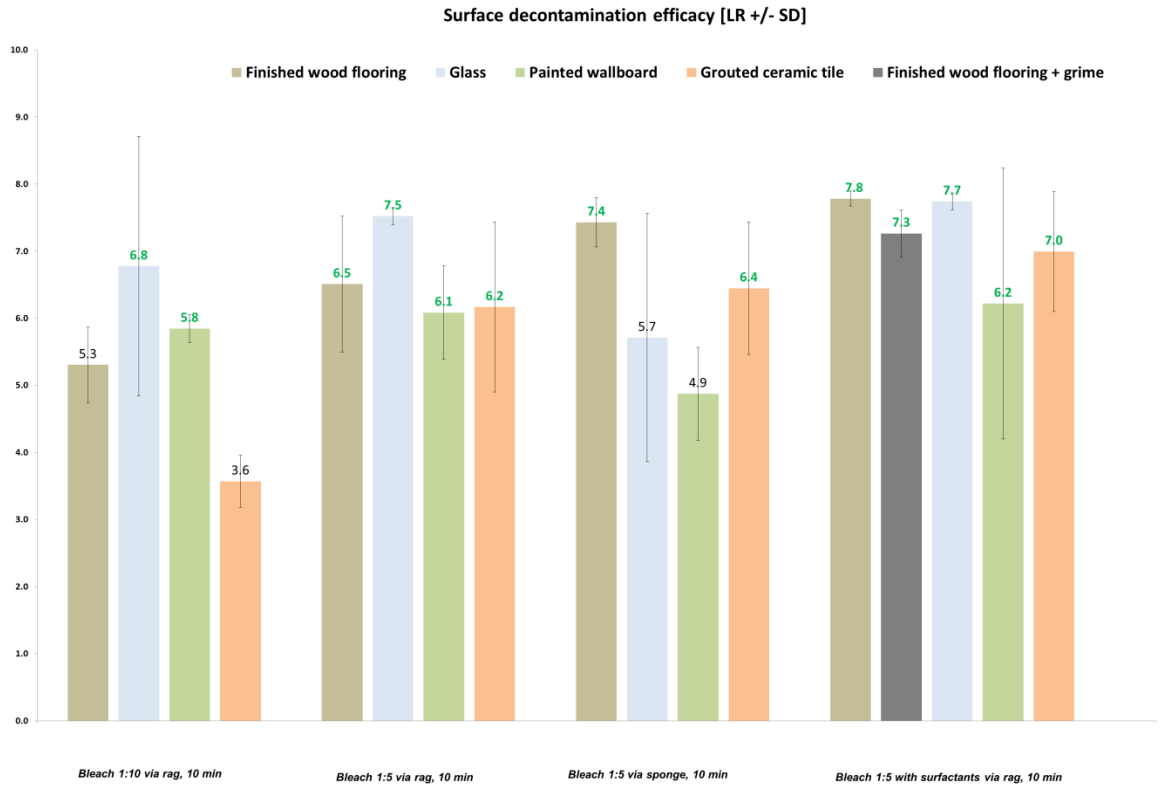


Figure 5-4. Decontaminant Specific LR values for Phase I (values in green – average target decontamination efficacy of LR>6)

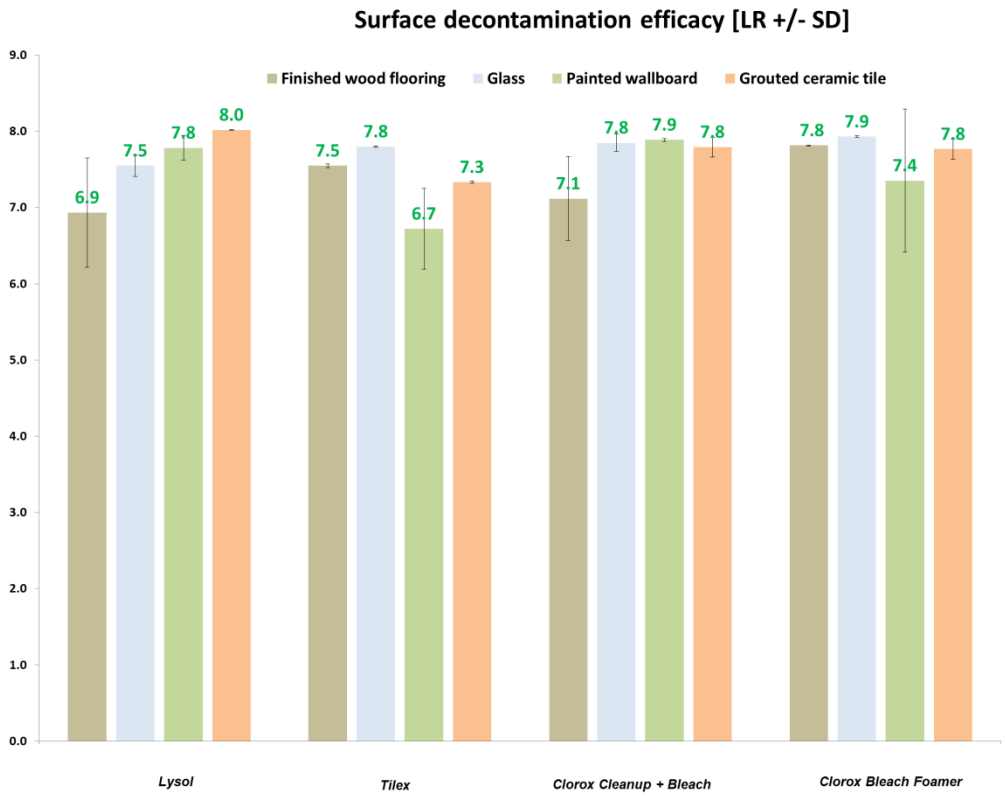


Figure 5-5. Decontaminant Specific LR values for Phase II (values in green – average target decontamination efficacy of LR>6)

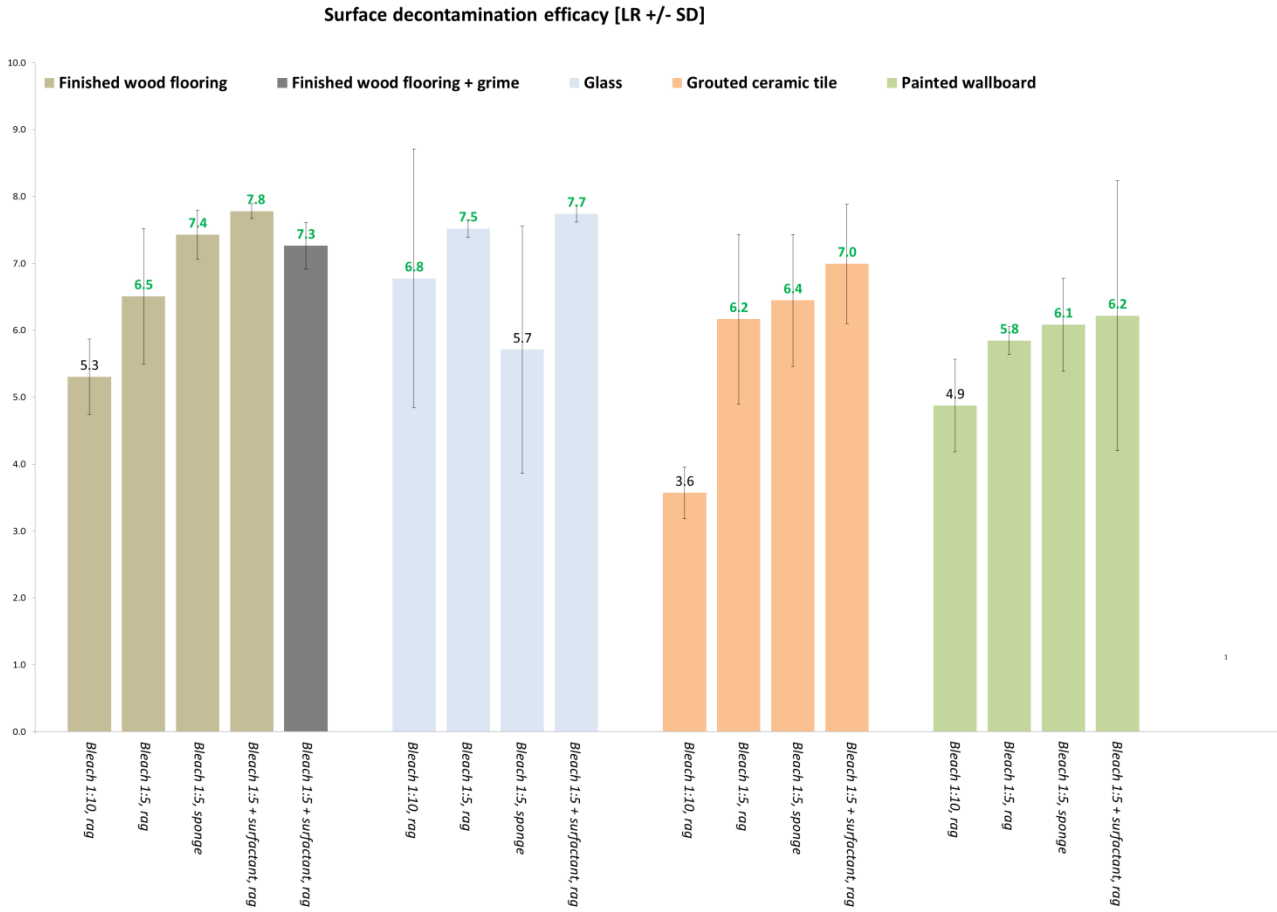


Figure 5-6. Material Specific LR Values for Phase I (values in green – average target decontamination efficacy of LR>6)

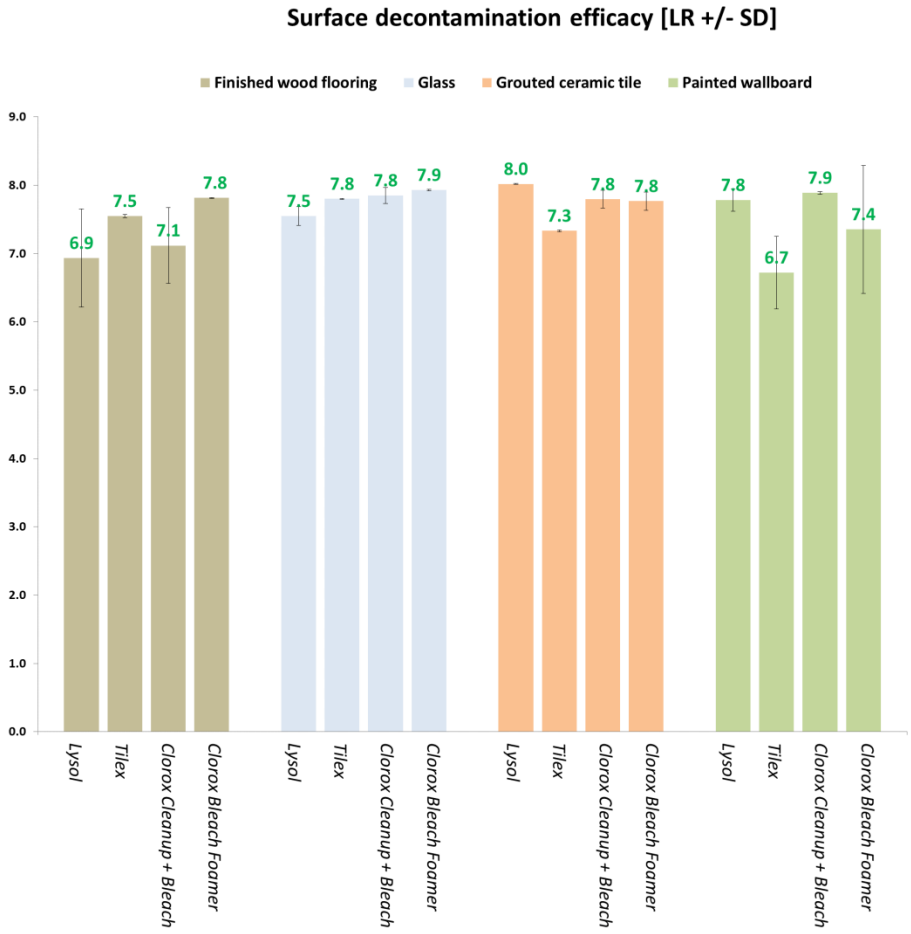


Figure 5-7. Material Specific LR Values for Phase II (values in green – average target decontamination efficacy of LR>6)

A comparison between Phase I and Phase II results suggest that RTU-COTS offer superior decontamination efficacy (>> 6 LR) for all building materials tested. The efficiency of RTU-COTS is not statistically significant among tested products (one-way ANOVA, p-value=0.58, $F=0.66<F_{crit}=3.49$). The results indicate that a decontamination approach using RTU-COTS products may be a better option for self-help decontamination approaches as spray application reduces contact with contaminated material prior to deactivation of spores. The second step (water rinse) is helpful (see Section 5.4.2 for results of spore-transfer to liquid effluents) is helpful with achieving a desired decontamination value (>6 LR). This step is also important in terms of method compatibility with bleach-sensitive materials (e.g., stainless steel). Results show a 1-2 Log level in rinsates for Phase 1 and Phase II.

5.4. Fate of Spores

The exposure to *B. anthracis* re-aerosolized during the decontamination operations is a potential source of exposure to personnel performing cleaning. The lethal dose where 50% of the population would be killed (LD50) for *B. anthracis* in humans is not definitively known, but based on animal data is estimated to be in the range of 4,100-10,000 inhaled spores. The survival of spores in the post-decontamination waste brings into consideration other possible routes of accidental exposure of the person performing decontamination through cuts and scrapes, i.e., via a cutaneous pathway.

5.4.1. Exhaust air concentrations

The composite air samples were taken using ViaCell® BioAerosol cassettes during application and exposure (processing) and rinsing phases of each decontamination test (i.e., results in Table 5.5.are for spores re-aerosolized from three or five coupons, respectively). Table 5-1 shows CFU detected for each type of material decontaminated in Phase I and Phase II (test specific results are given in Table C-1 and C-2 in Appendix C). These results suggest that decontamination procedures in Phase I and II have similar spore re-aerosolization rates. The highest number of viable spores detected in all air samples was approximately 10 CFU per cubic foot (Table C-1 and C-2, Appendix C). Although this value appears to be low, it should be emphasized that it represents the re-aerosolized spores from five 14x14 inch coupons (total area of approximately 6.0 ft^2). The decontamination cycle phase-specific aerosolization rates were not systematically investigated in this study, but the initial scrubbing or spraying stage is presumably the phase when most of the spores are aerosolized.

Table 5-1. Air Sample Results

	Phase I	Phase II
Material type (n=number of tests)	CFU/ft^3 (± SD)	
Finished wood flooring (n = 4)	7.9E-01(±1.4E+00)	3.5E+00 (±3.2E+00)
Grimed finished wood flooring (n = 1)	3.84+00	NA*
Glass (n = 4)	1.3E+00(±1.7E+00)	4.0E+00 (±4.4E+00)
Painted wallboard (n = 4)	2.3E+00 (±3.4E+00)	1.2E+00 (±1.6E+00)
Grouted ceramic tile (n = 4)	1.0E+00 (±1.0E+00)	2.0E+00 (±3.2E+00)

** not applicable - grimed surfaces were not tested in Phase II*

5.4.2. Liquid waste concentrations

Tables 5-2 and 5-3 show CFU recovered from the liquid waste (run-off and rinsate) samples; test specific results are given in Table C-1 and C-2 (Appendix C).

Table 5-2. Run-off Samples Results

Material type (n = number of tests)	Phase I	Phase II
	CFU/sample (± SD)	
Finished wood flooring (n = 4)	1.3E+03 (±1.1E+03)	2.3E+02 (±4.5E+02)
Grimed finished wood flooring (n = 1)	6.6E+01	NA*
Glass (n = 4)	1.1E+03 (±1.4E+03)	6.5E+00 (±6.7E+00)
Painted wallboard (n = 4)	2.6E+03 (±2.8E+03)	5.3E+00 (±5.3E+00)
Grouted ceramic tile (n = 4)	1.8E+03 (±3.2E+03)	4.0E+00 (±6.3E+00)

** not applicable - grimed surfaces were not tested in Phase II*

Table 5-3. Rinsate Sample Results

Material type (n = number of tests)	Phase I	Phase II
	CFU/sample (± SD)	
Finished wood flooring (n=4)	1.2E+01(±1.9E+01)	3.2E+01 (±4.9E+01)
Grimed finished wood flooring (n=1)	3.6E+01	NA*
Glass (n=4)	5.6E+01(±1.4E+01)	9.7E+00 (±6.9E+00)
Painted wallboard (n=4)	1.2E+02(±2.2E+02)	2.4E+00 (±2.9E+00)
Grouted ceramic tile (n=4)	1.4E+01(±2.1E+01)	3.4E+00 (±3.4E+00)

** not applicable -grimed surfaces were not tested in Phase II*

The average number of spores detected in the Phase I liquid waste was at about an average 3 log level in runoff samples and an average 1-2 log level in rinsate samples. In Phase II, the liquid waste was less contaminated, at an average 1-2 log level for runoff and average 0.5-1 log level in rinsates. These results were anticipated, since the Phase I procedures - with one extra step that involves mechanical wiping/scrubbing – were expected to result in a greater rate of physical removal of spores/higher spore transfer to the rinsate. In real-life decontamination scenarios, runoff and rinsate would not be neutralized, hence the sporicidal action of the decontaminant would have continued, resulting in reduction or complete deactivation of biological burden in the waste.

5.4.3. Solid waste concentrations

Table 5-4 shows CFU recovered from the post-decontamination solid waste samples (expended rags and sponges); test specific results are given in Table C-1 and C-2 (Appendix C).

Table 5-4. Solid-waste samples results

Material type (n = number of tests)	Phase I	Phase II
	CFU/sample (± SD)	
	Wiping medium # 1 (application of decontaminant step)	
Finished wood flooring (n = 4)	3.4E+03(±6.9E+03)	NA*
Grimed finished wood flooring (n = 1)	4.4E+01	NA*,**
Glass (n = 4)	1.4E+04(±2.4E+04)	NA*
Painted wallboard (n = 4)	7.7E+04(±1.0E+05)	NA*
Grouted ceramic tile (n = 4)	3.5E+04(±2.9E+04)	NA*
	Wiping medium # 2 (rinse step)	
Finished wood flooring (n = 2-4)	9.0E+01 (±1.2E+02)	4.0E+02 (±5.1E+02)
Grimed finished wood flooring (n = 1)	3.4E+00	NA**
Glass (n = 2-4)	8.3E+01 (±1.1E+02)	8.5E+01 (±1.5E+02)
Painted wallboard (n = 2-4)	4.8E+03 (±6.8E+03)	5.2E+02 (±1.0E+03)
Grouted ceramic tile (n = 2-4)	1.1E+03 (±1.5E+03)	4.6E+01 (±7.9E+01)

** not applicable – decontaminants were sprayed on in Phase II; ** not applicable - grimed surfaces were not tested in Phase II*

The contamination of expended wiping media used in Phase I and II is similar to or higher than the levels observed in the liquid waste (typically around 1-4 log level). In Phase I, rags and sponges used for application of bleach were more contaminated than rinse media. Similarly to contaminated liquid waste, the residual biocontamination of solid waste generated in real-life scenarios would most likely decrease over time (rags and sponges in this study were neutralized to allow qualitative analysis of spores). Still, it is extremely important to emphasize the importance of use of appropriate personal protective equipment and proper disposal of these waste (e.g., triple bagging) after the self-help decontamination procedure is concluded.

6. Summary

Most tests performed during Phase I and II achieved the target efficacy from surfaces of > 6 LR. The decontamination was achieved by a synergistic action of inactivation of spores by oxidizing agent (bleach) and mechanical removal. Of the procedures tested, those incorporating RTU-COTS containing bleach with surfactant/surfactants were more effective (>> 6LR). The results indicate that the Phase II decontamination approaches were comparable in decontaminating all types of tested materials (both porous and nonporous).

The fate of the biological spores showed secondary fugitive emissions of spores into aerosol fractions, which suggests that spores may be expected in indoor environments during self-help decontamination and potentially spread contamination throughout a residential dwelling and/or heating, ventilation and air

conditioning (HVAC) system. Viable spores were also found in liquid and solid post-decontamination waste that can therefore be a source of cross-contamination during decontamination and a potential human exposure hazard to persons performing self-help cleaning. It is noted that the reaerosolization of spores was measured for spore surface concentrations where these methods would not be recommended.

Impact of Study

One of the primary goals of this project was to demonstrate the effectiveness of RTU-COTS products for inactivating *Bacillus* spores on a variety of surfaces. The products identified in this project could be used by a homeowner as an option that could be used as a risk reduction measure in the event of a wide area release of *Bacillus anthracis*. The RTU-COTS products could also be used in conjunction with high tech methods (e.g., fumigation) to treat hot spots that remain after treatment.

Safety Guidelines

• Spores can also be released to the air, and can therefore be a significant source of cross-contamination during a remediation and can pose health risks to persons performing decontamination. The final “self-help” method tutorial/ instructional document resulting from this project should therefore emphasize the importance of using the required personal protective equipment (PPE).

• Users of this guidance should follow PPE recommendations on the product label when using these products. When using bleach or other disinfectants and cleaners follow the instructions on the labels for precautions, use, and personal protective equipment (PPE) recommendations.

• Personal Protective Equipment (PPE) should be used when using cleaning and disinfectant products. PPE may include protection for skin and eyes such as gloves and goggles. Other PPE should be employed only per product labels.

• Never mix cleaning products together, unless specifically allowed by the product label.

7. References

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Appendix A. Characterization of decontaminants and decontamination procedures

Table A-1. Phase I Decontamination Solution Recipes

Clorox® Concentrated Germicidal Bleach **1:10 dilution**	
DI water [mL]	9000 mL
Clorox® Concentrated Germicidal Bleach [mL]	1000 mL
Clorox® Concentrated Germicidal Bleach **1:5 dilution**	
DI water [mL]	8000 mL
Clorox® Concentrated Germicidal Bleach [mL]	2000 mL
Clorox® Splashless Bleach **1:5 dilution equivalent**	
DI water [mL]	5860 mL
Clorox® Splashless Bleach [mL]	4140 mL

Table A-2. Characterization of decontamination solutions and process parameters in Phase I

Test ID	Material Type	Decontamination Agent	Application Mode	Decontamination Solution Characterization					Run-off Characterization (neutralized)			Rinsate Characterization			Process Characterization					
				FAC [ppm]	Cl2 [g/L]	NaClO [%]	pH	T [°C]	NaClO [%]	pH	T [°C]	NaClO [%]	pH	T [°C]	Average Surface Loading Volume [mL+/- SD]		Average Application Time [h:min:sec +/- SD]		Average Processing Time [h:min:sec +/- SD]	
Initial Characterization		Bleach 1:10		6400	6.40	0.67	11.4	22.4												
1	Finished wood flooring	Bleach 1:10	Rag	6570	6.57	0.69	11.4	21.5	ND	4.27	21.90	ND	10.33	22.10	25.9	0.95	0:00:14	0:00:03	0:23:00	0:04:00
2	Glass	Bleach 1:10	Rag	6570	6.57	0.69	11.4	21.5	ND	9.34	22.00	ND	10.69	21.90	26.8	10	0:00:19	0:00:01	0:11:00	0:02:00
3	Painted wallboard	Bleach 1:10	Rag	6770	6.77	0.71	11.4	21.6	ND	3.86	21.70	ND	9.43	21.90	37.4	5.3	0:00:17	0:00:00	0:10:00	0:00:00
4	Grouted ceramic tile	Bleach 1:10	Rag	6770	6.77	0.71	11.4	21.6	ND	5.21	21.70	ND	9.91	21.80	21.9	9.6	0:00:17	0:00:01	0:10:00	0:00:00
Initial Characterization		Bleach 1:5		13000	13.0	1.36	11.6	21.7												
5	Finished wood flooring	Bleach 1:5	Rag	13962	13.96	1.47	11.6	21.7	ND	3.89	22.00	ND	7.26	22.10	16.7	6.2	0:00:15	0:00:02	0:10:00	0:00:00
6	Glass	Bleach 1:5	Rag	13962	13.96	1.47	11.6	21.7	ND	3.18	22.60	ND	9.49	22.30	28.2	4.1	0:00:16	0:00:02	0:10:00	0:00:00
7	Painted wallboard	Bleach 1:5	Rag	13581	13.58	1.43	11.6	21.7	ND	5.64	22.30	ND	8.47	21.80	27.03	13.7	0:00:14	0:00:02	0:10:00	0:00:00
8	Grouted ceramic tile	Bleach 1:5	Rag	13581	13.58	1.43	11.6	21.7	ND	3.57	21.90	ND	5.58	22.30	16.3	9.7	0:00:15	0:00:02	0:10:00	0:00:00
17	Finished wood flooring	Bleach 1:5	Sponge	14102	14.10	1.48	11.3	24.4	ND	4.15	24.30	ND	6.87	24.40	31.6	14.8	0:00:07	0:00:01	0:10:00	0:00:00
18	Glass	Bleach 1:5	Sponge	14102	14.10	1.48	11.3	24.4	ND	4.01	24.10	ND	9.51	24.20	85.3	42.2	0:00:07	0:00:01	0:10:00	0:00:00
19	Painted wallboard	Bleach 1:5	Sponge	14302	14.30	1.50	11.3	23.5	ND	6.12	24.10	ND	9.69	23.90	34.7	23.5	0:00:07	0:00:02	0:10:00	0:00:00
20	Grouted ceramic tile	Bleach 1:5	Sponge	14302	14.30	1.50	11.3	23.5	ND	4.11	23.90	ND	10.21	23.80	61.2	8	0:00:06	0:00:00	0:10:00	0:00:00
Initial Characterization		Bleach with surfactant 1:5		14800	14.8	1.55	11.8	22.8												
21	Finished wood flooring	Bleach with surfactant 1:5	Rag	16265	16.27	1.71	12.1	24.6	ND	9.72	25.60	ND	8.93	25.50	27.1	9.4	0:00:12	0:00:02	0:10:00	0:01:00
22	Glass	Bleach with surfactant 1:5	Rag	16265	16.27	1.71	12.1	24.6	ND	9.14	25.7	ND	5.15	25.7	42.5	4.1	0:00:13	0:00:01	0:10:00	0:01:00
23	Painted wallboard	Bleach with surfactant 1:5	Rag	15364	15.36	1.61	12.0	24.7	ND	5.64	22.3	ND	8.47	21.8	29.7	15	0:00:14	0:00:01	0:10:00	0:00:00
24	Grouted ceramic tile	Bleach with surfactant 1:5	Rag	15365	15.37	1.61	12.0	24.7	ND	3.57	21.9	ND	5.58	22.3	31.4	4.5	0:00:13	0:00:01	0:10:00	0:00:00
25	Finished wood flooring + grime	Bleach with surfactant 1:5	Rag	14702	14.70	1.54	12.2	24.6	ND	8.86	25.1	ND	6.77	25.2						

Table A-3. Characterization of Decontamination Solutions and Process Parameters in Phase II

Test ID	Material type	Decon Agent	Application Mode	Decontamination Solution Characterization					Run-off Characterization (neutralized)			Rinsate Characterization			Process Characterization					
				FAC [ppm]	Cl2 [g/L]	NaClO [%]	pH	T [°C]	NaClO [%]	pH	T [°C]	NaClO [%]	pH	T [°C]	Average Surface Loading		Average Application Time [h:min:sec +/- SD]		Average Processing Time [h:min:sec +/- SD]	
Initial Characterization				24024	24.02	2.52	12.2	24.3												
1	Finished wood flooring	Lysol® Mold & Mildew Blaster	Spray/Rag						ND	7.24	22.30	ND	9.96	21.10	16.80	2.60	0:00:09	0:00:01	0:10:30	0:00:36
2	Glass	Lysol® Mold & Mildew Blaster	Spray/Rag						ND	6.77	22.40	ND	9.59	22.30	16.80	2.60	0:00:09	0:00:01	0:11:00	0:00:48
3	Painted wallboard	Lysol® Mold & Mildew Blaster	Spray/Rag						ND	8.24	21.90	ND	9.16	22.60	21.10	0.80	0:00:08	0:00:01	0:10:00	0:00:00
4	Grouted ceramic tile	Lysol® Mold & Mildew Blaster	Spray/Rag						ND	3.68	23.10	ND	8.52	22.60	24.60	1.80	0:00:10	0:00:01	0:10:00	0:00:00
Initial Characterization				23743	23.74	2.49	12.6	24.6												
5	Finished wood flooring	Tilex® Mold & Mildew Remover	Spray/Rag						ND	9.50	22.60	ND	9.64	22.20	17.90	0.50	0:00:10	0:00:01	0:10:18	0:00:30
6	Glass	Tilex® Mold & Mildew Remover	Spray/Rag						ND	10.04	23.70	ND	9.91	23.80	17.20	0.80	-	-	0:10:00	0:00:00
7	Painted wallboard	Tilex® Mold & Mildew Remover	Spray/Rag						ND	9.66	22.50	ND	9.12	22.20	16.20	1.40	0:00:11	0:00:02	0:10:00	0:00:00
8	Grouted ceramic tile	Tilex® Mold & Mildew Remover	Spray/Rag						ND	9.50	22.60	ND	9.64	22.20	18.10	2.10	0:00:10	0:00:01	0:10:00	0:00:00
Initial Characterization				20117	20.12	2.11	12.6	24.8												
9	Finished wood flooring	Clorox® Clean-up Cleaner + Bleach	Spray/Rag						ND	10.08	21.40	ND	9.98	21.40	16.10	0.90	0:00:08	0:00:01	0:10:00	0:00:00
10	Glass	Clorox® Clean-up Cleaner + Bleach	Spray/Rag						ND	10.32	21.20	ND	10.00	21.50	16.10	0.90	0:00:07	0:00:01	0:10:00	0:00:00
11	Painted wallboard	Clorox® Clean-up Cleaner + Bleach	Spray/Rag						ND	NA	NA	ND	NA	NA	21.40	2.20	0:00:10	0:00:01	0:10:00	0:00:00
12	Grouted ceramic tile	Clorox® Clean-up Cleaner + Bleach	Spray/Rag						ND	NA	NA	ND	NA	NA	25.00	2.00	0:00:11	0:00:00	0:10:32	0:00:54
Initial Characterization				20518	20.52	2.15	12.6	25.4												
13	Finished wood flooring	Clorox® Disinfecting Bleach Foamer	Spray/Rag						ND	11.27	22.10	ND	9.48	22.30	42.60	3.40	0:00:20	0:00:01	0:10:00	0:00:00
14	Glass	Clorox® Disinfecting Bleach Foamer	Spray/Rag						ND	10.95	26.4	ND	8.82	22.8	39.30	2.20	0:00:18	0:00:01	0:10:00	0:00:00
15	Painted wallboard	Clorox® Disinfecting Bleach Foamer	Spray/Rag						ND	11.95	21.8	ND	9.93	17.5	40.90	3.50	0:00:16	0:00:01	0:10:00	0:00:00
16	Grouted ceramic tile	Clorox® Disinfecting Bleach Foamer	Spray/Rag						ND	11.07	21.6	ND	8.69	22	43.40	3.90	0:00:17	0:00:01	0:10:00	0:00:00

Appendix B. Miscellaneous Operating Procedures

MOP 3135

TITLE: Procedure for Sample Collection using BactiSwab™ Collection and Transport Systems

SCOPE: This MOP describes the procedure for collecting swab samples for Low Tech Decontamination Technique Testing

PURPOSE: The purpose if this MOP is to ensure all swab sampling is performed in a consistent manner.

Equipment/Reagents

- Disposable lab coat
- Nitrile examination gloves
- P95 Respirator
- Shoe covers
- Bouffant cap
- Safety glasses
- BactiSwab™ Collection and Transport System

1.0 PROCEDURE

1. Before starting the swabbing procedure, make sure you are wearing the appropriate, project-specific PPE (at a minimum gloves, lab coat, and safety glasses).
2. Through the sleeve, crush the BactiSwab™ ampule at midpoint.
3. Hold BactiSwab™ tip end up for at least five seconds to allow the medium to wet the swab.
4. Open the package and remove the BactiSwab™.
5. Label the plastic tube appropriately using the following scheme:

X-Y-N where,

X is the test number,
Y is the material abbreviation, and
N is the material number

6. Remove the cap-swab from the plastic tube.
7. Swab the surface while spinning the cap-swab between the thumb and index fingers. Swabbing should be conducted by following the recommend guidelines for each material as detailed in the project documentation (usually the QAPP).
8. Return cap-swab to tube.
9. Date and initial each sample tube. Enter this information into the lab notebook.
10. Complete the chain of custody form and relinquish the samples to the BioLab.

MOP-3144

TITLE: PROCEDURE FOR WIPE SAMPLING OF COUPONS

SCOPE: This MOP describes the procedure for wipe sampling both small and large coupons.

PURPOSE: The purpose of this MOP is to ensure consistent and representative sampling of such coupons.

EQUIPMENT (quantities are per sampling kit)

- Sterile sampling bag (10" x 14") – outer bag
- Sterile sampling bag (5.5" x 9 ") – inner "sample collection sterile sampling bag"
- Two sterile 50 mL Falcon Blue-Max™ Polypropylene Conical Tubes
- Sterile Kendall (ref. # 8402) 4-ply all-purpose sponge
- Sterile phosphate buffered saline with 0.005% TWEEN®-20, prepared according to MOP -6562
- Pipette or other method for aseptic dispensing of 5 mL liquid
- Sterile Posi-grip© forceps
- P-95 Particulate Respirators – to prevent contamination and for respiratory protection. (Specific projects may require additional respiratory protection and will be addressed in the project Quality Assurance Project Plan (QAPP), e.g., SAR)
- Powder-free Nitrile gloves (support person) and Kimtech Pure G3 Sterile Nitrile gloves (sampler)
- Dispatch® bleach wipes

1.0 PREPARATION

1. All materials needed for collection of each sample will be prepared in advance using aseptic technique. A sample kit for a single wipe sample will be prepared as follows:
    a. Two sterile sampling bags (10" x 14", 5.5" x 9") and a 50 mL conical tube, capped, will be uniquely labeled as specified in the project QAPP. These bags and conical tube will have the same label. The 5.5" x 9" labeled sterile sampling bag will be referred to as the sample collection sampling bag.

    b. A sterile all-purpose sponge will be placed in an unlabeled sterile 50 mL

conical tube using sterile forceps and aseptic technique. The all-purpose sponge will be moistened by adding 2.5 mL of sterile phosphate buffered saline with 0.005% TWEEN®-20. The tube will then be capped.

c. The labeled 50 mL conical tube (capped), the unlabeled conical tube containing the pre-moistened all-purpose sponge, and the 5.5" x 9" labeled sampling bag will be placed into the 10" x 14" labeled sampling bag. Hence, each labeled sampling bag will contain a labeled 50 mL conical tube (capped), an unlabeled capped conical tube containing a pre-moistened all-purpose sponge, and an empty labeled sampling bag.

d. Each prepared bag is one sampling kit.

2.0 SAMPLING PROCEDURE FOR SMALL (2"x2" or 14"x14") COUPONS

1. A three person team will be used, employing aseptic technique throughout. The team will consist of a sampler, sample handler, and support person.
2. Throughout the procedure, the support person will log anything they deem to be significant into the laboratory notebook.
3. In general, the team works from the least contaminated sample set (i.e., control blanks) towards the most contaminated sample set (i.e., positive controls).
4. The sampling team will each don a pair of sampling gloves (a new pair per sample, non-sterile, as they will only be handling non-sterile items); the sampler's gloves shall be sterile sampling gloves as they are the only member of the team in contact with the sample. All members shall wear dust masks to further minimize potential contamination of the samples. Depending on the situation, respiratory protection beyond a dust mask may be required to protect the sampling team (e.g., SAR; this will be specified in the project QAPP). New disposable lab coats are required for the sample handler when changing between different types of materials or when direct contact between the coupon and lab coat occurs.
5. The sample handler will remove the coupon from the appropriate cabinet (if necessary) and place it on the sampling area, being careful to handle the coupon only around the edges.
6. The support person will record the coupon code on the sampling log sheet.
7. For some coupons the sampling area is evident from pre-drawn outlines. For these coupons, proceed to step 10, as steps 8-9 are not necessary.
8. The support person will remove a template from the bag and aseptically unwrap it such that the sampler may grab it wearing sterile gloves.
9. The sampler will place the template onto the coupon surface and align it such that the edges of the coupon are visible through the holes on the template.
10. The support person will remove a sample kit from the sampling bin and record the sample tube number on the sampling log sheet next to the corresponding coupon code just recorded.

11. The sampler and support person will verify the sample code and ensure that the correct coupon and location are being sampled.
12. The support person will:
    a. Open the outer sampling bag touching the outside of the bag.
    b. Touching only the outside of the (10" x 14") bag, remove and open the unlabeled conical tube and pour the pre-moistened all-purpose sponge onto the sample or into the sampler's hands.
    c. Discard the unlabeled conical tube.
    d. Remove the sample collection sample bag (5.5" x 9"), being careful to not touch the inside of the outer sampling bag, and open it touching only the outside.
    e. Maneuver the labeled 50 mL conical tube to the end of the outer sterile sampling bag and loosen the cap.
    f. Remove the cap from 50 mL conical tube immediately preceding the introduction of the sample into the tube.
13. The sampler will:
    a. Wipe the surface of the sample horizontally using S-strokes to cover the entire sample area of the coupon using a consistent amount of pressure.
    b. Fold the all-purpose sponge concealing the exposed side and then wipe the same surface vertically using the same technique.
    c. Fold the all-purpose sponge over again and roll up the folded sponge to fit into the conical tube.
    d. Carefully place the all-purpose sponge into the 50 mL conical tube that the support person is holding, being careful not to touch the surface of the 50 mL conical tube or plastic sterile sampling bag.
14. The support person will then immediately close and tighten the cap to the 50 mL conical tube and slide the tube back into the sample collection sampling bag and seal it.
15. The support person will then wipe the sample collection sampling bag with a Dispatch® bleach wipe and place it into the outer sampling bag.
16. The support person will then seal the outer sample collection bag now containing the capped 50 mL conical tube (containing the all-purpose sponge) inside a sealed 5.5" x 9" sample collection bag.
17. The support person will then decontaminate the outer sample bag by wiping it with a Dispatch® bleach wipe.
18. The support person will then place the triply contained sample into the sample collection bin.
19. All members of the sampling team will remove and discard their gloves.
20. Steps 2 – 19 will be repeated for each sample to be collected.

3.0 SAMPLING METHOD FOR LARGE (4'x4' or larger) COUPONS

3.1 Sample Layout

The sampling of large coupons is carried out using a sample grid to divide the large coupons into representative sections. These sections are then numbered and selected to be sampled at different times during the course of the experiment as a blank, a control, or an experimental group sample. This selection grid is pre-determined and the Project Quality Assurance Project Plan (QAPP) may overrule the template shown in Figure 1 if otherwise specified.

As in the example below, the first cell is sampled as a Blank before contamination. Starting in cell 3, every third cell is sampled as a positive Control. This sample is to be taken post-contamination and before decontamination. Every cell directly following a Control cell is sampled as Experimental and is taken following decontamination. The sample kit labeling will be based on this grid and the sampling team must ensure to correctly sample the coupons based on this template.

1 Blank	2	3 Control	4 Experimental
5	6 Control	7 Experimental	8
9 Control	10 Experimental	11	12 Control
13 Experimental	14	15 Control	16 Experimental

Figure 1. 4' x 4' Material Section Template and Sample Grid

3.2 Sampling Procedure

1. A two-person team will be used, employing aseptic technique throughout. The team will consist of a sampler and a support person.
2. Throughout the procedure, the support person will log anything they deem to be significant into the laboratory notebook.
3. The sampling team will each don a pair of sampling gloves (a new pair per sample, non-sterile, as they will only be handling non-sterile items); the sampler's gloves shall be sterile sampling gloves as they are the only member of the team in contact with the sample. All members shall wear dust masks to further minimize potential contamination of the samples. Depending on the situation, respiratory protection beyond a dust mask may be required to protect the sampling team (e.g., SAR; this will be specified in the project QAPP).
4. The support person will record the coupon code on the sampling log sheet.
5. The sampler will place the template onto the coupon surface (using clamps as necessary).
6. The support person will remove a sample kit from the sampling bin and record the sample tube number on the sampling log sheet next to the corresponding coupon code just recorded.
7. The sampler and support person will verify the sample code and ensure that the correct coupon and location (cell) is being sampled.
8. The support person will:
    a. Open the outer sampling bag touching the outside of the bag.
    b. Touching only the outside of the (10" x 14") bag, remove and open the unlabeled conical tube and pour the pre-moistened all-purpose sponge onto the sample or into the sampler's hands.
    c. The unlabeled conical tube is retained for Step 9.
    d. Remove the sample collection sample bag (5.5" x 9") being careful to not touch the inside of the outer sampling bag and open it touching only the outside.
    e. Maneuver the labeled 50 mL conical tube to the end of the outer sterile sampling bag and loosen the cap.
    f. Remove the cap from 50 mL conical tube immediately preceding the introduction of the sample into the tube.
9. The sampler will:
    a. For a vertical coupon, the sampler will squeeze excess moisture from the sampling sponge to prevent dripping down the sampling surface. The excess moisture is caught in the unlabeled conical tube from Step 8c, and is then discarded.
    b. Wipe the surface of the sample using S-strokes to cover the entire sample area of the coupon (inside the grid) using a consistent amount of pressure.

c. Fold the all-purpose sponge concealing the exposed side and then wipe the same surface vertically using the same technique.

d. Fold the all-purpose sponge over again and roll up the folded sponge to fit into the conical tube.

e. Carefully place the all-purpose sponge into the 50 mL conical tube that the support person is holding being careful not to touch the surface of the 50 mL conical tube or plastic sterile sampling bag.

10. The support person will then immediately close and tighten the cap to the 50 mL conical tube and slide the tube into the sample collection sampling bag and seal it.

11. The support person will then wipe the sample collection sampling bag with a Dispatch® bleach wipe and place it into the outer sampling bag.

12. The support person will then seal the outer sample collection bag now containing the capped 50 mL conical tube (containing the all-purpose sponge) inside a sealed 5.5” x 9” sample collection bag.

13. The support person will then decontaminate the outer sample bag by wiping it with a Dispatch® bleach wipe.

14. The support person will then place the triply contained sample into the sample collection bin.

15. All members of the sampling team will remove and discard their gloves.

16. Steps 2 – 15 will be repeated for each sample to be collected.

MOP-3155

TITLE PROCEDURE FOR VIA-CELL AIR SAMPLING

SCOPE: This MOP describes the procedure for air sampling using the Via-Cell Bioaerosol Sampling Cassette.

PURPOSE: The purpose of this MOP is to ensure consistent and representative air sampling.

EQUIPMENT

- Zefon Via-Cell® Bioaerosol Sampling Cassette (p/n VIA010)
- EPA Method 5 Dry Gas meter box within annual calibration
- ½" I.D. vacuum tubing
- Labeled 5.5" x 15" sterile bag for tertiary containment
- Writing pen
- Project notebook

1.0 PREPARATION

1. Verify from the package that the Via-Cell cassette is within its expiration date. If not, discard.
2. Label the Via-Cell cassette with the appropriate sample I.D according to the test plan or Quality Assurance Project Plan (QAPP).
3. In the project laboratory notebook, record the cassette sample ID, lot number, and expiration date.

2.0 AIR SAMPLING

There are two areas of operation for Via-Cell air sampling: 1) at the cassette and 2) at the meter box. These two areas of operation may be performed by the same person or different people depending on the situation. When air sampling inside COMMANDER while occupied, for instance, a two-person team will be necessary; one person inside to connect the cassette and the other outside to start and stop the dry gas meter.

1. Wearing nitrile gloves, tear open the foil package using the tear strip on top. Use care when opening, as this package is re-sealable and is required to be used after sampling for transport to the laboratory for analysis.
2. Remove Via-Cell from the package (see Figure 1).

Figure 1. Disassembled Via-Cell, showing cap (far left), package, and cassette with outlet plug.

3. Remove the blue outlet plug from the cassette and place it into the foil for safe keeping (seen at the bottom of the green Via-Cell cassette in Figure 1).
4. Connect the Via-Cell sampler outlet to the dry gas meter using vacuum tubing and position the cassette in the desired location. The Via-Cell sampler is capable of operating in any vertical or horizontal orientation and in confined spaces.
5. Perform a leak-check on the cassette by pulling a vacuum on the inlet cap. The meter box flow rate should be zero.
6. Remove the large blue inlet cap for the cassette and place into the foil package for safekeeping.
7. Record the dry gas meter's initial volume.
8. At the meter box, turn on the pump and set the sampling pump to a flow rate of 15 lpm. Over the pressure drop of the Via-Cell cassette, this is at a delta H of 1.1" water as read on the front of the meter box. Pull a sample for the desired amount of time, monitoring the delta H every ten minutes. When sampling is completed, and with new gloves, replace the blue plug in the outlet and the blue cap over the inlet.
9. Record the sampling time and final volume. Check to ensure flow rate was 15 lpm.
10. Place the Via-Cell cassette into the special foil bag and zip it closed. Apply the red safety seal label over the top of the foil bag opening to ensure sample integrity until analysis.
11. Place foil bag containing cassette inside a pre-labeled 5.5" x 15" sterile bag for tertiary containment.
12. Submit the cassette to the Biocontaminant Laboratory along with a Chain-of-Custody (COC); the cassette should be analyzed according to MOP 6571 within 24 hours of collection. The COC form should include the collection time and the analysis by time and date in the comments section.

13. Each sample should be associated with a laboratory blank (plain, unused Via-Cell cassette) and a field blank of at least 150 liters of clean air in the same area as samples are collected.

MOP-3163

TITLE: AEROSOL APPLICATION OF GRIME ON MATERIAL COUPONS

SCOPE: This MOP describes the procedure for applying grime in a reproducible manner.

PURPOSE: The purpose of this MOP is to standardize grime application to material coupons using a HVLP sprayer.

EQUIPMENT:

- HVLP Sprayer with can
- Croix CH-10 Turbine with air line
- Grime (2.5g + 4.5g per coupon set)
- 95% Ethanol (50mL + 100mL / coupon set)
- Safety glasses
- Lab coat
- Nitrile Gloves
- Non-sterile cups
- Measuring tape
- Calibrated scale
- Spatula
- Coupons to be sprayed
- Timer
- Dispatch® wipes
- Kimwipes

Note: You will need a buddy during spraying.

1.0 GRIME PREPARATION

1.1 Solution Preparation

Prepare the grime/ethanol solution using the balance and a non-sterile cup.

a. Tare an empty cup and add 7.0 g of grime.
b. Pour the grime into the can (seen in Figure 1) inside a fume hood.
c. Add 150mL 95% ethanol to the can.

Figure 1. HVLP Sprayer can

d. Weigh the can and contents and record in the lab notebook. Empty can weight is 146.5g.
e. Connect the can to HVLP sprayer and seal using the lever on the top of the can.

1.2 Coupon Setup

a. Check the QAPP, or other controlling document, to determine which coupons will be sprayed.
b. Remove the coupons from their sterilization pouch and place the coupons against the back baffle in the walk-in hood in H122 as seen in Figure 2.
c. Note their position in the hood during spraying on the coupon tracker using coupon IDs.
d. Mark a line on the table in the hood 9” away from the bottom of the coupons with tape to use as a guide during spraying. The spray nozzle should be 9” from the bottom of the coupons during spraying.

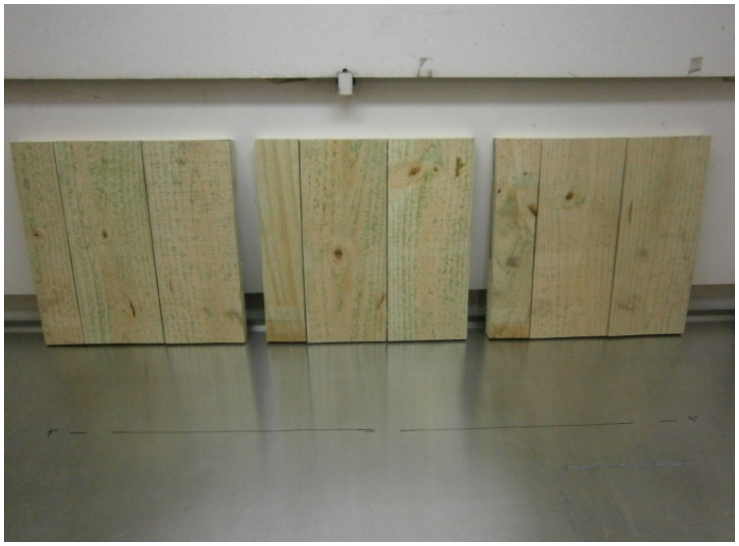


Figure 2. Coupon Setup

1.3 Sprayer Settings

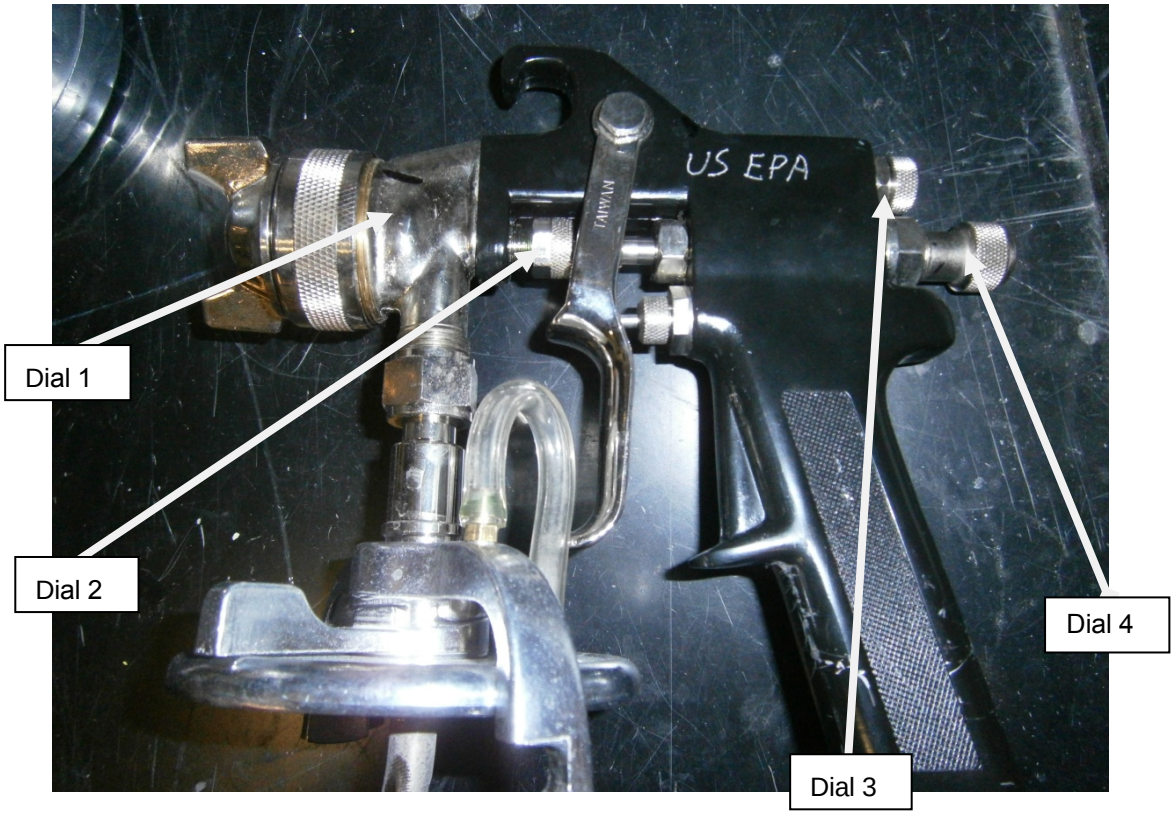


Figure 3. HVLP Sprayer

Dial 1 – The default position is seen in the Figure 4 below. This orients the fan in a diagonal spray. Line up all sharpie marks on the nozzle.

Dial 2 – Turn the dial clockwise to closed. The sharpie marks should line up when fully closed. From the closed position, turn the dial two complete turns counter-clockwise. (The sharpie mark will pass and then line up with the mark on the body of the sprayer). This is the default position. If this is not set up properly no liquid will be sprayed.

Dial 3 – Turn the dial clockwise to closed. The sharpie marks should line up when fully closed. From the closed position, turn the dial two complete turns counter-clockwise. (The sharpie mark will pass and then line up with the mark on the body of the sprayer). This is the default position.

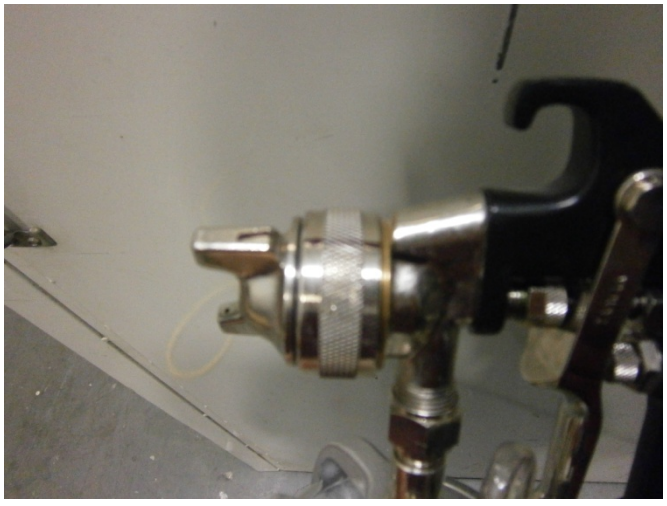


Figure 4. Dial 1

Dial 4 – Turn the dial clockwise to closed. The sharpie marks should line up when fully closed. From the closed position, turn the dial six complete turns counter-clockwise. (The sharpie mark will pass five times and then line up with the mark on the body of the sprayer). This is the default position.

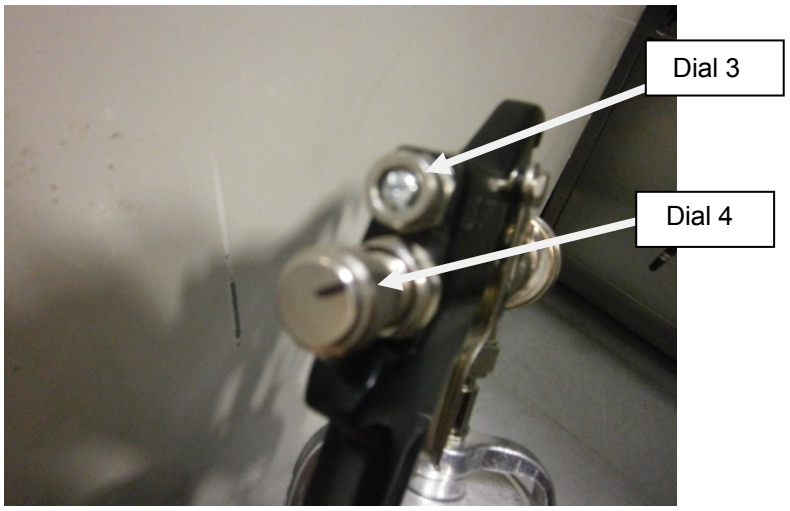


Figure 5. Dial 3 and 4

Set all dials to their default position to prepare for grime application.

2.0 SPRAY PROCEDURE

a. Ensure all sprayer settings are correct (as per Section 1.3).

b. Don all appropriate PPE (i.e., labcoat, gloves, safety glasses).

c. Connect the turbine hose to the bottom of the HVLP sprayer using the quick-connect fitting.

d. Ensure the valve on the quick connect fitting is turned on and have a buddy power on the turbine.

Note: The HVLP sprayer will constantly spray air whenever the turbine is on. Make sure you have the sprayer pointed into the hood at all times when the turbine is powered on.

e. Shake can for 15 seconds prior to beginning spray.

f. Start spray and timer simultaneously. Spray two 1.5-min applications with a 1 min break in between sprays. Record time in lab notebook. The spray pattern is shown below in Figure 6.

g. Spray top to bottom in 8 passes in the upright position (as seen in Figure 6, Steps 1 and 2).

h. Once at the bottom of the coupon, spray 4 passes bottom to top turning the sprayer sideways (as seen in Figure 6, Steps 3 and 4). This is increases the flowrate.

i. Repeat Steps g and h until 90 secs have elapsed. Stop spraying and stop timer.

j. After a 1 min break, shake the can for 15 secs, then resume spraying and start timer.

k. Repeat Steps g and h for an additional 90 secs. Stop spraying and stop timer. Record stop time.

l. Once spraying is complete, weigh can and contents and record the value in the lab notebook.

m. Determine the volume sprayed based on an average solution density of 0.85 g/mL.

n. Mark the appropriate column on the coupon tracker to indicate the specified coupons have been coated with grime.

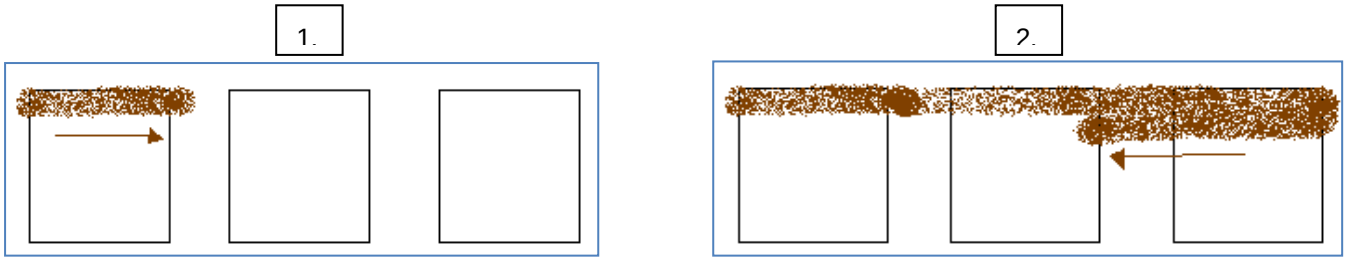
o. Allow 15 minutes for the coupons to dry before removing them from the hood.

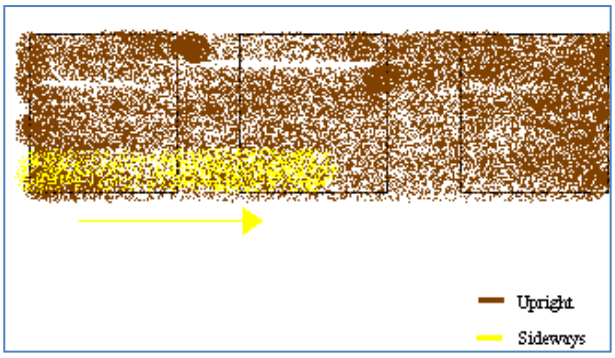
p. Unless stated otherwise by a QAPP or other controlling document, the coupons should be handled without touching the front surface, placed horizontally, and covered with a cleaned (decontaminated) puffing pyramid with gasket (see MOP 3161).

q. Before Section 3.0 or in between each sample set, ensure the sharpie marks on the HVLP sprayer are clear and bold. If unclear, redraw them.

r. For each additional coupon set:

1) Add 95% ethanol to the can equal to the volume sprayed in the previous test.
2) Add 1g of grime for every 21 mL of ethanol added.
3) Weigh the can and contents and record the value in the lab notebook.
4) Attach the can to the HVLP sprayer.
5) Repeat Section 2.0 of this MOP.





3

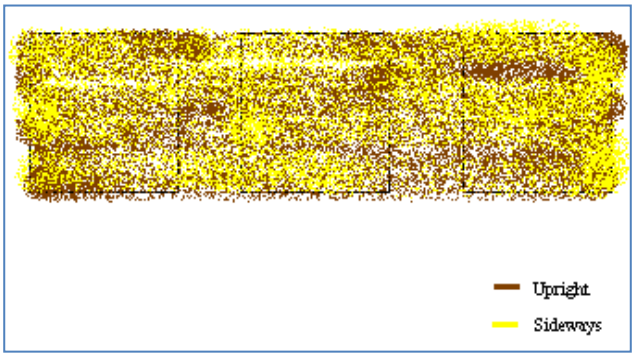


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Figure 6. Spray pattern

3.0 CLEAN UP

a. Record final weight of spray can.

b. Rinse spray can with ethanol, then DI water. Wash the can with soapy water, rinse, and allow to dry.

c. Disconnect sprayer from hose.

d. Turn off turbine blower, coil hose, and move to a convenient location.

e. Wipe down the empty hood with Dispatch and Kimwipes, until no black residue remains.

f. Rinse with DI water.

g. Allow to dry.

MOP 3198

TITLE: PROCEDURE FOR PREPARING DILUTED BLEACH SOLUTION FOR WA 4-59 PARAMETRIC BLEACH TESTING

SCOPE: This MOP describes a procedure for reproducibly preparing diluted bleach solutions for use in WA 4-59 testing.

PURPOSE: The purpose of this MOP is to ensure the solution meets quality assurance (QA) specifications for each test.

1.0 EQUIPMENT/REAGENTS

- Clorox® Concentrated Germicidal Bleach; - sodium hypochlorite concentration 8.25%

  NOTE: This is a new product of the Clorox Company that will replace Regular Germicidal Bleach

- Clorox® Splash-less Concentrated Bleach - sodium hypochlorite concentration 3.95 %
- HACH Test Kit – Hypochlorite (Bleach) D.T (5-15% as Cl_2 Model CN-HRDT; Cat No. 26871-00)
- 15 L Carboy (sterilized)
- 5000 mL Graduated cylinder
- 1000 mL Graduated cylinder
- 250 mL Erlenmeyer flask
- Deionized (DI) water
- Oakton Acorn Series pH 5 meter or equivalent
- Triple-rinsed container suitable for transporting hazardous solutions
- Oakton pH 4, 7 and 10 (pH = 4.00 ± 0.01, 7.00 ± 0.01 and 10.00 ± 0.01 @ 25 °C) buffer or equivalent
- Dispatch® wipes

NOTE: Diluted Bleach solution should be prepared fresh each day.

NOTE: Sterile equipment needs to be sterilized prior to testing either by autoclaving (in Biocontaminant Laboratory or H-122A) or by ethylene oxide (EtO) fumigation using the EtO sterilization system located in H-222.

Δ **Safety Requirements**:

- PPE Required: Safety glasses, lab coat, nitrile gloves
- All work performed in a chemical fume hood

2.0 PROCEDURE

2.1 Calibrate pH Meter

1. Turn meter on (Figure 1).
2. Rinse electrode thoroughly with DI water. DO NOT wipe the electrode.

Figure 1. Acorn pH5 Meter

3. Dip both the electrode and temperature sensor into pH 4.00 buffer solution. The glass bulb must be completely immersed into the sample. Stir gently and wait for the reading to stabilize (about 40 seconds).

4. Press the **CAL** key to enter the calibration mode. The display will momentarily flash "**CA**" to indicate Calibration. The display will show the current uncalibrated reading, blinking while in calibration mode.

5. Allow the reading to stabilize. The meter will automatically recognize 4.00, 7.00 or 10.00 buffers.

6. Record the uncalibrated value in the laboratory notebook. Press the **Enter** key once to confirm calibration for the 4.00 buffer. The LCD displays "**CO**" to indicate the calibration point has been confirmed. The meter exits calibration mode and returns to measurement mode.

7. Record the pH buffer measurement and temperature (press **MODE** key to select parameter) in the appropriate lab notebook.

8. Repeat step 4 and 7 for the 7.00 and 10.0 buffers.

2.2 Diluted Bleach Preparation

2.2.1 1:10 Dilution (theoretical concentration of hypochlorite 0.83%)

1. Add 9000 mL of DI water to sterilized (autoclaved) carboy of at least 15 L capacity.

2. Prepare 10 L of diluted bleach by adding 1000 mL of Clorox Concentrated Germicidal Bleach® to a carboy. Cap the container. Mix solution (by hand) for 1 min. This will result in a 1:10 ratio solution (target concentration = 0.83% sodium hypochlorite). Record the time and date on the carboy and in the laboratory notebook.

3. Test the pH and temperature of bleach solution in a carboy. Record in the laboratory notebook. Compare pH to the pH-threshold value established for the 1:10 dilution in preliminary testing. If the % difference >10%, re-prepare decontamination solution.

4. Measure the Free Available Chlorine (FAC) concentration of the bleach solution using the HACH kit (Section 2.4). Record in the laboratory notebook. Compare the FAC to the FAC

threshold value established for the 1:10 dilution in preliminary testing. If the % difference >10%, re-prepare decontamination solution.

5. Compare to pH with the threshold value established for the 1:1 dilution in preliminary testing. If the % difference >10%, re-prepare decontamination solution.

2.2.2 1:5 Dilution (theoretical concentration of hypochlorite 1.65%)

1. Add 8000 mL of DI water to sterilized (autoclaved) carboy of at least 15 L capacity.

2. Prepare 10 L of diluted bleach by adding 2000 mL of Clorox Concentrated Germicidal Bleach® to a carboy. Cap the container. Mix solution (by hand) for 1 min. This will result in a 1:5 ratio solution (target concentration = 1.65 % sodium hypochlorite). Record the time and date on the carboy and in the laboratory notebook.

3. Test the pH and temperature of bleach solution in a carboy. Record in the laboratory notebook. Compare the pH to the pH-threshold value established for the 1:5 dilution in preliminary testing. If the % difference >10%, re-prepare decontamination solution.

4. Measure the FAC concentration of the bleach solution using the HACH kit (Section 2.4). Record total digits in the laboratory notebook. Compare the FAC to the FAC threshold value established for the 1:5 dilution in preliminary testing. If the % difference >10%, re-prepare decontamination solution.

2.2.3 1:1 Dilution (theoretical concentration of hypochlorite 4.13%)

1. Add 5000 mL of DI water to sterilized (autoclaved) carboy of at least 15 L capacity.

2. Prepare 10 L of diluted bleach by adding 5000 mL of Clorox Concentrated Germicidal Bleach® to a carboy. Cap the container. Mix solution (by hand) for 1 min. This will result in a 1:1 ratio solution (target concentration = 4.13 % sodium hypochlorite). Record the time and date on the carboy and in the laboratory notebook.

3. Test the pH and temperature of bleach solution in a carboy. Record in the laboratory notebook. Compare the pH to the pH threshold value established for the 1:1 dilution in preliminary testing. If the % difference >10%, re-prepare decontamination solution.

4. Measure the FAC concentration of the bleach solution using the HACH kit (Section 2.4). Record total digits in the laboratory notebook. Compare the FAC to the FAC threshold value established for the 1:1 dilution in preliminary testing. If the % difference >10%, re-prepare decontamination solution.

2.3 Diluted Bleach with Surfactant Preparation

2.3.1 1:10 Dilution Equivalent with Surfactant (theoretical concentration of hypochlorite 0.83%)

1. Add 7930 mL of DI water to sterilized (autoclaved) carboy of at least 15 L capacity.

2. Prepare 10 L of diluted bleach with surfactant by adding 2070 mL of Clorox Splash-less Concentrated Bleach® to a carboy. Cap the container. Mix solution (by hand) for 1 min. This will result in an equivalent of 1:10 ratio regular bleach solution (final concentration of hypochlorite 0.83%). Record the time and date on the carboy and in the laboratory notebook.

3. Test the pH and temperature of bleach solution in a carboy. Record in the laboratory notebook. Compare the pH to the pH threshold value established for the 1:10 dilution with surfactant in preliminary testing. If the % difference >10%, re-prepare decontamination solution.

4. Measure the FAC concentration of the bleach solution using the HACH kit (Section 2.4). Record in the laboratory notebook. Compare the FAC to the FAC threshold value established for the 1:10 dilution with surfactant in preliminary testing. If the % difference >10%, re-prepare decontamination solution.

2.3.2 1:5 Dilution Equivalent with Surfactant (theoretical concentration of hypochlorite 1.65%)

1. Add 5860 mL of DI water to sterilized (autoclaved) carboy of at least 15 L capacity.

2. Prepare 10 L of diluted bleach with surfactant by adding 4140 mL of Clorox Splash-less Concentrated Bleach® to a carboy. Cap the container. Mix solution (by hand) for 1 min. This will result in an equivalent of 1:5 ratio regular bleach solution (final concentration of sodium hypochlorite 1.65 %). Record the time on the carboy and in the laboratory notebook.

3. Test the pH and temperature of bleach solution in a carboy. Record in the laboratory notebook. Compare the pH to the pH threshold value established for the 1:5 dilution with surfactant in preliminary testing. If the % difference >10%, re-prepare decontamination solution.

4. Measure the FAC concentration of the bleach solution using the HACH kit (Section 2.4). Record in the laboratory notebook. Compare the FAC to the FAC threshold value established for the 1:5 dilution with surfactant in preliminary testing. If the % difference >10%, re-prepare decontamination solution.

2.3.3 1:1 Dilution Equivalent with Surfactant (theoretical concentration of hypochlorite 4.125%)

NOTE: Concentration of sodium hypochlorite in Splash-less Clorox Concentrated Bleach® is lower than 4.13% hence a 1:1 dilution equivalent with surfactant will be prepared with 4.29% solution of Clorox Concentrated Regular Bleach® added to Splash-less Clorox Concentrated Bleach® to adjust the concentration of sodium hypochlorite.

1. Add 4800 mL of DI water to sterilized (autoclaved) carboy of at least 15 L capacity.

2. Prepare 10 L of diluted bleach at 4.29% sodium hypochlorite by adding 5200 mL of Clorox Concentrated Germicidal Bleach® (8.25%). Mix solution (by hand) for 1 min.

3. Take 4750 mL of 4.29% solution of Clorox Concentrated Germicidal Bleach® just prepared and add it to another sterilized (autoclaved) carboy of at least 15 L capacity.

4. Add 5250 mL of Clorox Splash-less Concentrated Bleach® to a carboy from step 3. Cap the container. Mix solution (by hand) for 1 min. This will result in an equivalent of 1:1 ratio regular bleach solution (final concentration of sodium hypochlorite 4.13 %). Record the time on the carboy and in the laboratory notebook.

5. Test the pH and temperature of bleach solution in a carboy. Record in the laboratory notebook. Compare the pH to the pH threshold value established for the1:1 dilution with surfactant in preliminary testing. If the % difference >10%, re-prepare decontamination solution.

6. Measure the FAC concentration of the bleach solution using the HACH kit (Section 2.4). Record in the laboratory notebook. Compare the FAC to the FAC threshold value established for the 1:1 dilution with surfactant in preliminary testing. If the % difference >10%, re-prepare decontamination solution.

2.4 Titration Using the HACH Kit

1. Insert a clean delivery tube into the 2.26 N Thiosulfate Titrant Solution cartridge. Attach the cartridge to the titrator body.
2. Flush the delivery tube by turning the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe off the tip.
3. Fill the Erlenmeyer flask to about 150 mL with DI water.
4. Add the contents of one potassium Iodide Power Pillow to the flask and stir to mix.
5. Add the contents of one Acid Reagent Powder Pillow to the flask and stir to mix.
6. Dispense 5 mL of the bleach sample below the solution level in the flask. The solution will turn yellow – dark brown in color depending on the amount of chlorine present.
7. Place the delivery tube tip into the solution and stir the solution flask while titrating with the thiosulfate titrant until the solution is light yellow.
8. Add 1 mL of starch indicator solution to the flask and stir to mix. The solution will turn a dark blue color.
9. Continue titration until the solution becomes colorless.
10. Record the number of digits used.

2.5 Calculations

800 digits = 1 mL of 2.26 N STS dispensed

PPM Calculation:

$$ppm = \frac{\text{Digits} \times 2.26 \times 35453}{800 \times \text{V}}$$

Where, V is volume (mL) of bleach sample used in titration.

PPM / 1000 = g/L Chlorine

FINAL CHECKLIST:

- ☐ Calibrate pH meter
- ☐ Measure pH and temperature of solution
- ☐ Measure FAC concentration of the solution

MOP 6568

TITLE: ASEPTIC ASSEMBLY OF WIPE KITS

SCOPE: This MOP outlines the procedure for the aseptic assembly of wipe kits.

PURPOSE: To aseptically assemble kits that will be used to collect wipe samples from which quantifiable data will be derived.

Materials:

- PPE (gloves, lab coat, safety goggles)
- Biological Safety Cabinet (Class II)
- pH-adjusted bleach
- Deionized water
- 70% solution of denatured ethanol
- Kimwipes
- Sterile, sealed Twirl-em bags in two sizes, 10”x15” and 5.5”x9”
- Sterile Kendall 4-ply all-purpose sponges
- Sterile, disposable thumb forceps
- 50mL conical tubes containing 5mL PBST tubes (MOP 6562)
- Sharpie
- Wire or foam rack for 50mL conical tubes
- Secondary containment such as a large Tupperware bin
- Lab notebook
- QAPP for project that is utilizing the wipe samples

1.0 PROCEDURE

1.1 Preparation for Wipe Kit Assembly

Prior to wipe kit assembly, 50mL sterile conical tubes containing 5mL of sterile PBST and a sterile 2-ply sponge must be put together. They are assembled in the following manner:

1. Begin by donning PPE (gloves, lab coat, and protective eyewear)**.**

2. Clean the workspace and biological safety cabinet by wiping surfaces with pH-adjusted bleach, followed by deionized water, and then with a 70% solution of denatured ethanol. Wipe the surfaces with a kimwipe to remove any excess liquid. Make sure the workspace is clean and free of debris. Gather all necessary items to perform the task, place these items on a clean cart beside the biological safety cabinet, within arm's reach so that, once the procedure has begun, the task may be performed without interruptions.

3. Discard gloves and replace with fresh pair.

4. Place the sterile 50mL conical tubes containing 5mL PBST tubes under the biological safety cabinet in a foam or wire rack designed to hold 50mL conical tubes. Using two sterile, disposable thumb forceps, aseptically transfer one half of a 4-ply, sterile, all-purpose sponge to each of the tubes. Complete the transfer by using the two forceps together to first separate the 4-ply sponge in half to create two 2-ply sponges. Then remove a cap from one of the tubes, carefully fold one of the 2-ply sponges using the forceps together and aseptically place it in the opening of the tube so that it sits at the top portion of the tube, while the 5mL of PBST remain at the bottom of the tube. Replace the cap to the tube. Repeat this process until all of the tubes have sponges in them. Once all of the tubes contain sterile sponges, then label the tube rack appropriately with the action completed, the date and your initials and place the tubes on the shelf. These tubes are shelf stable for up to three months.

1.2 Assembly of Wipe Kits

Wipe kits are assembled in the following manner:

1. No more than 48 hours prior to testing or collecting samples, assemble the wipe kits. Wipe kits can be assembled outside of the biological safety cabinet, in a dry, clean area. Make certain to use proper PPE, including gloves, while handling all wipe kit materials. Gather all materials to assemble the kits before assembly. These materials include:

    - 50mL conical tubes containing both a sterile wipe sponge and 5mL PBST
    - Twirl-em bags in two sizes, 10”x15” and 5.5”x 9”

    - Sharpie
    - Vortex mixer

2. Obtain a copy of the labeling scheme for the samples. This may be detailed in the QAPP. For each wipe kit, use a Sharpie and label a large 10” x 15” Twirl-em bag and a 50mL conical tube containing the sponge and PBST.

3. Once all of the tubes are labeled, use the vortex mixer on the highest setting to agitate the tube. This will mix the sponge, which was placed at the top of the tube, with the 5mL of PBST.

4. Open the labeled, 10” x 15” Twirl-em bags one at a time. Place the labeled, agitated tubes in the 10” x 15” Twirl-em bags that have the corresponding label (that matches the tube). Add a non-labeled, sealed 5.5” x 9” Twirl-em bag into the 10”x 15” Twirl-em bag, along with the tube containing the wipe sponge. This completes the wipe kit assembly. Record the time and date in which the wipe kits were assemble in the lab notebook; include the labeling schematic for the wipe kits.

5. Place the assembled wipe kits into a secondary containment, such as a large Tupperware bin. Use within 48 hours. When moving the kits to a sampling location, always have them in secondary containment.

Appendix C. Decontamination Efficiency Results

Table C-1. Decontamination Efficiency Results for Phase I

Test ID	Material Type	Decontamination Agent	Application mode	Log Reduction (surface)		% Non-detect (n=3)	Positive Controls			Test Coupons				Wiping Medium Step 1 (bleach solution/rag or sponge)			Runoff			Wiping MediumSstep 2 (water rinse/rag or sponge)			Rinsate			Aerosol	Procedural Blank	Negative control
				Average LR	STD		Average CFU	STD	RSD	Average CFU	Median CFU	STD	RSD	Average CFU	STD	RSD	Average CFU	STD	RSD	Average CFU	STD	RSD	Average CFU	STD	RSD	CFU/ft3	CFU/sample	CFU/sample
1	Finished wood flooring	Bleach 1:10	Rag	5.30	0.57	0%	1.95E+07	6.56E+06	34%	1.52E+02	7.50E+01	1.74E+02	110%	4.22E+00	8.79E-02	2%	2.23E+03	1.76E+03	79%				4.06E+01	3.24E+01	80%	<1	<1	<1
2	Glass	Bleach 1:10	Rag	6.78	1.93	33%	1.60E+07	1.00E+06	6%	1.45E+03	1.47E+01	2.50E+03	170%	5.00E+04	3.34E+04	67%	9.73E+02	4.08E+02	42%				1.48E+02	2.24E+02	150%	<1	<1	<1
3	Painted wallboard	Bleach 1:10	Rag	5.85	0.21	33%	2.64E+07	1.08E+07	41%	3.68E+01	3.37E+01	1.14E+01	31%	8.46E+04	8.62E+04	102%	2.78E+03	7.02E+02	25%				5.10E+00	1.45E-01	2.8%	1.0E+00	<1	<1
4	Grouted ceramic tile	Bleach 1:10	Rag	3.57	0.39	0%	2.33E+07	7.93E+06	34%	7.62E+03	4.70E+03	6.50E+03	85%	6.74E+04	6.40E+04	95%	6.53E+03	5.53E+03	85%				4.53E+01	1.79E+01	39%	<1	<1	<1
5	Finished wood flooring	Bleach 1:5	Rag	6.51	1.01	33%	2.49E+07	1.16E+07	47%	1.98E+01	1.61E+01	2.14E+01	108%	4.21E+00	6.60E-02	2%	6.54E+02	2.89E+02	44%				4.98E+00	9.14E-02	1.8%	<1	<1	<1
6	Glass	Bleach 1:5	Rag	7.52	0.13	100%	1.90E+07	5.12E+06	27%	<1	<1	0.00E+00	0%	3.65E+03	6.29E+03	172%	1.82E+02	2.60E+02	140%				2.34E+00	2.75E-02	1.2%	1.2E+00	<1	<1
7	Painted wallboard	Bleach 1:5	Rag	6.08	0.70	0%	1.39E+07	1.09E+07	78%	7.12E+00	7.14E+00	2.67E+00	37%	2.17E+05	1.71E+05	79%	6.40E+03	6.22E+03	97%				3.72E+00	4.38E-02	1.2%	1.4E+00	<1	<1
8	Grouted ceramic tile	Bleach 1:5	Rag	6.17	1.27	0%	2.64E+07	4.56E+06	17%	1.31E+02	1.28E+01	2.15E+02	160%	4.68E+04	4.23E+04	90%	6.70E+00	5.71E+00	85%				5.74E+00	6.67E-02	1.2%	<1	2.3E+00	<1
17	Finished wood flooring	Bleach 1:5	Sponge	7.43	0.37	67%	2.48E+07	6.25E+06	25%	1.14E+00	1.14E+00	9.84E-01	87%	1.37E+04	1.79E+04	130%	2.10E+03	3.56E+02	17%	1.77E+02	1.98E+02	112%	4.74E+00	1.16E-01	2.4%	2.9E+00	<1	<1
18	Glass	Bleach 1:5	Sponge	5.71	1.85	33%	2.95E+07	1.15E+07	39%	7.72E+02	1.34E+02	1.22E+03	160%	1.87E+03	2.64E+03	141%	3.03E+03	2.53E+03	84%	1.62E+02	2.23E+02	137%	6.96E+01	8.91E+01	130%	3.8E+00	<1	<1
19	Painted wallboard	Bleach 1:5	Sponge	4.87	0.69	0%	2.05E+07	3.75E+06	18%	4.75E+02	5.10E+02	4.14E+02	87%	3.30E+03	3.62E+03	110%	1.17E+03	6.20E+02	53%	9.60E+03	1.58E+04	164%	4.52E+02	1.13E+02	25%	7.3E+00	<1	<1
20	Grouted ceramic tile	Bleach 1:5	Sponge	6.45	0.99	33%	2.30E+07	1.43E+06	6.2%	2.54E+01	1.21E+01	3.33E+01	130%	2.58E+04	2.64E+03	10%	6.58E+02	6.12E+02	93%	2.11E+03	3.62E+03	172%	4.54E+00	2.88E-02	0.63%	3.8E+00	<1	<1
21	Finished wood flooring	Bleach with surfactant 1:5	Rag	7.78	0.11	100%	3.40E+07	8.11E+06	24%	<1	<1	1.03E-02	1.9%	4.15E+00	8.02E-02	2%	3.60E+00	7.70E-02	2.%	3.51E+00	8.61E-02	2%	2.41E+00	4.97E-02	2.1%	<1	<1	<1
22	Glass	Bleach with surfactant 1:5	Rag	7.74	0.13	100%	3.19E+07	9.74E+06	31%	<1	<1	1.65E-02	2.9%	4.20E+00	1.81E-01	4%	4.60E+00	3.22E-02	1%	3.51E+00	1.80E-02	1%	4.60E+00	3.27E-02	0.71%	<1	<1	<1
23	Painted wallboard	Bleach with surfactant 1:5	Rag	6.22	2.02	33%	1.90E+07	3.00E+06	16%	7.94E+02	1.18E+00	1.37E+03	170%	1.60E+03	2.11E+03	131%	7.28E+01	5.74E+01	79%	5.52E+00	1.84E+00	33%	4.23E+00	2.97E-02	0.70%	<1	<1	<1
24	Grouted ceramic tile	Bleach with surfactant 1:5	Rag	7.00	0.90	67%	2.04E+07	6.34E+05	3.1%	7.83E+00	<1	1.25E+01	160%	8.13E+02	7.97E+02	98%	3.86E+00	4.65E-02	1%	3.44E+00	9.88E-02	3%	4.88E+00	0.00E+00	0.00%	<1	<1	<1
25	Finished wood flooring + grime	Bleach with surfactant 1:5	Rag	7.26	0.35	67%	1.42E+07	3.66E+06	26%	<1	<1	3.30E-01	41%	4.14E+01	9.09E-01	2%	6.60E+01	2.86E+01	43%	3.44E+00	1.41E-01	4%	3.55E+01	0.00E+00	0.00%	3.8E+00	<1	<1

Table C-2. Decontamination Efficiency Results for Phase II

Test ID	Material type	Decon agent	Application Mode	LogRreduction (surface)		% Non-detect (n = 5)	Positive Controls			Test Coupons				Wiping Medium Step 2 (water rinse rag)			Runoff			Rinsate			Aerosol	Procedural Blank	Negative Control
				Average LR	STD		Average CFU	STD	RSD	Average CFU	Median CFU	STD	RSD	Average CFU	STD	RSD	Average CFU	STD	RSD	Average CFU	STD	RSD	CFU/ft3	CFU/ Sample	CFU /Sample
1	Finished wood flooring	Lysol® Mold & Mildew Blaster	Spray/Rag	6.93	0.72	20%	3.40E+07	2.64E+07	78%	8.67E+00	1.33E+00	1.11E+01	130%	1.08E+03	1.77E+03	160%	3.68E+00	4.55E-02	1.2%	1.04E+02	9.89E+01	95%	7.32E+00	<1	3.85E+00
2	Glass	Lysol® Mold & Mildew Blaster	Spray/Rag	7.55	0.14	80%	2.94E+07	1.61E+07	55%	<1	<1	3.01E-01	39%	3.04E+02	5.67E+02	190%	5.12E+00	9.64E-02	1.9%	6.70E+00	1.95E-01	2.9%	3.01E+00	<1	<1
3	Painted wallboard	Lysol® Mold & Mildew Blaster	Spray/Rag	7.78	0.16	60%	4.55E+07	2.69E+06	5.9%	<1	<1	3.07E-01	39%	3.43E+00	1.19E+00	35%	4.44E+00	8.56E-02	1.9%	6.46E+00	4.56E-02	0.71%	3.53E+00	<1	<1
4	Grouted ceramic tile	Lysol® Mold & Mildew Blaster	Spray/Rag	8.02	0.008	100%	6.05E+07	8.28E+06	14%	<1	<1	1.00E-02	1.7%	3.40E+00	6.68E-02	2.0%	8.44E+00	5.99E-02	0.71%	5.56E+00	6.86E-02	1.2%	6.80E+00	<1	<1
5	Finished wood flooring	Tilex® Mold & Mildew Remover	Spray/Rag	7.55	0.023	100%	3.58E+07	2.88E+07	80%	<1	<1	3.21E-02	5.6%	4.16E+00	1.58E+00	38%	6.72E+00	9.77E+00	150%	3.39E+00	4.04E-02	1.2%	4.89E+00	<1	1.11E+00
6	Glass	Tilex® Mold & Mildew Remover	Spray/Rag	7.80	0.009	100%	4.07E+07	4.01E+06	10%	<1	<1	1.41E-02	2.2%	4.16E+00	1.54E+00	37%	1.39E+00	1.67E-02	1.2%	6.70E+00	1.71E-01	2.6%	1.04E+01	3.41E+00	<1
7	Painted wallboard	Tilex® Mold & Mildew Remover	Spray/Rag	6.72	0.53	20%	1.94E+07	2.25E+06	12%	5.96E+00	4.69E+00	5.39E+00	90%	2.06E+03	2.04E+03	99%	3.65E-01	1.38E-02	3.8%	1.10E+00	4.49E-02	4.1%	<1	1.54E+00	<1
8	Grouted ceramic tile	Tilex® Mold & Mildew Remover	Spray/Rag	7.33	0.013	100%	1.67E+07	1.53E+06	9.2%	<1	<1	2.31E-02	3.0%	1.64E+02	3.32E+02	200%	5.78E+00	4.97E-02	0.86%	1.22E+01	2.18E-15	0.0%	<1	1.38E+01	1.71E+01
9	Finished wood flooring	Clorox® Clean-up Cleaner + Bleach	Spray/Rag	7.12	0.55	20%	4.82E+07	1.71E+07	35%	5.68E+00	6.33E+00	4.57E+00	81%	5.12E+02	6.31E+02	120%	9.11E+02	3.43E+02	38%	3.28E+02	4.59E+02	140%	1.48E+00	<1	1.28E+00
10	Glass	Clorox® Clean-up Cleaner + Bleach	Spray/Rag	7.85	0.11	80%	5.23E+07	1.87E+07	36%	<1	<1	2.25E-01	31%	2.00E+01	3.70E+01	190%	3.12E+00	4.06E-02	1%	7.50E+00	1.07E-01	1.4%	2.75E+00	<1	<1
11	Painted wallboard	Clorox® Clean-up Cleaner + Bleach	Spray/Rag	7.89	0.019	100%	5.10E+07	4.41E+06	8.6%	<1	<1	2.82E-02	4.3%	4.70E+00	1.87E+00	40%	3.72E+00	1.63E+00	44%	6.45E+00	0.00E+00	0.0%	<1	<1	<1
12	Grouted ceramic tile	Clorox® Clean-up Cleaner + Bleach	Spray/Rag	7.79	0.13	80%	4.80E+07	2.76E+06	5.8%	<1	<1	2.87E-01	36%	1.17E+01	1.62E+01	140%	1.34E+02	2.27E+02	170%	1.11E+01	4.89E+00	44.1%	<1	<1	1.33E+00
13	Finished wood flooring	Clorox® Disinfecting Bleach Foamer	Spray/Rag	7.81	0.007	100%	4.36E+07	6.93E+06	16%	<1	<1	1.05E-02	1.6%	3.35E+00	5.62E-02	1.7%	8.85E+00	7.66E+00	87%	1.77E+01	0.00E+00	0.0%	<1	<1	<1
14	Glass	Clorox® Disinfecting Bleach Foamer	Spray/Rag	7.93	0.008	100%	5.57E+07	8.18E+06	15%	<1	<1	1.25E-02	1.9%	9.60E+00	1.38E+01	140%	1.63E+01	0.00E+00	0.0%	1.89E+01	0.00E+00	0.0%	<1	<1	<1
15	Painted wallboard	Clorox® Disinfecting Bleach Foamer	Spray/Rag	7.35	0.94	60%	4.71E+07	8.16E+06	17%	1.95E+01	6.76E-01	4.18E+01	210%	3.46E+00	5.58E-02	1.61%	1.28E+01	3.08E-01	2.4%	2.71E+00	3.87E-02	1.4%	<1	<1	<1
16	Grouted ceramic tile	Clorox® Disinfecting Bleach Foamer	Spray/Rag	7.77	0.13	80%	4.67E+07	2.65E+06	5.7%	<1	<1	3.04E-01	36%	3.42E+00	2.15E-02	0.63%	9.16E+00	5.85E-02	0.64%	7.00E+00	4.48E-02	0.64%	<1	5.26E+00	1.33E+00

Appendix D: Quality Assurance

This project was performed under an approved Category III Quality Assurance Project Plan (QAPP) titled Evaluation of Bioagent Decontamination Options for Owner/Occupants (January 2014) and Amendment 1 to this QAPP.

Quality control (QC) samples such as procedural blank coupons (coupons that underwent the fumigation process but which were not inoculated) and negative controls (which did not undergo the fumigation process) were included to monitor for cross-contamination. Results are given in Appendix C (Table C-1 and C-2).

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 of time required for each decontamination step or procedure, any deviations from the QAPP, and physical impacts on the materials. All the tests were conducted in accordance with developed Decontamination Technologies Research Laboratory (DTRL) and BioLab MOPs, listed in Appendix B, to ensure repeatability and adherence to the data quality validation criteria set for this project.

D-1. Calibration Procedures

All equipment was verified as being certified calibrated or having the calibration validated by the Metrology Laboratory prior to use. Calibration of instruments was done at the frequency shown in Table D-1. Any deficiencies were noted, and the instrument was adjusted to meet calibration tolerances and recalibrated within 24 h. No deviations were noted.

Table D-1. Instrument Calibration Frequency

Equipment	Calibration/Certification	Expected Tolerance	Frequency
pH meter/pH calibration	Perform a 3-point calibration with non-expired standard buffers that bracket the target pH before each use.	± 0.1 pH units	Daily
pH meter/temperature calibration	Per manufacturer's instructions, compare displayed value to a NIST certified thermometer	± 0.5 °C	Monthly
Stopwatch	Compare against NIST Official U.S. time per MOP	±1 min/30 days	Monthly
Scale	Calibration by the EPA Metrology Laboratory/Check calibration with Class 2 weights prior to weighing	± 0.1% weight	Yearly/Daily
Micropipettes	Certified as calibrated at time of use/Recalibrated by gravimetric evaluation of pipette performance to manufacturer's specifications every year	± 5%	Yearly
Burettes	Manufacturer certified	0.1 mL	NA

D-2. QA/QC Checks

D-2.1. Data Quality Objectives & Data Quality Indicators

The data quality objectives (DQOs) were used to identify the critical measurements needed to address the objectives of the test program, and specify tolerable levels of potential errors associated with data collection as well as the limitations of the use of the data. Data quality indicators (DQIs) for these critical measurements were used to determine if the collected data met the quality assurance (QA) objectives. A list of these data quality indicators can be found in Table D-2. Failure to provide a measurement method or device to meet these goals resulted in a rejection of results derived from the critical measurement, no results were rejected.

Table D-2. Data Quality Indicators for the Critical Measurements

Measurement Parameter	Analysis Method	Accuracy	Precision/ Repeatability
Dilution ratio	Volumetric	0.01%	NA
FAC	Titration	±2%	NA
pH	pH meter /NIST-traceable buffer solutions	± 0.01 pH units	NA
Temperature drift	pH meter /NIST-traceable thermometer	±0.5 °C	NA
Time	NIST calibrated stopwatch	± 1 minute per hour	NA
Neutralizer volume	Volumetric	1 mL	NA
Volume	Volumetric	1 mL	NA
Plated Volume	Pipette	2%	1%
Temperature of Incubation Chamber	NIST traceable thermometer (daily)	+2 °C	NA
Volumes	Serological pipette tips	0.1 mL	NA
	Burettes	0.1 mL	NA
Counts of CFU/Plate	Manual counting	±10% of all CFUs per plate between first and second count	100% RSD between triplicates for each plate

The quantitative acceptance criteria for each critical measurement are shown in Table D-3. Tests with conditions falling outside of these criteria were rejected and repeated upon approval by the EPA WAM.

Table D-3. Acceptance Criteria for Critical Measurements

Measurement Parameter	Target Values	Acceptance criteria
Decontamination solution FAC between batches*	6500-15,000 ppm	± 10% of target value
Decontamination solution pH between batches*	11.4-11.8	± 10% of target value
Decontamination solution temperature drift (Task 1)	< 5°C	< 5°C between mixing and application of decontaminant
Volume of neutralizer	varied	± 1 mL
Interaction/processing time for Task 1	10 min	± 1 min

**FAC and pH of all decontamination solutions were established experimentally prior to testing in a series of preliminary experiments. Triplicate measurements of these parameters were performed for each liquid decontaminant to be tested. Measurements were performed per MOP 3198. The averages from these measurements were then used as the target threshold for FAC and pH for each decontamination formulation (Table A-2).*

An additional set of biological data quality indicators was applied to the laboratory blanks, procedural blanks, positive controls, test coupons and supplies used for microbiological analyses. These data quality indicators are listed in Table D-4. The acceptance criteria were all met.

Table D-4. Additional Data Quality Indicators Specific to Microbiological Data

Coupon or Sample Type	Acceptance Criteria	Information Provided	Corrective Action
Positive Control Coupons *Sample from material coupon contaminated with biological agent but not subjected to the test condition*	Target recovery of 1 10^7 CFU per coupon (sample) with a standard deviation of < 0.5. (5 10^6 – 5 10^7 CFUs/coupon); 90% recovery of inoculum; Grubbs outlier test (or equivalent).	Initial contamination level on the coupons; allows for determination of log reduction; controls for confounds arising from history impacting bioactivity; controls for special causes. Shows viability of sampling technique and ability of the plate to support growth.	Outside target range: discuss potential impact on results with EPA WACOR; check inoculum and prepare new inoculum if necessary. Outlier: evaluate/exclude value
Procedural Blank *Coupon without biological agent*	Non-detect	Controls for sterility of materials and methods used in the procedure	Reject results upon approval of WACOR, otherwise analyze data with procedural blank results as test minimum, identify and remove source of contamination if possible.
Blank Plating of Microbiological Supplies	No observed growth following incubation	Controls for sterility of supplies used in dilution plating	Sterilize or dispose of source of contamination. Replate samples.
Blank Tryptic Soy Agar (TSA) Sterility Control *Plate incubated, but not inoculated*	No observed growth following incubation	Controls for sterility of plates.	All plates are incubated prior to use, so any contaminated plates will be discarded.
Exposed Field Blank Samples *A wipe kit will be handled, a vacuum sock kit will sample ambient air.*	Non-detect	The level of contamination present during sampling	Clean up environment. Sterilize sampling materials before use.
Unexposed Field Blank Samples *A wipe kit will be transferred without being handled, a vacuum sock kit will be transferred without switching on the vacuum*	Non-detect	The level of contamination present during sampling	Clean up environment. Sterilize sampling materials before use.
Background Swabs	Non-detect	Determines sterility of materials and equipment before use	Clean up environment. Sterilize sampling materials before use.

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