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Enhancing Activated-Peroxide Formulations for Porous Materials: Test Methods & Results

Report for "Expanding Low-technology Decontamination Options with Activated Peroxide-based Materials" (WARRP07)

Paula Krauter, Matthew Tezak, Raymond Boucher, Mark Tucker

Prepared by
Sandia National Laboratories
Albuquerque, New Mexico 87185 and Livermore, California 94550

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Paula Krauter and Mark Tucker
Chemical and Biological Systems
Sandia National Laboratories
P.O. Box 6632
Albuquerque, New Mexico 87185-0734

Matthew Tezak and Raymond Boucher
Research & Development, LMATA Government Service, LLC
Sandia National Laboratories
P.O. Box 6632
Albuquerque, New Mexico 87109

Abstract

During an urban wide-area incident involving the release of a biological warfare agent, the recovery/restoration effort will require extensive resources and will tax the current capabilities of the government and private contractors. In fact, resources may be so limited that decontamination by facility owners/occupants may become necessary and a simple decontamination process and material should be available for this use. One potential process for use by facility owners/occupants would be a liquid sporicidal decontaminant, such as pH-amended bleach or activated-peroxide, and simple application devices. While pH-amended bleach is currently the recommended 'low-tech' decontamination solution, a less corrosive and toxic decontaminant is desirable. The objective of this project is to provide an operational assessment of an alternative to chlorine bleach for 'low-tech' decontamination applications – activated hydrogen peroxide. This report provides the methods and results for activated-peroxide evaluation experiments. The results suggest that the efficacy of an activated-peroxide decontaminant is similar to pH-amended bleach on many common materials.

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1. INTRODUCTION

The United States Environmental Protection Agency (USEPA) has responsibility under the National Response Framework (NRF) for the cleanup of the environment and critical infrastructure in the event of chemical, biological, or radiological terrorist attacks, industrial spills and accidents or natural disasters and thus acceptance of decontamination materials and methods by the USEPA and other stakeholder is of fundamental importance. During an urban wide-area biological incident the recovery/restoration effort will likely require extensive resources and tax the capabilities of local, state, regional, and federal authorities. In such an event, the number of private decontamination contractors available may not be sufficient to respond to the decontamination needs. In fact, resources may be so limited that decontamination by the owner/occupant may become necessary. The American National Standards Institute's Homeland Security Standards Panel consensus document *American National Standard for Disaster/ Emergency Management and Business Continuity Programs,* developed in response to *The 9/11 Commission Report,* stated that the private sector, which controls 85% of the critical infrastructure in the nation, remains largely unprepared for a terrorist attack.

A simple decontamination process and material should be available for decision-makers to recommend in certain circumstances, e.g., areas where a low-tech strategy is applicable or in facilities with tertiary contamination. A simple decontamination process for building owners/occupants is the process using a liquid sporicidal decontamination material, such as pH-amended bleach or activated-peroxide and simple application devices. While pH-amended bleach is currently the recommended 'low-tech' decontamination solution, a less corrosive and toxic decontaminate is desirable. The objective of this project is to provide an operational assessment of an alternative to bleach for 'low-tech' decontamination applications.

The purpose of this evaluation is to generate objective performance data that can subsequently be used by building and facility managers, first responders, groups responsible for building decontamination, and other technology buyers and users to make informed purchase and application decisions. The objective of this document is to describe procedures to determine the efficacy of activated-peroxides for killing a surrogate of the biological agent *Bacillus anthracis* spores, on a range of representative surfaces typical of those found in or around a public building. The ultimate goal of this project is to provide a technology for restoring a contaminated building to a usable state following a biothreat incident.

The performance parameters by which the decontamination technologies will be evaluated under this effort include

- Log-kill or efficacy.
- Surface damage caused by the decontamination technology (qualitative).
- Decontaminant off-gasses (chlorine gas, hydrogen peroxide vapors) or breakdown products.

This project is a collaboration between Sandia National Laboratories (SNL) and the USEPA. It is a follow-on to an effort that began under a previous DHS- and DoD-funded project – the Interagency Biological Restoration Demonstration (IBRD) project. Initial work to evaluate activated-peroxide formulations has been conducted at Sandia with the objective of evaluating activated-peroxide solutions to select an optimal formulation. Following this initial evaluation and selection of optimal formulations, further work was conducted at the USEPA research

facility in Research Triangle Park, NC with the objective to evaluate the selected formulation against *Bacillus anthracis* spores. Results from this work are contained in a separate USEPA report.

2 QUALITY OBJECTIVES

The performance parameters to be evaluated under this test/QA plan include:

- Quantitative assessment of decontamination efficacy of sporicidal spray decontaminants.
- Qualitative assessment of residual viable organisms on test surfaces.
- Changes in appearance of test coupons based upon visual observation.

The quantitative assessment of decontamination efficacy is impacted by uncertainty in four measurements: the volume of stock suspension spiked onto coupons, the number of viable spores in the stock suspension and in the coupon extracts, the temperature, and the contact time. Critical data required to achieve performance objectives are summarized in Table 1.

Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) registration requires that sporicides demonstrate a predetermined level of efficacy when tested against *B. anthracis* spores, or spores of a suitable surrogate, using one of several validated test methods. These methods are often test tube based and involve submersion of replicate coupons of contaminated test material (e.g., silk suture loops, porcelain penicylinders, ceramic tile, glass, etc.) in the liquid product to be evaluated. These tests are not designed to predict product performance on all complex surfaces. By utilizing surface materials, this study offers operationally-relevant insight into the expected performance of activated-peroxide decontaminants on surfaces contaminated with spores of *B. anthracis*.

The spore challenge level utilized in this study (~1 x 10^8) was determined to be appropriate to demonstrate a 6-log-unit kill efficacy, generally considered acceptable as a performance criterion for registration of sporicidal products for *B. anthracis* spores under the Federal Insecticide, Fungicide, and Rodenticide Act (U.S. Environmental Protection Agency, 2007).

Table 2.1. Critical data quality objectives

Data Requirements	Method	Unit	Acceptable Uncertainty in Data	Corrective Action
Application volume	Micropipette	ml, microliter (µl)	±1%	Replace with calibrated and sufficiently accurate micropipette; Pipettes are recalibrated by gravimetric evaluation.
Viable spores	Manual count and automatic plate reader	CFU	±10% (controls)	Provide training; test performance; re-count questionable plates
Temperature	National Institute of Standards and Technology (NIST) traceable certification (±0.4°C @25°C)	°C	±2°C	Replace with calibrated and sufficiently accurate thermometer.
Time	Data logger manufacturer's specifications indicate time accuracy of 61 seconds/ month (±0.000023%)	Hr	±0.05% (2 seconds hour)	Replace with calibrated and sufficiently accurate clock.

The quantitative measurement that is critical to this evaluation is a differential measurement; that is, the test coupons are spiked with spores from the same batch and, subsequently, the coupons are treated with the decontamination technology. The number of viable spores recovered from decontaminated coupons and the coupons that were spiked in the same fashion but not decontaminated are used in calculating log-kill (or efficacy). The mean ±SD of the log-kill values are calculated for each coupon type and biological agent combination.

3 TEST METHOD

3.1 Test Overview

The test investigated the performance of several activated-peroxide formulations with the intent to improve the performance on porous surface materials. Table 2 lists the factors used during this evaluation. The procedure provides 1) a standardized immersion test, and 2) a method to evaluate the efficacy on several common surface materials.

Table 3.1. Test Factors to evaluate formulations on common surface materials

Factor	Test Levels
Surface materials	1. Glazed tile 2. Carpet- supplied by USEPA 3. Unpainted wood, (plywood, pressure treated) 4. Painted wood, molding 5. Linoleum- supplied by USEPA 6. Stainless steel or Glass- supplied by USEPA 7. Mortar (cement) http://quikrete.com/ProductLines/MortarMix.asp (50 coupons per material plus extras)
Coupon size	• Vinyl, carpet unpainted wood, painted wood (Approximately 1.9 cm by 7.5 cm) • Stainless Steel (60mm x 20mm x 1mm) **Glass (51mm x 27mm x 2mm)** • Cement (mold measurements – may decrease a bit when cured) - 40mm x 20mm x 13mm
Coupon sterilization	Biocabinent UV 1 hr. each side
Contaminant surface concentrations	$1x10^8$ CFU/coupon (6-log kill standard). Dispersed in 10 droplets/coupon. Use DPG (silica coated) *B. atrophaeus* for titer.
Number of tests	Select the top performing formulas to conduct a repeat test due to number of samples requiring extraction and analysis. Determine after initial test results.
Number of coupons/ material	6 (confidence), 4 positive controls (neutralizer + decontaminant)
Personnel for sample collection, processing, and analysis	1 technologist.

Factor	Test Levels
Number of formulations to test	15 activated-peroxide formulations. Plus a neutralized formulation for each of the 15 test solutions for baseline data.
Number of positive control/test	10% process controls. Additional positive controls- direct inoculum onto plates (titer).
Decon Dispersal	Pipette a known quantity onto coupons (about 10^8 CFU/ml). Allow to dry onto each coupon
Exposure time (wet contact time)	60 min for both porous materials. 30 min for nonporous materials.
Temperature & Relative Humidity	Room temperature, <50% RH.

3.2 Decontaminant Inactivation (Neutralization)

Inactivator Test: sporicide neutralization tests were carried out to determine the concentration of chemical neutralizer required to completely inactivate each sporicide and to prevent biocidal or biostatic effects of the sporicides during extraction, processing, and culturing of *B. atrophaeus* following the exposure period. Neutralization tests were based upon method ASTM E 1054-02; however, instead of 30 to 100 cells ml^{-1}, 1 x 10^8 CFU per assay were used in order to increase sensitivity. The procedure is shown below:

1. **Titer Preparation:** Using aseptic technique in a bio-cabinet make the spore suspension (in PBS buffer with 0.001% Tween 80) from Powdered DPG stock to ~1 X 108 cfu/ml. The starting DPG stock was approximately 1 x 1012 CFU/g.
    a. In a 50ml conical filled with 35ml of sterile distilled water with 0.001% tween add 5 mg of spore stock; Vortex for 5 – 60s cycles. Re-Vortex for 2 – 60s cycles to suspend the spores then determine concentration by serial dilution plating on 3M Petrifilm (48 hour incubation at 37°C).
    b. Create a large batch of required titer (1 x 10^8 CFU ml^{-1}). In a sterilized 500 ml Erlenmeyer flask (autoclave with a stir bar inside and cap off with 100 ml beaker), add 400 ml of sterilized (autoclaved) and filtered DDI with 0.001% tween. Calculate amount of above stock solution to add to 400 ml to create required titer (1 x 10^8 CFU ml^{-1}). Wrap the beaker to flask with Parafilm and place the flask with titer on a stir plate and stir with enough force to create a vortex that reaches the bottom of the flask (not more than that as foam will start to form). Mix overnight; determine concentration by serial dilution. Adjust titer if necessary by either adding more of the initial spore stock or adding more sterilized DDI. For future use of the titer, place flask on stir plate and stir for 30 min before use (again, with enough force to create a vortex that touches the bottom of the flask).

2. **Prepare Decontaminant Formulations:** Make up decontamination formulations. Use appropriate chemical safety procedures and work in the chemical hood. Make up formula #4 and #9 (see Table 3).
3. Measure the pH for each formulation. The target pH is approximately 9.5. Record change in formulation and all pH measurements for each formulation.
4. Test for neutralization for formulations in Table 2 using 0.4% thiosulfate (STS) and 0.005% catalase; 0.5% STS and 1.5% STS. Make up the neutralization solutions.
5. Determine the amount of decontaminant liquid to be used to completely cover a slide coupon by pipetting the decontaminant onto the coupon in a weigh boat until the coupon is covered with liquid. This is the volume of decon to add to the buffer (decontaminant only). Test on each coupon type; determine the volume of decontaminant used then average for all porous and nonporous materials. The average volume will be used for the inactivator tests.
6. Prepare 4 test tubes for each formulation by adding 10 ml of phosphate-buffered saline (PBS) (Sigma, St. Louis, MO) amended with 0.1% Triton X-100 (Sigma) and the predetermined volume of sporicide formulations (see 5.).

Table 3.2. Number of samples for neutralizer test

Formula #	Neutralizers			
	0.4% thiosulfate (STS) and 0.005% catalase	0.5% STS	1.5% STS	No neutralizer (negative controls)
4	3	3	3	3
9 (buffer only)				3

7. Add 0.4% thiosulfate (STS) and 0.005% catalase or, 0.5% STS or, 1.5% STS into one of the 4 test tubes and no neutralizer into the 4th tube (carefully label each test tube and check total volumes).
    a. Start with 15% STS add 1.5 ml to 8 (7.5 ml) of decon, vortex 60 sec then add 1 ml spore titer.
    b. 5% STS add 1.5 ml to 8 ml decon, mix 60sec, then add 0.5 ml spore titer.
    c. Make 0.005% catalase and 4% STS solution, add 1.5 ml of the mixture to 8 ml decon, mix for 60 sec, then add 0.5 ml spore titer.
    d. Solution formula #4 (because it is the most concentrated).
8. Contact time of 30 min. for all conical (for the no-neutralizer test set).
9. Add 1 x 10^8 CFU *B. atrophaeus* spores to each test tube (0.5 ml).
10. Vortex for (2) 15 sec bursts.
11. Serial dilution and plate in triplicate following the exposure. Incubate for 48 hr. at 37°C then visually and on the automatic plate reader.

12. Evaluate neutralized recovery efficiency against formula #9 for % recovery. Note that an acceptable range is 90% and above. Percent recovery is determined by comparison to control samples in which all constituents except the sporicide were added. The concentration of neutralizer that yielded the highest recovery will be used in the decontamination tests.
13. Measure pH.
14. Repeat with greater concentration of STS until complete neutralization is obtained (measured by CFUs).
15. In 50-ml conical add 27 ml DI water, 3 ml decontaminant and 5 ml of 30% STS.

3.3 Decontaminant Formulation Preparation

3.3.1 Amended Bleach Formulation (gold standard decontaminant)

Amended Bleach Solution: pH-7, 5,000 to 6,000 ppm bleach (1 part bleach, 1 part vinegar, 8 parts water), 20°C, for 30 or 60 min contact. Source: USEPA website.

1. A bleach solution close to but not above pH 7 (neutral), as tested with a paper test strip, and at a concentration of 5,000 to 6,000 parts per million (ppm) was prepared by mixing: one part bleach (with a 5.25% - 6.00% sodium hypochlorite concentration) one part white vinegar, and eight parts water. Bleach and vinegar are not combined together directly. Water was first added to the bleach (e.g., two cups water to one cup of bleach), then vinegar (e.g., one cup), and then the remaining water (e.g., six cups).
2. Treated surfaces should remain in contact with the bleach solution for 30 or 60 minutes. Repeated applications were necessary to keep the surfaces wet.
3. Decontaminant should be prepared fresh each day (efficacious for approximately 4-hr).

3.3.2 Activated-Peroxide Formulations

1. Collect all materials
2. Prepare the formulas listed in Table 4a or 4b below.
3. Prepare solution in 3 parts:
    a. Part A is the water, buffer, Variquat 80MC or Maquat 1412, and ethanol or Triton X-100.
    b. Part B is the peroxide and water solution.
    c. Part C in the activator—TAED (g) or Triacetin (mL).
4. Add Part B to Part A and stir for 30 sec.
5. Add activator to Part A/B and stir for 30-60 min for TAED and 1 min for Triacetin
6. Test the pH of each formula (target is 9.5).
7. Decontaminant should be prepared fresh each day (Efficacious for ~4-hr).

Table 3.3. Activated-peroxide formulations for Preliminary Test 1

	Formula #1 (%)	Formula #2 (%)	Formula #3 (%)	Formula #4 (%)	Formula #5(%)	Formula #6 (%)	Formula #7 (%)	Formula #8 (%)	Formula #9 (%), positive control
H_2O_2	4	6	4	6	4	6	4	6	0
TAED	2	3			2	3	2	3	0
Triacetin			2	3					
Variquat	0.1	0.1	0.1	0.1	0.1	0.1	0	0	0
Triton-X100	0	0	0	0	0	0	0.1	0.1	0
Ethanol	5	5	5	5	0	0	0	0	0
Potassium carbonate	3*	3	3	3	3	3	3	3	3
Water	Up to 100%	Up to 100%	Up to 100%	Up to 100%	Up to 100%	Up to 100%	Up to 100%	Up to 100%	Up to 100%
pH @ 0 hr	9.75	9.34	9.78	9.45	9.66	9.28	9.74	9.40	11.55

Table 3.4. Activated-peroxide formulations for Preliminary Test 2

	Formula #10 (%)	Formula #11 (%)	Formula #12 (%)	Formula #13 (%)	Formula #14 (%)	Formula #15 (%)	Formula #9 (%), positive cntl
H_2O_2	6	6	6	6	6	6	0
Triacetin	3	3	3	3	3	3	0
Variquat 80MC *or* Maquat 1412	0.1	0	0.1	0	0	0	0
Triton-X100	0	0.1	0	0.1	0	0	0
Ethanol	5	5	0	0	5	0	0
Potassium carbonate	3	3	3	3	3	3	3
Water	Up to 100%	Up to 100%	Up to 100%	Up to 100%	Up to 100%	Up to 100%	Up to 100%
pH @ time 0	9.26	9.22	9.13	9.11	9.21	9.18	11.53

3.3.3 Chemical sources

- Hydrogen peroxide (50%): Fisher Scientific, CAS: 7722-84-1.
- Tetraacetylethyldiamine (TAED): Warwick B637 TAED (smaller granules and goes into solution faster).
- Triacetin: ACROS Organics, New Jersey, USA, CAS: 102-76-1.
- Variquat 80 MC: Goldschmidt Chemical Corporation, CAS: 68424-85-1.
- Triton X-100: LABCHEM, Inc., CAS: 9002-93-1.
- Ethanol (99.5%): ACROS Organics, CAS: 64-17-5.
- Potassium Carbonate: Sigma Aldrich, CAS: 584-08-7.

3.4 Preliminary Test #1

Test Objectives:

- Determine if efficacy is better with 4 or 6% H_2O_2;
- Determine if efficacy is the same or better using Triacetin instead of TAED (also a comparison for time required to prepare formula);
- Determine if efficacy improves with the addition of ethanol;
- Determine if efficacy improves with triton-x100 instead of ethanol and Variquat.

3.5 Preliminary Test #2

Test Objectives:

- Determine if Variquat improves formulation.
- Determine if ethanol improves formulation.
- Determine if Triton-X improves formulation.

Methods for Preliminary Tests

1. Use 27 unpainted wood coupons (replicate of 3 for each formulation).
2. Prepare decon formula s #1 through #9. Allow to sit for 1 hr. Then mix for 1 min.
3. Pipette 1 x 108 spores (1 ml) on each coupon and allow to dry. The coupons + spores are contained within a weigh boat.
4. Pipette 3 ml of decontaminant onto coupon so that it is saturated (note that the conical contains 27 ml H2O w/Tween + 3 ml decon + 5 ml neutralizer for controls).
5. Keep coupon wet for 60 min. Record total ml of decontaminant used and how many applications were used.

6. Add coupon to 50 ml conical containing 30.0 ml of water + 0.001% Tween + 3% (5.0 ml, 30% STS) STS. Use neutralizer to rinse weigh boat if necessary to collect all spores.
7. Dilute up to 1 x 105 for controls and plate on Petrifilm.
8. Incubate at 37°C for 48 hours and count.
9. Record data and determine log-kill (see section on calculations, page 17).

Pre-test on Porous Materials (carpet, cinder block (grout/cement) and unpainted pressure-treated wood)

1. Prepare test coupons: sterilize using UV light for 1 hr. on each side.
2. Record temperature and RH% during tests. Attempt to keep lab<70% RH and 20 to 25°C.
3. Lay coupons flat in a weigh boat in an area protected from HVAC inlets/outlets.
4. Spike spores onto test coupons (1 ml of 1 x 108 CFU ml-1 titer). Allow to dry (about 1 hr.).
5. Add required volume of decon formulation onto coupon using sterile pipette; thoroughly wet each coupon.
6. Keep coupons wet for 60 min. Record if, and how many applications are necessary to keep coupons wet for contact time, and total volume of decontaminant used on each coupon. (Preliminary tests used 3 ml of decontaminant: 2 ml at time 0 and 1 ml at time 30 min).
7. Immediately upon completion of the contact time, transfer test coupons, along with any collected runoff, to 50 ml conical tubes containing 30 ml of sterile PBS amended with 0.001% Triton X-100, and the amount of STS determined from the neutralization tests (5 ml). Total volume should be 35 ml.
8. Extract concrete coupons by sonication for 30 min; extract other materials by vortexing in two 15-sec bursts.
9. Conduct 10-fold dilutions and plate on. One ml of the combined coupon extract and decon run-off will be removed. Prepare serial dilutions in sterile water. Plates should be incubated at 37°C for 48 hr.
10. Number of CFU ml-1 is determined by multiplying the average number of colonies by the reciprocal of the dilution. The total number of spores recovered can then be calculated by multiplying the CFU ml-1 by the total volume of the extract.
11. It is assumed that 100% recovery of spores from the spiked test coupons will not be achieved; therefore, viable spores may remain on the test coupons. A qualitative assessment will be performed to determine whether viable spores remain on the decontaminated test coupons. Following the extraction process described above, each coupon will be transferred into a sterile 50 ml conical tube containing 20 ml of sterile tryptic soy broth culture medium. These vials will be incubated at 37°C for 7 days. The tubes will be visually assessed qualitatively for viability. A cloudy culture medium may indicate "growth" of viable spores, vegetative cells, or other microorganisms. A clear culture medium indicates "no growth," consistent with a complete kill of all microorganisms.

3.6 Methods for Down-Select Tests for Optimal Formulations

1. Prepare test coupons: sterilize using UV light for 1 hr. on each side.
2. Record temperature and RH% during tests. Attempt to keep laboratory <70% RH and 20 to 25°C. Record RH and temperature.
3. Lay coupons flat in a weigh boat in an area protected from HVAC inlets/outlets.
4. Spike spores onto test coupons (1 ml of 1 x 10^8 CFU ml^{-1} titer). Allow to dry (about 1 hr).
5. Apply decon formulation to coupon surface using a sterile pipette. Thoroughly wet each coupon.
6. Keep the coupons wet for 60 min. Record if, and how many applications are necessary to keep the coupons wet for the contact time and the total volume of decontaminant used on each coupon.
7. Immediately upon completion of the contact time, test coupons along with any collected runoff should be transferred to 50 ml conical tubes containing 30 ml of sterile PBS amended with 0.001% Triton X-100, and the amount of 30% STS determined from the neutralization tests (5 ml, equivalent to 5% STS in final volume). Total liquid volume should be 35 ml.
8. Extract concrete coupons by sonication for 30 min, extract other materials by vortexing in two 15-sec bursts.
9. Conduct 10-fold dilutions and plate on Petrifilm. One ml of the combined coupon extract and decon run-off will be removed. Prepare serial dilutions in sterile water. Plates should be incubated at 37°C for 48 hr.
10. Number of CFU/ml will be determined by multiplying the average number of colonies by the reciprocal of the dilution.
11. It is assumed that 100% recovery of spores from the spiked test coupons will not be achieved; therefore, viable spores may remain on the test coupons. A qualitative assessment will be performed to determine whether viable spores remain on the decontaminated test coupons. Following the extraction process described above, each coupon will be transferred into a sterile 50-ml conical tube containing 20 ml of sterile tryptic soy broth culture medium. These vials will be incubated at 37°C for 7 days. The tubes will be visually assessed qualitatively for viability. A cloudy culture medium may indicate "growth" of viable spores, vegetative cells, or other microorganisms. A clear culture medium indicates "no growth," consistent with a complete kill of all microorganisms.

Table 3.5. Matrix of decontaminant formulations and coupon materials

Decontaminant Solution Activated-peroxide Formulate #	Coupon material	Material Test Coupon (spiked, decontaminated)	Reference Method Material Test Coupon Neutralized (spiked, neutralizer buffer, decontaminated)	Laboratory Blank (not spiked, not Decontaminated, n=3); Lab positive control, PBST, n=6
10	Carpet	6	4	3
12	Carpet	6	4	
Amended Bleach	Carpet	6	4	
PBST	Carpet	--	--	6
10	unpainted wood	6	4	3
12	Unpainted wood	6	4	
Amended Bleach	Unpainted wood	6	4	
PBST	Unpainted wood	--	--	6
10	Cinder block	6	4	3
12	Cinder block	6	4	
Amended Bleach	Cinder block	6	4	
PBST	Cinder block	--	--	6
10	Painted wood	6	4	3
12	Painted wood	6	4	
Amended Bleach	Painted wood	6	4	
PBST	Painted wood	--	--	6
10	Vinyl	6	4	3
12	Vinyl	6	4	
Amended Bleach	Vinyl	6	4	
PBST	Vinyl	6	--	
10	Stainless steel	6	4	3
12	Stainless steel	6	4	
Amended Bleach	Stainless steel	6	4	
PBST	Stainless Steel	--	--	6
10	Glazed tile	6	4	3
12	Glazed tile	6	4	
Amended Bleach	Glazed tile	6	4	
PBST	Glazed tile	--	--	6

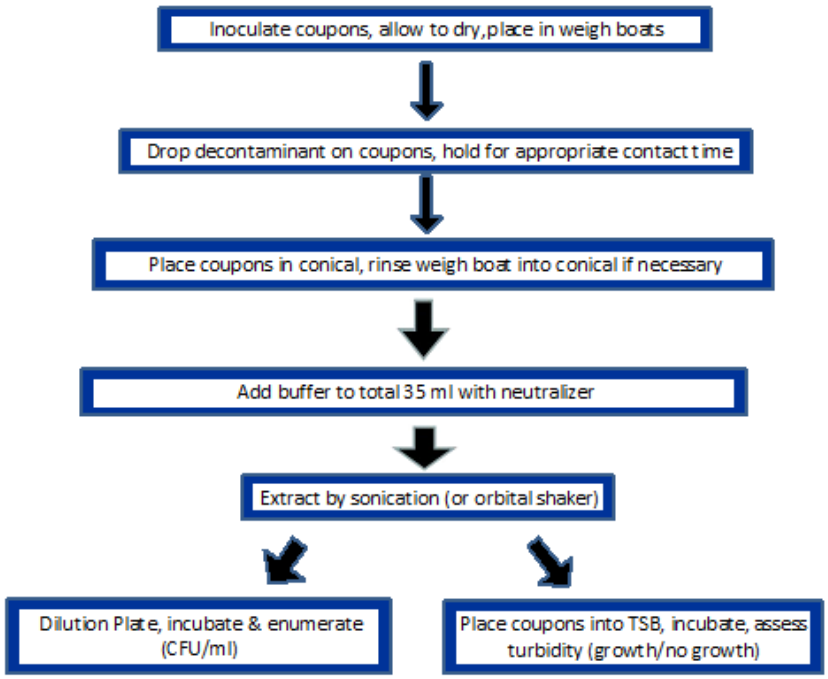

Figure 3.1. Flow chart for the activated-peroxide decontaminant evaluation

Calculations: Percent recovery was determined for each material according to equation (1): Mean Percent Recovery = [Mean $CFU_{positive}$ control / $CFU_{inoculum}$] x 100 (1) The efficacy of each sporicide was quantified by determining the difference in recovered viable spores between positive control coupons and test coupons for each coupon material and expressed as "Log Reduction". Five replicates of each were used to determine Log Reduction (LR) values for each sporicide on each material according to equation (2): $Efficacy = (\log CFUc) - (\log CFUt)$ (2) where CFUc is the abundance of colonies observed on positive control plates, and CFUt is the abundance of colonies observed on test coupon plates.

Temperature and Relative Humidity Conditions: During testing, the temperature and the relative humidity will be maintained at ambient conditions (i.e., 20 to 26°C and <70% relative humidity). When using the spraying system, relative humidity may increase due to aerosolizing the decontaminant. If this occurs, the glove box will be evacuated using a vacuum pump to reduce the humidity to below 70%. Temperature and relative humidity will be monitored using a calibrated thermometer/hygrometer.

Surface Damage: Following decontamination of the test surfaces prepared as described in Section 3, each test surface will be examined visually to establish whether use of the decontamination approach caused any obvious damage to the surface. Surface damage will be observed before extraction. The test surface will be allowed to dry before inspection for damage. Visual inspection of the surface will then take place through side-by-side comparison of the decontaminated test surface and control coupons of the same test material. Differences in color, reflectivity, and roughness will be assessed qualitatively; and observations will be made by the evaluation staff and recorded**.** Observed damage will be confirmed by a second evaluator.

3.7 Large-scale Test

A large-scale test was also conducted as part of this project. This test was conducted in the 8'x8'x8' test chamber located at Sandia. Twenty-one coupons or test locations (each approximately 1' x 1') were spiked with *B. atrophaeus* spores and placed in the chamber. Formulation #10 was applied to the coupons through a garden sprayer. The decontaminant was left on the coupons for 30 minutes after which they were sampled using a sterile wipe. The wipes were analyzed for surviving spores following a standard culturing method previously described. The coupons consisted of the following materials:

- Ceramic Tile
- Vinyl Tile
- Tabletop (wood)
- Chair (plastic)
- Stainless Steel

The layout of the coupons and test locations in the test chamber is shown below:

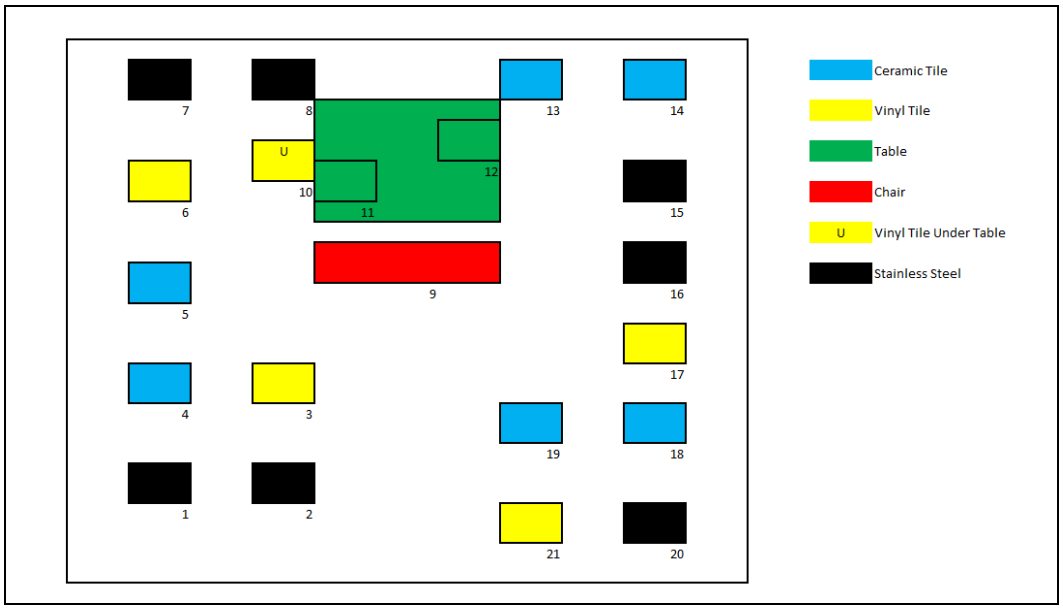

Figure 3.2. Chamber layout for large-scale test.

The test protocol is shown below:

1. Clean coupons: Bleach sterilize and rinse with DI water.
2. Lay coupons out in pre-determined pattern throughout chamber.
3. Spike coupons with spores at a concentration of ~$1x10^7$ spores/cm^2.
4. Let spores dry for 30 minutes.
5. Spray decontaminant (2L) onto coupons and let decontaminant remain for 30 minutes.

6. Sample each coupon with a sterile wipe and place into 50 mL centrifuge tube containing 30 mL of sterile DI water with tween.
7. In laboratory, vortex each sample for 1 minute and sonicate for 30 minutes.
8. Vortex each sample again for 1 minute, serial dilute, and plate onto petrifilm.
9. Place into incubator for 24 hours and enumerate.

4. SURFACE CHARACTERISTIC MEASUREMENTS

Part of the evaluation process included a measurement of the effect of the decontaminant on surface characteristics of the test coupons. The following characteristics were measured on 2 representative coupons for each sample surfaces before and after decontaminant treatment.

1. Electrostatics: The force between two point charges using Coulomb's law equations. (Simco, Model FMX-003 Electrostatic field meter).
2. Surface Roughness Index: A measure of the texture of a surface. Piezoelectric pick-up stylus with diamond tip measures within tolerances that conform to ASME B46.1. (Phase II Surface Roughness Gage Model # SRG-1000). See page 30 for roughness index results.

5. RESULTS

The two best activated-peroxide decontaminants were tested for efficacy against 7 common surface materials contaminated with about log-8 to -9 CFUs. Formula #10 was composed of H_2O_2 (6%), Triacetin (3%), Variquat 80MC (0.1), Ethanol (5%), Potassium carbonate (3%) and water. The pH of the solution was 9.26. Formula #11 was composed of H_2O_2 (6%), Triacetin (3%), Triton-X100 (0.1), Ethanol (5%), Potassium carbonate (3%) and water. The pH of the solution was 9.22.

The results summary for the decontaminant selection tests (Table 6) provides data suggesting that the efficacy of activated- peroxide decontaminant is similar to amended bleach on some common surface materials. The exception is cement where amended bleach decontamination resulted in a 5.38-log reduction and activated-peroxide decontaminants were not efficacious. Carpet resulted in similar efficacy of about a 3-log reduction. Large deviations in the results were seen in these tests so there is no statistical significance between the 2 activated-peroxide formulations. The difference between amended bleach and the activated-peroxide formulations is significant with bleach being more efficacious on painted wood, cement and tile. Activated-peroxide decontaminants were more efficacious on vinyl and unpainted wood.

The results for the large-scale test (Table 7) show that the formulation had high efficacy on all tested materials. No surviving spores were detected on any material.

Table 5.1. Efficacy of 3 decontaminants on surface materials

Test Material	Log Reduction, Activated-peroxide Formula #10	Log Reduction, Activated-Peroxide Formula #12	Log Reduction, Amended Bleach	Material Uptake (difference between titer and buffer control, Log reduction)	Vol. of decon used (mL)
Unpainted wood	2.92	2.95	1.20	0.80	3.0
Painted wood	4.08	4.21	7.68	0.49	3.0
Vinyl	8.25	4.85	4.40	0.07	1.0
Carpet	2.92	3.27	2.52	1.06	10.0
Cement	0.00	0.00	5.38	0.76	5.0
Tile	2.14	2.80	7.65	0.06	1.0
Stainless steel	4.31	6.49	6.45	0.34	1.0

Table 5.2. Efficacy of Formulation #10 on surface materials in large-scale test

Test Material	Starting Concentration (Log spores/cm^2)	Final Concentration (Log spores/cm^2)	Log Reduction, Activated-Peroxide Formulation #10
Ceramic tile #1	7.01	ND	7.01
Ceramic tile #2	7.01	ND	7.01
Ceramic tile #3	7.01	ND	7.01
Ceramic tile #4	7.01	ND	7.01
Ceramic tile #5	7.01	ND	7.01
Ceramic tile #6	7.01	ND	7.01
Vinyl tile #1	7.01	ND	7.01
Vinyl tile #2	7.01	ND	7.01
Vinyl tile #3	7.01	ND	7.01
Vinyl tile #4	7.01	ND	7.01
Vinyl tile #5	7.01	ND	7.01
Tabletop #1	7.01	ND	7.01
Tabletop #2	7.01	ND	7.01
Chair #1	7.01	ND	7.01
Stainless Steel #1	7.01	ND	7.01
Stainless Steel #2	7.01	ND	7.01
Stainless Steel #3	7.01	ND	7.01
Stainless Steel #4	7.01	ND	7.01
Stainless Steel #5	7.01	ND	7.01
Stainless Steel #6	7.01	ND	7.01
Stainless Steel #7	7.01	ND	7.01

Sample ID (formula, replicate #/dilution)	Dilution	Red Raw Count	Red Calculated Count	calc. results	AVG	SD	Log (avg)	Log SD	Log reduction	comments
T 7	1 : 1E+07	21	210000000	2.10E+08			8.32			
Blank 1 0	1 : 1	7	7	2.45E+02						
Blank 2 0	1 : 1	3	3	1.05E+02						
Blank 3 0	1 : 1	7	7	2.45E+02	1.98E+02	6.60E+01	2.30	1.82		
10C 1 5	1 : 100000	11	1100000	3.85E+07						
10C 2 5	1 : 100000	12	1200000	4.20E+07						
10C 3 5	1 : 100000	10	1000000	3.50E+07						
10C 4 5	1 : 100000	4	400000	1.40E+07	3.24E+07	1.09E+07	7.51	7.04		neutralized formula 10
12C 1 5	1 : 100000	8	800000	2.80E+07						
12C 2 5	1 : 100000	12	1200000	4.20E+07						
12C 3 5	1 : 100000	12	1200000	4.20E+07						
12C 4 5	1 : 100000	10	1000000	3.50E+07	3.68E+07	5.80E+06	7.57	6.76		neutralized formula 12
BleachC 1 5	1 : 100000	7	700000	2.45E+07						
BleachC 2 5	1 : 100000	8	800000	2.80E+07						
BleachC 3 5	1 : 100000	15	1500000	5.25E+07						
BleachC 4 5	1 : 100000	13	1300000	4.55E+07	3.76E+07	1.17E+07	7.58	7.07		neutralized bleach
10 1 2	1 : 100	11	1100	3.85E+04						
10 2 2	1 : 100	9	900	3.15E+04						
10 3 2	1 : 100	15	1500	5.25E+04						
10 4 2	1 : 100	15	1500	5.25E+04						
10 5 2	1 : 100	7	700	2.45E+04						
10 6 2	1 : 100	10	1000	3.50E+04	3.91E+04	1.04E+04	4.59	4.02	2.92	10 diff neut decon & test decon
12 1 2	1 : 100	10	1000	3.50E+04						
12 2 2	1 : 100	7	700	2.45E+04						
12 3 2	1 : 100	16	1600	5.60E+04						
12 4 2	1 : 100	11	1100	3.85E+04						
12 5 2	1 : 100	15	1500	5.25E+04						
12 6 2	1 : 100	11	1100	3.85E+04	4.08E+04	1.06E+04	4.61	4.03	2.95	12 diff neut decon & test decon
PBST 1 5	1 : 100000	8	800000	2.80E+07						
PBST 2 5	1 : 100000	12	1200000	4.20E+07						
PBST 3 5	1 : 100000	6	600000	2.10E+07						
PBST 4 5	1 : 100000	11	1100000	3.85E+07						
PBST 5 5	1 : 100000	5	500000	1.75E+07						
PBST 6 5	1 : 100000	15	1500000	5.25E+07	3.33E+07	1.23E+07	7.52	7.09	0.80	diff titer & positive cntls
Bleach 1 4	1 : 10000	9	90000	3.15E+06						
Bleach 2 4	1 : 10000	7	70000	2.45E+06						
Bleach 3 4	1 : 10000	8	80000	2.80E+06						
Bleach 4 4	1 : 10000	3	30000	1.05E+06						
Bleach 5 4	1 : 10000	8	80000	2.80E+06						
Bleach 6 4	1 : 10000	6	60000	2.10E+06	2.39E+06	6.83E+05	6.38	5.83	1.20	Bleach neut decon & test decon

Figure 5.1. Evaluation test data for unpainted wood

Sample ID	Dilution	Red Raw Count	Red Calculated Count	calc. results	AVG	SD	Log (avg)	Log SD	Log reduction	comments
T 7	1 : 1E+07	16	1.60E+08				8.20			
Blank 1 0	1 : 1	1	1.00E+00							
Blank 2 0	1 : 1	2	2.00E+00							
Blank 3 0	1 : 1	1	1.00E+00		1.33E+00	4.71E-01	0.00			
10C 1 5	1 : 100000	19	1.90E+06	6.65E+07						
10C 2 5	1 : 100000	15	1.50E+06	5.25E+07						
10C 3 5	1 : 100000	27	2.70E+06	9.45E+07						
10C 4 5	1 : 100000	26	2.60E+06	9.10E+07	7.61E+07	4.97E+05	7.88	5.70		neutralized formula 10
12C 1 5	1 : 100000	41	4.10E+06	1.44E+08						
12C 2 5	1 : 100000	29	2.90E+06	1.02E+08						
12C 3 5	1 : 100000	13	1.30E+06	4.55E+07						
12C 4 5	1 : 100000	17	1.70E+06	5.95E+07	8.75E+07	1.10E+06	7.94	6.04		neutralized formula 12
BleachC 1 5	1 : 100000	23	2.30E+06	8.05E+07						
BleachC 2 5	1 : 100000	5	5.00E+05	1.75E+07						
BleachC 3 5	1 : 100000	15	1.50E+06	5.25E+07						
BleachC 4 5	1 : 100000	12	1.20E+06	4.20E+07	4.81E+07	6.46E+05	7.68	5.81		neutralized bleach
10 1 1	1 : 10	12	1.20E+02	4.20E+03						
10 2 1	1 : 10	21	2.10E+02	7.35E+03						
10 3 1	1 : 10	20	2.00E+02	7.00E+03						
10 4 1	1 : 10	20	2.00E+02	7.00E+03						
10 5 1	1 : 10	17	1.70E+02	5.95E+03						
10 6 1	1 : 10	15	1.50E+02	5.25E+03	6.30E+03	3.20E+01	3.80	1.51	4.08	10 diff neut decon & test decon
12 1 1	1 : 10	9	9.00E+01	3.15E+03						
12 2 1	1 : 10	10	1.00E+02	3.50E+03						
12 3 1	1 : 10	10	1.00E+02	3.50E+03						
12 4 1	1 : 10	18	1.80E+02	6.30E+03						
12 5 1	1 : 10	17	1.70E+02	5.95E+03						
12 6 1	1 : 10	17	1.70E+02	5.95E+03	5.43E+03	3.86E+01	3.73	1.59	4.21	12 diff neut decon & test decon
3 1 5	1 : 100000	14	1.40E+06	4.90E+07						
3 2 5	1 : 100000	15	1.50E+06	5.25E+07						
3 3 5	1 : 100000	13	1.30E+06	4.55E+07						
3 4 5	1 : 100000	17	1.70E+06	5.95E+07						
3 5 5	1 : 100000	17	1.70E+06	5.95E+07						
3 6 5	1 : 100000	13	1.30E+06	4.55E+07	5.19E+07	1.67E+05	7.72	5.22	0.49	diff titer & positive cntls
Bleach 1 3	1 : 1000	27	2.70E+04	9.45E+05						
Bleach 2 3	1 : 1000	29	2.90E+04	1.02E+06						
Bleach 3 3	1 : 1000	37	3.70E+04	1.30E+06						
Bleach 4 3	1 : 1000	16	1.60E+04	5.60E+05						
Bleach 5 3	1 : 1000	21	2.10E+04	7.35E+05						
Bleach 6 3	1 : 1000	14	1.40E+04	4.90E+05	2.40E+04	7.92E+03	0.00	4.26	7.68	Bleach neut decon & test decon

Figure 5.2. Evaluation test data for painted wood

Sample ID	Dilution	Red Raw Count	Red Calculated Count	calc. results	AVG	SD	Log (avg)	Log SD	Log reduction	comments
T 7	1 : 1E+07	18	1.8E+08	2.10E+08			8.26			
Blank 1 0	1 : 1	3	3	1.05E+02						
Blank 2 0	1 : 1	2	2	7.00E+01						
Blank 3 0	1 : 1	5	5	1.75E+02	1.17E+02	4.37E+01	2.07			
10C 1 5	1 : 100000	44	4400000	1.54E+08						
10C 2 5	1 : 100000	47	4700000	1.65E+08						
10C 3 5	1 : 100000	58	5800000	2.03E+08						
10C 4 5	1 : 100000	55	5500000	1.93E+08	1.79E+08	2.00E+07	8.25	7.30		neutralized formula 10
12C 1 5	1 : 100000	56	5600000	1.96E+08						
12C 2 5	1 : 100000	47	4700000	1.65E+08						
12C 3 5	1 : 100000	55	5500000	1.93E+08						
12C 4 5	1 : 100000	57	5700000	2.00E+08	1.88E+08	1.39E+07	8.27	7.14		neutralized formula 12
BleachC 1	1 : 100000	13	1300000	4.55E+07						
BleachC 2	1 : 100000	10	1000000	3.50E+07						
BleachC 3	1 : 100000	36	3600000	1.26E+08						
BleachC 4	1 : 100000	49	4900000	1.72E+08	9.45E+07	5.67E+07	7.98	7.75		neutralized bleach
10 1 1	1 : 10	0	0	0.00E+00						
10 2 1	1 : 10	0	0	0.00E+00						
10 3 1	1 : 10	0	0	0.00E+00						
10 4 1	1 : 10	0	0	0.00E+00						
10 5 1	1 : 10	0	0	0.00E+00						
10 6 1	1 : 10	0	0	0.00E+00	0.00E+00	0.00E+00	0.00	0.00	8.25	10 diff neut decon & test decon
12 1 1	1 : 10	8	80	2.80E+03						
12 2 1	1 : 10	10	100	3.50E+03						
12 3 1	1 : 10	10	100	3.50E+03						
12 4 1	1 : 10	8	80	2.80E+03						
12 5 1	1 : 10	3	30	1.05E+03						
12 6 1	1 : 10	7	70	2.45E+03	2.68E+03	8.25E+02	3.43	2.92	4.85	12 diff neut decon & test decon
PBST 1 5	1 : 100000	21	2100000	7.35E+07						
PBST 2 5	1 : 100000	19	1900000	6.65E+07						
PBST 3 5	1 : 100000	24	2400000	8.40E+07						
PBST 4 5	1 : 100000	123	12000000	4.20E+08						
PBST 5 5	1 : 100000	29	2900000	1.02E+08						
PBST 6 5	1 : 100000	48	4800000	1.68E+08	1.52E+08	1.24E+08	8.18	8.09	0.07	diff titer & positive cntls
Bleach 1 1	1 : 10	0	0	0.00E+00						
Bleach 2 1	1 : 10	0	0	0.00E+00						
Bleach 3 1	1 : 10	0	0	0.00E+00						
Bleach 4 1	1 : 10	0	0	0.00E+00						
Bleach 5 1	1 : 10	24	240	8.40E+03						
Bleach 6 1	1 : 10	41	410	1.44E+04	3.79E+03	5.63E+03	3.58	3.75	4.40	Bleach neut decon & test decon

Figure 5.3. Evaluation test data for vinyl tile

Sample ID	Dilution	Red Raw Count	Red Calculated Count	calc. results	AVG	SD	Log (avg)	Log SD	Log reduction	comments
T 7*	1 : 1E+07	130	1300000000	2.10E+08			9.11			
Blank 1 0	1 : 1	1	1	3.50E+01						
Blank 2 0	1 : 1	0	0	0.00E+00						
Blank 3 0	1 : 1	0	0	0.00E+00	1.17E+01	1.65E+01	1.07			
10C 1 5	1 : 10000	32	320000	1.12E+07						
10C 2 5	1 : 10000	52	520000	1.82E+07						
10C 3 5	1 : 10000	23	230000	8.05E+06						
10C 4 5	1 : 10000	36	360000	1.26E+07	1.25E+07	3.67E+06	7.10	6.57		neutralized formula 10
12c 1 5	1 : 10000	32	320000	1.12E+07						
12C 2 5	1 : 10000	35	350000	1.23E+07						
12C 3 5	1 : 10000	42	420000	1.47E+07						
12C 4 5	1 : 10000	47	470000	1.65E+07	1.37E+07	2.06E+06	7.14	6.31		neutralized formula 12
BleachC 1	1 : 10000	186	1900000	6.65E+07						
BleachC 2	1 : 10000	29	290000	1.02E+07						
BleachC 3	1 : 10000	29	290000	1.02E+07						
BleachC 4	1 : 10000	160	1600000	5.60E+07	3.57E+07	2.58E+07	7.55	7.41		neutralized bleach
10 1 1	1 : 10	31	310	1.09E+04						
10 2 1	1 : 10	22	220	7.70E+03						
10 3 1	1 : 10	23	230	8.05E+03						
10 4 1	1 : 10	38	380	1.33E+04						
10 5 1	1 : 10	70	700	2.45E+04						
10 6 1	1 : 10	71	710	2.49E+04	1.49E+04	7.18E+03	4.17	3.86	2.92	10 diff neut decon & test decon
12 1 1	1 : 10	36	360	1.26E+04						
12 2 1	1 : 10	25	250	8.75E+03						
12 3 1	1 : 10	24	240	8.40E+03						
12 4 1	1 : 10	12	120	4.20E+03						
12 5 1	1 : 10	14	140	4.90E+03						
12 6 1	1 : 10	14	140	4.90E+03	7.29E+03	2.96E+03	3.86	3.47	3.27	12 diff neut decon & test decon
PBST 1 5	1 : 100000	38	3800000	1.33E+08						
PBST 2 5	1 : 100000	35	3500000	1.23E+08						
PBST 3 5	1 : 100000	24	2400000	8.40E+07						
PBST 4 5	1 : 100000	32	3200000	1.12E+08						
PBST 5 5	1 : 100000	33	3300000	1.16E+08						
PBST 6 5	1 : 100000	32	3200000	1.12E+08	1.13E+08	1.49E+07	8.05	7.17	1.06	diff titer & positive cntls
Bleach 1 2	1 : 100	36	3600	1.26E+05						
Bleach 2 2	1 : 100	27	2700	9.45E+04						
Bleach 3 2	1 : 100	32	3200	1.12E+05						
Bleach 4 2	1 : 100	38	3800	1.33E+05						
Bleach 5 2	1 : 100	23	2300	8.05E+04						
Bleach 6 2	1 : 100	29	2900	1.02E+05	1.08E+05	1.80E+04	5.03	4.26	2.52	Bleach neut decon & test decon

Figure 5.4. Evaluation test data for carpet

Sample ID	Dilution	Red Raw Count	Red Calculated Count	calc. results	AVG	SD	Log (avg)	Log SD	Log reduction	comments
T 7	1 : 1E+07	21	210000000	2.10E+08			8.32			
BL 1 2	1 : 1	5	5	1.75E+02						
BL 2 2	1 : 1	9	9	3.15E+02						
BL 3 2	1 : 1	8	8	2.80E+02	2.57E+02	5.95E+01	2.41			
10C 1 5	1 : 100000	24	2400000	8.40E+07						
10C 2 5	1 : 100000	28	2800000	9.80E+07						
10C 3 5	1 : 100000	34	3400000	1.19E+08						
10C 4 5	1 : 100000	38	3800000	1.33E+08	1.09E+08	1.88E+07	8.04	7.28		neutralized formula 10
12C 1 5	1 : 100000	29	2900000	1.02E+08						
12C 2 5	1 : 100000	21	2100000	7.35E+07						
12C 3 5	1 : 100000	22	2200000	7.70E+07						
12C 4 5	1 : 100000	34	3400000	1.19E+08	9.28E+07	1.86E+07	7.97	7.27		neutralized formula 12
BC 1 5	1 : 100000	11	1100000	3.85E+07						
BC 2 5	1 : 100000	7	700000	2.45E+07						
BC 3 5	1 : 100000	42	4200000	1.47E+08						
BC 4 5	1 : 100000	87	8700000	3.05E+08	1.29E+08	1.12E+08	8.11	8.05		neutralized bleach
10 1 3	1 : 1000	49	4900000	1.72E+08						
10 2 3	1 : 1000	59	5900000	2.07E+08						
10 3 3	1 : 1000	65	6500000	2.28E+08						
10 4 3	1 : 1000	44	4400000	1.54E+08						
10 5 3	1 : 1000	42	4200000	1.47E+08						
10 6 3	1 : 1000	41	4100000	1.44E+08	1.75E+08	3.16E+07	8.24	7.50	-0.21	10 diff neut decon & test decon
12 1 3	1 : 1000	31	3100000	1.09E+08						
12 2 3	1 : 1000	26	2600000	9.10E+07						
12 3 3	1 : 1000	35	3500000	1.23E+08						
12 4 3	1 : 1000	46	4600000	1.61E+08						
12 5 3	1 : 1000	59	5900000	2.07E+08						
12 6 3	1 : 1000	51	5100000	1.79E+08	1.45E+08	4.06E+07	8.16	7.61	-0.19	12 diff neut decon & test decon
PBST 1 5	1 : 100000	23	2300000	8.05E+07						
PBST 2 5	1 : 100000	9	900000	3.15E+07						
PBST 3 5	1 : 100000	11	1100000	3.85E+07						
PBST 4 5	1 : 100000	8	800000	2.80E+07						
PBST 5 5	1 : 100000	5	500000	1.75E+07						
PBST 6 5	1 : 100000	7	700000	2.45E+07	3.68E+07	2.06E+07	7.57	7.31	0.76	diff titer & positive cntls
Bleach 1 1	1 : 1	30	30	1.05E+03						
Bleach 2 1	1 : 1	30	30	1.05E+03						
Bleach 3 1	1 : 1	32	32	1.12E+03						
Bleach 4 1	1 : 1	0	0	0.00E+00						
Bleach 5 1	1 : 1	0	0	0.00E+00						
Bleach 6 1	1 : 1	0	0	0.00E+00	5.37E+02	5.37E+02	2.73	2.73	5.38	Bleach neut decon & test decon

Figure 5.5. Evaluation test data for cement

Sample ID	Dilution	Red Raw Count	Red Calculated Count	calc. results	AVG	SD	Log (avg)	Log SD	Log reduction	comments
T 7	1 : 1E+07	22	2.2E+08				8.34			
10 1 1	1 : 10	1	10	3.50E+02						
10 2 1	1 : 10	1	10	3.50E+02						
10 3 1	1 : 10	6	60	2.10E+03						
10 4 1	1 : 10	2	20	7.00E+02						
10 5 1	1 : 10	6	60	2.10E+03						
10 6 1	1 : 10	4	40	1.40E+03	1.17E+03	7.47E+02	3.07	2.87	4.31	formula 10
12 1 1	1 : 10	0	0	0.00E+00						
12 2 1	1 : 10	0	0	0.00E+00						
12 3 1	1 : 10	0	0	0.00E+00						
12 4 1	1 : 10	1	10	3.50E+02						
12 5 1	1 : 10	1	10	3.50E+02						
12 6 1	1 : 10	1	10	3.50E+02	1.75E+02	5.00E+00	2.24	0.70	6.49	formula 12
B 1 1	1 : 10	0	0	0.00E+00						
B 2 1	1 : 10	0	0	0.00E+00						
B 3 1	1 : 10	0	0	0.00E+00						
B 4 1	1 : 10	1	10	3.50E+02						
B 5 1	1 : 10	0	0	0.00E+00						
B 6 1	1 : 10	0	0	0.00E+00	5.83E+01	3.73E+00	1.77	0.57	6.45	amended bleach
PBST 1 5	1 : 100000	18	1800000	6.30E+07						
PBST 2 5	1 : 100000	27	2700000	9.45E+07						
PBST 3 5	1 : 100000	34	3400000	1.19E+08						
PBST 4 5	1 : 100000	25	2500000	8.75E+07						
PBST 5 5	1 : 100000	41	4100000	1.44E+08						
PBST 6 5	1 : 100000	29	2900000	1.02E+08	1.02E+08	7.19E+05	8.01	5.86	0.34	PBST
BLANK 1 0	1 : 1	0	0	0.00E+00						
BLANK 2 0	1 : 1	0	0	0.00E+00						
BLANK 3 0	1 : 1	0	0	0.00E+00	0.00E+00	0.00E+00	0.00	0.00		Negative control
10C 1 5	1 : 100000	16	1600000	5.60E+07						
10C 2 5	1 : 100000	19	1900000	6.65E+07						
10C 3 5	1 : 100000	12	1200000	4.20E+07						
10C 4 5	1 : 100000	24	2400000	8.40E+07	6.21E+07	1.53E+07	7.79	7.19		Neutralized formula 10
12C 1 5	1 : 100000	15	1500000	5.25E+07						
12C 2 5	1 : 100000	12	1200000	4.20E+07						
12C 3 5	1 : 100000	9	900000	3.15E+07						
12C 4 5	1 : 100000	12	1200000	4.20E+07	4.20E+07	7.42E+06	7.62	6.87		Neutralized bleach
BLEACHC 1 5	1 : 100000	21	2100000	7.35E+07						
BLEACHC 2 5	1 : 100000	18	1800000	6.30E+07						
BLEACHC 3 5	1 : 100000	17	1700000	5.95E+07						
BLEACHC 4 5	1 : 100000	10	1000000	3.50E+07	5.78E+07	1.41E+07	7.76	7.15		Neutralized formula 12

Figure 5.6. Evaluation test data for stainless steel

Sample ID	Dilution	Red Raw Count	Red Calculated Count	calc. results	AVG	SD	Log (avg)	Log SD	Log reduction	comments
T 7	1 : 1E+07	15	1.5E+08				8.18			
Blank 1 0	1 : 1	0	0							
Blank 2 0	1 : 1	0	0							
Blank 3 0	1 : 1	0	0		0.00E+00	0.00E+00	0.00			
10C 1 4	1 : 10000	83	8.30E+05	2.91E+07						
10C 2 4	1 : 10000	89	8.90E+05	3.12E+07						
10C 3 4	1 : 10000	110	1.10E+06	3.85E+07						
10C 4 4	1 : 10000	107	1.10E+06	3.85E+07	3.43E+07	1.22E+05	7.54	5.09		neutralized formula 10
12C 1 4	1 : 10000	137	1.40E+06	4.90E+07						
12C 2 4	1 : 10000	237	2.40E+06	8.40E+07						
12C 3 4	1 : 10000	184	1.80E+06	6.30E+07						
12C 4 4	1 : 10000	251	2.50E+06	8.75E+07	7.09E+07	4.49E+05	7.85	5.65		neutralized formula 12
BleachC 1	1 : 10000	167	1.70E+06	5.95E+07						
BleachC 2	1 : 10000	37	3.70E+05	1.30E+07						
BleachC 3	1 : 10000	123	1.20E+06	4.20E+07						
BleachC 4	1 : 10000	185	1.80E+06	6.30E+07	4.44E+07	5.66E+05	7.65	5.75		neutralized bleach
10 1 2	1 : 100	43	4.30E+03	1.51E+05						
10 2 2	1 : 100	61	6.10E+03	2.14E+05						
10 3 2	1 : 100	55	5.50E+03	1.93E+05						
10 4 2	1 : 100	79	7.90E+03	2.77E+05						
10 5 2	1 : 100	62	6.20E+03	2.17E+05						
10 6 2	1 : 100	85	8.50E+03	2.98E+05	2.46E+05	1.41E+03	5.39	3.15	2.14	10 diff neut decon & test decon
12 1 2	1 : 100	42	4.20E+03	1.47E+05						
12 2 2	1 : 100	31	3.10E+03	1.09E+05						
12 3 2	1 : 100	21	2.10E+03	7.35E+04						
12 4 2	1 : 100	29	2.90E+03	1.02E+05						
12 5 2	1 : 100	37	3.70E+03	1.30E+05						
12 6 2	1 : 100	42	4.20E+03	1.47E+05	1.13E+05	7.52E+02	5.05	2.88	2.80	12 diff neut decon & test decon
PBST 1 5	1 : 100000	39	3.90E+06	1.37E+08						
PBST 2 5	1 : 100000	39	3.90E+06	1.37E+08						
PBST 3 5	1 : 100000	39	3.90E+06	1.37E+08						
PBST 4 5	1 : 100000	35	3.50E+06	1.23E+08						
PBST 5 5	1 : 100000	38	3.80E+06	1.33E+08						
PBST 6 5	1 : 100000	34	3.40E+06	1.19E+08	1.31E+08	2.05E+05	8.12	5.31	0.06	diff titer & positive cntls
Bleach 1 1	1 : 10	0	0	0.00E+00						
Bleach 2 1	1 : 10	0	0	0.00E+00						
Bleach 3 1	1 : 10	0	0	0.00E+00						
Bleach 4 1	1 : 10	0	0	0.00E+00						
Bleach 5 1	1 : 10	0	0	0.00E+00						
Bleach 6 1	1 : 10	0	0	0.00E+00	0.00E+00	0.00E+00	0.00	4.26	7.65	Bleach neut decon & test decon

Figure 5.7. Evaluation test data for glazed tile

Surface roughness testing results. The treated samples were immersed in a solution of tween buffer and neutralizer, removed and allowed to air dry for four to five hours.

Coupon Type	Treated	Sampling	Replicate	Replicate	Replicate 3	Avg	Difference between treated & untreated	
Linoleum		0.8	0.98	0.42	0.8	0.73		
Linoleum	x	0.8	0.75	0.75	0.85	0.78	0.05	no change
Stainless Steel		0.25	0.13	0.13	0.1	0.12		
Stainless Steel	x	0.25	0.08	0.1	0.13	0.10	-0.02	no change
Unpainted Wood		2.5	2.88	3.45	4.14	3.49		
Unpainted Wood	x	0.8	1.61	0.73	4.36	2.23	-1.26	flater
Concrete		2.5	3.03	0.6	3.14	2.26		
Concrete	x	2.5	6.27	4.93	10.29	7.16	4.91	rougher
Carpet		2.5	9.3	6.85	3.57	6.57		
Carpet	x	2.5	11.14	4.75	5.1	7.00	0.42	no change
Tile		0.8	0.52	0.65	0.55	0.57		
Tile	x	0.8	0.81	0.65	0.62	0.69	0.12	
Painted Wallboard		2.5	3.4	3.19	3.81	3.47		
Painted Wallboard	x	0.8	0.57	0.62	0.7	0.63	-2.84	flater

Figure 5.8. Surface roughness test results

Pores in wood materials tended to swell and the roughness index decreased.

6. PROPOSED APPLICATION PROTOCOL

A proposed application protocol has been developed for use of an activated-peroxide decontaminant. Decontamination processes are subject to oversight by the Unified Command and may require site-specific modification.

Liquid Decon Application Technique[1, 2, 7]
Pre-Application Steps
1. Obtain an efficacious liquid sporicidal—Peridox™ (USEPA Reg. No. 81073-2) or pH-amended bleach— that has received USEPA registration for use against with *B. anthracis*. Use unregistered peroxide-based products, such as SporKlenz RTU™, Easy Decon 200™, MinCare™ and Oxonia™, or a simple blend of buffered peroxide and activator, only after they have been approved under a FIFRA exemption[3, 4, 5]. 2. If possible, replace all filters in the ventilation units and with high efficiency filters. Wet the contaminated filters with a decontaminant, place in a labeled double bag for removal. Alternatively, turn off the ventilation system and place a HEPA-filtered portable air scrubber with ductwork running from the system to somewhere outside of the project area in order to maintain negative pressure in relation to the cold zones (non-contaminated zones). Remove non-essential materials and porous materials, such as fabric drapes, sofas and chairs. Consider cost efficiency of replacing non-essential items rather than decontamination. Wet these materials with decontaminant prior to removing to reduce cross-contamination. Place in double bags and label. 3. Identify valuable materials that may be damaged by decontamination with liquid materials. Double bag and label these for off-site non-destructive decontamination methods (e.g., ethylene oxide). Apply liquid sporicidal decontaminant (see 2 and 3 above) to the outer bag for the required contact time prior to their removal.
Pre-Cleaning (optional)
4. Pre-clean, if visibly dirty, all surfaces with a wet/dry vacuum fitted with a HEPA-rated filter. Insure that the HEPA filters are correctly installed in the filter frame. Dispose of HEPA filters as contaminated waste. 5. On heavily soiled surfaces only, wash off dried on dirt, oils, or grease by scrubbing with a brush, soap and water. Rinse the surface with water. Vacuum standing water from horizontal surfaces with the wet/dry vacuum[6].
Decontamination
6. Keep the surfaces wetted with the decontaminant for the required time period (e.g., 30 minutes), re-applying solution as needed to maintain wetness and follow the FIFRA-registered instructions for the decontaminant product. 7. Wet-vacuum or mop any residual standing liquid.
Re-application (if needed)
8. Pending results of post-treatment sampling, reapply the decontamination solution if needed.

[1] USEPA/NDT Decontamination Analytical and Technical Service (DATS) Contract Number: EP-W-06-089 TDD No. TO-02-07-12-0016. After Action Report Danbury Anthrax Incident. September 19, 2008. Kelly Smith (Dynamac Corp.) Michael J. Nalipinski, EPA/OSC

[2]Shawn P. Ryan, Worth Calfee, Sang Don Lee, and Russell Wiener, Stella Payne, Rob Delafield, Calvin Whitfield, Nicole Griffin Gatchalian, Matt Clayton, and Abderrahmane Touati in the presentation entitled "Assessment of Liquid and Physical Decontamination Methods for Environmental Surfaces Contaminated" (5/28/09).

[3] See the USEPA website for the latest information on products registered as sporicidal decontaminants (http://www.epa.gov).

[4] Several commercially available products (peroxide/PAA or activated-peroxide solutions) are efficacious for spores; see Wood, Joseph P. *Technology Evaluation Report: Evaluation of Liquid and Foam Technologies for the Decontamination of B. anthracis and B. subtilis Spores on Building and Outdoor Materials: DioxiGuard™ (Frontier Pharmaceutical), pH-Amended Bleach, Calcium Polysulfide, CASCAD™ Surface Decontamination Foam (Allen-Vanguard), Oxonia Active® (Ecolab Inc.), Minncare® Cold Sterilant (Minntech Corp.) and SanDes (DTI-Sweden AB)*. EPA/600/R-09/150, November 2009, www.epa.gov/ord. http://www.epa.gov/nhsrc/pubs/600r09150.pdf.

[5] Note that regular household (3%) hydrogen peroxide is not efficacious on spores (SAND2010-2584C).

[6] Vacuum and washing may cause contaminant spread. These steps should only be used on heavily soiled surfaces (see Ryan *et al*., 5/28/09).

[7]Updated from Figure 2 of Krauter *et al*., Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science. September 2011, 9(3): 301-309. doi:10.1089/bsp.2011.0025.

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