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Evaluation of Diperadipic Acid as a Surface Decontaminant for Spore-Forming Biological Agents

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Evaluation of Diperadipic Acid as a Surface Decontaminant for Spore-Forming Biological Agents

Evaluation Report

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Acronyms and Abbreviations

BioLab	NHSRC Research Triangle Park (RTP) Microbiology Laboratory
Bg	*Bacillus atrophaeus* var. *globigii*
CFU	colony forming unit(s)
DE	Dey Engley
DHS	U.S. Department of Homeland Security
DI	deionized
DPAA	diperadipic acid
DQI	data quality indicator
DQO	data quality objective
EPA	U.S. Environmental Protection Agency
ETC	environmental test chamber
EtO	ethylene oxide
HSRP	Homeland Security Research Program
ID	identification
LR	log reduction
NHSRC	National Homeland Security Research Center
NIST	National Institute for Standards and Technology
ORD	Office of Research and Development
pAB	pH-adjusted bleach
PBST	phosphate-buffered saline with 0.05% Tween® 20
QA	quality assurance
QC	quality control
RH	relative humidity
RSD	relative standard deviation
RTP	Research Triangle Park
STS	sodium thiosulfate
TSA	tryptic soy agar

Executive Summary

An environmental contamination incident involving an infectious or contagious biological agent may pose significant risks to human health. Currently, there are a limited number of sporicidal chemicals that are approved for use against *Bacillus anthracis*, the causative agent of anthrax. Characterization of potential decontamination options, ahead of a contamination incident, are important to ensure response and remediation operations initiate promptly and are effective. The current laboratory-scale study was undertaken to evaluate the decontamination efficacy of diperadipic acid, under application conditions that were found to be effective for pH-adjusted bleach (a common sporicidal liquid). The results showed that diperadipic acid demonstrated >6 Log Reduction on glass surfaces, but <2 Log Reduction on concrete. Like studies with pH-adjusted bleach, viable spores were found in runoff samples, indicating that relocation of contaminants from surfaces may be possible during decontamination.

1 Introduction

This project was conducted by the U.S. Environmental Protection Agency (EPA) Office of Research and Development's (ORD's) National Homeland Security Research Center (NHSRC) and supports the mission of the EPA's Homeland Security Research Program (HSRP) by providing relevant information pertinent to the decontamination of contaminated areas resulting from a biological incident. The key objective of this project is to estimate the potential *reduction of viable bacterial spores* (effectiveness) as a function of the remediation activities applied under challenging situations and under conditions in field operations.

This study was conducted to evaluate the decontamination efficacy of a diperadipic acid formulation (Lynntech, 2017), when spray-applied to relevant building materials (concrete and glass) contaminated with *Bacillus anthracis* surrogate spores. (*Bacillus anthracis* is the causative agent for anthrax.) Test conditions (spray duration, spray flow rate, spray distance, contact time, temperature, materials, test chamber, etc.) previously shown to be effective when pH-adjusted bleach was evaluated were selected for the current evaluation of diperadipic acid (DPAA).

1.1 Background

The EPA 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 other contamination incidents. To carry out the HSRP mission, the NHSRC conducts research to provide expertise and guidance on the selection and implementation of decontamination methods that could provide the scientific basis for a significant reduction in the time and cost of remediating contaminated sites.

This project addresses a direct need expressed by the EPA's Office of Land and Emergency Management's Chemical, Biological, Radiological, and Nuclear Consequence Management Advisory Division (CMAD). This need consists of evaluating the effectiveness of innovative decontamination formulations and procedures, including spraying surfaces with sporicidal liquids under such conditions.

1.2 Project Objectives

The present effort, described in this data report, evaluated the effectiveness of a spray-based "low-tech" decontamination method to inactivate/remove spores from building materials (concrete and glass) using a DPAA formulation developed by Lynntech, Inc. The DPAA biological agent decontamination formulation is a stabilized powder form, claimed to be sporicidal and effective against a wide range of microorganisms including aerobic and anaerobic bacteria and algae. For this effort, "low-tech" is defined as a decontamination approach that can use off-the-shelf material or equipment, readily available at a local hardware store.

Test coupons were prepared from typical urban building materials (glass and concrete), inoculated with the target organism *Bacillus atrophaeus* var. *globigii* (*Bg*) using an aerosol deposition method (U.S. EPA, 2017), and sprayed with the test solution at 25 °C. Tests were conducted in an environmental test chamber (ETC) so that the environmental conditions (temperature and relative humidity [RH]) could be tightly controlled. To simulate large-scale

outdoor operations, all test components (spray nozzles, coupons, runoff collection, decontaminant reservoir, decontaminant supply tubing, etc.) were located within the chamber and were acclimated to test conditions prior to testing. The surface decontamination efficacy of each formulation was measured based on the reduction of viable spores on the surface of the test coupons. Relocation of viable spores from the contaminated coupon surfaces into the overspray runoff during each decontamination event was also investigated. Spray pressure, volumes, duration, and angle of application were selected based upon conditions previously shown effective (complete kill) when using pH-adjusted bleach (U.S. EPA, 2017).

2 Experimental Approach

2.1 Overall Experimental Approach

Testing was conducted at EPA's Research Triangle Park (RTP) facility in North Carolina. The general experimental approach used to meet the project objectives is described in the previously published report entitled "Evaluation of Spray-Based, Low-Tech Decontamination Methods under Operationally Challenging Environments: Cold Temperatures," (Lee et. al., 2017) and is summarized below.

1. **Test Coupon Preparation**: The 18-mm diameter coupons shown in Figure 2-1 were prepared from two target materials: concrete and glass. The glass coupon was made of an 18-mm glass disc that was affixed to an aluminum stub using a carbon-based adhesive. The concrete coupon was fabricated from Sakrete® Top' N Bond Patcher (Sakrete, Cincinnati, OH), with a drywall nail in the center of the back for handling.

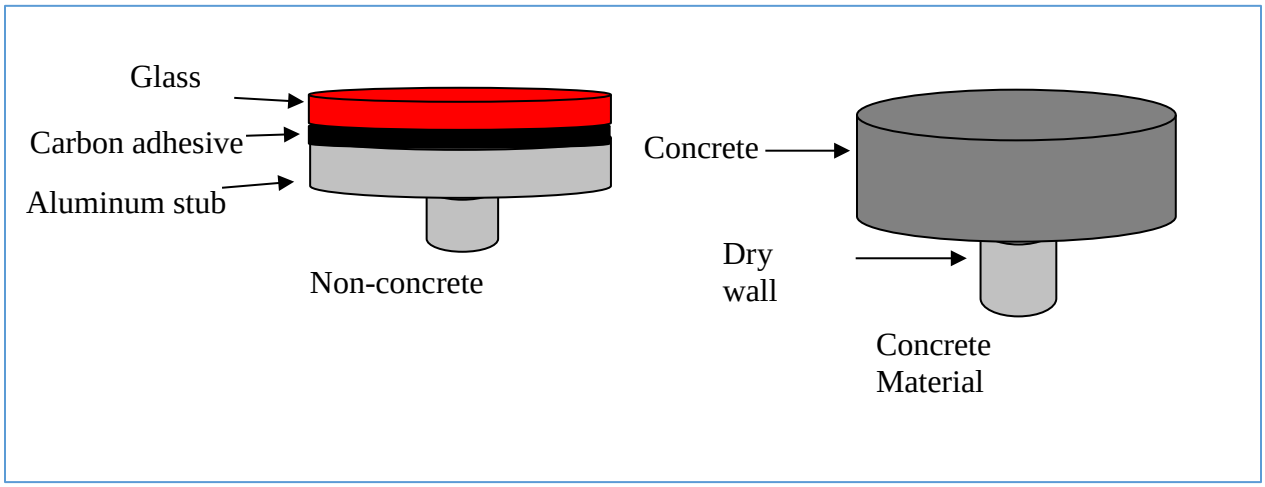

Figure 2-1. Coupon diagram of non-concrete and concrete materials.

2. **Test Material Sterilization**: The coupons, funnels, and stages for storing coupons and plastic spray bottles were sterilized using an Andersen ethylene oxide (EtO) sterilizer system (EOGas®, Part No. 333, ANPRO, Haw River, NC), and a sterilization kit (Kit #6, Part No. AN1006, ANPRO) that includes a cartridge, a humidichip®, a dosimeter, and a bag.
3. **Test Material Inoculation**: The test coupons were inoculated using an aerosol deposition method (U.S. EPA, 2017) that delivered a known concentration of spores in a repeatable fashion. Approximately 1×10^7 spores of *Bg*, a surrogate organism for *Bacillus anthracis*, were deposited onto each coupon.
4. **Decontamination Solution Preparation**: A powdered concentrate of DPAA, manufactured by Lynntech, Inc. (College Station, TX), and provided by the Department of Homeland Security (DHS), was dissolved in deionized (DI) water (130 grams of DPAA in 1 liter of DI water), and stored at 4 °C before use.

5. **Decontamination Procedure**: Test coupons (five coupons per test material) were decontaminated using the ETC, equipped with an automated spraying system.
6. **Sample neutralization:** Dey Engley (DE) broth (Dey and Engley, 1994) was selected as the neutralizing agent, as discussed previously (U.S. EPA, 2017, Section 3.6). The neutralizing agent was applied to stop the decontamination activity after a prescribed exposure time. After the prescribed exposure times, coupons were collected and deposited into a tube containing the neutralizing agent. Sodium thiosulfate (STS) was also evaluated as a neutralizer.
7. **Runoff Collection:** Liquid runoff from each coupon was also collected through sterile funnels into sample tubes that contained pre-determined volumes of neutralizer.
8. **Sample extraction and analysis**: Viable *Bg* spores were extracted from the test samples (coupon and runoff), and aliquots were analyzed using an automated spiral plating system (Autoplate 5000, Advanced Instruments Inc., Norwood, MA). Viable spore recovery was quantified in terms of colony forming units (CFU) present in each sample.
9. **Determination of decontamination efficacy:** Decontamination efficacy was expressed as log reduction (LR) of viable *Bg* spores recovered. Decontamination efficacy for each coupon was determined by comparison to positive control sample results. The transfer of viable organisms to post-decontamination liquid waste was evaluated through quantitative analysis of decontamination procedure residues (such as decontamination solution runoff samples).

2.2 Test Matrix

The test matrix for this effort is detailed in Table 2-1. The test conditions denote the temperature and humidity conditions surrounding the coupons at the time of the spray test. The test chamber temperature was set at 25 °C, while the RH inside the chamber was recorded at the set temperature with no corrections. The decontaminant application conditions were set to a duration, flow rate, and reapplication frequency that achieved >6 LR when pH-adjusted bleach (pAB) was used as the decontaminant in the same test chamber under the same environmental conditions (U.S. EPA, 2017). This test design allows results from tests with DPAA and other decontaminants, to be compared with the baseline performance of a well-characterized decontaminant (pAB). Test coupons were sprayed with a 5-second spray at time zero, followed by another 5-second spray at the 10-minute mark. Two 10-minute contact times, one after each spray, were rendered. The total exposure time (wetted contact time) was 20 minutes.

Table 2-1. Test Matrix

Test	Decontaminant /Formulation*	Spray Number/Duration	Contact time (Minutes)	Temperature (°C)	Humidity (%RH)	Material Type
1	Diperadipic Acid	5-second spray, one at 0 minutes, one at 10 minutes	20	25	Ambient	Concrete
2						Glass

RH, relative humidity

3 Sampling and Analytical Procedures

The primary results from this study will be from the analysis of samples resulting in recovered viable spores (measured as CFU) per sample expressed on a log-10 scale. Additional measurements prior to or during the decontamination procedure application are also required to ensure quality control in the testing. These measurements include quality control checks on the reagents and equipment being used in the decontamination procedure.

A sampling data log sheet was maintained for each sampling event (or test) that included each sampling event, the date, test name, sample IDs (identifications), and other test details such as test temperature, final rinsate volume, and sample extraction time. The sample IDs were pre-printed on the sampling data log sheet before sampling began. Digital photographs were taken to document activities throughout the test cycle.

3.1 Microbiological Analysis

This section discusses the project sampling and analytical procedures, including sample quantities, sample types, and coupon sample extraction and analysis.

3.1.1 Sample Quantities

For each decontamination solution, there were five replicates of coupon samples, five liquid rinsate samples, three positive control samples, one procedural blank and one negative control sample per material. Table 3-1 lists the total numbers of samples of each type for each test.

Table 3-1. Sample Types and Numbers for Each Decontamination Solution

Sample Type	Number of Samples
Test coupon sample (decontaminated)	5
Liquid rinsate sample	5
Positive control sample	3
Procedural blank	1
Negative control sample	1

3.1.2 Sample Types

The three major sample types for this project are discussed below.

- **Surface test coupon samples:** Each coupon sample was aseptically transferred, using sterile forceps, from the stage in the ETC to a 50-mL conical tube containing 10 mL of phosphate buffered saline with 0.05% Tween® 20 (PBST) (TWEEN®, Croda International PLC, Snaith, UK) and 1.5 mL of DE broth.

- **Liquid rinsate samples:** These samples were collected in 250 mL conical tubes, which were pre-loaded with a set amount of neutralizer, to assess the potential for viable microorganisms that were washed off the coupon surfaces. Samples were collected from all liquid runoff during spray applications, and the collection funnels were subsequently rinsed with sterile DI water. Rinsate samples were collected in the same vials as runoff and together constituted a single sample. After collection, test coupon and liquid rinsate samples were sealed in secondary containment and transported to the NHSRC Research Triangle Park (RTP) Microbiology Laboratory (BioLab) for quantitative analysis.

3.1.3 Sample Extraction and Analysis

The EPA RTP BioLab analyzed all samples for the presence of spores (sterility check samples) and quantified the number of viable spores per sample (test coupon and liquid runoff samples). For all sample types, PBST was used as the extraction buffer. After the extraction procedure, each sample was aliquoted and plated in triplicate using a spiral plater (Autoplate 5000, Advanced Instruments, Inc., Norwood, MA), which deposits the sample in exponentially decreasing amounts across a rotating agar plate in concentric lines to achieve three 10-fold serial dilutions on each plate. Plates were incubated at 35 ± 2 °C for 16 to 19 hours. The colonies on each plate were enumerated using a QCount® colony counter (Advanced Instruments, Inc., Norwood, MA).

Positive control samples were diluted 100-fold (10^{-2}) in PBST before spiral plating, and samples of unknown concentration were plated undiluted and after a 100-fold dilution. Samples with known low concentrations were plated undiluted. The QCount® colony counter automatically calculates the CFU/mL in a sample based on the dilution plated and the number of colonies that develop on the plate. The QCount® records the data in an Microsoft® Excel® spreadsheet.

Only plates meeting the threshold of at least 30 CFU were used for spore recovery estimates. Samples below the 30-CFU threshold were either spiral-plated again with a more concentrated sample aliquot, filter-plated, or spread-plated in triplicate on tryptic soy agar (TSA) plates using 1-mL aliquots per plate to achieve a lower detection limit. The plates were incubated at 35 ± 2 °C for 20 to 24 hours before manual enumeration.

3.2 Data Reduction

The overall effectiveness of a decontamination technique is a measure of the ability of the method to inactivate and/or remove the spores from material surfaces while considering viable spores that might be relocated from the test surface. Such fugitive biological emissions could result in secondary contamination that would necessitate additional remediation strategies.

Data reduction was performed on measurements of the total viable spores (measured as CFU) recovered from each replicate coupon. The average recovered viable spores and standard deviation for each group of coupons was determined. The groups of coupons included the following for each combination of material type and extracted sample type:

- Positive control areas (replicates, average, standard deviation)

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

4 Results and Discussion

This section summarizes the test results for the decontamination efficacy (surface and total) of the DPAA decontaminant. Relocation of viable spores from the contaminated coupon surfaces into the overspray runoff during each decontamination event is also reported.

4.1 Neutralization Results

The presence of the decontamination solution on the sample surface or in the liquid effluent following a decontamination event could negatively bias recovery and efficacy results. Based on previous studies, sodium thiosulfate (STS) (Calfee et al., 2011), and DE broth were shown to be effective neutralizers for various antimicrobial agents. Both STS and DE broth were evaluated for their respective effectiveness in neutralizing the DPAA solution using glass and concrete building materials.

The volume of DPAA solution used for the test coupons in this test was derived from the results obtained for previously performed volume determination test. Table 4-1. indicates the volumes of DPAA solution and neutralizers used in this test.

The results of the neutralization test series, shown in Table 4-1, and illustrated in Figure 4-1 for the liquid effluent, confirm the effectiveness of both neutralizers and the residual decontamination of the DPAA formulation, when not neutralized. To remain consistent with concurrent studies undertaken by EPA to evaluate low tech/low cost decontamination formulation effectiveness to inactivate spores, DE broth was used as a neutralizer for the decontamination testing.

Table 4-1. Results of Neutralizer Effectiveness Tests

Description	Neutralizer	Material	Average DPAA Volume (mL)	Average Neutralizer Volume (mL)	Spore Recovery	
					Avg. CFU	RSD (%)
Test coupon	DE Broth	Concrete	0.6	1.5	3.48E+06	18.79
	STS		0.6	0.4	3.90E+06	8.23
	DE Broth	Glass	0.2	1.5	8.16E+05	8.60
	STS		0.2	0.2	1.14E+06	5.44
Rinsate	DE Broth	-	5.0	5.0	2.76E+06	15.54
	STS		5.0	3.7	1.58E+07	18.86
	DE Broth		5.0	10.0	1.13E+07	56.11
	STS		5.0	7.3	1.43E+07	18.60
	No neutralizer		5.0	0.0	8.66E-01	15.24
Inoculum Control					1.18E+07	

CFU, colony forming units; DE, Dey Engley; DPAA, diperadipic acid; RSD, relative standard deviation; STS, sodium thiosulfate

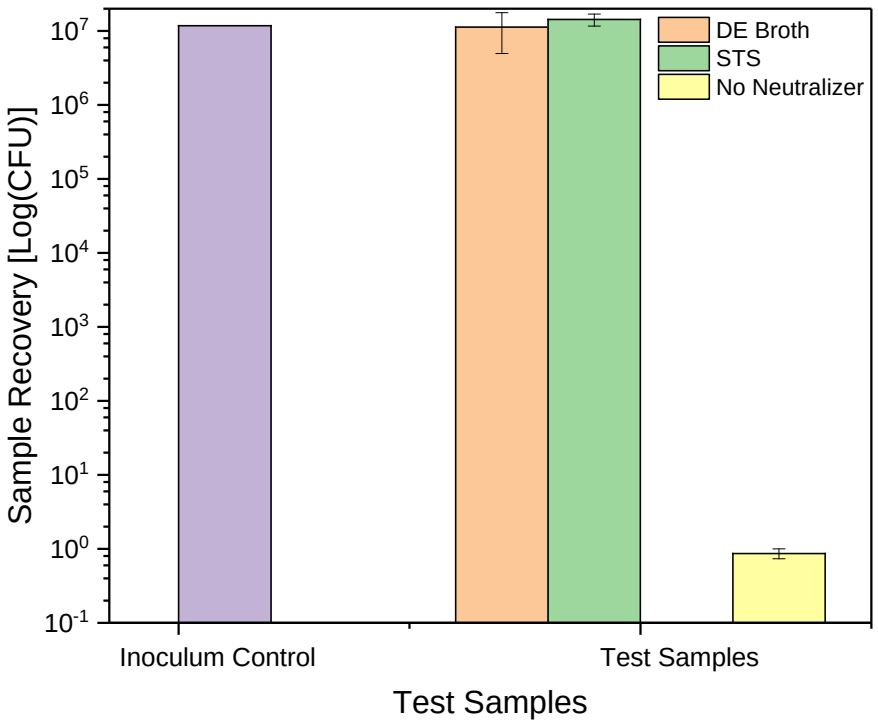

Figure 4-1. Neutralizer effectiveness.

4.2 Decontamination Results

Decontamination efficacy (Figure 4-2 and Table 4-2) was expressed as a log reduction (LR) of the viable *Bg* spores (CFU) recovered. Typically, for laboratory assessments of decontamination efficacy, for a 1 x 10^6 CFU) or greater, the LR ≥ 6 is considered effective (U.S. EPA, 2007), and when no viable spores are recovered (complete kill) after decontamination treatment, the method is considered highly effective. Decontamination efficacy for each test material was determined by comparison to positive control sample results, and calculated as follows:

Decontamination efficacy = Mean (*Log* CFU positive control sample) – Mean (*Log* CFU Post Decontamination test coupon sample)

Quantitative assessment of residual (background) contamination was performed by sampling procedural blanks (non-inoculated coupons exposed to the same decontamination process as the test coupons). The transfer of viable organisms to post-decontamination liquid waste also was evaluated through quantitative analysis of decontamination solution runoff samples.

The tests were set up for a five-second spray duration, with one repeat application (two total applications). The total solution contact time was 20 minutes (10 minutes after the first spray and 10 minutes after the second spray). After the spraying operation was complete, test coupons were immersed in a neutralizing agent to quench the decontamination reaction. Samples were then sent to the BioLab for analysis. The results show that the formulation is very effective for

nonporous glass material but much less effective with the more alkaline, porous concrete material.

To assess the potential fate of the spores, immediately after a decontamination event, all liquids used in the decontamination test process were collected and quantitatively analyzed. To provide a conservative estimate of spore fate and transport, rinsates were neutralized immediately upon collection by pre-loading collection tubes with a neutralizing agent. As expected, the post-decontamination spore recoveries in the rinsates were on the same order of magnitude for both the concrete and glass and are results of a physical removal of the spores from the materials.

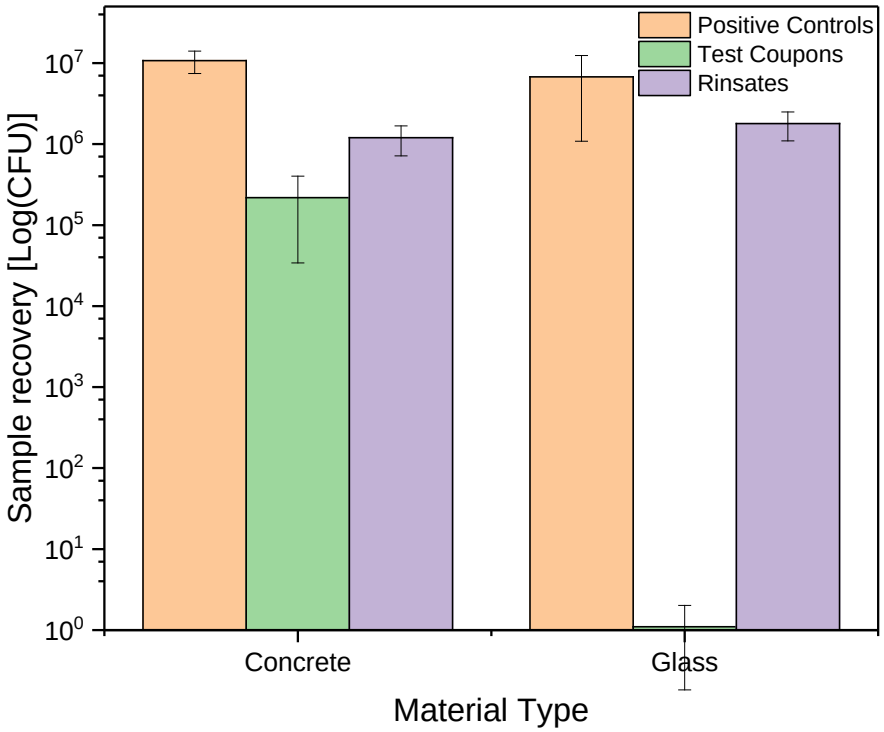

Figure 4-2. Test sample recoveries for concrete and glass following a DPAA decontamination event.

Table 4-2. Sample Types and Numbers for Each Decontamination Solution

Material Type	Positive Control Recovery (CFU)		Test Coupon Recovery (CFU)		Rinsate Recovery (CFUs)		Surface Decon Efficacy(LR)	
	Average	Standard Deviation	Average	Standard Deviation	Average	Standard Deviation	Average	Standard Deviation
Concrete	1.07E+07	3.29E+06	2.18E+05	1.83E+05	1.20E+06	4.80E+05	1.83	0.42
Glass	6.76E+06	5.67E+06	1.10E+00	9.17E-01	1.80E+06	7.02E+05	6.78	0.27

CFU, colony forming units; LR, log reduction

5 Quality Assurance and Quality Control

All test activities were documented in laboratory notebooks and digital photographs. The documentation included, but was not limited to, a record for each decontamination procedure, any deviations from the quality assurance project plan, and physical impacts on materials. All tests were conducted in accordance with established EPA Decontamination Technologies Research Laboratory and BioLab procedures to ensure repeatability and adherence to the data quality validation criteria set for this project. These procedures are maintained with the facility manual.

The following sections discuss the criteria for the critical measurements and parameters, data quality indictors (DQIs), and the quality assurance (QA) and quality control (QC) checks for the project.

5.1 Criteria for Critical Measurements and Parameters

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

- ETC temperature
- Flow rate of spray nozzles of the automated spray system
- Sample volume collected
- Exposure time
- Temperature of the incubation chamber
- CFU counts
- Plated volume
- Neutralizer volume

5.2 Data Quality Indicators

Table 5-1 lists the DQIs for the critical measurements and parameters. These DQIs were used to determine if the collected data met the QA objectives. Volumes of components were measured as accurately as possible using appropriate measuring equipment (such as volumetric flasks and graduated cylinders).

Table 5-1. Data Quality Indicators for Critical Measurements and Parameters

Critical Measurement	Measurement Device	Accuracy or Precision Target	Detection Limit
ETC temperature	Temperature control sensor	± 0.5 °C	-73 to +175 °C
Sprayer flow rate	Volume collected in graduated cylinder per time	± 10%	1 mL per minute

Critical Measurement	Measurement Device	Accuracy or Precision Target	Detection Limit
Rinsate volume collected	Conical vial	12.5 mL	+ 0.1 mL
Spray time	Timer	± 1 second	1 second
Exposure time	Timer	± 1 second	1 second
Temperature of incubation chamber	NIST-traceable thermometer	± 2 °C	NA
CFU counts	QCount	Calibration of spiral plater with instrument standard 2.0×10^4 must yield QCount output of 1.82×10^4 to 2.30×10^4	20 CFU/plate
Plated volume	Spiral plater	NA	NA
Neutralizer volume	Serological pipette tips	0.1 mL	0.05 mL
Pressure of automated spray system	Compressed air regulator	± 1 psi	0 psi

CFU, colony forming unit; ETC, environmental test chamber; NIST, National Institute of Standards and Technology; NA, Not Applicable

5.3 Quality Assurance/Quality Control Checks

The critical measurements and parameters listed in Table 5-1 were measured before testing. If the measurements obtained did not meet the DQI goals, the test was stopped. Tests proceeded only when the DQI criteria were met.

Many QA/QC checks were used in this project to ensure that the data collected met all the critical measurements listed in Table 5-1. The measurement and parameter criteria were set at the most stringent levels routinely achievable. The acceptance criteria for the microbiological analysis also were set at the most stringent levels routinely achievable, and decisions to accept or reject test results were based on analytical judgment to assess the likely impact of the failed criterion on the conclusions drawn from the data.

All the critical measurements and parameters met the DQI target acceptance criteria listed in Table 5-1. Control samples and procedural blanks were included along with the test samples so that well-controlled quantitative values were obtained. Background checks for the presence of bacterial spores were included as part of the standard protocol. Replicate coupons of both materials were included for each set of test conditions. Specific QC checks performed under this project included a check of the integrity of samples and supplies, BioLab control checks and QA assessments and corrective actions are described below.

5.3.1 Check of Integrity of Samples and Supplies

Samples were carefully maintained and preserved to ensure their integrity. Samples were stored away from standards or other samples that could cross-contaminate them. In addition, project personnel carefully checked supplies and consumables before use to verify that they met specified project quality objectives. All pipettes were calibrated yearly by an outside contractor (Calibrate, Inc., Carrboro, NC). Incubation temperature was monitored using National Institute of Standards and Technology (NIST)-traceable thermometers, and the EPA Metrology Laboratory calibrated the balances yearly.

5.3.2 Microbiology Laboratory Control Checks

Quantitative standards do not exist for biological agents. Viable spores were counted using an Advanced Instruments QCount® colony counter. Counts greater than 300 or less than 30 CFU were considered outside the quantitation range. If the CFU count did not fall within the acceptable quantitation range, the sample was re-plated at a different volume or dilution and then re-counted.

Before each batch of plates was enumerated, a QC plate was analyzed, and the result was verified to be within the range indicated on the back of the QC plate. As the plates were counted, a visual inspection of colony counts made by the QCount® colony counter was performed. Obvious count errors made by the software were corrected by adjusting the settings (such as colony size, light, and field of view) and by recounting using an edit feature of the QCount® software that allows manual removal of erroneously identified spots or shadows on the plate or by adding colonies that the QCount® software may have missed.

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

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

- **Positive control coupons:** Coupons inoculated in tandem with the test coupons to demonstrate the highest level of contamination recoverable from a specific inoculation event.
- **Procedural blank coupons:** Material coupons sampled in the same fashion as test coupons but not inoculated with the surrogate organism before sampling.
- **Blank TSA sterility controls:** Plates incubated but not inoculated.
- **Replicate plates of diluted microbiological samples:** Replicate plates for each sample.
- **Unexposed field blank:** Material coupons sampled in the same fashion as test coupons but not inoculated with the surrogate organism before sampling, or exposed to the decontamination process.

Table 5-2 lists the additional QC checks built into the BioLab procedures designed to provide assurances against cross-contamination and other biases in the microbiological samples.

Table 5-2. Additional Quality Control Checks for Biological Measurements

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

Bg, *Bacillus atrophaeus* var. *globigii;* CFU, colony forming units; RSD, relative standard deviation; TSA, tryptic soy agar

5.3.3 QA Assessments and Corrective Actions

The QA assessments and corrective actions for this project were intended to provide rapid detection of data quality problems. Mild contamination in QC procedural blank samples was observed after the completion of testing. However, this contamination was very minimal and had little to no effect on the project results. Project personnel were intimately involved with the data on a daily basis so that any data quality issue became apparent soon after it occurred. Blank and negative samples in which spores were present were at or near the detection limit. Table 5-3 summarizes the QA/QC assessment of spore recoveries for the various control sample types.

Table 5-3. Quality Assurance/Quality Control Assessment of Spore Recoveries (CFU) for Various Control Samples

Procedural Blanks		Procedural Blank Rinsates		Negative Controls	
Concrete	Glass	Concrete	Glass	Concrete	Glass
6	ND	6	6	ND	5

CFU, colony forming units; ND, non-detect

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