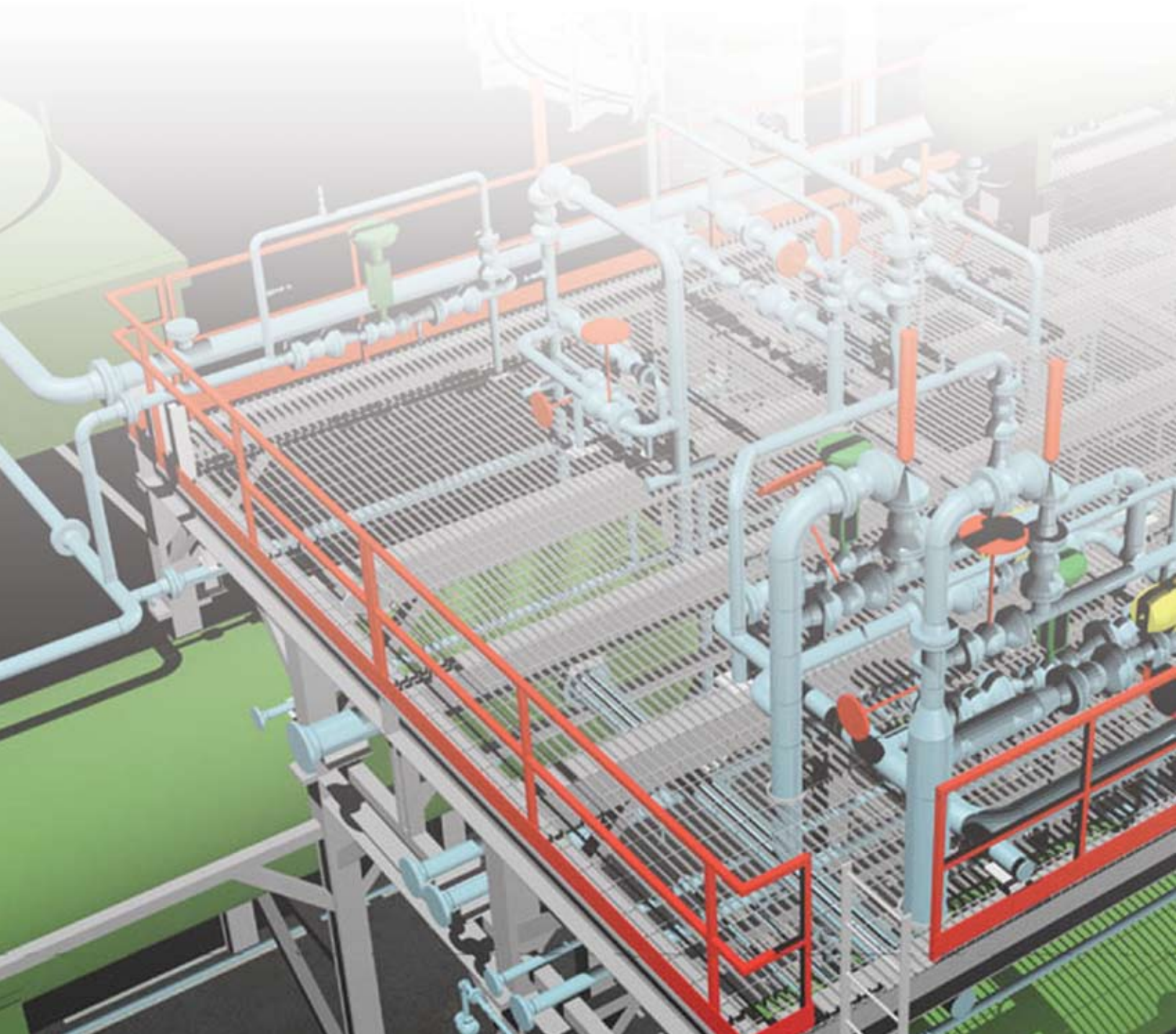
United States
Environmental Protection
Agency

TEST/QA PLAN FOR

Evaluating Liquid and Foam Sporicidal Spray Decontaminants

Office of Research and Development
National Homeland Security
Research Center



EPA/600/R-06/066

Test/QA Plan

for

Evaluating Liquid and Foam Sporicidal Spray Decontaminants

March 2006

Battelle
505 King Avenue
Columbus, OH 43201

A PROJECT MANAGEMENT

A1 TITLE AND APPROVAL PAGE

EPA/Battelle Approval of Test/QA Plan

for

Evaluating Liquid and Foam Sporicidal Spray Decontaminants

March 2006

Joseph P. Wood USEPA Task Order Project Officer	3/3/2006 Date
Eletha Brady-Roberts NHSRC Quality Assurance Manager	3/3/06 Date
Karen Riggs Battelle TTEP Manager	3/6/06 Date
Zachary Willenberg Battelle Quality Assurance Manager	3-6-06 Date

BATTELLE
505 King Avenue
Columbus, OH 43201

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LIST OF ACRONYMS

ANOVA	two-way analysis of variance
AOAC	AOAC International (formerly Association of Official Analytical Chemists)
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
BSC	biosafety cabinet
BSL	biosafety level
BWD	bare wood
C	Celsius
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CFU	colony forming unit
cm	centimeter
DL	decorative laminate
EPA	U.S. Environmental Protection Agency
FDA	Food and Drug Administration
GM	galvanized metal ductwork
GS	glass
in	inch
IC	industrial grade carpet
ISO	International Organization for Standardization
mL	milliliter
µL	microliter
MREF	Medical Research and Evaluation Facility
NHSRC	National Homeland Security Research Center
NIST	National Institute of Standards and Technology
PBS	phosphate-buffered saline
PC	painted concrete cinder block
PW	painted wallboard paper
QA	quality assurance
QC	quality control
QMP	Quality Management Plan
rpm	revolutions per minute
SD	standard deviation
SOP	standard operating procedure
STS	sodium thiosulfate
TOPO	Task Order Project Officer
TSA	technical systems audit
TTEP	Technology Testing and Evaluation Program

A3 DISTRIBUTION LIST

Ms. Karen Riggs
Mr. Zachary Willenberg
Ms. Elisha Morrison
Dr. James Rogers
Battelle
505 King Avenue
Columbus, OH 43201-2693

Dr. Michael Taylor
Battelle
10300 Alliance Rd, Ste 155
Cincinnati, OH 45242

Mr. Eric Koglin
USEPA National Homeland Security
Research Center
944 East Harmon Avenue
Las Vegas, NV 89119

Ms. Eletha Brady-Roberts
USEPA National Homeland Security
Research Center
26 W. Martin Luther King Drive
Cincinnati, OH 45268

Mr. Joseph P. Wood
U.S. Environmental Protection Agency
U.S. EPA Mailroom, E343-06
Research Triangle Park, NC 27711

A4 TECHNOLOGY EVALUATION ORGANIZATION

The technology evaluation will be performed by Battelle under the direction of the U.S. Environmental Protection Agency's (EPA) National Homeland Security Research Center (NHSRC) through the Technology Testing and Evaluation Program (TTEP). The majority of the information in this test/quality assurance (QA) plan is based on a previously approved test/QA plan by the Task Order Project Officer (TOPO). The organization chart in Figure 1 shows the individuals from Battelle, the vendor(s), and EPA who will have responsibilities in the technology evaluation. The responsibilities of these organizations and individuals are summarized in the following subsections.

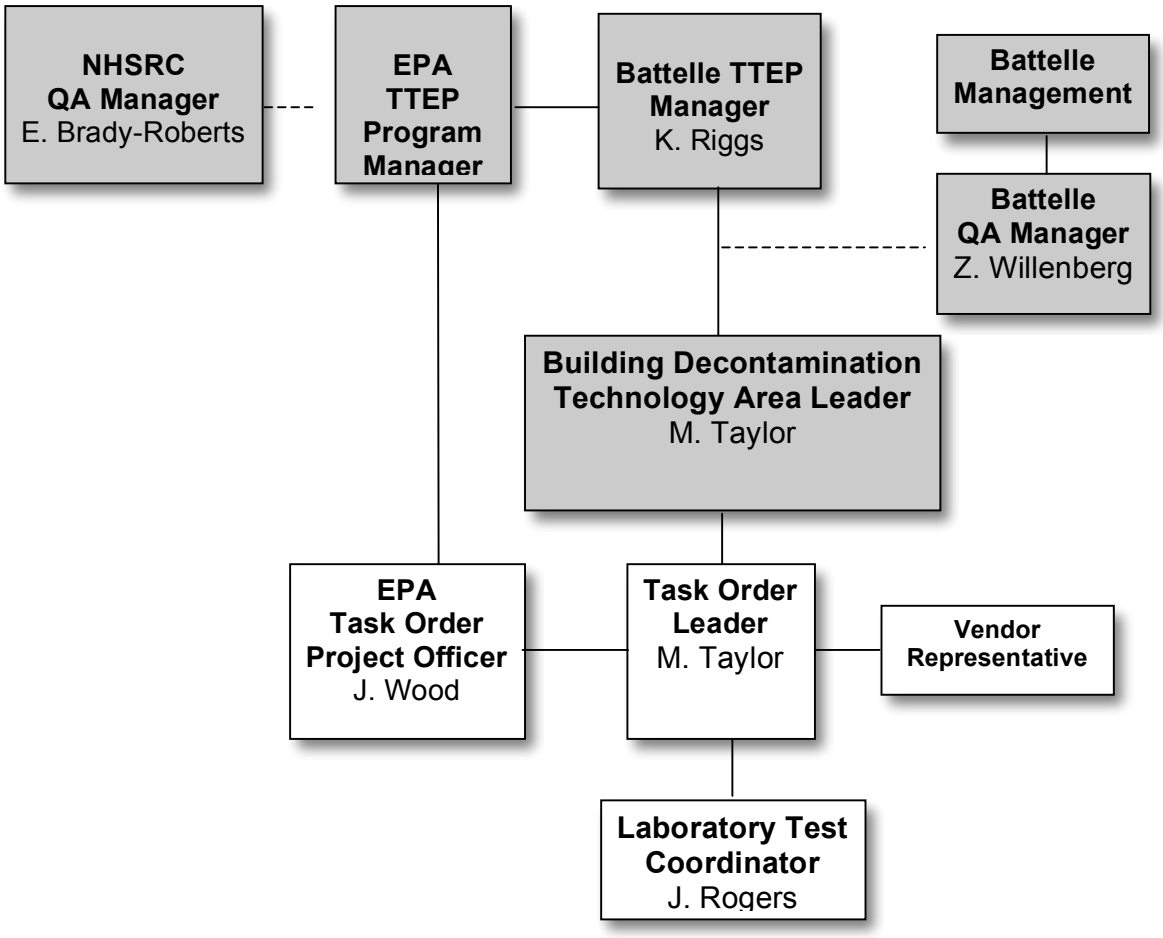

Figure 1. Organization Chart for the Spray Decontamination Evaluation

A4.1 Battelle

Dr. Michael Taylor is Battelle's Building Decontamination Technology Area Leader and Task Order Leader for this technology evaluation. He will have overall responsibility for ensuring that the technical, schedule, and cost goals established for testing and evaluation is met, and that the procedures employed for the evaluation are consistent with TTEP guidelines. Dr. Taylor will serve as the primary interface for the TOPO. Dr. Taylor's responsibilities are to

- Ensure that TTEP procedures are being followed
- Select the appropriate laboratory or location for the evaluation
- Prepare the draft test/QA plan and evaluation reports
- Establish a test schedule
- Revise this test/QA plan and evaluation reports in response to reviewers' comments
- Keep the Battelle TTEP Manager informed of the progress and difficulties in planning and conducting the evaluation
- Coordinate with the Battelle Quality Assurance Manager for the technical and performance audits as required by Battelle or EPA Quality Management staff
- Have overall responsibility for ensuring that this test/QA plan is followed
- Respond to any issues raised in assessment reports and audits, including instituting corrective action as necessary
- Establish a budget and schedule for the technology evaluation and direct the effort to ensure that budget and schedule are met
- Coordinate distribution of the final test/QA plan and evaluation reports.

Ms. Karen Riggs is Battelle's TTEP Manager. As such, Ms. Riggs will

- Maintain communication with the EPA TTEP Program Manager on all aspects of the program
- Monitor adherence to budgets and schedules in this work
- Provide the TOPO with monthly technical and financial progress reports
- Review and approve the draft and final test/QA plan
- Review the draft evaluation reports

- Ensure that necessary Battelle resources, including staff and facilities, are committed to the technology evaluation
- Ensure that vendor confidentiality is maintained
- Support Dr. Taylor in responding to any issues raised in assessment reports and audits
- Issue a stop work order if audits indicate that data quality is being compromised.

Mr. Zachary Willenberg is Battelle's Quality Assurance Manager for TTEP. As such, Mr. Willenberg will

- Review and approve the draft and final test/QA plan
- Maintain communication with EPA Quality Management staff for this program
- Conduct a technical systems audit (TSA) at least once during the technology evaluation
- Audit at least 10% of the evaluation data
- Prepare and distribute an assessment report for each audit
- Verify implementation of any necessary corrective action
- Notify Battelle's TTEP Manager to issue a stop work order if internal audits indicate that data quality is being compromised. Notify the Task Order Leader if such an order is requested
- Provide a summary of the QA/quality control (QC) activities and results for the evaluation reports
- Review the draft evaluation reports
- Ensure that all quality procedures specified in this test/QA plan and in the TTEP Quality Management Plan[1] (QMP) are followed
- Maintain training records.

Dr. James Rogers is Battelle's Laboratory Test Coordinator for this evaluation. His responsibilities are to

- Coordinate with vendor representatives to facilitate the performance of the evaluation
- Assist in preparing the draft test/QA plan
- Arrange for using the test facility and establishing evaluation schedules
- Arrange for the availability of qualified staff to conduct the evaluation

- Assure that the evaluation is conducted in accordance with this test/QA plan
- Provide input into revision of this test/QA plan and evaluation report in response to reviewers' comments
- Update the Battelle TTEP Manager and Task Order Leader on progress and difficulties in planning and conducting the evaluation
- Coordinate with the Battelle Quality Assurance Manager for the performance of TSAs as required by Battelle or EPA Quality Management staff.

Dr. Stephen R. Rohrer is the Battelle Biosafety Officer for this project.

A4.2 Vendors

Vendors of the sporicidal decontamination technologies may

- Provide input for preparing the draft test/QA plan
- Review this test/QA plan and approve the final version prior to the evaluation of their technology
- Sign a vendor agreement specifying the respective responsibilities of the vendor and of Battelle in the evaluation
- Provide information on the quantitative response of their sporicidal decontamination technologies to aid in planning the evaluation
- Provide additional equipment (if applicable) used for their decontamination technologies in the technology evaluation
- Train Battelle and/or test facility staff in implementing their sporicidal decontamination technologies
- If available, provide information regarding contact time, spray distance, and spray deposition requirements
- Provide support, if needed, in using their sporicidal decontamination technologies during testing
- Review their respective draft evaluation reports.

A4.3 EPA

Mr. Eric Koglin is the EPA TTEP Program Manager for the EPA contract with Battelle, "Testing and Evaluation of Homeland Security-Related Technologies for the Measurement,

Sampling, Removal, and Decontamination of Chemical and Biological Agents" under which TTEP has been established.

Mr. Joseph Wood is the EPA TOPO for TTEP Task Order 1113. As such, Mr. Wood will

- Have overall responsibility for directing the evaluation process
- Review the draft test/QA plan
- Approve the final test/QA plan and any subsequent versions
- Review the draft evaluation reports
- Oversee the EPA review process on the draft test/QA plan and evaluation reports
- Coordinate submission of evaluation reports for final EPA approval.

Ms. Eletha Brady-Roberts is the NHSRC QA Manager for TTEP. As such, Ms. Brady-Roberts will

- Review and approve the draft test/QA plan and any subsequent versions
- Perform, at her option, one external TSA during the technology evaluation
- Notify the EPA TOPO to contact the Battelle TTEP Manager to issue a stop work order if an external audit indicates that data quality is being compromised
- Prepare and distribute an assessment report summarizing the results of the external audit, if one is performed
- Review the draft evaluation reports.

A5 PROBLEM DEFINITION/BACKGROUND

Among its responsibilities related to homeland security, EPA has the goal of identifying methods and equipment that can be used for decontaminating both indoor and outdoor environments following a terrorist attack using chemical or biological agents. In January 2003, EPA established the NHSRC to manage, coordinate, and support a wide variety of homeland security research and technical assistance efforts. The NHSRC is, through TTEP, conducting tests to evaluate the performance of both developmental and commercially available products, methods, and equipment for decontaminating porous (e.g., carpet) and non-porous (e.g., glass) surfaces contaminated with biological and chemical agents.

One or more biological agents (e.g., spores, vegetative cells, biotoxins) may be released in or around a building during a terrorist attack. Indoor surfaces (e.g., carpet, laminate, concrete) representing those found in a typical office building or mass transit station have been selected for use in evaluating the decontamination technology. The indoor surfaces selected include both porous and non-porous materials (see Section B1.3).

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. All potential users need unbiased, high quality, objective third-party data and information to assess how well the available decontamination tools will meet their performance objectives while protecting human health and the environment. All testing and evaluation conducted through the TTEP is under the direction of EPA and is subject to the TTEP QMP.[1] In performing each evaluation, Battelle will follow the general procedures described in the QMP[1] and Battelle has (as stipulated in the QMP) developed this test/QA plan. This test/QA plan has been prepared for the evaluation of sporicidal liquid or foam decontamination technologies that are applied with a spray applicator. The appendix of this test/QA plan contains a detailed description of the semi-automated spray system used to evaluate spray decontamination technologies. The appendix also includes a description of how the semi-automated spray system is used to identify spray-applied sterilants that have sporicidal activity and therefore should be considered for evaluation.

The objective of this test/QA plan is to describe procedures to determine the efficacy of decontamination technologies for killing the biological agent, *Bacillus anthracis*, Ames spores, or surrogate spores on a range of representative surfaces typical of those found in or around a public building, with the ultimate goal of providing technologies for restoring the building to a usable state. Decontamination of personnel or large equipment items (e.g., manufacturing equipment) is not covered in this test/QA plan. Decontamination technology testing and evaluation are being performed to generate data indicative of the technology performance or efficacy. For the evaluation conducted under this test/QA plan, performance is quantitatively assessed by sampling and analysis of viable spores before and after using the decontamination technology. The body of this test/QA plan provides the general framework under which this decontamination technology class will be evaluated.

A6 TECHNOLOGY EVALUATION DESCRIPTION AND SCHEDULE

This test/QA plan focuses on the evaluation of commercially available technologies for decontaminating indoor surfaces in or around a typical office building or mass transit station. This plan specifically focuses on decontamination in the building environment, in the context of use by personnel responsible for decontamination of the area after a terrorist attack. The overall objective of the evaluation called for under this plan is to determine the efficacy of sporicidal decontamination technologies for inactivating biological agents in or on typical indoor surfaces. Each technology will be evaluated by careful monitoring of contact time and temperature. For this evaluation, the performance of each of the decontamination technologies will be evaluated as described in Section B2.2.5.

A technology to be evaluated under this test/QA plan is a sodium hypochlorite formulation, which has been pH-adjusted with 5% acetic acid to 7.0 ± 0.2, according to the procedure outlined on the U.S. EPA Web site (http://www.epa.gov/pesticides/factsheets/chemicals/bleachfactsheet.htm). This amended bleach will be used as the baseline formulation for comparative testing of commercial or developmental technologies. Control coupons will be sprayed with a benign liquid (e.g., sterile water or phosphate-buffered saline [PBS]). Evaluations of additional commercial or developmental liquid or foam technologies under this test/QA plan may be described in an amendment to this test/QA plan.

The performance of technologies for decontaminating indoor surfaces spiked with a biological agent (*B. anthracis* Ames) or surrogates will be assessed at temperatures and relative humidity representative of those that could be found or established in a building decontamination situation.

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

- Log kill or efficacy
- Residual viable microorganisms (qualitative)
- Surface damage caused by the decontamination technology (qualitative).

A brief qualitative assessment of overall ease of preparation, application, handling, and storage will be included in the final report.

The decontamination evaluation to be conducted under this plan is limited to a biological warfare agent and surrogates in or on individual samples (test coupons) of building materials. Applications of decontaminants and subsequent evaluations involving the biological warfare agent will be performed in the Medical Research and Evaluation Facility (MREF) Biosafety Level 3 (BSL-3) facility.

The evaluation described in this test/QA plan is expected to begin 2 to 4 weeks after this test/QA plan has been approved. Two trials are required. Each trial includes applying spores (spiking) to test coupons (day 1); decontaminating test coupons, extracting spores, and dilution plating (day 2); and counting colonies (day 3). The two trials required to complete all testing for a single decontamination technology require two weeks of elapsed laboratory time.

A7 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 at a given temperature and contact time 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.

Table 1. Critical Data Quality Objectives

Data Required	Method	Unit	Acceptable Uncertainty in Data	Corrective Action
Application volume	Micropipette	mL, micro-liter (µL)	±5%	Replace with calibrated and sufficiently accurate micropipette
Viable spores	Manual count	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) and/or a NIST-traceable thermometer/ hygrometer	°C	±2°C	Replace with calibrated and sufficiently accurate thermometer and note variance in study file
Time	Data logger manufacturer's specifications indicate time accuracy of 61 seconds/ month (±0.000023%)	Hour	±0.05% (2 seconds/ hour)	Replace with calibrated and sufficiently accurate clock; note variance in study file

Quality control requirements are summarized in Table 5 in Section B5. Two of the critical QC measurements are the spore density (colony-forming units [CFU]/milliliter [mL] in the stock suspension) and recovery (mean percentage of spores extracted from a material compared to spores applied to the coupon). The number of colony-forming spores spiked onto coupons will be acceptable if the spore density measured for the spike controls (shown in Table 5) is within ± 25% of the target level (approximately 1 x 10^9 spores per 1 mL). Recoveries will be acceptable if they are >1% and <300% of the spores applied to the coupon. Recoveries below 10% or greater than 150% will be discussed with the TOPO prior to decontamination testing.

A7.1 Recovery

The percent recovery of viable spores from each test sample (control and decontaminated) will be determined in order to ascertain the differential number of spores recovered from test coupons following initial spiking and completing the testing process. Here, recovery is defined as the number of viable CFUs extracted from each test and control coupon relative to the number of CFUs in the inoculum used to spike each coupon.

The number of CFU spiked onto the control coupons is calculated as:

CFU spike/coupon = spore density (CFU/mL) x 0.1 mL spike (1)

The number of CFU extracted from a coupon is calculated as:

CFU extracted coupon = (mean CFU plate count x 1/dilution factor)
x [(volume extraction buffer) + (volume sprayed $decon_{cr}$)] (2)

Where

$decon_{cr}$ is the total decontaminant on the coupon and run-off; this value is determined from the spray and weigh test (see appendix). Recovery will be calculated for the *j*th control coupon (an individual test coupon) within the *i*th test material (a specific test material) as:

$$\text{Recovery}_{ij} = \frac{x_{ij0}}{x_{ij}} \quad (3)$$

Where

x_{ij0} are the CFU values in extract samples for the *i*th of six control coupons within the *i*th test material after the drying period

x_{ij} are the CFU values spiked onto the *j*th replicate coupon of the *i*th test material

The percent recovery data will be discussed with the TOPO and at the TOPO's discretion the following statistical analysis of the results will be performed. To determine the differential recoveries (to assess whether the type of test material influences recovery) of spores from various coupon types, statistical methods will be employed. The methods for calculating the differential recovery and assessing outliers are summarized below.

The recovery data for each agent will be fit to a one-way analysis of variance (ANOVA) model of the form:

$$\text{Recovery}_{ij} = \mu_{(r)} + \tau_{(r)i} + \varepsilon_{(r)ij} \quad (4)$$

Where

$\mu_{(r)}$ is the overall mean recovery obtained for spores of a specific type spiked onto a specific test material

$\tau_{(r)i}$ is the average effect on mean recovery due to the *i*th test material

$\varepsilon_{(r)ij}$ are the error terms for the *j*th replicate of the *i*th test material group; the errors are assumed to be $N(0,\sigma^2)$

Model diagnostics will be examined to determine whether there are any difficulties with outliers or the model assumptions of constant variance and normality of the residuals. If the data are not adequate for the model, appropriate transformations or more general statistical models (e.g., non-parametric) will be considered. The Grubbs test[2] will be used to identify outliers. Outliers will be further investigated; but, unless an error in recording or processing the data can be identified, the outlier will be excluded in the final analysis and noted in the report. No more than one outlier can be excluded for results to be acceptable. More than one outlier will result in the samples being rerun.

The recovery results from the coupon testing will be a matrix table in which each entry shows the mean percentage of recovery of viable spores, along with a 95% confidence interval for each surface material.

Statistical analysis will consist of pairwise comparisons of recovery percentages between materials. Both point estimates and corresponding p-values will be produced for each comparison. The modeling and analysis will be carried out with PROC Mixed in SAS v9.2.

A7.2 Efficacy

The number of CFU of *B. anthracis* Ames or a surrogate in extracts of control and test coupons will be determined. The first step in calculating overall decontamination efficacy is to calculate decontamination efficacy for each coupon in a given set of replicates. Differential efficacy is defined as the extent (by log reduction) to which the viable spores extracted from the test coupons after the decontamination treatment were less than the viable spores extracted from positive control coupons that were exposed only to water spray at the same temperature and dwell time as the treatment. Efficacy will be calculated for each test coupon for each test material as:

$$Efficacy_{ij} = \log_{10}(\bar{x}_{ij}) - \log_{10}(x_{(t)ij}) \quad (5)$$

Where

$\bar{x}_{ij}$ is the arithmetic mean of the CFU values of the control coupons of the *i*th test material

$x_{(t)ij}$ are the measured CFU values on the *j*th replicate coupon of the *i*th test material

The efficacy data will be discussed with the TOPO and at the TOPO's discretion the following statistical analysis of the results will be performed. To calculate the differential efficacy for a specific type of spores on a specific type of test material, statistical methods will be employed. The efficacy data will be fit to a one-way ANOVA of the form:

$$Efficacy_{ij} = \mu + \tau_i + \varepsilon_{ij} \quad (6)$$

Where

μ is the overall mean recovery

τ_i is the average effect on mean recovery due to the *i*th test material

ε_{ij} are the error terms for the *j*th replicate of the *i*th test material group; the errors are assumed to be $N(0,\sigma^2)$

B. anthracis Ames and surrogates will be combined under one ANOVA to facilitate comparisons among the spore types. A main effect for specific spore type will be added to this model to compare mean efficacy differences due to the spore type.

Model diagnostics will be examined to determine whether there are any difficulties with outliers or the model assumptions of constant variance and normality of the residuals. If the data are not adequate for the model, appropriate transformations or more general statistical models (e.g., non-parametric) will be considered. The Grubbs test[2] will be used to identify outliers. Outliers will be further investigated; but, unless an error in recording or processing the data can be identified, the outlier will be excluded in the final analysis and noted in the report.

The primary decontamination efficacy results from the coupon testing will be a matrix table in which each entry shows the mean log reduction in viable spores.

Statistical analysis will consist of comparing whether the differential efficacy of the decontamination treatment at a particular temperature, contact time, and test material was statistically significantly different from zero. Additional comparisons will be made of mean efficacy between materials. Both point estimates and corresponding p-values will be produced for each comparison. The modeling and analysis will be carried out with PROC Mixed in SAS v9.2.

Cases may exist in which a very small number of CFUs are found on the replicate coupons after a particular treatment. In these cases, the data may be modeled using methods consistent with rare events, such as Poisson distributions.

Laboratory blanks will control for sterility, and procedural blanks will control for viable spores inadvertently introduced to test coupons. The procedural blanks will be spiked with an equivalent amount of 0.1 mL of "stock suspension" that does not contain the biological agent or surrogate. As noted in Table 5, there can be no CFU from extracts of laboratory or procedural blanks for the corresponding test results to be accepted.

A8 SPECIAL TRAINING/CERTIFICATION

The evaluation will be conducted at Battelle's laboratories. The MREF, in West Jefferson, Ohio, has chemical and biological surety agent laboratories certified for the use of chemical and biological warfare agents. Battelle test facilities at 505 King Avenue in Columbus, Ohio, may be used for decontamination of biological agents where a BSL-2 is sufficient. The Battelle Eastern Science and Technology Center in Aberdeen, Maryland, has both BSL-3 and BSL-2 facilities and could be used if necessary, depending on the availability and capability of the facilities. Alternative facilities would only be used if all the requirements for safety, security, and testing capability established by this plan were met.

A8.1 General Site Description

Sporicidal decontamination technologies will be evaluated at Battelle's MREF. The evaluation will be performed in accordance with Battelle's facility-specific methods and the standard operating procedures (SOPs) that are cited where appropriate throughout this test/QA plan.

The MREF specializes in research, development, testing, and evaluation of medical countermeasures against highly pathogenic biological and highly toxic chemical materials. This facility is one of a very limited number of U.S. laboratories capable of studying aerosolized etiological agents in animal models under BSL-3 containment. This facility maintains state-of-the-art equipment and professional and technical staffing expertise to safely conduct testing and evaluation of hazardous biological materials under the Food and Drug Administration's (FDA) Good Laboratory Practices Guidelines (21 *Code of Federal Regulations* [CFR] Part 58). The MREF operates in compliance with all applicable federal, state, and local laws and regulations, including U.S. Army regulations, and is routinely inspected by personnel from the appropriate government agency. Battelle operates the MREF in compliance with requirements contained in 32 CFR 626 and 627, Biological Defense Research Programs. The MREF is International Organization for Standardization (ISO) 9001 certified, accredited by the American Association for the Accreditation of Laboratory Animal Care, and inspected by and compliant with regulations of the U.S. Department of Agriculture, FDA, Drug Enforcement Agency, Ohio EPA, U.S. Army Safety Team, U.S. Army Inspector General, U.S. Army Medical Research Institute of Chemical Defense Safety and Chemical Operations Branch, U.S. Army Medical Research and Materiel Command Office of Animal Care and Use Review, Madison County Health Department, and Battelle's Institutional Animal Care and Use Committee. The MREF is licensed to ship, receive, and handle select agents, as defined by the Centers for Disease Control and Prevention (CDC).

Testing outlined in this test/QA plan will be performed in the MREF BSL-3 facility, which was completed in 1995 and expanded to 31,000 square feet in 2002. The containment area within the facility is designed to meet or exceed the BSL-3 facility guidelines published by the CDC and National Institute of Health entitled *Biosafety in Microbiological and Biomedical Laboratories*.[3] Included are seven BSL-3 microbiology laboratories that contain multiple

Class III biosafety cabinets (BSCs) and two autoclaves. Additional laboratories within this area include multiple microbiology laboratories equipped with Class II BSCs. Test procedures at the MREF are governed by established SOPs that are specified by facility, number, and title.

A8.2 Training

Because of the hazardous materials involved in this technology evaluation, documentation of proper training and certification of the test personnel is mandatory before testing takes place. The Battelle Quality Assurance Manager, or designee, must assure that documentation of such training is in place for all evaluation personnel before allowing evaluation to proceed.

All participants in this evaluation (i.e., Battelle, EPA, and vendor staff) will adhere to the security, health, and safety requirements of the Battelle facility in which testing will be performed. Vendor staff may offer instruction to Battelle evaluation personnel using their decontamination technology, but will not be the technology users during the evaluation. To the extent allowed by the test facility, vendor staff may observe, but may not conduct, any of the technology evaluation activities identified in this test/QA plan.

Access to restricted areas of the test facility will be limited to staff who have met all the necessary training and security requirements. The existing access restrictions of the test facility will be followed, i.e., no departure from standard procedures will be needed for this evaluation. All visiting staff at the test facility will be given a site-specific safety briefing prior to the start of any test activities. This briefing will include a description of emergency operating procedures and the identification and location and operation of safety equipment (e.g., fire alarms, fire extinguishers, eyewashes, exits). Evaluation procedures must follow all safety practices of the test facility at all times. Any report of unsafe practices in this evaluation, by those involved in the evaluation or by other observers, shall be grounds for stopping the evaluation until the Quality Assurance Manager and evaluation personnel are satisfied that unsafe practices have been corrected.

A9 DOCUMENTATION AND RECORDS

Documentation of training related to technology testing, field-testing, data analysis, and

reporting is maintained for all Battelle technical staff in training files at their respective locations. The Battelle Quality Assurance Manager may verify the presence of appropriate training records prior to the start of testing. If Battelle staff operate and/or maintain a vendor-owned decontamination system during the technology evaluation, the vendor will be required to train those staff prior to the start of testing. Battelle will document this training with a consent form, signed by the vendor, that states which Battelle staff have been trained on their technology. Battelle technical staff will have a minimum of a bachelor's degree in science/engineering or have equivalent work experience.

As stated in Section 5.1.1 of the TTEP QMP,[1] program requirements and the technology evaluation records needed to reconstruct evaluation activities and verify that reported data were collected in a quality manner reconciled to the QMP will be retained for at least seven years after final payment under the Blank Purchase Agreement for the TTEP. These records consist of

- Test/QA plan
- Chain-of-custody forms
- Laboratory record books
- Data collection forms
- Electronic files (both raw data and spreadsheets)
- Technology evaluation report
- Quality assessment reports.

All of these records will be maintained by the Task Order Leader or designee (with the exception of the quality assessment reports) during the evaluation and transferred to Battelle's Records Management Office for storage at the conclusion of the evaluation. All written records must be in ink. Any corrections to entries, or changes in recorded data, must be made with a single line through the original entry. The correction is then to be entered, initialed, and dated by the person making the correction.

B MEASUREMENT AND DATA ACQUISITION

B1 EXPERIMENTAL DESIGN

B1.1 General Test Design

This test/QA plan specifies procedures for bench-scale testing to evaluate sprayed liquid/foam sporicidal decontaminants under specified operating conditions and ambient conditions for decontaminating porous and non-porous surfaces consisting of small pieces (i.e., test coupons) of building materials to which a biological agent or surrogates have been added.

Treatments for a given biological agent and building material will be defined in terms of the organism and material. Inactivation will refer to the log reduction in biological agent or surrogate compared to the respective controls. Differential efficacy of inactivation for various indoor surface materials may also be determined.

A pretest-posttest control group design will be used for each material and biological agent or surrogate:

R O_1 X O_2

R O_1 O_3

where time passes from left to right and

R signifies random selection of the test coupons for control, experiment, and type of biological organism.
O represents the mean of measurement of the spores extracted from the coupons, and
X represents the experimental variable, in this case the decontamination process

At a given point in time, the effect of the experimental variable is $(O_2 - O_1)-(O_3 - O_1)$, or simplified, $(O_2 - O_3)$.

The experimental design will allow the following null (H_O) and alternate (H_A) hypotheses to be statistically tested:

$$H_O : R_{Treatment} - R_{Control} = 0$$

$$H_A : R_{Treatment} - R_{Control} > 0$$

Where:

$R_{Treatment}$ is the geometric mean reduction in viable spores extracted from coupons in the treatment group

$R_{Control}$ is the geometric mean reduction in viable spores extracted from the positive control coupons

The experimental design will allow testing the hypothesis (H_O) that there is no significant difference ($p \leq 0.05$) in the log reduction in viable spores between the treatment and the positive control groups or the alternate hypothesis (H_A) that a significant difference exists between the treatment and positive control groups. Treatment will be defined in terms of the quantity of viable agent or surrogate, identity of the decontaminant, operational implementation of the decontaminant (e.g., concentrations, contact time), sprayer conditions (e.g., spray distance, air pressure, spray time), and ambient conditions (e.g., temperature, humidity). Decontamination efficacy will be calculated as described in Section A7.

Fourteen replicate coupons—six spiked test coupons [spiked, decontaminated], six positive controls [spiked, not decontaminated], one laboratory blank [not spiked, not decontaminated], one procedural blank [not spiked, decontaminated]—will be included for each coupon material and each biological agent tested (see the test matrix in Table 2). Unique sample identification codes will link each coupon to corresponding MREF standard data report forms; for example, MREF Data Report Form BioDecon-018-01 for the Liquid Decontamination Spraying System (Figure 2).

Practical constraints prevent all of the coupons from simultaneous decontamination. Therefore, randomly selected coupons will be decontaminated in each of two decontamination periods (trials).

Table 2. Matrix of Materials, Organisms, and Treatments

Organism	Material	Test Coupon (spiked, decontaminated) (n=6)	Reference Method (spiked, decontaminated with amended bleach) (n=6)	Positive Control Coupons (spiked, not decontaminated) (n=6)	Laboratory Blank (not spiked, not decontaminated) (n=1)	Procedural Blank (not spiked, decontaminated) (n=1)
B. anthracis (Ames)	Carpet	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
B. anthracis (Ames)	Bare wood	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
B. anthracis (Ames)	Glass	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
B. anthracis (Ames)	Decorative laminate	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
B. anthracis (Ames)	Galvanized metal	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
B. anthracis (Ames)	Painted wallboard paper	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
B. anthracis (Ames)	Painted concrete block	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
B. anthracis (Sterne)	Carpet	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
B. anthracis (Sterne)	Bare wood	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
B. anthracis (Sterne)	Glass	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date

Organism	Material	Test Coupon (spiked, decontaminated) (n=6)	Reference Method (spiked, decontaminated with amended bleach) (n=6)	Positive Control Coupons (spiked, not decontaminated) (n=6)	Laboratory Blank (not spiked, not decontaminated) (n=1)	Procedural Blank (not spiked, decontaminated) (n=1)
B. anthracis (Sterne)	Decorative laminate	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
B. anthracis (Sterne)	Galvanized metal	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
B. anthracis (Sterne)	Painted wallboard paper	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
B. anthracis (Sterne)	Painted concrete block	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
B. subtilis	Carpet	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
B. subtilis	Bare wood	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
B. subtilis	Glass	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
B. subtilis	Decorative laminate	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
B. subtilis	Galvanized metal	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
B. subtilis	Painted wallboard paper	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date

Organism	Material	Test Coupon (spiked, decontaminated) (n=6)	Reference Method (spiked, decontaminated with amended bleach) (n=6)	Positive Control Coupons (spiked, not decontaminated) (n=6)	Laboratory Blank (not spiked, not decontaminated) (n=1)	Procedural Blank (not spiked, decontaminated) (n=1)
B. subtilis	Painted concrete block	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
G. stearothermophilus	Carpet	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
G. stearothermophilus	Bare wood	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
G. stearothermophilus	Glass	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
G. stearothermophilus	Decorative laminate	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
G. stearothermophilus	Galvanized metal	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
G. stearothermophilus	Painted wallboard paper	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date
G. stearothermophilus	Painted concrete block	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Six sample IDs, °C, %RH, test date	Sample ID, °C, %RH, test date	Sample ID, °C, %RH, test date

535-CG667913
Liquid Decon Spraying System

Performed By/Date: _____

QC/Tech Reviewed By/Date: _____

Sample ID	Liquid Decon Sprayer			Contact Time			% RH*
	Time (sec)	Distance (in)	Pressure (psi)	Start Time	End Time	Total (min)	
Rug Spray Blank							
Concrete Spray Blank							
Rug 1 H2O							
Rug 2 H2O							
Rug 3 H2O							
Rug 4 H2O							
Rug 5 H2O							
Rug 6 H2O							
Concrete 1 H2O							
Concrete 2 H2O							
Concrete 3 H2O							
Concrete 4 H2O							
Concrete 5 H2O							
Concrete 6 H2O							
Rug 1 NaOCl							
Rug 2 NaOCl							
Rug 3 NaOCl							
Rug 4 NaOCl							
Rug 5 NaOCl							
Rug 6 NaOCl							
Concrete 1 NaOCl							
Concrete 2 NaOCl							
Concrete 3 NaOCl							
Concrete 4 NaOCl							
Concrete 5 NaOCl							
Concrete 6 NaOCl							

*Relative humidity recorded immediately prior to each spray replicate

Figure 2. MREF Data Report Form BioDecon-018-01 for the Liquid Decontamination Spraying System

B1.2 Scale of Testing, Testing Apparatus

The liquid decontamination spraying system developed at the Battelle MREF will be used inside Compact Glove Box 830-ABC (Plas Labs, Inc., Lansing, MI; see Figure 3) to contain the decontamination spray and biological agents. This test chamber is 71 centimeters (cm) wide by 59 cm deep by 74 cm high (28 inches [in] x 23 in x 29 in) and has outer dimensions of 110 cm (width) by 61 cm (depth) by 79 cm (height) (43 in x 24 in x 31 in). The chamber has a total volume of 317 liters (11.2 cubic feet). The test chamber also has a top opening of 43 cm by 58 cm (17 in x 23 in) and an attached transfer chamber that is 30 cm (12 in) long and an inner diameter of 28 cm (11 in). Glove ports, integral to the test chamber, are available for working in the glove box. The glove box will be modified with high efficiency particulate air-filtered vent valves to relieve potential pressure buildup during spraying. The decontaminant will be administered as a spray onto the test surfaces at a spray time, distance, and contact time recommended by the product manufacturer, if available. If these spray parameters are not provided by the manufacturer, then default parameters will be implemented; for example, a spray time of 10 seconds, a distance of 30 cm (12 in), and a contact time of 10 minutes. These default spray time and distance parameters are derived from the recommended default parameters given in the Association of Analytical Communities (AOAC) 961.02 Germicidal Spray Products as Disinfectants (Table 3). Test coupons will be approximately 1.9 cm by 7.5 cm; multiple coupons of each material will be spiked with the agent/surrogate, placed into the test chamber on the spraying system, and sprayed with the decontaminant. Laboratory blank (i.e., not spiked, not decontaminated) and positive control (i.e., spiked, not decontaminated) coupons will also be prepared for each test material, and results obtained for these coupons will be used with the data resulting from the analyses of post-treatment samples to calculate decontamination efficacy. This evaluation methodology is a highly controlled, reproducible approach to assess decontamination efficacy that simulates a realistic, small-scale spray application of the

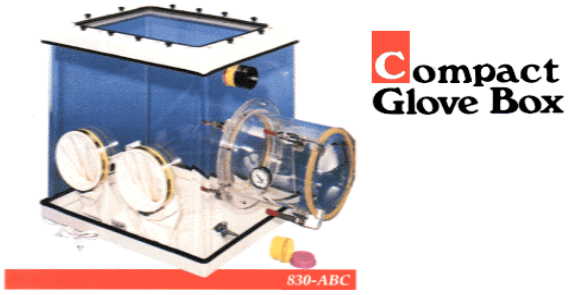

Figure 3. Compact Glove Box for Sporicidal Decontamination Technology Evaluation

decontamination technology. A detailed description of the spraying system is provided in the appendix.

Table 3. Default Spray Parameters

Parameter	Setting
Spray Time[a]	10 seconds
Spray Distance[a]	30 cm (12 in)
Contact Time[a]	10 minutes
Spray Air Pressure[b]	40 pounds per square inch

[a] AOAC 961.02, Section C.
[b] May be subject to change.

B1.3 Test Surfaces

Various structural, decorative, and functional surfaces typically found inside an office building or a mass transit station will be used to evaluate sporicidal decontamination technologies. The surface materials that will be used include both non-porous and porous surfaces. Test coupons (typically measuring 1.9 cm by 7.5 cm) will be prepared from larger pieces of stock materials. The representativeness and uniformity of the test materials are critical to assuring reliable evaluation results. Representativeness means that the materials are typical of those used in buildings in terms of quality, surface characteristics, structural integrity, etc. Uniformity means that all test pieces are essentially equivalent for evaluation purposes. Representativeness will be assured by selecting test materials that meet industry standards or specifications for indoor use and by obtaining those materials from appropriate suppliers. Uniformity will be maintained by obtaining a large enough quantity of material that multiple test samples with uniform characteristics can be prepared (e.g., test coupons will be cut from the interior rather than the edge of a large piece of material) or by using standardized coupons where available. Details of the test surfaces listed below are shown in Table 4.

Non-porous materials:

- Decorative laminate (DL)
- Galvanized metal ductwork (GM)
- Glass (GS)

Porous materials:

- Industrial-grade carpet (IC)

- Painted (latex, semi-gloss) concrete cinder block (PC).
- Painted (latex, flat) wallboard paper (PW)
- Bare wood (pine lumber) (BWD)

B1.4 Biological Agents and Surrogates

The biological agent to be used under this test/QA plan was selected based on an evaluation of potential threats to buildings.[4, 5] The evaluation considered availability, lethality, potential delivery pathways, and persistence of potential agents.

The biological agent used in evaluating the sporicidal decontamination technology will be *Bacillus anthracis* Ames strain spores. Biological surrogates will be used to establish correlations between the decontamination efficacy of surrogates and agents. To provide correlations with the *B. anthracis* results, the surrogates *B. anthracis* Sterne strain (animal vaccine strain), *B. subtilis* (American Type Culture Collection [ATCC] 19659), and *Geobacillus stearothermophilus* (ATCC 12980) will be used. *B. anthracis* spores and surrogate spores will be prepared and characterized according to MREF SOPs.[6-10]

Table 4. Material Characteristics

Material	Lot, Batch, or ASTM No., or Observation	Manufacturer/ Supplier Name	Approximate Coupon Size, L x W, inches	Material Preparation
DL	Laminate/ Formica/ White Matte Finish	Solid Surface Design	1.9 cm x 7.5 cm	None; sterilized with gamma irradiation
GM	Industry Standard 24-Gauge Galvanized Steel	Accurate Fabrication	1.9 cm x 7.5 cm	Autoclaved; cleaned with acetone
GS	C1036, 1/8 in thick	Brooks Brothers	1.9 cm x 7.5 cm	Autoclaved; cleaned with acetone
IC	ShawTek, EcoTek 6. Color: mottled gray/dark brown/ black	Shaw Industries, Inc	1.9 cm x 7.5 cm	Sterilized with gamma irradiation
PC	American Society for Testing and Materials C90	Wellnitz	1.9 cm x 7.5 cm	Brush and roller painted all sides. One coat Martin Senour latex primer (#71-1185) and one coat Porter Paints latex semi-gloss finish (#919); autoclaved.
PW	05-16-03; Set-E-493; Roll-3	United States Gypsum Company	1.9 cm x 7.5 cm	Roller painted on one side using Martin Senour Paints. One primer (#71-1185) and two finish (flat, #70-1001) coats; sterilized with gamma irradiation.
BWD	Screen Molding, ¼ in thick (Pine Wood)	Kingswood Lumber	1.9 cm x 7.5 cm	Sterilized with gamma irradiation

B. subtilis and *G. stearothermophilus* have commonly been used as surrogates for *B. anthracis* in decontamination technology testing; *B. anthracis* Sterne has not been typically used for decontamination testing. The *G. stearothermophilus* surrogate exhibits comparatively high resistance to various sporicidal decontaminants. The *B. subtilis* (ATCC 19659) surrogate is the most commonly used surrogate for *B. anthracis*.

Spores will be prepared according to established MREF procedures.[11, 12] Working stock suspensions of each spore type will be prepared at a target density of approximately $1x10^9$ CFU/milliliter (mL).

B1.5 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.

B1.6 Surface Damage

Following decontamination of the test surfaces prepared as described in Section B2.2, 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.

B2 METHODS REQUIREMENTS AND PROCEDURES

B2.1 Agents

B. anthracis (Ames and Sterne), *B. subtilis* (ATCC 19659), and *G. stearothermophilus* (ATCC 12980) spores will be prepared according to established MREF procedures.[6, 7] The SOPs used for production also provide for identification and purity. Strains will be confirmed by third-party genotyping. Identity of organisms received from other sources will be confirmed in documentation from the source of the organism. Working stock suspensions of each spore type will be prepared at a target density of approximately $1x10^9$ CFU/mL.

B2.2 Coupon-Scale Testing

B2.2.1 Preparation of Test Materials

Each of the test coupons will be cut to 1.9 cm by 7.5 cm from the interior of a large piece of test material. Edges and damaged areas will be avoided when cutting test coupons. The coupons will be sterilized prior to use. A methods demonstration will determine an appropriate method of sterilization for each material type. Autoclaving, either using wet heat or dry heat, will be used where possible to sterilize these materials. Other non-chemical sterilization methods (e.g., gamma irradiation) will be evaluated for materials that cannot be sterilized by autoclaving. Packaging and storage conditions will preserve sterility until the coupons are ready for use. To prevent contamination of test surfaces, aseptic technique, following Battelle policies and guidelines[8-10] will be exercised during all phases of handling the test coupons.

The test coupons will be visually inspected prior to spiking with the biological agents. Coupons with anomalies on the test surface will not be used. An anomaly is any obvious difference in a coupon when compared with similar coupons, such as an unpainted concrete surface, an oil smudge on a metal surface, or chipped laminate. Coupons will be used once. On each evaluation day, each coupon will be assigned a unique identifier code by the evaluation staff. The identifier code will be placed on the coupons, vials, and plates in indelible ink. Prior to applying the biological agent or surrogate, the surface of each coupon will be disinfected by wiping with 70% isopropanol to minimize contamination by microorganisms other than those being evaluated.

B2.2.2 Spiking of Biological Agents onto Test Coupons

Biological agent/surrogates will be spiked onto test coupons in an appropriate BSC (BSC-III or BSC-II) according to established MREF procedures.[8-12] Spiked coupons will be prepared fresh for each day of experimental work. Test coupons will be placed flat in the BSC and spiked at approximately 1 x 10^8 CFU per coupon. A 100-µL aliquot of a stock suspension (approximately 1 x 10^9 CFU/mL) of spores will be dispensed (using a micropipette) as small droplets across the surface of the test coupon. After spiking with biological agent or surrogate suspension, the test coupons will remain undisturbed overnight in the BSC-III (*B. anthracis* Ames) or BSC-II (*B. anthracis* Sterne, *B. subtilis, G. stearothermophilus*) to dry.

B2.2.3 Confirmation of Spore Density in Stock Suspensions

To confirm the spore density (number of spores per volume) of biological agents and surrogates, the respective stock spore suspensions used to spike the coupons will be re-enumerated on each day of use. An aliquot (0.1 mL) of the stock suspension and each serial dilution to 10^{-7} will be plated onto tryptic soy agar plates and incubated overnight at 35ºC to 37ºC for *B. anthracis* and *B. subtilis* and at 55ºC to 60ºC for *G. stearothermophilus*. Plates will be enumerated within 18 to 24 hours of plating as described in the MREF SOPs.[13, 14] The number of CFU/mL will be determined by multiplying the average number of colonies per plate by the reciprocal of the dilution.

B2.2.4 Application of the Decontaminant and Monitoring of Test Procedures

On the day following spiking, test coupons intended for decontamination (including blanks and controls) will be transferred into the glove box (test chamber) where the spraying system is located. The decontaminant will be applied in accordance with the vendor's instructions (if available) with respect to spray distance and contact time. The decontaminant contact time, spray distance, and temperature will be controlled and monitored/recorded. The spraying system to be used for this testing automatically controls for parameters such as spray distance, liquid decontaminant flow rate, air pressure for spraying, and product deposition, thereby eliminating differences in these parameters that may be associated with human error. Where applicable, the respective numeric values for each of these parameters will be recorded

for each decontaminant tested. Spray parameters can readily be adjusted to meet vendor recommendations or requirements. The design of the spraying system enables the user to adjust any necessary parameters to ensure the accuracy and repeatability of sprays. Following decontamination, the test chamber will be cleared using the vendor-supplied method for neutralizing the decontamination reagent. If no instructions for neutralization are provided, the test chamber will be cleaned following procedures established under the Battelle MREF Facility Safety Plan.[12]

B2.2.5 Determination of Decontamination Efficacy

The performance or efficacy of the sporicidal decontaminants will be assessed by determining the number of viable organisms remaining on the test coupons, as well as the number of viable organisms in the liquid run-off after decontamination. These data will be compared with the number of viable organisms extracted from the control coupons sprayed with a benign liquid (e.g., sterile water or PBS).

This test/QA plan includes application of laboratory methods in novel combinations of test organisms, materials, decontamination technologies, and neutralization methods. Therefore, before executing this test/QA plan, a methods demonstration based on a modification of a testing scheme for neutralization described in American Society for Testing and Materials (ASTM) E 1054-02,[15] will be used to determine (1) decontamination technology effectiveness (add spores to decontamination liquid; determine CFU without neutralization), (2) neutralizer and/or dilution effectiveness at terminating decontamination (add spores to decontamination liquid; determine CFU with neutralization), (3) neutralizer toxicity (add spores to neutralizer; determine CFU), and (4) decontamination control effectiveness (add spores to extraction buffer without neutralizer; determine CFU). One modification is that approximately 1×10^8 CFU will be used for testing instead of the 30 to 100 CFU/mL outlined in ASTM E 1054-02. The increased concentration of CFU is anticipated to provide better sensitivity for this neutralization evaluation. For this test/QA plan, it is anticipated that sodium thiosulfate (STS) will be used as the neutralizer for baseline comparative technology, amended bleach. It is known that STS can inhibit bacterial growth; therefore, when evaluating the use of STS as a neutralizer, step 3 (described above) may not be performed. Findings will be discussed with the TOPO and will also guide the selection of the neutralization method. The neutralization results will be summarized in the final report as mean

(±SD) total spores recovered and percent neutralization efficacy. Neutralization efficiency will be calculated by dividing the total spores recovered from test samples (e.g., spores + decontaminant + neutralizer in extraction buffer) by the total number of spores recovered from the controls (spores added to extraction buffer without decontaminant or neutralizer) and expressed as a percentage.

In the methods demonstration, whether dilution in the extraction step is sufficient to terminate decontamination will be determined, or whether adding a neutralizer to the extraction fluid can be used to terminate decontamination. If neutralization can be accomplished in the extraction step, the replicate test coupons and a procedural blank that was decontaminated will be transferred aseptically to sterile 50-mL conical tubes after decontamination. For each type of biological agent, the decontaminated, control, and blank coupons (except the procedural blank used for evaluating obvious damage) will be placed individually in conical vials containing 10 mL of sterile extraction buffer to which, if necessary, a neutralizer to stop the decontamination has been added. The tubes will be agitated on an orbital shaker for 15 minutes at approximately 200 revolutions per minute (rpm) at room temperature.

If the method demonstration shows that neutralization, separate from extraction, is needed, the test coupons and procedural blanks will be removed from the decontamination fluid and placed into an individual container or well holding sufficient neutralizer to cover the spiked surface of the coupon. The spiked surface will remain in contact with the neutralizer for the period determined by the method demonstration. The neutralizer contact time will be monitored and recorded.

The neutralized coupons will then be transferred individually into sterile 50-mL conical vials containing 10 mL of sterile PBS extraction buffer. The tubes will be agitated on an orbital shaker for 15 minutes at approximately 200 rpm at room temperature.

For spore extraction, the tubes will be agitated for 15 minutes on an orbital shaker at approximately 200 rpm at room temperature. Following extraction, 1 mL of the coupon extract (plus any decontaminant run-off from the coupon) will be removed, and a series of dilutions through 10^{-7} will be prepared in sterile water. An aliquot (0.1 mL) of the undiluted extract (and coupon run-off) and each serial dilution will be plated onto tryptic soy agar plates and incubated overnight at 35ºC to 37ºC for *B. anthracis* and *B. subtilis* and at 55ºC to 60ºC for *G. stearothermophilus*. Plates will be enumerated within 18 to 24 hours of plating as described in

MREF SOPs.[13, 14] The number of CFU/mL will be determined by multiplying the average number of colonies per plate by the reciprocal of the dilution. The volume of neutralizer will be included in the determination of the total CFU, where CFU/mL will be multiplied by the volume of extraction buffer plus neutralizer to determine total CFU. Dilution data representing the greatest number of individually definable colonies will be expressed as mean ± standard deviation of the numbers of CFU observed. Figure 4 shows the spray decontamination evaluation procedure.

Decontamination efficacy will be calculated as discussed in Section A7. The efficacy calculations will be performed using the total of the spores extracted from the test coupons as well as any collected decontaminant run-off from the coupons.

Based on previous decontamination studies, 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 cultured at the appropriate temperature for *B. anthracis* or surrogates to encourage viable spore germination and subsequent proliferation of vegetative bacteria.

At 1 and 7 days post-decontamination, 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.

B2.2.6 Observation of Surface Damage

Following application of the decontaminants, each test surface will be examined visually to establish whether the decontaminant caused any obvious damage to the surface. Observation of surface damage will be performed immediately after the designated contact time with the decontaminant, but before post-decontamination sampling to assess efficacy. Visual inspection of the surface will take place through side-by-side comparison of the decontaminated test surface and the control coupons of the same test material. Differences in color, reflectivity, and

roughness will be assessed qualitatively, and observations will be recorded by the evaluation staff and recorded.

B2.2.7 Observation of Ease of Use

A non-critical qualitative assessment will be made in the final report to document the ease of preparation, application, handling, and storage that was experienced. Shelf life, if any, provided by the manufacturer will be noted in the final report.

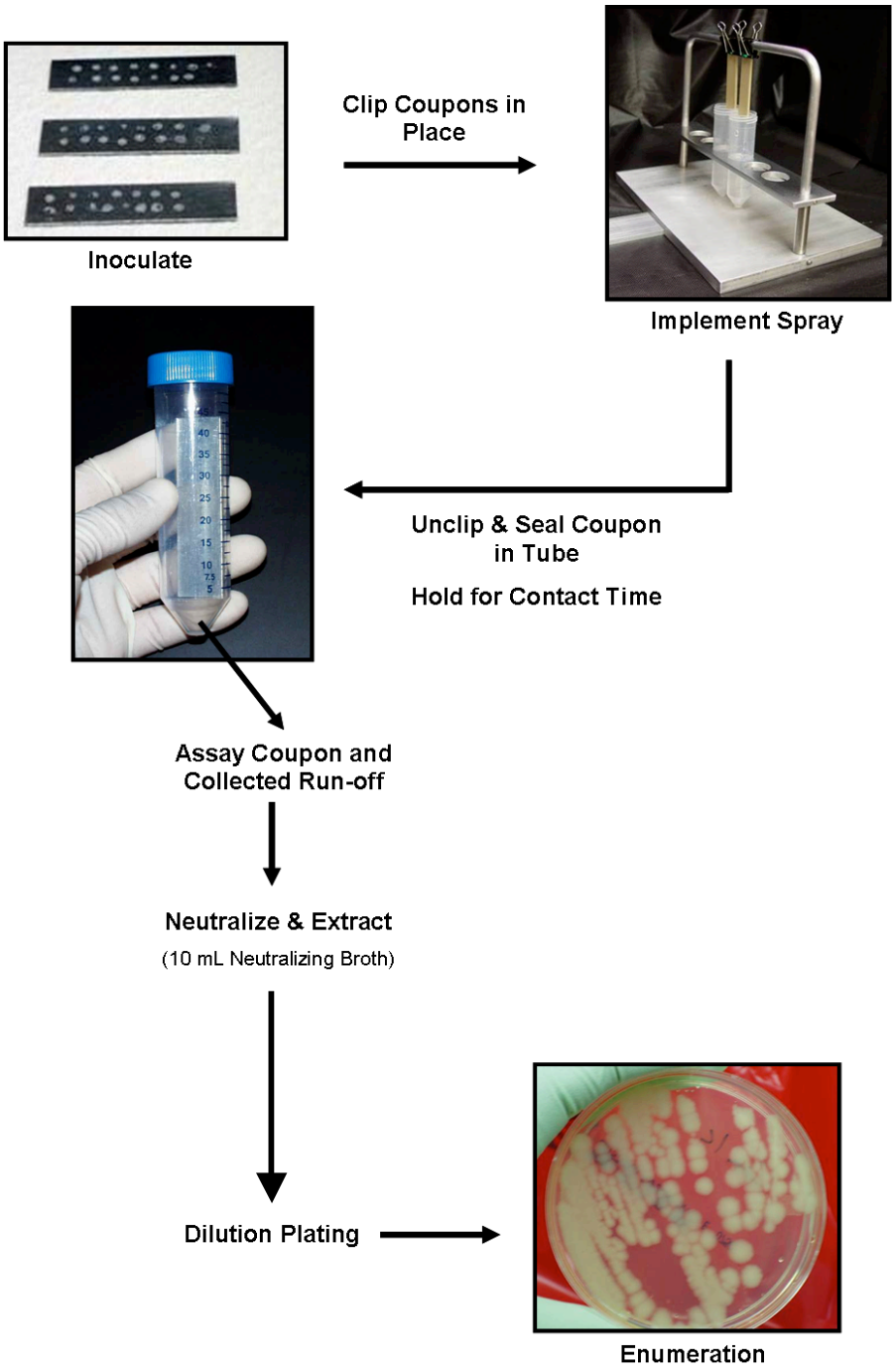

Figure 4. Flow Chart of Spray Decontamination Evaluation

B3 SAMPLE HANDLING AND CUSTODY REQUIREMENTS

Testing will occur within a secure area. The test materials in labeled containers will be delivered directly to the testing laboratory where coupons are spiked. Test coupons will be spiked with biological agent/surrogates in an appropriate BSC (BSC-III or BSC-II) according to established MREF procedures[8-12] Each coupon will be assigned a unique identifier code by the evaluation staff for traceability.

B4 ANALYTICAL METHODS REQUIREMENTS

No analytical methods are required in this evaluation.

B5 QUALITY CONTROL REQUIREMENTS

Quantitative standards do not exist for biological agents and surrogates. 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 CFUs enumerated on decontaminated coupons and the coupons that were spiked in the same fashion but not decontaminated are used in calculating log kill (or efficacy) (see Section B2.2.5). The mean ±SD of the log kill values will be calculated for each coupon type and biological agent combination. The QC samples that are analyzed in each group (see Table 2) include six test coupons (spiked, decontaminated), six positive controls, one laboratory blank, and one procedural blank. In addition, one TSA plate will be evaluated for sterility with each group; and the measurement of spore density, described in Section B2.2.3, will be used as a QC for growth on the medium.

For each liter of sterile tryptic soy broth prepared, one vial containing 10 mL of broth that is not spiked (negative control) and one vial containing 10 mL of broth spiked with a positive control organism will be incubated at 35 to 37ºC for 7 days. Additionally, tryptic soy broth will be visually examined for cloudiness or contamination prior to use.

The QC samples, acceptance criteria, and corrective actions are provided in Table 5.

Table 5. Quality Control Sample Requirements

QC Sample	Information Provided	Acceptance Criteria	Corrective Action[a]
Spike Control	Calculated value of spore density in stock suspensions.	±25% of target spike level of 10^9 spores per mL (10^8 spores per 0.1 mL).	Reject results; prepare stock suspensions meeting target spore density level in spike control.
Laboratory Blank (coupon spiked with diluent without biological agent and not subjected to the decontamination treatment)	Controls for sterility of the coupon material.	No observed CFU.	Reject results; identify and remove source of contamination.
Procedural Blank (coupon spiked with diluent without biological agent and subjected to the decontamination treatment)	Controls for contamination during decontamination treatment.	No observed CFU.	Reject results; identify and remove source of contamination.
Positive Control (coupon spiked with biological agent but not subjected to the decontamination)	Controls for recovery and confounds arising from history impacting spore viability; controls for special causes.	Mean CFU >1% and ≤300% of spiked spores (10^8 spores per 0.1 mL in target spike); Grubbs outlier test with no more than one outlier.	Mean CFU <1% or >300% recovery = reject results; evaluate/exclude values for outliers.
Test Coupons (spiked and subjected to decontamination)	Replicate coupons control for special causes.	Grubbs outlier test with no more than one outlier.	Evaluate/exclude values for outliers.
Blank tryptic soy agar plate (plate incubated, but not spiked)	Controls for sterility of plates.	No observed growth following incubation	Incubate additional 10 plates. If any additional growth is observed, reject results from the lot.
Growth Control (tryptic soy agar plate incubated after spiking with organisms)	Controls for ability to support growth.	In the verification of spore density, described in B2.2.3, ±50% of nominal spore density observed.	Incubate additional 10 plates, including 5 from a different lot. If significant differences in growth are observed between the lots, reject results from the lot that is not supporting growth.
Blank Tryptic Soy Broth (autoclaved tube of medium, not spiked incubated for seven days)	Controls for sterility.	No observed growth following 7-day incubation.	If remaining batch appears clear, re-autoclave the remaining batch and retest; if remaining batch appears cloudy do not use that batch.
Positive Control, Tryptic Soy Broth, (autoclaved tube of spiked medium incubated for seven days)	Controls for ability to support growth.	Growth observed following 7-day incubation.	Determine whether the medium batch or lot is causing no growth. Replace medium to achieve positive growth.

[a]Testing results will be discussed with the TOPO prior to implementing corrective action

B6 INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE REQUIREMENTS

The equipment needed for the evaluation is listed in Table 6 with the data quality indicators and corrective actions that will be taken in the event of substandard performance. All instruments utilized in the technology evaluation will be calibrated as stipulated by the manufacturer or, at a minimum, annually.

Table 6. Measurement Parameters and Data Quality Indicators

Parameter	Measurement Method	Data Quality Indicators	Corrective Action
Temperature	NIST-traceable thermo hygrometer	Compare against calibrated thermometer before and after evaluation testing, agree ±10%	Replace
Relative Humidity	NIST-traceable hygrometer	Compare against calibrated hygrometer before and after evaluation testing, agree ±10%	Replace
Pressure	Certified gauge	Compare against NIST-traceable calibrated gauge before and after evaluation testing, agree ± 10%	Replace
Micropipette	All micropipettes will be certified as calibrated at time of use	±1%; pipettes are recalibrated by gravimetric evaluation of pipette performance to manufacturer's specifications every 6 months by supplier (Rainin Instruments)	Recalibrate
Time	Compare to U.S. Naval Observatory time values	±14 seconds per day, evaluate semiannually	Replace
Volume of Decontaminant	Pipette	Check calibration gravimetrically over the range of volumes used before and after evaluation testing, agree ±5%	Recalibrate

B7 INSTRUMENT CALIBRATION AND FREQUENCY

The equipment needed for the evaluation will be maintained and operated according to the quality requirements and documentation of the evaluation facility. All equipment used at the time of evaluation will be verified as being certified, calibrated, or validated. Calibration of instruments will be done at the frequency specified in Section B6 and checked according to Table 6. Any deficiencies will be noted. The instrument will be adjusted to meet calibration tolerances and recalibrated. If tolerances are not met after recalibration, additional corrective action will be taken, including replacement of the equipment.

B8 INSPECTION/ACCEPTANCE REQUIREMENTS FOR SUPPLIES AND CONSUMABLES

Supplies and consumables will be acquired from reputable sources. The source and purity of reagent-grade chemicals and standards will be documented and documentation retained in the file. Supplies and consumables will be examined for evidence of tampering or damage upon receipt and prior to use, as appropriate. In addition, expiration dates will be noted and recorded.

Solutions will be prepared following MREF protocols and will be documented in reagent preparation forms. These forms include preparation instructions, suppliers, catalog numbers, lot number expiration dates for components, calculated and actual amounts used, and specific equipment used with calibration information. A lot number and expiration date is assigned to each reagent. All documents are initialed and dated.

Tryptic soy agar plates are manufactured by Remel (Lenexa, KS). Each box of tryptic soy agar plates includes a certification of lot number and expiration date. Tryptic soy agar plates will be examined for evidence of tampering or damage upon receipt and prior to use, as appropriate.

B9 DATA ACQUISITION REQUIREMENTS

No data needed for this project implementation are obtained from non-measurement sources such as computer databases, programs, literature files, or historical databases.

B10 DATA MANAGEMENT

Data acquisition during the evaluation includes proper recording of the procedures used to assure consistency in the evaluation and adherence to this test/QA plan; documenting sampling/evaluation conditions; recording observations regarding the condition of the surface of each coupon before and after the decontamination process; and recording efficacy results and evaluation conditions. Data will be acquired by the Battelle testing staff manually and recorded immediately in a consistent format throughout the evaluation. All written records will be in ink, and any corrections to recorded data will be made with a single line through the original entry. The correction will then be entered, initialed, and dated by the person making the correction. Any non-obvious correction will include a reason for the correction. Strict confidentiality of evaluation data will be maintained.

B10.1 Efficacy Calculations

Prior to analyzing data for the inactivation of biological agents from indoor surface materials, a separate analysis will be conducted to determine the recovery of viable spores from the time of initial spiking of the control coupon to the completion of the drying period. Recovery will be calculated as described in Section A7.1 to indicate the number of CFUs extracted from the coupons after the drying time relative to the number of CFUs spiked onto the control coupon.

An ANOVA will be fitted to the data as described in Section A7.1. Model diagnostics will be examined to assess whether there are any difficulties with outliers or the model assumptions of constant variance and normality of the residuals. If the data are not adequate for the model, appropriate transformations or more general statistical models (e.g., non-parametric) will be considered. The Grubbs test[2] will be used to identify outliers. Unless an error in recording or processing the data can be identified and/or corrected, the outlier will be excluded in the final analysis and noted in the report.

Once final statistical models have been fit, pre-planned comparisons will be performed. This will consist of comparing whether there are statistically significant differences in recovery between pairs of materials. The overall error rate for the set of comparisons will be controlled at 5%.

To measure the effects of decontamination on the inactivation of biological agents, decontamination efficacy will be calculated (as described in Section A7.2) to indicate the relative reduction in viable spores achieved by the decontamination technology.[12, 14, 15] In cases where no (or very small numbers of) CFU remain after decontamination, modeling will be conducted using Poisson distribution theory.

The efficacy data will be fit to an ANOVA to evaluate the differential efficacy due to test material on log reduction (described in Section A7.2). There will be a separate ANOVA for each of *B. anthracis* (Ames), *B. anthracis* (Sterne), *B. subtilis*, and *G. stearothermophilus*. The *B. anthracis* (Ames), *B. anthracis* (Sterne), *B. subtilis*, and *G. stearothermophilus* will be combined under one ANOVA to facilitate pre-planned comparisons among the spore types.

Model diagnostics will be examined to assess whether there are any difficulties with outliers or the model assumptions of constant variance and normality of the residuals. If the data are not adequate for the model, appropriate transformations or more general statistical models

(e.g., non-parametric) will be considered. The Grubbs test[2] will be used to identify outliers. Unless an error in recording or processing the data can be identified and/or corrected, the outlier will be excluded in the final analysis and noted in the report.

Statistical analysis will consist of performing a set of pre-planned comparisons. Once final statistical models have been fit, the main effects and interactions will be examined to determine if they are statistically significant. Based on these results, appropriate estimates will be determined for the set of pre-planned comparisons. They include

- Comparing whether the efficacy of the decontamination treatment at a particular temperature and contact time was statistically significantly different from zero for each surface material
- Comparing the estimated mean decontamination efficacy to determine whether significant differences exist among the seven surface materials
- Comparing the calculated mean decontamination efficacy for *B. anthracis* Ames to *B. anthracis* Sterne, *B. subtilis,* and *G. stearothermophilus* to determine whether significant differences exist between anthrax agents and the surrogate.

The overall error rate for these pre-planned comparisons will be controlled at 5%.

The evaluation results will be compiled in a report. The report will briefly describe TTEP and the evaluation procedures utilized, as well as all evaluation data and observations. The preparation of the draft report, review of the draft report, revision of the draft report, final approval, and distribution of the final report will be conducted as stated in the QMP.

C ASSESSMENT/OVERSIGHT

C1 ASSESSMENT AND RESPONSE ACTIONS

Battelle's Quality Assurance Manager will audit at least 10% of the evaluation data. Battelle's Quality Assurance Manager will trace the data from initial acquisition, through reduction and statistical comparisons, to final reporting. All data calculations will be checked.

C1.1 Technical Systems Audit

Battelle's Quality Assurance Manager or designee will perform at least one TSA during the evaluation. The TSA is to ensure that the evaluation is performed in accordance with the TTEP QMP,[1] the test/QA plan, and any SOPs to ensure that the necessary QA/QC procedures are implemented. Battelle's Quality Assurance Manager may review evaluation methods, compare test procedures to those specified in this test/QA plan, and review data acquisition and handling procedures. Battelle's Quality Assurance Manager will prepare a TSA report, and the findings must be addressed either by modifications of test procedures or by documentation in the evaluation records and final report. At EPA's discretion, EPA QA staff may also conduct an independent on-site TSA during the evaluation. The EPA TSA findings will be communicated to evaluation staff at the time of the audit and documented in a TSA report. These findings must be addressed as stated above.

C1.2 Performance Evaluation Audits

No performance evaluation audit will be performed for biological agents and surrogates, as quantitative standards for these biological materials do not exist. The confirmation procedure, controls, blanks, outlier test, and method validation efforts will be the basis of support for biological evaluation results. Calibration of instruments used for measuring temperature, humidity, pressure, and flow velocity will be monitored according to the processes and schedule identified in Table 6 and documented. As a performance check of the spraying system, a gravimetric assessment of the total amount of decontaminant deposited on each coupon and collected in the run-off will be performed (see Appendix A).

C1.3 Data Quality Audit

The Battelle Quality Assurance Manager will audit at least 10 percent of the evaluation data. The Quality Assurance Manager will trace the data from initial acquisition, through reduction and statistical comparisons, and to final reporting. All data analysis calculations will be checked.

C2 REPORTS TO MANAGEMENT

Each assessment and audit will be documented in accordance with Section 9.5 in the TTEP QMP[1] Assessment reports will include the following:

- Identification of any adverse findings or potential problems
- Space for response to adverse findings or potential problems
- Possible recommendations for resolving problems
- Citation of any noteworthy practices that may be of use to others
- Confirmation that solutions have been implemented and are effective.

During the course of any assessment or audit, Battelle's Quality Assurance Manager will identify to the technical staff performing experimental activities any immediate corrective action that should be taken. If serious quality problems exist, Battelle's Quality Assurance Manager will contact the TTEP Manager to request a stop work order. Once the assessment report has been prepared, the Building Decontamination Technology Area Leader or Task Order Leader will ensure that a response is provided for each adverse finding or potential problem and will implement any necessary follow-up corrective action. Battelle's Quality Assurance Manager will ensure that follow-up corrective action has been taken.

D DATA VALIDATION AND USABILITY

D1 DATA REVIEW, VALIDATION, AND VERIFICATION REQUIREMENTS

Records generated during the evaluation will receive a QC/technical review before being used to evaluate or report results. This review will be performed by a Battelle technical staff member other than the person who originally generated the record. Evaluation staff will be consulted as needed to clarify any issues about the data records. The review will be documented by the person performing it by adding his/her initials and date to a hard copy of the record being reviewed. This hard copy will then be returned to the Battelle staff member who generated or will be storing the record.

D2 VALIDATION AND VERIFICATION METHODS

To ensure that data generated meet the goals of the evaluation, a number of data validation procedures will be performed. Section C of this test/QA plan provides a description of the validation safeguards employed for evaluations. Data validation efforts include completing QC activities and performing TSA audits. An audit of data quality will be conducted by the Battelle Quality Assurance Manager to ensure that data review, verification, and validation procedures were completed and to assure the overall quality of the data.

Data verification is conducted as part of data review. Handwritten data will be visually inspected to ensure that all entries were properly recorded or transcribed and that any erroneous entries were properly noted (i.e., single line through the entry, with an error code and the initials of the recorder and date of entry). All calculations used to transform the data will be reviewed to ensure their accuracy and appropriateness. Calculations performed manually will be reviewed and repeated using a handheld calculator or commercial software (e.g., Excel). Calculations performed using standard commercial software will be reviewed by inspecting the equations used for the calculations and verifying selected calculations by handheld calculator.

D3 RECONCILIATION WITH DATA QUALITY OBJECTIVES

The data comparisons described in Section B2.2.5 will be conducted separately for each sporicidal decontaminant undergoing evaluation. Separate evaluation reports will then be prepared, each addressing one sporicidal decontaminant. Each evaluation report will present the

test data, as well as the results of the evaluation of those data. The evaluation reports will briefly describe the TTEP program and will present the procedures used in the evaluation. These sections will be common to each report. The results of the technology evaluation will then be stated quantitatively. The preparation of draft evaluation reports, review of reports by vendors and others, revision of reports, final approval, and distribution of the reports will be conducted as stated in the program QMP.[1]

Data obtained will be assessed by comparing them with the data quality objectives contained in Section A7. In the case of the data collected for calculating efficacy, the data quality objective will not be met if the positive controls (spiked, not decontaminated) fail to yield extracts that contain viable organisms. In cases where no detectable growth occurs (such as when decontamination reagent contacts the spiked controls), the test must be repeated.

E REFERENCES

1. Battelle, *Quality Management Plan (QMP) for the Technology Testing and Evaluation Program* (TTEP). 2005.
2. Grubbs, F., "Procedures for detecting outlying observations in samples." *Technometrics,* 1969. 11(1): p. 1-21.
3. *Biosafety in Microbiological and Biomedical Laboratories.* 1999, Centers for Disease Control and Prevention and National Institute of Health: Washington, D.C.
4. Raber, E., Jin, A., Noonan, K., McGuire, R., and Kirvel, R.D., "Decontamination Issues for Chemical and Biological Warfare Agents: How Clean is Clean Enough?" *Int. J. Environ. Health Res.*, 2001(11):p. 128-148.
5. Battelle, *Decontamination Technology Testing and Evaluation: Task 1: Technology Identification and Selection,* prepared for the U.S. EPA under Task Order 1113 by Battelle. 2004.
6. Battelle, Battelle MREF SOP Number: MREF. X-074, *Standard Operating Procedure (SOP) for the Production of* Bacillus anthracis *Spores*: West Jefferson, Ohio.
7. Battelle, Battelle MREF SOP Number MREF.X-093, *Standard Operating Procedure for the Production of* Bacillus anthracis *Spores in a Small Fermentor.*
8. Battelle, FSP Annex 5 to Appendix B, *Guidelines for Safe Handling and Storage of Etiologic Agents at the MREF.* Columbus, Ohio.
9. Battelle, *Battelle MREF Facility Safety Plan (FSP) Annex 6 to Appendix B of the FSP,* "Guidelines for the Control of Etiologic Agents in the MREF Biofacility."
10. Battelle, *FSP Annex 7 to Appendix B,* "Guidelines for Disinfection/Decontamination of Etiological Agents at the MREF Biofacilities." West Jefferson, Ohio.
11. Battelle, *Battelle MREF Facility Safety Plan Annex 12 to Appendix B,* "Guidelines for the Use of Class II and Class III Biological Safety Cabinets in the MREF Biofacility."
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13. Battelle, Battelle MREF SOP Number: MREF. X-112, *Standard Operating Procedure (SOP) for Interpreting and Calculating Enumeration Data for Biological Decontamination Testing.* West Jefferson, Ohio.
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15. ASTM, Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents. *ASTM International Designation E 1054-02.* ASTM International Designation E, 2002. 1054-02.

APPENDIX

DETAILED DESCRIPTION OF THE DECONTAMINATION SPRAYING SYSTEM AND PERFORMANCE PARAMETER EVALUATION

Liquid Decontamination Spraying System Developed at the Battelle Medical Research and Evaluation Facility

General Description:

The liquid decontamination spraying system enables the user to precisely control time of spray, air pressure, product deposition, and spray distance. Moreover, the design of this sprayer enables the user to easily adjust the setting(s) for one or all of these parameters to accommodate the physical properties of the liquid/foam product being tested. Such precise control of these parameters minimizes the potential for human error associated with spraying, enables control of product deposition, and minimizes sample-to-sample variability. This spray methodology may enhance the comparability of data obtained in different laboratories that are each following the same testing protocol.

Specific Description Controlled Parameters

The main features/components of this spraying system include

- Aluminum and stainless steel construction
- Adjustable coupon holder for variable spray distance
- Pneumatic spray nozzle
- Liquid reservoir for decontaminants
- Needle valves (pressure and liquid flow)
- Pressure gauge
- Digital timer
- Pneumatic valve.

The aluminum and stainless steel construction was chosen to minimize the potential damaging effects (e.g., oxidation, corrosion) of some of the decontaminants used during testing. By sliding the base of the coupon holder using a tongue and groove design, the coupon holder

easily adjusts for spray distances of 6 to 12 inches from the tip of the spray nozzle to the coupon surface. The coupons are held in place on the coupon holder so that they are oriented vertically.

The pneumatic spray nozzle is positioned to spray the decontaminants in a conical pattern perpendicular to the coupon surface. A pneumatic nozzle was chosen to achieve precise, remotely activated on/off spray control. The tip of the nozzle can be easily removed and exchanged with an alternate tip that provides a different spray pattern. The spray nozzle is connected to two stainless steel lines—one line for the decontaminant tested and one line for the pressurized air (supplied by a compressed air cylinder). The decontaminant is contained in a stainless steel reservoir, where the decontaminant is fed to the spray nozzle by gravity. In the nozzle, the liquid decontaminant is mixed with pressurized air fed from the second inlet line, resulting in an atomized spray coming out of the nozzle. All lines, valves, and spray nozzles in contact with decontaminant or PBS are flushed with sterile water followed by air between each solution change.

The pressurized air feeding the nozzle is controlled in two ways. First, the air flow to the nozzle (on/off) is controlled by the use of a pneumatic valve that is opened and closed using a digital timer. The pressurized air line feeding the sprayer is connected to the pneumatic valve that is normally in the “closed” position (zero air flow to the nozzle). This pneumatic valve is connected to a digital timer that can be set to operate from 0.1 second to minutes. When the digital timer is set for the appropriate spray time, the timer is activated, which opens the pneumatic valve allowing air to flow to the spray nozzle. Once the timer counts down from the set time to zero, the timer stops, thereby returning the pneumatic valve to the “closed” position. The second control feature is the adjustable needle valve that regulates the air pressure to the spray nozzle. A pressure gauge between the needle valve and the nozzle enables monitoring of the air pressure (in psi) that is distributed to the spray nozzle. By turning the needle valve, the pressure to the nozzle can be adjusted without the need to adjust air pressure at the air source. Figure A-1 is a flow diagram of the spray system. Figure A-2 shows photographs of side and top views of the spray system set-up inside the compact glove box.

Use of the Liquid Decontamination Spraying System for Assessing Retention of Liquid/Foam Decontaminant in Test Material and Screening Sterilant and Disinfectant Formulation for Sporicidal Activity

The retention of a spray-applied liquid formulation on a porous or non-porous test material can be determined by performing a "spray and weigh" test using a minimum of one porous and one non-porous material. For this test, the combined weight (grams) of the target test coupon and conical vial beneath the coupon will be recorded prior to and after spraying. The difference in weight (grams) between the before and after spraying represents the mass of the liquid spray deposited on the surface and the collected run-off in the conical vial. Five periods of spraying will be evaluated: 1, 5, 10, 15, and 20 seconds (N = 4/time period). The time periods are controlled automatically by the spraying system, eliminating the element of human error. The distance of the spray from the nozzle to the coupon surface will be 12 inches. The coupons will be suspended vertically, and the spray stream will be 90 degrees to the surface of the coupons. Eight replicate samples will be sprayed during each time period. The average and standard deviation of the deposited spray and run-off during each period will be determined and plotted on a graph. The correlation coefficient (R^2) will also be calculated for the total recovery. On each testing day, a performance check of the spraying system will be performed for each decontaminant. This performance evaluation will consist of a spray and weigh (described above) for a specified time period (e.g., 10 seconds) at a distance of 12 inches. The total decontaminant mass from this spray will be plotted against the spray and weigh profile for the specific decontaminant determined above. If the total mass from this performance test is within ±10% of the previously determined spray and weigh profile, the results will be considered acceptable. If the total mass from this performance test is >10% of the pre-determined spray and weigh profile, the results will be considered unacceptable. If unacceptable, the spraying system will be adjusted and the spray and weigh test repeated. Once acceptable results are obtained, the decontamination testing will be initiated.

The sporicidal effects of spray-applied liquid/foam technologies such as sterilants or disinfectants can be rapidly screened using the liquid decontamination spray system as follows:

1. Apply 1 x 10^8 *B. anthracis* Ames spores in aqueous suspension to each of eight glass coupons.

2. Allow to stand overnight to dry.

3. Apply liquid/foam formulation to four coupons (retain four as positive controls) using technology supplied by or purchased from the vendor. The automated spray apparatus will be used to apply the liquid/foam.

4. Using extraction and culture techniques described in the test/QA plan for verifying hydrogen peroxide vapor technologies, [1] quantitate viable and extractable spores.

5. Express results as log kill calculated using Equation 5 in the test/QA plan for evaluating sporicidal spray decontaminants.

For each spray time (1, 5, 10, 15, and 20 seconds) and coupon material, data will be expressed as mean (±SD) total mass sprayed decontaminant and summarized in the final report. The correlation coefficient for total recovery as a function of the five time periods will also be reported. For each technology evaluated, the calculated decontamination efficacy will be reported with respect to the spray time, total mass deposited (per spray & weigh), and contact time.

At the discretion of the TOPO and pending time/resource availability, additional "spray & weigh" analyses will be performed on selected technologies and coupon test materials. This analysis will differentiate between the mass of sprayed technology deposited on the coupon from the mass that is collected as run-off. For each coupon test material and technology evaluated, data will be expressed as mean (±SD) total mass sprayed decontaminant deposited on the coupon and mean (±SD) total mass sprayed decontaminant collected as run-off.

References

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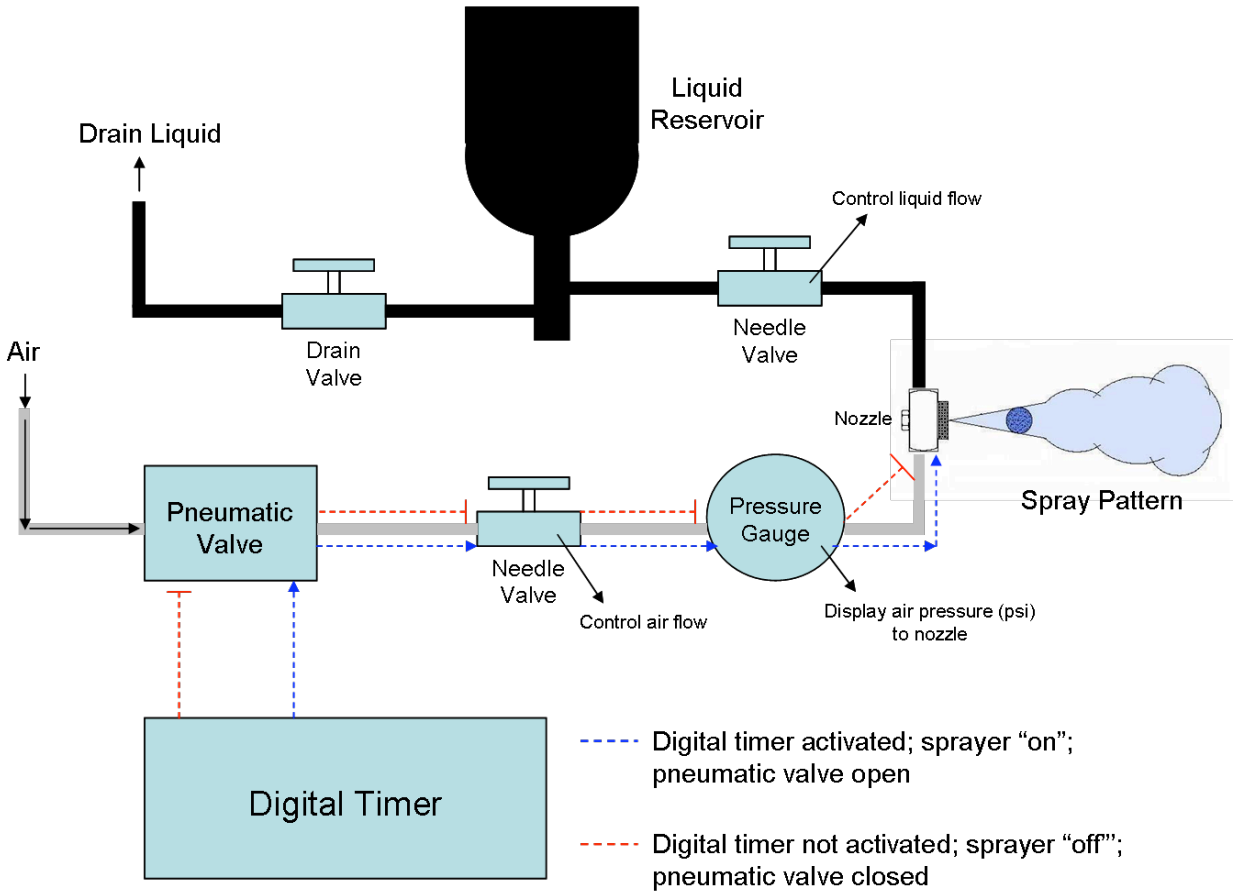

Figure A-1. Flow Diagram of Spray System

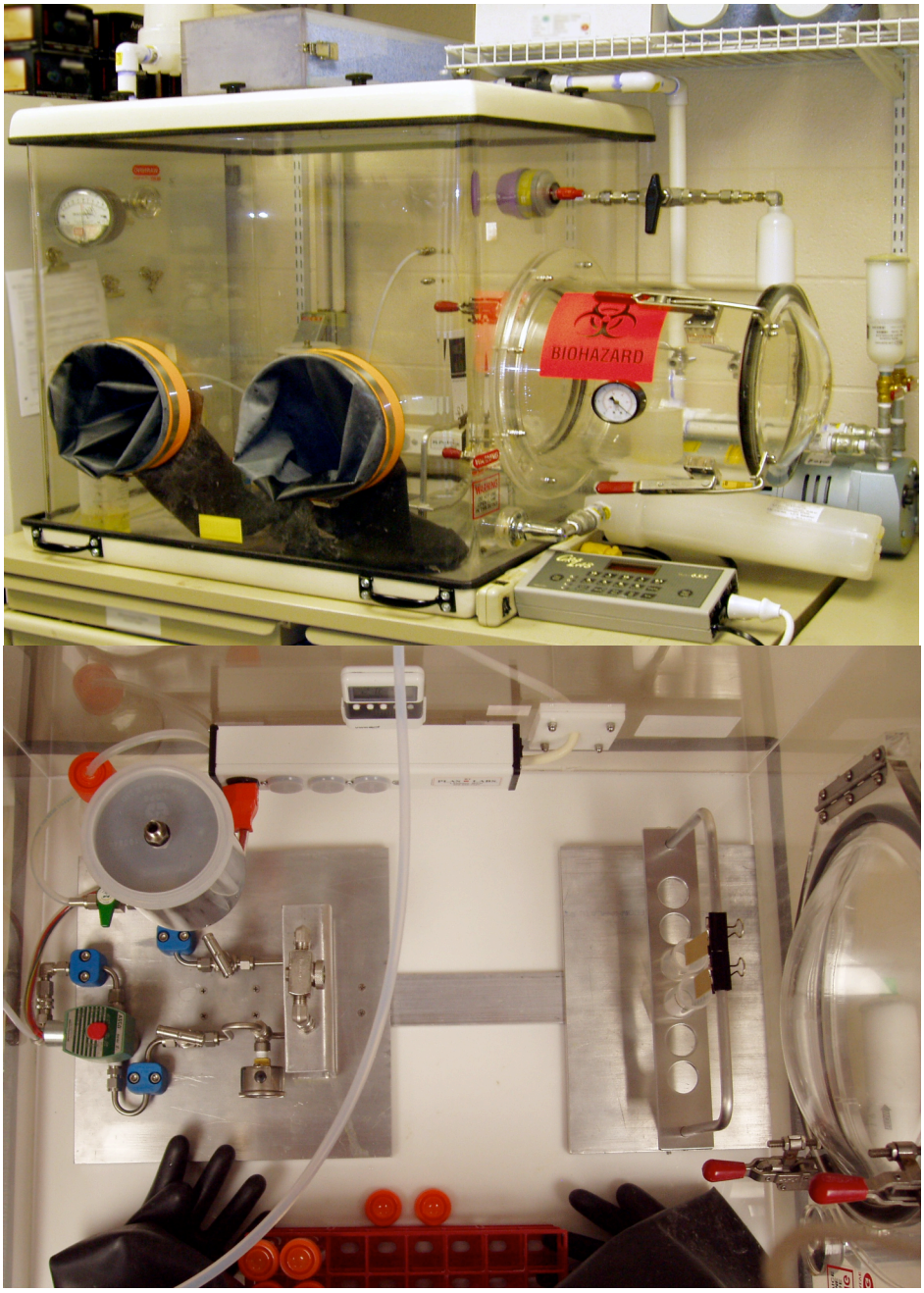

Figure A-2. Sprayer Set-up Inside the Compact Glove Box