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14. ABSTRACT
This TOP provides a quantitative sampling-based biological decontamination protocol to analyze the efficacy of liquid and gaseous/vaporous decontaminants on military-relevant surfaces. The procedures in this TOP were developed for use in research and development and early developmental testing in the test and evaluation phase of an acquisition program, and are not intended for operational testing.

15. SUBJECT TERMS
Decontaminant; spore-forming; vegetative; virus; decontamination efficacy

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U.S. ARMY TEST AND EVALUATION COMMAND
TEST OPERATIONS PROCEDURE

*Test Operations Procedure 08-2-065 11 February 2016
DTIC AD No: AD1003462

DEVELOPMENTAL TESTING OF LIQUID AND GASEOUS/VAPOROUS DECONTAMINATION ON BACTERIAL SPORES AND OTHER BIOLOGICAL WARFARE AGENTS ON MILITARY-RELEVANT SURFACES

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1. SCOPE.

1.1 Purpose.

a. The purpose of this Test Operations Procedure (TOP) is to provide a quantitative sampling-based biological decontamination protocol to assess the efficacy of gaseous/vaporous and liquid decontaminants. This TOP provides basic information necessary to plan, conduct, and report biological decontamination efficacy testing. Required facilities, equipment, procedures, test parameters, and data obtained using these test methods are discussed. Decontamination efficacy is determined by measuring contamination levels on military-relevant surfaces before and after decontamination. The protocol in this TOP is based on the developed test methodologies from Edgewood Chemical Biological Center (ECBC), Edgewood, Maryland[1,2**], and Naval Surface Warfare Center Dahlgren Division (NSWCDD), Dahlgren, Virginia[3].

b. The procedures in this TOP use coupons or panels to simulate military-relevant surfaces. The rationale for using coupons is that testing is less expensive and more efficient with small pieces of a surface material than with full-size test items. The rationale for using panels is that larger test items more closely represent fielded military equipment surfaces that may not be adequately represented by small coupons.

c. The procedures in this TOP were developed for use in research and development (R&D) and early developmental testing (DT) in the test and evaluation (T&E) phase of an acquisition program. The procedures in this TOP will allow a determination of the efficacy of gaseous/vaporous or liquid decontaminants applied to the surfaces of selected test materials that are contaminated with bacteria (spores and vegetative cells) and viruses that can be used as biological warfare agents. Decontamination efficacy provides relative ranking or screening information for decontaminant comparison.

d. This TOP provides guidance on test design issues and data requirements. The TOP guidance will be focused by information found in the test documentation including, but not limited to, the operational evaluation plan (OEP), the test and evaluation master plan (TEMP), the capability development document (CDD), the capability production document (CPD), and the system threat assessment report (STAR).

NOTE: The results from testing using these procedures will not be reflective of operational testing because it does not involve realistic application methods or field procedures.

1.2 Test Information.

a. For the purposes of this TOP, the term biological agent will be used to refer to bacteria (spore-forming and vegetative) and viruses that are simulants or select agents.

** Superscript numbers correspond to those in Appendix E, References.

b. Specific criteria for biological agent preparation must be met.

(1) Spore and Vegetative Bacterial Cell Stock Preparation. Bacterial stocks will be expanded from a single colony containing the correct morphology and pigmentation. Viability and concentration will be monitored by performing plate counts for each organism. With each plate count, purity will be assessed by examining and evaluating colony morphology for any stock contamination. Biological agent purity will also be evaluated (where applicable) by microscopic examination of Gram stain. The stock concentration cannot drop below the highest inoculation density required for surface deposition for a given test. Quantitative polymerase chain reaction (qPCR) should also be employed to verify the presence of the target organism and quantify its concentration in genome equivalents (GE)/mL. Concentrations of endotoxin protein will also be assessed with a standard method such as bicinchoninic acid (BCA) assay, Lowry assay, or Bradford assay to determine if there is contamination from Gram-negative bacteria.

(a) Spore preparations should yield at least 1.0×10^{10} colony-forming units (CFU)/mL in storage medium and contain 95 percent refractile spores with less than 5 percent cellular debris as measured by phase-contrast microscopy. The 95 percent concentration can be achieved by combining spores from multiple plates into a centrifuge bottle and centrifuging in sterile water. The step should be repeated two times, with re-suspension of the pellet each time. Stock assessment will be performed by microscopy, and enumeration will be performed using appropriate instrumentation, such as a Coulter counter (Beckman Coulter Incorporated***, Indianapolis, Indiana). The challenge concentration must be documented in the test plan and report.

(b) Vegetative cell preparations should yield at least 1.0×10^{11} CFU/mL on storage medium. Microscopy will be used to verify that at least 95 percent of the vegetative cells are free of spore-forming inclusion bodies. The challenge concentration must be documented in the test plan and report. **NOTE**: The specified yield for vegetative cell stock preparations accounts for a possible 1- to 3-log reduction from die-off caused by sample capture. The positive control will indicate the degree of die-off and whether the stock concentration must be adjusted.

(2) Virus preparations should contain at least 1.0×10^{11} plaque-forming units (PFU)/mL as determined by a standardized plaque assay for the virus species or strain used. Viability and concentration will be monitored by performing plaque assays for each viral stock. With each plaque assay, purity will be assessed by examining plaque morphology for any stock contamination. The stock concentration cannot drop below the highest inoculation density required for surface deposition for a given test. qPCR should be used to verify the presence of the target deoxyribonucleic acid (DNA) species or strain and quantify the concentration in GE/mL. Reverse transcriptase-qPCR (RT-qPCR) should be used to verify the presence of the target ribonucleic acid (RNA) species or strain and quantify the concentration in GE/mL. The challenge concentration must be documented in the test plan and report. **NOTE**: The specified concentration of the virus stock preparation accounts for a possible 1- to 3-log reduction of PFUs from die-off caused by sample capture. The positive control will indicate the degree of die off and whether the stock concentration must be adjusted.

*** The use of brand names does not constitute endorsement by the Army or any other agency of the Federal Government, nor does it imply that it is best suited for the intended application.

c. The procedures in this TOP require (based on the research work referenced) coupons (at least 2 × 5 cm) or panels (at least 30.5 × 30.5 cm) cut from military-relevant materials. If other coupons sizes are used, the rationale, coordination taken, and methodology must be documented in the test plan and test report as appropriate. Some examples of military-relevant surfaces are glass, butyl rubber (representative of seals), polycarbonate (representative of mask lenses), and chemical agent-resistant coating (CARC)-painted steel (representative of military equipment). **NOTE**: Four representative materials were chosen for the methodologies described in this TOP[1,2,3]; however, other military-relevant materials may be tested using these procedures.

d. It is important to understand that the physical characteristics of the materials to be tested will have a significant impact on the recovery of biological agent from those materials. Some of the test material or biological agent characteristics that affect biological agent recovery include, but are not limited to, static charge, inherent stickiness, hydrophobicity, hydrophilicity, porosity, and permeability. A recovery study must be done on each material and biological organism combination to be tested if there is no pre-existing information available.

e. The following types of decontaminants were used to develop the procedures in this TOP:

(1) Gaseous/vaporous decontaminants [e.g., chlorine dioxide (ClO_2); vaporized hydrogen peroxide (VHP®, Steris Corporation, Mentor, Ohio); and hot, humid air (HHA)].

(2) Liquid decontaminants [e.g., high-test hypochlorite (HTH); Easy DECON™ 200 Decontamination Solution (DF-200, Envirofoam Technologies, Huntsville, Alabama); Clorox® Ultra Bleach (The Clorox Company, Oakland, California); and Spor-Klenz® (Steris Corporation, Mentor, Ohio)].

f. The procedures in this TOP were developed using the following contaminants: *Bacillus (B.) subtilis (Bg), B. anthracis (Ba), Pantoea (P.) agglomerans (Pa)*, and Vaccinia virus [Paragraphs 1.2.f(1) through (4)]. If other biological agent species or strains are selected for a test, modifications to portions of these procedures may be necessary depending on the strain chosen. The strain(s) used will depend on availability and the discretion of the testing facility in coordination with the test sponsor, and must be documented. The sources for the strains used for development of this TOP were:

(1) *Bg* [catalog number 19659™, American Type Culture Collection (ATCC®), Manassas, Virginia]. **NOTE**: *B. subtilis* has been reclassified as *B. atrophaeus* since the initial research for this TOP was conducted and will be referred to as *B. atrophaeus* (*Bg*) throughout this document. The abbreviation *Bg was* originally derived from the name *B. globigii,* which was first reclassified to *B. subtilis* and then to *B. atrophaeus*. *Bg* is a species of spore-forming bacteria.

(2) *Ba* ΔSterne. This strain was obtained from the Unified Culture Collections at the U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID, Fort Detrick, Maryland). **NOTE**: *Ba* is a species of spore-forming bacteria; ΔSterne is the specific strain used.

(3) *Pa*, ATCC® catalog number 33243™[4]. *Pa* was formerly known as *Erwinia herbicola* (*Eh*). **NOTE**: *Pa* is a species of vegetative bacteria.

(4) Vaccinia virus [Western Reserve (WR) strain] was obtained from ATCC® (catalog number VR-119). The Vaccinia virus WR strain was a commonly used laboratory strain, derived from the original virus isolated by the New York City Board of Health (NYCBOH, New York, New York) using intracerebral passages in mice followed by tissue culture adaptation. The resulting virus is neurovirulent in mice and is called the WR strain. **NOTE**: The WR strain is not currently available (as of TOP publication), but a tissue-culture-adapted version can be ordered from ATCC® (catalog number VR-1354).

g. The minimum required challenge concentrations selected for this TOP are 5.0×10^8 CFU per sample area (e.g., 10 cm^2) for spore-forming organisms, 5.0×10^9 CFU per sample area (e.g., 10 cm^2) for vegetative cells, and 5.0×10^9 PFU per sample area (e.g., 10 cm^2) for viruses. If a different concentration is used (based on requirements documents or test sponsor requirements), the rationale for the change must be documented in the test plan and report.

1.3 Limitations.

a. The procedures in this TOP alone may not be sufficient to assess the efficacy of all decontaminants and decontamination processes. These procedures, as currently developed, are designed to be used only for R&D and early DT when evaluating the efficacy of gaseous/ vaporous and liquid decontaminants on coupons or panels of test materials in laboratories.

b. The test procedures in this TOP are based on gaseous/vaporous and liquid decontaminants. The procedures may need modification to accommodate other types of decontaminants (e.g., dry powder).

c. The results obtained by using these test procedures are not designed to correlate with specific medical or toxicological values or system performance.

d. The data obtained by these procedures cannot necessarily be correlated with specific field conditions.

e. The spore preparation method employed in this TOP is not necessarily representative of a military threat.

1.4 Method of Evaluation.

a. The following procedures will be used to determine the efficacy of a decontaminant to meet the program-specific criteria for biological decontaminability.

(1) Decontamination efficacy via log reduction will be determined using Equation 1:

$$Log_{10}(DE) = Log_{10}(CD_i) - Log_{10}(CD_p) \qquad \text{Equation 1}$$

Where,
DE = decontamination efficacy
CD_i = initial contamination density in CFU or PFU
CD_p = post-decontamination density in CFU or PFU

(2) The decontamination efficacy is then expressed as the log reduction and compared with the appropriate threshold as stated in this TOP. The thresholds are specific to decontamination requirements and are as follows: If the decontaminant achieves a 6-log or greater reduction for spore-forming organisms or a 7-log or greater reduction for vegetative cells and viruses (or the reduction required in the test documentation), then the decontaminant has successfully met the requirement for efficacy as a decontaminant.

2. FACILITIES AND INSTRUMENTATION.

2.1 Facilities.

Item	Requirement
Biosafety level (BSL)-1, BSL-2 and/or BSL-3 laboratories **NOTE**: The risk factors associated with the organism tested will determine the BSL required. Depending on the organism and its strain, a surety facility may be required for testing.	The laboratory provides the general support needed for work with biological agents, including analysis support, emergency response provisions, and hazardous waste storage and disposal. The biological laboratory must be constructed to ensure safe and secure storage, handling, general and specialized biological analysis, and decontamination of biological agents and/or simulants used for T&E. The laboratory is required to store and prepare test quantities of biological agent simulant materials, to charge disseminating devices, to prepare samplers, and to analyze all biological agent simulant.
Class II biological safety cabinet (BSC) for biological simulants	All BSCs must be certified and operating within the manufacturer's parameters. BSCs must be equipped with an air intake system and an exhaust system that exhausts through high-efficiency particulate air (HEPA) filters (i.e., capable of retaining 99.7 percent of particles 0.3 µm in diameter or larger). The

Item	Requirement
	BSC must be large enough to allow free air circulation around the test item. All applicable biological surety regulations will be followed. BSCs or environmental chambers will be used to house the test item(s) during agent or simulant contamination/decontamination (C/D) and sampling. Airflow control is required.

2.2 Instrumentation.

Testing with gaseous/vaporous or liquid decontaminants requires a contained (fully enclosed and under engineering controls) environment. Gaseous/vaporous decontaminants will most likely require environmental control to ensure optimal efficacy. Liquid decontaminants do not require the same environmental restrictions. Environmental control during the test process may also be required to represent an operational scenario.

Parameter	Measuring Device	Permissible Error of Measurement
Air temperature	Thermocouple or other.	±1.0°C.
Relative humidity (RH)	Humidity probe or other.	±2 percent.
Airflow speed	Anemometer.	±0.1 m/seconds.
Photographs	Still color camera.	Resolution must be high enough to record typical test procedures, details of contamination techniques, and contamination density (including any discrepancies from planned procedures necessitated by operational conditions).
Flow control of decontaminant	Mass flow controller or equivalent.	±10 percent at 20°C.

Parameter	Measuring Device	Permissible Error of Measurement
Contamination measurements (background contamination levels, post-contamination verification, and post-decontamination levels)	Microscopes and automatic colony/plaque counters or equivalent.	±10 percent CFU or PFU per sample.

3. REQUIRED TEST CONDITIONS.

3.1 Preparations for Test.

3.1.1 Documentation.

a. A test plan will be developed using the test documentation and other program-specific requirements/documents. The test plan will include a detailed statistical design, an execution matrix, and a data management plan. The test plan must be approved before testing can begin.

b. The development of test plans requires the following:

(1) Review of this TOP and applicable test documentation.

(2) Establishment of test objectives and criteria.

(3) Selection of appropriate military-relevant surface materials, decontamination methods, and test equipment.

c. Relevant test center/institution standing operating procedures (SOPs) and other procedures will be reviewed for applicability, completeness, and adequacy. These documents must be updated, as required.

d. All pertinent documentation will be maintained as part of the test record.

3.1.2 Biological Organism Strain Selection.

a. The determination of whether to use biological agents or simulants will be made during the planning phase. It is always preferable to use actual biological agents when cost, schedule, and resources allow.

b. To reduce personnel exposure risks, logistical burdens, and costs, it is common practice to use simulants in place of agents for testing purposes. When testing is conducted using simulants for biological agents, a corresponding agent-to-simulant relationship (ASR) study will be performed to verify the validity of using a simulant for testing. The ASR study will be coordinated with the test sponsor and the system evaluators and then documented in the test

report. Information on the physical parameters used for simulant selection will be included in the test report.

(1) Spore-Forming Bacteria. *Bg* and *Ba* ΔSterne are Gram-positive spore formers used in separate experiments to determine the variability of different spore species for the decontamination[1] and recoverability[3] studies. *Bg* and *Ba* ΔSterne are used as suitable simulants for *Ba*. Other Gram-positive *B.* species or strains such as *B. thuringiensis kurstaki* (*Btk*) and *B. cereus* (*Bc*) may be used with these test procedures. The strain used and results must be fully documented in the test report.

(2) Vegetative-Cell Bacteria. *Pa* is a Gram-negative, non-spore-forming bacterium. *Pa* is a suitable simulant for both *Yersinia pestis* (*Yp*) and *Francisella tularensis* (*Ft*) bacterial vegetative cells[2].

(3) Virus. Vaccinia virus WR is a suitable simulant for the Variola virus.

3.1.3 Sample Size.

The number of coupons (sample size) of test articles for test methods identified in this TOP may be determined based on design of experiment (DOE), confidence required by the customer, test article size, availability, cost, or other factors. The recommended number of replicates is the number from the DOE when a joint acquisition program is under test. If the program is not a joint acquisition program and a DOE is not established, then the minimum number of test coupons must be five. The minimum number of replicates for the dose confirmation samples per contamination set will be five. If the sample size is less than recommended, a test execution matrix will be devised to maximize the ability to meet stated objectives and criteria. Statistical confidence limits will be calculated and reported.

3.1.4 Coupon and Panel Use.

a. Coupons must measure at least 2 × 5 cm, and panels must measure at least 30.5 × 30.5 cm (multiple areas will be sampled on each panel). If a different size of coupon or panel will be used based on requirements documents or test sponsor requirements, the rationale for the size used will be documented in the test plan and test report. **NOTE**: The limit on panel size is determined by chamber and hood dimensions. Larger panels may be used if the hood and chamber can accommodate them.

b. Before use in testing, coupons and panels must be autoclaved. Materials that would be destroyed or degraded by autoclaving may be sterilized by other means (e.g., chemical).

c. The entire upper surface of each coupon will be used for contamination, decontamination, and sampling. When testing liquid decontaminants, each coupon must be placed in a container (e.g., Petri dish) before the decontaminant is applied to prevent loss of the decontaminant.

d. Panel testing requires identification and selection of multiple sample areas of predetermined size (e.g., 10 cm^2 each) on each panel. A minimum of eight sample areas (not

counting all control areas) will be selected for contamination and sampling. Each sample area will have the boundary marked and the location labeled; no markings will be placed inside the boundaries of the sample area.

e. When testing liquid decontaminants on panels, a barrier mechanism will be used to create wells or dams around each sample area to contain the liquid contaminant and decontaminant and prevent cross-contamination between sampling areas. For example, a barrier mechanism with corresponding sampling areas cut out can be laid onto the panel. The barrier material will form a seal with the panel surface (e.g., via adhesive backing) to prevent decontaminant from leaking under the edges, be sufficiently thick to prevent overflow, and be sterile (can be autoclaved) to avoid cross-contamination. An example of such a material is extreme-temperature silicone rubber translucent, adhesive back, 0.16 cm thick, 30.5 × 30.5 cm. Any adhesive backed material must be checked with the decontaminant(s) for effect on the adhesive that could allow penetration from one well to another.

3.2 Environmental Documentation (United States Only).

All local, state, and federal environmental regulations will be followed, and appropriate documentation will be prepared and submitted.

3.3 Safety.

a. Throughout testing, the primary emphasis will be placed on operator and test safety. Applicable safety and surety regulations will be reviewed to ensure that all test procedures comply with the regulations.

b. The determination of which BSL is required will be based on the most current edition of the Centers for Disease Control and Prevention (CDC) publication, Biosafety in Microbiological and Biomedical Laboratories (BMBL)[5], which establishes the level of containment based on the risk associated with the species or strains tested.

c. Personnel will have adequate safety and hazard training for working in BSL-2 and BSL-3 laboratories and will have the necessary clearance for handling select agents. All personnel working in a BSL-3 laboratory will be enrolled in the Special Immunization Program (SIP) and will be immunized as appropriate for the tests.

3.4 Quality Assurance (QA)/Quality Control (QC).

a. A QA plan must be prepared for each test program to ensure that all controllable variables are controlled and that appropriate records are kept throughout the duration of testing. Variables that cannot be controlled must be identified in the test plan. Test variables may include, but are not limited to: viability and stability of biological agents and simulants, purity and stability (pot life and shelf life) of decontaminants, calibration and maintenance of instrumentation and disseminators, accuracy and precision of laboratory analysis, and quality and uniformity of all coupons/panels.

b. The test equipment will be calibrated in accordance with (IAW) the manufacturer's specifications for each piece of applicable equipment. All instrumentation will be covered by a valid certificate of calibration traceable to a national standard and operated in strict accordance with the manufacturer's handbook.

c. The condition of the test material at the time of testing is an important test variable. Test materials must be inspected IAW TOP 08-2-500[6] or as otherwise specified in the receipt inspection subtest in the test plan. Inspection data, certificates of compliance, or similar documentation will be reviewed to ensure that exterior surfaces, finishes, and packaging meet specifications. Generally, materials will be tested in an as-received condition.

4. TEST PROCEDURES.

4.1 Objective.

Assess the decontamination efficacy of gaseous/vaporous and/or liquid decontaminants against bacterial spores, vegetative bacterial cells, or viruses representative of select, biological agents or agent simulants.

4.2 Criterion and Conditions.

4.2.1 Criteria.

a. Spore-Forming Bacteria. The decontaminant must achieve a 6-log or greater reduction (or the reduction required in the test documentation) from a minimum starting challenge as defined in the CDD, TEMP, or this TOP concentration of 5.0×10^8 CFU per sample area (e.g., 10 cm^2) to successfully meet the requirement for decontamination efficacy.

b. Vegetative Bacteria. The decontaminant must achieve a 7-log or greater reduction (or the reduction required in the test documentation) from a minimum starting challenge as defined in the CDD, TEMP, or this TOPconcentration of 5.0×10^9 CFU per sample area (e.g., 10 cm^2) to successfully meet the threshold for decontamination efficacy.

c. Virus. The decontaminant must achieve a 7-log or greater reduction (or the reduction required in the test documentation) from a minimum starting challenge as defined in the CDD, TEMP, or this TOP concentration of 5×10^9 PFU per sample area (e.g., 10 cm^2) to successfully meet the threshold for decontamination efficacy.

NOTE: Example calculations for starting contamination concentrations are in Table 1.

TABLE 1. EXAMPLE CALCULATIONS FOR CONTAMINATION CONCENTRATIONS

Spore Calculations (CFU)	Vegetative Cell (CFU) and Virus (PFU) Calculations
Stock: 1×10^{10} CFU/mL Conversion to µL: 1×10^{10} CFU/1000 µL = 1×10^{7} CFU/µL (1×10^{7} CFU/µL) × 50 µL[a] = starting challenge concentration of 5×10^{8} CFU/10 cm^2	Stock: 1×10^{11} CFU or PFU/mL Conversion to µL: 1×10^{11} CFU or PFU/1000 µL = 1×10^{8} CFU or PFU/µL (1×10^{8} CFU or PFU/µL) × 50 µL[a] = starting challenge concentration of 5×10^{9} CFU or PFU/10 cm^2

[a]Represents five, 10-µL drops applied to test surface.

4.2.2 Conditions.

a. Number of Test Replicates. During testing, coupons or panel sample areas will be used only once.

b. Biological agent/simulant viability, titer, and/or purity must be confirmed before use.

c. Liquid Application. The precision dispensing device (e.g., pipette) used to apply the biological agent must be capable of dispensing the drops in a uniformly repeatable volume on the surface of the test material(s). Precision dispensing device calibration must be current and compliant with the required performance specifications listed in the current versions of the International Organization for Standardization (ISO) 8655[7] Parts 1 and 2, the American Society for Testing and Materials (ASTM) E 1154-89[8] (for the volumes delivered), in applicable SOPs, or in the manufacturer's instructions.

d. Gas/Vapor Generator. The gas/vapor generator will disseminate a controlled concentration of decontaminant into the air. The flow of decontaminant within the gas/vapor generation system will be controlled with mass flow controllers or an equivalent control device. The generator control system will continuously monitor and control the gas/vapor temperature and RH.

4.3 Controls.

a. The procedures in this TOP will be conducted using experimental controls prepared on the same day as testing to ensure the reliability and repeatability (within the constraints of working with a living and growing organism) of the analytical procedure for each batch of test samples.

b. The positive control will consist of a coupon or a panel sample area that has been contaminated but not decontaminated. There will be at least three positive control coupons or panel sample areas per trial. The positive controls will be sampled concurrently with the test

coupons or panel sample areas in the trial. The positive controls must be sampled before the decontamination process or isolated from the decontamination process and then sampled. If the positive control results show no growth (e.g., 0 CFU) or growth that is less than or equal to that of the decontaminated surfaces, then the trial will be considered to have failed and will need to be repeated. The total number of positive controls will be specific to the test and documented.

c. The negative control will consist of at least one coupon or panel sample area that will not be contaminated. The negative control must be sampled after the decontamination process. If the negative control shows growth, then the trial failed and must be repeated. The total number of negative controls will be specific to the test and documented.

d. Additional QCs may be used for liquid decontaminant testing. The total number and types of additional controls will be specific to the test and documented in the test plan and report. Additional controls that may be used include, but are not limited to, the following:

(1) A water/water control may serve as a positive control to provide data on the sampling efficiency from the material or sampling method employed.

(2) An additional negative control coupon or sample area will be wetted with water or phosphate-buffered saline (PBS), or whatever solution in which the simulant/agent is suspended, to determine if the suspension solution became contaminated.

(3) A neutralizer/decontamination control is another important control and is intended to verify neutralization efficacy in the test process and ensure that the neutralizer does not have an adverse effect on the biological agent and its ability to grow. The neutralization procedure and validation of the neutralizer will be clearly documented in the final test report. The total number and types of additional controls will be specific to the test and documented in the test plan and report.

(4) A rinse control may be necessary if a rinse procedure is used after the decontamination period.

e. Sample and analysis controls include, but are not limited to, the following:

(1) Swab control (unused swab).

(2) Swab of a non-contaminated surface.

(3) Diluent control.

(4) Plate control (e.g., qPCR).

(5) Slurry subsample to qPCR for a quantification of reduction in GE/mL.

(6) Other controls as needed and specified by the type of test and analysis performed.

f. Collected samples must be cultured within 18 hours (maximum) of collection. If the samples are not immediately cultured, they must be stored at 4 °C.

4.4 Methods and Procedures.

4.4.1 Test Method Outline.

This test method outline briefly lists the test procedures for C/D when using gaseous/vaporous and liquid decontaminants and measuring pre- and post-decontamination levels of biological agents/simulants. Not all subtests are applicable to all types of coupon or panel materials. Many test procedures are identical for both liquid and gaseous decontaminants; however, when a procedure differs by decontaminant type, the difference will be clearly designated in the specific paragraph.

a. Bacterial and viral strains (Paragraph 4.5.1).

b. Media, culture, and biological agent preparation (Paragraph 4.5.2).

c. Coupon and panel handling and sterilization (Paragraph 4.5.3).

d. Viability, purity, and concentration assessment (Paragraph 4.5.4).

e. Dispensing device (Paragraph 4.5.5).

f. Inoculation (Paragraph 4.5.6).

g. Extraction buffers and media (Paragraph 4.5.7).

h. Panel surface sampling with wipes and swabs (Paragraph 4.5.8).

i. Extraction (Paragraph 4.5.9).

j. Dilution plating and titer enumeration (Paragraph 4.5.10).

k. Decontamination (Paragraph 4.5.11).

(1) Gaseous decontaminant generators and test chamber (Paragraph 4.5.11.a).

(2) Gaseous decontaminant exposures (Paragraph 4.5.11.b).

(3) Liquid decontaminant neutralizer preparation (Paragraph 4.5.11.c).

(4) Application of liquid decontaminant and neutralizer (Paragraph 4.5.11.d).

l. Post-decontamination surface sampling with wipes and swabs (Paragraph 4.5.12).

(1) Gaseous decontaminant post-decontamination surface sampling (Paragraph 4.5.12.a).

(2) Liquid decontaminant post-decontamination surface sampling (Paragraph 4.5.12.b).

m. Post-decontamination spore extraction (Paragraph 4.5.13).

n. Post-decontamination dilution plating and titer enumeration (Paragraph 4.5.14).

o. Decontamination efficacy determination (Paragraph 4.5.15).

4.4.2 Significance and Use.

The data collected using the procedures in this TOP will allow an analysis of the efficacy of liquid and gaseous decontaminants. The decontamination efficacy is determined by measuring pre- and post-decontamination levels of biological contamination on military-relevant surfaces.

4.4.3 Hazards.

a. BSL-2 microorganisms and decontaminants (e.g., VHP®, calcium hypochlorite/HTH, and sodium hypochlorite) used in testing are considered hazardous.

b. All safety protocols will be followed to address the hazards inherent in working with the selected biological agents, simulants, and decontaminants. Biological safety guidelines are found in Department of the Army (DA) Pamphlet (PAM) 385-69[9], other service-specific guidelines, or CDC guidelines[5].

c. A test plan will be developed with a safety section identifying and addressing all safety concerns for each test conducted. The safety section of the test plan will be coordinated with the test site's safety office.

4.5 Test Procedures for Gaseous and Liquid Decontaminants Testing.

4.5.1 Bacterial and Viral Strains.

The necessary strain(s) that represent or simulate the threat will be selected, obtained, and defined in the test documentation. QC tests will be performed to confirm the identity of the microorganism if required by the test program.

4.5.2 Media, Culture, and Biological Agent Preparation.

a. General.

(1) The seed stocks will be kept at -80 °C for long-term storage (up to 24 months).

(2) All new seed stocks will be quantified by standard plate count or plaque assay and qPCR. qPCR will contain a 5-point standard curve, target markers, reference negative controls (also known as non-target markers), positive controls, and no-template controls. The standard curve slope must have a correlation coefficient greater than 0.990. The concentration of all working stocks (as determined by standard plate count) must be at least 1 log greater than the desired challenge concentration.

(3) Viability and titer must be confirmed before use and after storage by standard plate count or plaque assay. The stock must be enumerated by standard plate count/plaque assay in triplicate using standard microbiological techniques and incubated under optimal conditions for the organism.

(4) If a clean stock is desired to reduce experimental variability, then the preparation will be washed following a documented test procedure.

(5) The working stock concentration cannot drop below the highest inoculation density required for surface deposition for a given test. qPCR will be employed using the appropriate controls to verify the presence of the target organism and quantify concentration in GE/mL. If a multiplex qPCR is used, reference controls can be run on one plate per agent/ simulant. The qPCR and Gram staining procedures will be reported in test documentation.

b. Bacteria. The method used to propagate the bacterial agent or simulant will be described in detail in the test documentation. If the bacterial preparation is purchased, the supplier, the lot number, and any other pertinent information will be provided in the test documentation. Stocks will be expanded from a single colony containing the correct morphology and pigmentation. Stock viability and concentration will be monitored by performing plate counts for each bacterial agent. With each plate count, purity will be assessed by evaluating colony morphology for evidence of any stock contamination. Bacterial purity will also be evaluated by microscopic examination of Gram stain cultures. Concentrations of endotoxin protein will be assessed with a standard method, such as BCA assay, Lowry assay, or Bradford assay (for detection of contamination from Gram-negative bacteria).

(1) Spores. The working stock will include a yield of at least 1.0×10^{10} CFU/mL and contain at least 95 percent refractile spores with less than 5 percent cellular debris after growth and harvest. Assessment will be performed by microscopy and enumeration using instrumentation, such as a Coulter counter and standard plate count. The working spore stocks may be maintained for up to 6 months at 4 °C. The procedure(s) used to verify the percent of spores must be provided in the test documentation.

(2) Vegetative Cells. The working stock will include a yield of at least 1.0×10^{11} CFU/mL in storage medium, and microscopy will be used to verify that at least 90 percent of the vegetative cells are free of spore-forming inclusion bodies. The working vegetative stocks may be maintained for up to 30 days at 4 °C. The procedure(s) used to verify that the vegetative cells are free of spore-forming inclusion bodies must be provided in the test documentation.

c. Virus. The working stock of the virus preparation will contain a yield of at least 1×10^{11} PFU/mL. The virus strain can be propagated and assayed in the same cell line. For example, Vero cells can be used for propagation and determining virus titer by plaque assay. To avoid significant changes in viral titer, 50 to 100 aliquots (0.5 to 2 mL each in volume) of the working stock will be prepared and maintained at -80 °C. Each aliquot will be used immediately upon thawing and will not be refrozen. The specific procedures used to produce the working stock must be documented in the test plan and report. A qPCR assay will be used to verify the presence of the target DNA species or strain and quantify the concentration in GE/mL of

working stocks. RT-qPCR will be used to verify the presence of the target RNA species or strain and quantify the concentration in GE/mL.

d. The working stock of inoculant may contain up to 1 percent fetal-bovine serum (FBS), representing bio-burden. The inclusion of bio-burden is considered a worst-case scenario. The use of bio-burden must be coordinated with the threat and evaluator communities to ensure a thorough understanding of the implications in using bio-burden in testing. If bio-burden is to be used in testing, then the rationale for its use will be included in test documentation.

e. After working stocks are prepared or pulled from storage, aliquots will be collected for analysis. Viability and concentration will be assessed by performing plate counts for each organism. With each plate count, purity will be assessed by evaluating colony/plaque morphology for any stock contamination. Biological agent purity will also be evaluated by microscopic examination of Gram stain cultures. The stock concentration cannot drop below the highest inoculation density required for surface deposition for a given test.

4.5.3 Coupon and Panel Handling and Sterilization.

a. Coupon and Panel Specifications. Each type of coupon or panel material will be obtained from a single source to ensure uniformity during testing; however, different material types may be acquired from different sources. The supplier, manufacturer, lot number, coating specification, and any other identifying characteristics will be documented for all materials to be tested. Coupon dimensions will be at least 2 × 5 cm, and panel dimensions will be at least 30.5 × 30.5 cm.

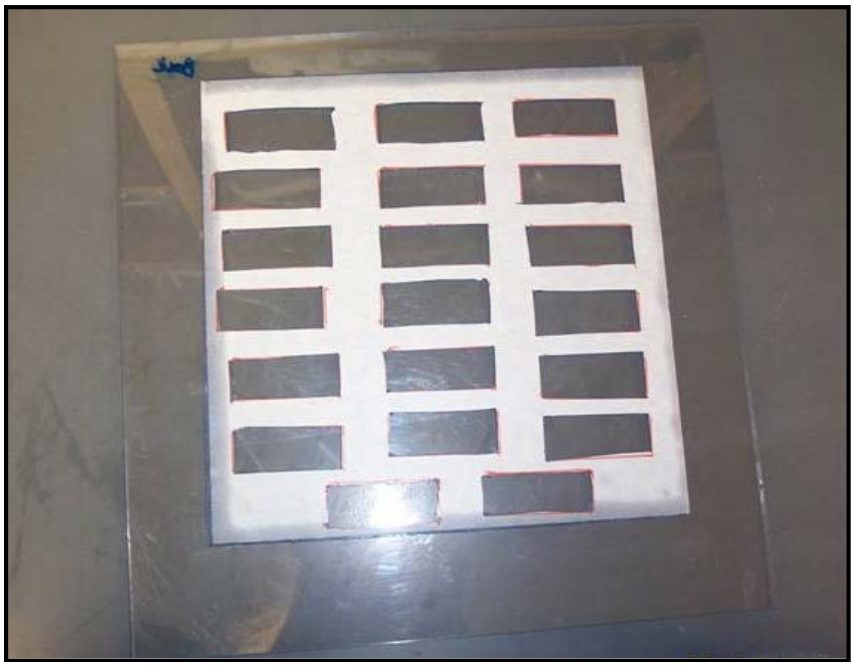
b. Coupon and Panel Inspection/Traceability. The test center will inspect each coupon or panel for uniformity and defects. Only coupons or panels that pass visual inspection will be used for testing. All coupons and panels used for testing will be marked or etched with a unique test item control number (TICN). The TICN must not interfere with or be located on the surface to be contaminated.

c. Coupon and Panel Preconditioning. Nonporous coupons and panels will be washed with soap and de-ionized Type I ultrapure water (conductivity of 0.055 µsiemens/cm), rinsed with ultrapure water, and then sprayed with 70 percent ethanol. The coupons and panels will be wrapped in aluminum foil and sealed with autoclave tape. Sterilization pouches may also be used. Appropriate procedures for porous materials will be developed and documented in the test plan and report.

d. Autoclaving at 121 °C for 30 minutes using a dry cycle is the recommended method for sterilizing coupon and panel material that may be autoclaved safely. After autoclaving, the coupons or panels will be allowed to dry at ambient room temperature (ART) in a BSC and stored in the autoclaved foil package in a clean environment at ambient temperature. Temperature and RH conditions in the storage environment will be recorded. An appropriate sterilization method for materials that cannot be autoclaved will be developed and described in the test plan. If there is no appropriate sterilization method available, then sufficient controls will be used to account for background contamination in the test results.

e. For liquid decontaminant testing, four coupons or four panel sample areas will be marked and used for controls (e.g., water/water, water/neutralizer, neutralizer/decontaminant, decontaminant/neutralizer). In addition, for all types of testing, positive and negative controls will be identified and marked. Information on controls is in Paragraph 4.3.

f. When panels are used for liquid decontaminant testing, a barrier material (e.g., butyl rubber or silicone gaskets with the sample areas cut out, or a line of butyl or silicone laboratory tacky tape, or similar material) will be used as a dam to contain the liquid in the sample areas during testing. This barrier must be placed on the panel before inoculation with a contaminant. Horizontal orientation of the panel must be maintained to prevent liquid spilling from the sample areas. The barrier must form a seal with the panel surface (e.g., via adhesive backing) to prevent leakage. Barrier materials must be sufficiently thick to prevent overflow and sterile to avoid cross-contamination. Also, barrier materials should be able to survive exposure to extreme-temperatures so that they can be autoclaved. An example of a panel with barrier overlay is shown in Figure 1.



NOTE: The white barrier material shows cut outs (forming the sample areas) removed before the barrier was applied to the panel.

Figure 1. Example of a polycarbonate panel (placed in a holder) with an adhesive-backed sheet of barrier material overlaying the surface[3].

4.5.4 Viability, Purity, and Concentration Assessment.

a. Biological organisms are sensitive to factors such as temperature, duration of storage, and transport. To mitigate biological stock degradation, appropriate storage and handling procedures will be followed. The biological agent viability, purity, and concentration will be verified on a regular basis for the duration of testing to monitor integrity.

b. Stock viability and concentration will be monitored by performing plate counts or plaque assays for each biological agent. With each plate count/plaque assay, purity will be assessed by evaluating colony/plaque morphology for any stock contamination. Biological agent purity will also be evaluated by microscopic examination of Gram stain cultures. Stock concentration cannot drop below the highest inoculation density required for surface deposition for a given test.

c. For bacterial QC checks, the stock will be plated in triplicate onto blood agar plates (BAPs) or tryptic soy agar (TSA) plates.

d. For virus QC checks, a serial 10-fold dilution of the stock virus will be tested for viability by plaque assay and the titer will be recorded. To avoid significant changes in viral titer, 50 to 100 aliquots (0.5 to 2 mL each in volume) of the working stock will be prepared and maintained at -80 °C. Each aliquot will be used immediately upon thawing and will not be refrozen.

e. A qPCR assay will be employed to verify the presence of the target DNA species or strain and quantify the concentration in GE/mL of the working stocks. RT-qPCR will be employed to verify the presence of the target RNA species or strain and quantify the concentration in GE/mL. Each assay microtiter plate must contain a standard curve, target markers, reference negative controls (also known as nontarget markers), positive controls, and no-template controls. If a multiplex qPCR is used, reference controls can be run on one plate per agent or simulant.

4.5.5 Dispensing Device.

It is of the utmost importance to ensure that biological agent is applied to the material surfaces accurately, consistently, and reproducibly for effective quantification of post-decontamination extraction recovery. Each coupon or panel sample area will be inoculated with 50 µL of biological agent in five distinct 10-µL spots on the surface (Figure 2).

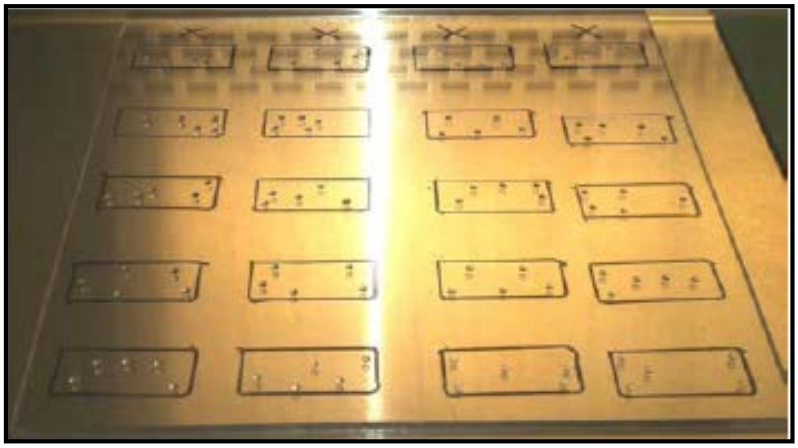


Figure 2. Example of an inoculated polycarbonate panel[1] showing the 2- × 5-cm sample areas clearly marked and containing five drops per sector.

4.5.6 Inoculation.

a. The coupons and panels will be contaminated with the biological agents/simulants inside a BSC dedicated for manipulation of only one organism at a time to prevent cross-contamination. Temperature and RH conditions in the laboratory will be recorded.

b. Coupons. The coupons will be individually placed in a sterile Petri dish using sterile forceps. The Petri dishes containing coupons will then be placed in a plastic container with an airtight lid. Working stocks of biological agent/simulant with a concentration that is at least 1 log greater than the desired challenge concentration [Paragraphs 1.2.b(1) and (2)] will be prepared to achieve the minimum challenge concentration densities of 5.0×10^8 CFU/10 cm^2 for spores and 5×10^9 PFU/10 cm^2 for vegetative bacteria and virus. This will be accomplished by depositing five distinct 10-µL aliquots (50 µL total) on the surface of the coupon. The contaminated coupons will be allowed to dry at ART and ambient RH in a BSC or in an environmental chamber set to 25±5 °C and 10 to 25 percent RH.

c. Panels. Panels will be divided into sample areas, as specified in the test plan, and inoculated with biological agent/simulant (Figure 2). Working stocks of biological agent/ simulant with a concentration that is at least 1 log greater than the desired challenge concentration will be prepared to achieve the minimum challenge concentration densities of 5.0×10^8 CFU/10 cm^2 for spores and 5×10^9 PFU/10 cm^2 for vegetative bacteria and virus. This will be accomplished by depositing five distinct 10-µL aliquots (50 µL total) in each test sample area on the surface of the panel. The contaminated panels will be allowed to dry at ART and ambient RH in a BSC or in an environmental chamber set to 25±5 °C and 10 to 25 percent RH.

d. Control coupons and panel sample areas must be incubated. The coupons and panels must be allowed to dry under the same conditions at which they were incubated. Microscopic examination will be performed to verify that spores remain phase bright during the drying process on the coupons and panels. A control coupon will verify the loss of viability for vegetative cells and virus during the drying period. This is especially important if additional components are added to the spore inoculums (e.g., bio-burden).

4.5.7 Extraction Buffers and Media.

a. An appropriate extraction buffer will be selected for testing. Any methodology conducted to maximize the ability of the buffer to extract the organism(s) from test surfaces will be reported. Using the appropriate extraction buffer is extremely important because it affects the recovery of the organism tested. The test plan and report will identify the buffer, include the rationale for selection of the buffer, and describe any surfactant or other additives.

b. Extraction buffers and cell culture media will be selected to maximize the extraction efficiency (EE) and percent recoveries for each biological agent. Previous work with extraction of *Ba* endospores on multiple coupon material types using PBS + 0.1 percent Triton X-100 (PBST) demonstrated excellent extraction efficiencies above 90 percent. Heart infusion broth (HIB) was used for extraction of *Pa*, and Dulbecco's Modified Eagles Medium (DMEM) was the extraction media for the Vaccinia virus WR[2].

NOTE: Extraction buffers such as, but not limited to, PBS and buffered peptone water (BPW), with and without 0.05 percent polysorbate 80 or 0.5 percent Triton X-100 may be used.

4.5.8 Panel Surface Sampling With Wipes and Swabs.

NOTE: This procedure is also followed for the control samples. Company and catalog numbers for polyester gauze pads and cotton swabs are included in Appendix B to help personnel find equivalent supplies. Recovery efficiencies were established using these supplies; if a different supplier or type of wipe or swab is selected for use, recovery efficiency studies must be conducted.

a. A combination of sterilized polyester gauze pads (wipes) and cotton swabs will be used to sample large coupons[1,2,3]. **NOTE**: Using wipes resulted in the greatest recovery efficiency[1]. Swabs may be used in addition to wipes to increase overall recovery.

b. Polyester wipes will be removed from the package and cut in half using flame-sterilized scissors[3].

(1) Wipes will be folded twice widthwise, turned 90 degrees, and then folded widthwise again. The ends of the wipe will be gathered together to facilitate holding using the thumb and fingers[3] (Figure 3).

(2) Using a pipettor, the wipes will be pre-moistened with 1 mL of the appropriate extraction buffer. The wetted wipes will be scrubbed lengthwise across the sample area (Figure 3).

(3) After sampling (scrubbing) has been completed, the wipe will be placed in a labeled 50-mL conical tube containing 24 mL of the appropriate buffer. A separate, labeled, 50-mL conical tube will be used for each sample area on the panel.

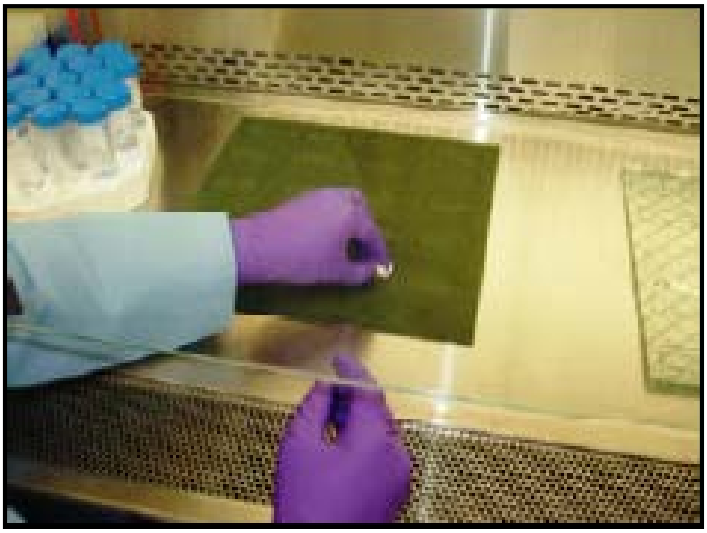


Figure 3. Example of surface sampling on a panel using a wipe[1].

c. Swabs will be premoistened by dipping them into a conical tube containing 10 mL of the appropriate recovery buffer. Each sample area will be swabbed in a horizontal direction with three consecutive cotton swabs. After swabbing the sector or sample area, the three swabs will be returned to the single tube containing the 10 mL of recovery buffer. **NOTE**: The swab applicator may have to be cut with flame-sterilized scissors in order to fit in the tube. It is recommended that 3-inch cotton swabs be used so that the applicator does not have to be cut[1,2,3].

4.5.9 Extraction.

a. Coupons. Each coupon will be placed in a labeled 50-mL conical tube containing 25 mL of extraction buffer. The tube will be vortexed at the appropriate speed and time for each biological agent (e.g., 750 rpm for 5 minutes for spores; 750 rpm for 15 minutes for vegetative bacteria and viruses; if rpm is not specified, medium to high setting should be used)[1,2]. The duration and speed must be documented.

b. Panels. The labeled 50-mL conical tube(s) containing the polyester wipe [in 25 mL of extraction buffer (1 mL from premoistened wipe plus 24 mL in the tube)] and swabs (in 10 mL of extraction buffer) will be vortexed at the appropriate speed (e.g., 2 minutes at approximately 1500 rpm)[1,2]. The duration and speed must be documented.

c. Some complex test materials may require sonication for 10 minutes for optimal spore extraction. If sonication is performed, then the rationale and all sonication procedures used will be reported.

4.5.10 Dilution Plating and Titer Enumeration.

a. General. Titer enumerations will be performed with each test run to verify starting biological agent challenge concentrations. After the extraction procedure is performed, 10-fold sample dilutions will be made, ranging from 10^{-1} to 10^{-4} for wipes and 10^{-1} to 10^{-3} for swabs. Dilutions are highly variable based on decontaminant effectiveness; therefore, a neat aliquot of extraction material (representative of 10^{0}) will be plated in addition to the diluted samples. The dilution range must be determined empirically for each new decontaminant.

(1) An aliquot of 100 µL from the dilutions will be placed (in triplicate) on BAP/TSA plates (or appropriate media) and spread with a sterile spreader. For samples that have been exposed to gas/vapor, an aliquot of 100 µL will be placed (in triplicate) on plates and spread with a sterile spreader.

(2) Additionally, a 3-mL aliquot will be pour-plated on BAP/TSA plates (in triplicate, 1 mL per plate), for samples exposed to gas/vapor. All spread and pour plates will be incubated at 37 °C for growth (12 to 16 hours or more, depending on the organism).

(3) The CFU and/or PFU will be counted from the replicate plates and will be averaged and multiplied by the dilution factor, volume factor, and total volume of the extraction media to calculate the corrected CFU and/or PFU.

(4) The values, log values, and percent relative standard deviation (RSD) for replicate dilutions generating 30 to 300 colonies/plaques will be computed and averaged to derive the mean log CFU or PFU. Dilutions that exhibit more than 300 colonies/plaques will be recorded as “too numerous to count” (TNTC) and excluded from the statistics. Dilutions with less than 30 colonies/plaques will be recorded as “no growth.” Because evaluating decontamination efficacy is the objective, all plates with less than 30 colonies/plaques will be counted and recorded to document any post-decontamination presence of biological agent.

b. Dilution, Plating, and Enumeration for Bacteria. The extracted material will be serially diluted 10-fold, ranging from 10^{-1} to 10^{-4} and plated onto BAP or TSA to determine the number of CFU recovered post-extraction. Specifically, 1.8-mL sterile polypropylene tubes will be labeled for each sample from 10^{-1} to 10^{-4}. Each tube will receive 900 µL of buffer. A 100-µL aliquot will be removed from the extraction material tube, added to the 10^{-1} tube, and vortexed. This procedure will continue until all 10-fold serial dilutions are prepared. A 100-µL aliquot of each dilution and the undiluted material will be plated in triplicate on BAP or TSA. The plates will be incubated for 12 to 16 hours at temperatures appropriate for each organism. Enumeration of the CFU will be performed after the incubation period or the following day.

c. Dilution, Plating, and Enumeration for Virus. A confluent monolayer of Vero cells will be counted using a hemacytometer. A 12-well, 35-mm^2 tissue culture plate will be seeded with 5×10^{5} cells per well in 2-mL minimum essential medium (MEM)-5.0. The culture plate will be incubated for 2 days in a humidified, 5 percent carbon dioxide (CO_2) incubator at 37°C to reach confluency. Ten-fold serial virus dilutions will be made with MEM-1.0 in duplicate with appropriate controls. The medium from Vero cells will be removed, and the cells will be infected in duplicate wells with 0.1 mL of the appropriate virus dilution (e.g., 10^{-4} to 10^{-9}). The

plate will be incubated 1 hour in a CO_2 incubator at 37 °C with a gentle circular motion at 15-minute intervals to spread virus uniformly without disturbing the cells. Each well will be overlaid with 2 mL MEM-1.0 and placed in a CO_2 incubator at 37 °C for 2 days. After 2 days, 2 mL of 10 percent formalin will be added to each well and allowed to rest undisturbed for a time period from 4 hours to 4 days to fix the monolayer. The medium will be removed; then 0.5 mL of 0.1 percent crystal violet will be added to each well and incubated for 5 minutes at room temperature. Crystal violet will be aspirated, and the wells will be rinsed with distilled water and allowed to dry. The viral titer will be determined by counting plaques within the wells and multiplying by the dilution factor. The plaque assay will be optimized to obtain 20 to 80 plaques. **NOTE**: The passage number will be documented and must not exceed the number of passes for the cell line that is used for testing.

4.5.11 Decontamination.

a. Gaseous Decontaminant Generators and Test Chamber.

(1) The coupons and panels will be placed in the test chamber and exposed to the desired gaseous decontaminant within the desired parameters. During the process, temperature, RH, pressure, and decontaminant sensors will be programmed, controlled, and monitored. Mixing fans in the chamber will ensure even distribution of decontaminant over the exposed surfaces.

(2) The gaseous decontaminant will be prepared and administered as described by the product manufacturer.

b. Gaseous Decontaminant Exposures.

(1) Coupons and/or panels will be fumigated with the desired decontaminant at the manufacturer-recommended conditions for concentration × time (CT).

(2) The manufacturer-recommended conditions may include:

(a) RH.

(b) Temperature.

(c) Concentration in parts per million volume (ppmv).

(d) Contact time (beginning when the target concentration is reached until the gas/vapor chamber is emptied of gas/vapor after the trial is complete).

c. Liquid Decontaminant Neutralizer Preparation.

(1) The use of a decontaminant neutralizer is essential when testing a liquid decontaminant. The selection of an appropriate neutralizer will depend on the active ingredient(s) contained in the decontaminant.

(2) A methodology study will be conducted with the selected neutralizer for a particular decontaminant. The methodology study will include information on the testing conducted to verify the efficacy of the neutralizer on the decontaminant, a description of any effects the neutralizer has on the microorganism decontaminated, and a description of any effects the neutralizer has on the materials or substrates tested. The test report must include all pertinent information from the neutralizer methodology study.

d. Application of Liquid Decontaminant and Neutralizer.

(1) A volume of 1 mL of control solution or decontaminant will be placed on each coupon or panel sample area. This will result in a 1-mL total volume of control solution or decontaminant in each sample area. Decontaminant or control solution will be added at predetermined intervals of time (e.g., 30 seconds) and allowed contact with the sample areas for 15 minutes or another decontamination time based on manufacturer specifications.

(2) A volume of 1 mL of appropriate neutralizer will be added to each sample area after the decontaminant contact time. This will result in a 2-mL total volume of solution (control solution or decontaminant + neutralizer) in each sample area. Adding equal volumes of decontaminant and neutralizer simplifies pipetting and prevents errors because the pipette volume is not changed during the protocol. Additionally, the 1-mL neutralizer volume is sufficient to cover the surface and minimize runoff. **NOTE**: In the neutralizer/decontaminant control area, the neutralizer will be added immediately after decontaminant application rather than allowing a 15-minute contact time. Nothing will be added at the 15-minute time point to the neutralizer/decontaminant control area.

(3) The neutralizer will be allowed to remain in contact with the decontaminant for an appropriate amount of time (e.g., 5 minutes). Table 2 provides an example schedule with detailed timing of solution addition to and sampling of materials. Actual timing and sampling procedures must be detailed in the test plan, and the actual conduct must be described in the test report.

(4) Table 3 shows an example of some decontaminants and neutralizers used in developing the procedures in this TOP. The test planning process should include a rationale for the selection of neutralizers for the decontaminant(s) tested. The test report must include a description of how well the selected neutralizers performed.

Table 2. EXAMPLE TIMING SCHEDULE FOR SOLUTION ADDITION TO AND SAMPLING OF PANEL SAMPLE AREAS 1 THROUGH 10.

Sample Area	First Addition		Second Addition		Sample Removal Time[b] (mm:ss)
	Test Time (mm:ss)[a]	Solution	Test Time (mm:ss)	Solution	
1	00:00	Water	15:00	Water	20:00
2	00:30	Water	15:30	Neutralizer	20:30
3	01:00	Neutralizer/ Decontaminant	16:00	None	21:00
4	01:30	Decontaminant	16:30	Neutralizer	21:30
5	02:00	Decontaminant	17:00	Neutralizer	22:00
6	02:30	Decontaminant	17:30	Neutralizer	22:30
7	03:00	Decontaminant	18:00	Neutralizer	23:00
8	03:30	Decontaminant	18:30	Neutralizer	23:30
9	04:00	Decontaminant	19:00	Neutralizer	24:00
10	04:30	Decontaminant	19:30	Neutralizer	24:30

[a]Minutes:seconds.
[b]Describes the time interval at which 1.5 mL of liquid is removed from the sample area as detailed in Paragraph 4.5.12 b(1).

Table 3. EXAMPLES OF LIQUID DECONTAMINANTS AND ASSOCIATED NEUTRALIZERS.

Decontaminant	Neutralizer
2 percent calcium hypochlorite/high-test hypochlorite (HTH).	2 percent sodium thiosulfate.
Easy DECON™ 200 Decontamination Solution (DF-200).	4 percent sodium metabisulfite.
10 percent sodium hypochlorite with no hydrogen ion concentration (pH) adjustment (pH ~10).	2 percent sodium thiosulfate.
10 percent sodium hypochlorite adjusted to a pH ~7.0.	1 percent sodium thiosulfate.
Ready-to-use Spor-Klenz®.	4 percent metabisulfite.

4.5.12 Post-Decontamination Surface Sampling With Wipes and Swabs.

NOTE: Company and catalog number for the polyester gauze pads and cotton swabs used in developing this TOP are included in Appendix B. Recovery efficiencies were established using these supplies; if a different supplier or type of wipe or swab is selected for use, recovery efficiency studies must be conducted.

a. Gaseous Decontaminant Post-Decontamination Surface Sampling. The procedures outlined in Paragraph 4.5.8 will be followed.

b. Liquid Decontaminant Post-Decontamination Surface Sampling. The procedures outlined in Paragraph 4.5.8 will be conducted, with the following procedural additions:

(1) Using a pipette, 1.5 mL of liquid (e.g., water/water control, neutralized decontaminant) will be removed from the sample area and placed into a labeled 50-mL conical tube containing 23 mL of an appropriate buffer. An example sample-removal timing schedule is provided in Table 2. **NOTE**: The gauze wipe will not be premoistened because there will be residual liquid (0.5 mL) on the coupon or panel area.

(2) Wipes will be folded twice widthwise, turned 90 degrees, and then folded widthwise again. The ends of the wipe will be folded together to facilitate holding with the thumb and fingers[3] (Figure 3).

(3) The wipe will be dabbed on the remaining 0.5 mL of liquid. All of the remaining liquid must be absorbed by the wipe.

(4) The wipe will be placed in the same labeled conical tube used in the step from Paragraph 4.5.12.b(1). A separate labeled 50-mL conical tube will be used for each coupon or panel sample area.

4.5.13 Post-Decontamination Spore Extraction.

a. The procedures outlined in Paragraph 4.5.9 will be followed.

b. After removing all aliquots from the conical tube, the tube will be sealed and the remaining liquid will be allowed to incubate.

c. The clarity of the liquid will be checked after 12 to 48 hours of incubation (depending upon the organism). If the liquid is clear, then all spores from the sampler/coupon have been killed. If the liquid is turbid, then the extraction method must be reexamined to determine means for improvement.

4.5.14 Post-Decontamination Dilution Plating and Titer Enumeration.

The procedures outlined in Paragraph 4.5.10 will be followed.

4.5.15 Decontamination Efficacy Determination.

a. After the decontamination process (gas/vapor or liquid), sampling will be conducted as soon as possible. Samples will be incubated for 12 to 48 hours (depending on the organism) and then the CFU or PFU will be counted.

b. The log CFU or PFU values, mean log CFU or PFU value, and log CFU or PFU standard deviation for replicate coupons will be computed.

c. Decontamination efficacy will be calculated using Equation 2.

$$DE_{sr} = \frac{CFU_r}{CFU_i} \qquad \text{Equation 2}$$

Where,
DE_{sr} = decontamination efficacy (sample recovery)
CFU_r = CFU or PFU recovered
CFU_i = CFU or PFU inoculated (titer enumeration control)

d. Log reduction values will be calculated using Equation 1 [Paragraph 1.4.a(1)].

5. DATA REQUIRED.

The following data will be reported in the units indicated:

a. Gas/Vapor Chamber Decontamination Data.

(1) Temperature (±2 °C).

(2) RH (±5 percent).

(3) Airflow speed through the chamber (±0.1 m/seconds).

(4) Elapsed time decontaminant was present (±1 minute).

(5) Contamination data for each sample area, including concentration before decontamination and residual concentration after decontamination (10 percent CFU or PFU).

(6) Calculated log reduction (±0.1 log).

(7) All control data (10 percent CFU or PFU).

b. Liquid Decontamination Data.

(1) Volume of decontaminant used (±0.1 mL).

(2) Decontaminant lot number.

(3) Time for decontaminant preparation (±1 min).

(4) Time from preparation to decontaminant application (±1 min)

(5) Time to disseminate (±1 sec)

(6) Decontaminant contact time (±1 min).

(7) Neutralizer contact time (±1 min).

(8) Contamination data for each sample area, including concentration before decontamination and residual concentration after decontamination (10 percent CFU or PFU).

(9) Calculated log reduction (±0.1 log).

(10) All control data (10 percent CFU or PFU).

c. Additional Data.

(1) Laboratory temperature.

(2) Laboratory humidity.

(3) Coupon material and any material treatments.

d. Biological Agent or Simulant.

(1) Name, control number/lot number, and biological agent manufacturer.

(2) Diluent used.

(3) Spore preparation yield of at least 95 percent spores (by phase-contrast microscopy) and particle size distribution using Coulter analysis.

(4) Vegetative bacterial cell preparation yield.

(5) Virus preparation yield.

(6) GEs.

(7) Protein assay results.

(8) Microscopic results (e.g., Gram stain and phase contrast).

(9) Date prepared or reconstituted.

(10) Date used.

(11) Inoculant density (10 percent CFU/mL).

(12) Dispensing device (e.g., pipettor) used.

(13) Volume of biological agent/simulant suspension applied (±1 mL).

(14) Dispensing time in seconds.

(15) Spore acceptance criteria.

(16) Sample area size (±1 cm^2).

e. Descriptions of decontamination solutions (e.g., manufacturer, formulation, active ingredients, lot number, pot life, shelf life, and age), methods, equipment, and item-specific procedures used.

f. Descriptions of decontaminant solution neutralizer (e.g., manufacturer, formulation, active ingredients, and lot number), methods, equipment, and item-specific procedures used.

g. Description and photographs (or video) of any degradation of materials (e.g., corrosion, swelling, etc.) resulting from the decontaminant. A scale of reference will be included in any photographs.

h. If applicable, a description and photographs (or video) of any special procedures performed during the decontamination process because of the nature of the decontaminant.

i. Any relevant safety findings as a result of testing.

6. PRESENTATION OF DATA.

a. C/D data will be presented in a format to show direct comparison of pre- and post-exposure concentrations of the test item.

b. Data will be reported as the geometric mean to reduce the influence of outliers and to give equal weight to all experimentally derived values. The standard error of the mean will be used to calculate the error bars around the geometric mean. **NOTE**: For data sets that give zero counts, an arbitrary number [e.g., 1 or minimum detection limit (MDL) for the assay] may need to be added to all data in that set and then subtracted after calculating the geometric mean. The rationale for adding 1 or MDL is to enable the calculation of the logarithm.

c. Data Comparison (When Using Normalization Techniques). To compare decontamination data across materials, the mathematical techniques found in DeVries and Hamilton, Estimating the Antimicrobial Log Reduction: Part 1[10] and Part 2[11] will be used as follows: the experimental decontaminant's geometric mean, positive geometric standard error,

and negative geometric standard error will be multiplied by a normalization value that would be required to convert the geometric mean of the water/water control to the maximum value of 5×10^8 for spores and 5×10^9 for vegetative bacteria and viruses.

d. Data Quality. A high coefficient of variation (CV) indicates greater dispersion in the variable measurements. If the CV from the replicate plate or replicate samples is too high, the data may be designated as invalid; but the data values will be reported.

e. Data Graphing. To graphically depict the raw and normalized data in terms of CFU or PFU, data bars will be shown as the geometric mean, and the geometric standard error of the mean will be used as error bars. For log reduction graphs, the geometric mean of the experimental values will be converted to $\log_{10}$.

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APPENDIX A. BACKGROUND.

a. A critical part of the biological decontaminant selection process in the Department of Defense (DOD, Washington, DC) is the review of available efficacy data. Because of Warfighter requirements, it is necessary that the candidate decontaminant is effective against bacterial endo-spores, particularly *Ba* spores, in 15 minutes or less. The availability of useful bioefficacy data is particularly challenging because of the following factors:

(1) DOD requirements specifying that candidate decontaminants must be effective against both chemical and biological agents within a short period of time.

(2) The lack of standardized biological efficacy test methods across the DOD laboratories.

(3) The lack of a standard test method for quantification of efficacy against endospore-forming bacteria.

b. Following the tragic events of September and October 2001, there was heightened concern and awareness about the deliberate introduction of chemical and biological (CB) agents into buildings by terrorists. Such an attack on airfield or shipboard control and command centers requires effective and prompt efforts to protect building occupants and to decontaminate these facilities for re-occupancy. The potential technologies currently available are loosely grouped into three categories:

(1) Surface-applied decontamination, which includes hypochlorite, aqueous hydrogen peroxide, aqueous chlorine dioxide, nanoemulsions, Sandia foam (Sandia National Laboratories, Albuquerque, New Mexico), Canadian Aqueous System for Chemical Biological Agent Decontamination (CASCAD™) surface decontaminating foam (SDF™)[Department of National Defence, Defence Research and Development Canada (DRDC), Suffield, Alberta, Canada], and L-gel (oxone).

(2) Gas and vapor-phase decontamination, which includes ethylene dioxide, ClO_2, VHP®, methyl bromide, ozone, and paraformaldehyde.

(3) Energy-based decontamination, which includes directed energy, photochemicals, and plasma.

c. Based on past experience in the cleanup of a number of federal and commercial buildings [such as the Hart Senate Office Building and the Curseen-Morris United States Postal Service (USPS) (Formerly Brentwood Processing & Distribution Center), Washington, DC; USPS Hamilton Processing & Distribution Center, Trenton, New Jersey; and American Media Inc (AMI) Building, Boca Raton, Florida], it appeared that fumigation or use of gases/vapors was the most desirable method for cleaning up three-dimensional building interiors. Currently, no DOD or Environmental Protection Agency (EPA, Washington, DC) standardized test method is available for quantitative testing of the efficacy of gases/vapors against biological contaminants on military-relevant surfaces.

APPENDIX A. BACKGROUND

d. Surface-applied technologies may be useful in field, personnel, or sensitive equipment decontamination. The Association of Official Analytical Chemists (AOAC)-Sporicidal Activity Test (SAT), currently employed by the EPA as a measure of sporicidal activity of liquid disinfectants, is constrained by two facts: 1) the test offers only a qualitative evaluation of decontaminant efficacy and 2) the test applies only to liquid disinfectants.

e. The unique requirements of DOD testing must still be addressed. Specifically, test requirements that must be considered include the ability to test both liquid and gaseous/vaporous decontaminants at an increased challenge level, with reduced contact times on a wide range of materials with samples larger than 1 cm^2, with minimal dilution and manipulation of the sample. Because of the wide range of materials on which DOD must evaluate the efficacy of biological decontaminants, it is of particular importance that the method developed for DOD Test and Evaluation (T&E) to accommodate materials larger than 1 cm^2. Additionally, current DOD methods do not offer potential advantages in terms of reduced sample manipulation and reduced time sensitivity.

f. The methodology studies[1,2,3] conducted at NSWCDD and ECBC included optimization of test protocols and testing of liquid and gaseous decontaminants. The methods were validated at the ECBC[1,2] and NSWCDD[3] laboratories. All results and test methods from each respective organization were presented in an informal brief to the Interagency Expert Panel (IEP). The final test method protocol was submitted to the Joint Science and Technology Office (JSTO)-Chemical and Biological Defense (CBD). Finally, the methodology reports[1,2,3] were used in the development of this TOP.

APPENDIX B. ADDITIONAL LABORATORY EQUIPMENT AND SUPPLIES.

NOTE: This list is not all-inclusive. Also, the supplies listed are not necessarily used in all TOP applications.

a. Ultraviolet/visible (UV/VIS) spectrometer.

b. Polymerase chain reaction equipment.

c. Ultracentrifuge.

d. Large-capacity incubator.

e. Incubator shaker.

f. Bioreactor.

g. Autoclave.

h. Imaging station.

i. Cell counter/enumerator.

j. Microscopes (e.g., inverted, phase contrast, etc.).

k. Automated plate-pourer.

l. Colony counter.

m. High-capacity vortexer.

n. Polyester gauze pads, 10.1 × 10.1 cm, (4 × 4 in), Fisher Scientific catalog number 19-808-937 (Fisher Scientific, Pittsburg, Pennsylvania). **NOTE**: Company and catalog number for the gauze pads used in developing this TOP is included to help personnel find equivalent supplies. Recovery efficiencies were established using the listed supplies; if a different supplier or type of pad/wipe is selected for use, recovery efficiency studies must be conducted.

o. Cotton swabs, 7.6 cm., (3 in), Colonial Scientific catalog number 162.98630.F16 (Colonial Scientific, Richmond, Virginia). **NOTE**: Company and catalog number for the cotton swabs used in developing this TOP is included to help personnel find equivalent supplies. Recovery efficiencies were established using these supplies; if a different supplier or type of swab is selected for use, recovery efficiency studies must be conducted.

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APPENDIX C. GLOSSARY.

Biological decontamination	The process of making a material safe by absorbing, destroying, neutralizing, rendering harmless, or removing biological agents.
Capability development document (CDD)	A document that reviews the information necessary to develop a proposed program(s), normally using an evolutionary acquisition strategy. The CDD outlines an affordable increment of militarily useful, logistically supportable, and technically mature capability.
Capability production document (CPD)	A document that addresses the production elements specific to a single increment of an acquisition program.
Sponsor	The organization responsible for drafting, staffing, and revising capabilities documents. For this document, sponsors include combat developers.
Spore density	Spore density (spore quantity per unit area) can result in layering and clustering over a surface area, which can impact spore recovery. In addition, spore recovery can be affected by three parameters: 1) the innate nature of the surface, e.g., porosity and surface charge; 2) the type and volume of extractant used; and 3) physical agitation, i.e., sonication and vortexing to dislodge the spores from the surface.

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APPENDIX D. ABBREVIATIONS.

AMI	American Media Inc
AOAC	Association of Official Analytical Chemists (renamed AOAC International)
ART	ambient room temperature
ASR	agent-to-simulant relationship
ASTM	American Society for Testing and Materials
ATCC®	American Type Culture Collection
B.	*Bacillus*
Ba	*B. anthracis*
BAP	blood agar plate
Bc	*B. cereus*
BCA	bicinchoninic acid
Bg	*Bacillus atrophaeus, formerly B. subtilis and B. globigii*
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BPW	buffered peptone water
BSC	biological safety cabinet
BSL	biosafety level
Btk	*B. thuringiensis, var kurstaki*
CARC	chemical agent-resistant coating
CASCAD™	Canadian Aqueous System for Chemical/Biological Agent Decontamination
CB	chemical and biological
CBD	chemical and biological defense
C/D	contamination/decontamination
CDC	Centers for Disease Control
CDD	capability development document
CFU	colony forming units
ClO_2	chlorine dioxide
CO_2	carbon dioxide
CPD	capability production document
CT	concentration × time

APPENDIX D. ABBREVIATIONS.

CV	coefficient of variation
DA	Department of the Army
DF-200	Easy DECON™ 200 decontamination solution
DMEM	Dulbecco's Modified Eagles Medium
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	design of experiment
DRDC	Defence Research and Development Canada
DT	developmental testing
ECBC	Edgewood Chemical Biological Center
EE	extraction efficiency
Eh	*Erwinia herbicola*
EPA	Environmental Protection Agency
FBS	fetal-bovine serum
Ft	*Francisella tularensis*
GE	genomic equivalents
HEPA	high-efficiency particulate air (filter)
HHA	hot, humid air
HIB	heart infusion broth
HTH	high-test hypochlorite
IAW	in accordance with
IEP	interagency expert panel
ISO	International Organization for Standardization
JSTO	Joint Science and Technology Office

APPENDIX D. ABBREVIATIONS.

MDL	minimum detection limit
MEM	minimum essential medium
NSWCDD	Naval Surface Warfare Center Dahlgren Division
NYCBOH	New York City Board of Health
OEP	operational evaluation plan
P.	*Pantoea*
Pa	*Pantoea agglomerans*
PAM	pamphlet
PBS	phosphate-buffered saline
PBST	PBS + 0.1 percent Triton X-100
PFU	plaque-forming units
pH	hydrogen ion concentration
PPMV	parts per million volume
QA	quality assurance
QC	quality control
qPCR	quantitative polymerase chain reaction
R&D	research and development
RH	relative humidity
RNA	ribonucleic acid
RSD	relative standard deviation
RT-qPCR	reverse transcriptase-qPCR
SAT	sporicidal activity test
SDF™	surface decontaminating foam
SIP	Special Immunization Program
SOP	standing operating procedure
STAR	system threat assessment report

APPENDIX D. ABBREVIATIONS.

T&E	test and evaluation
TEMP	test and evaluation master plan
TICN	test item control number
TNTC	too numerous to count
TOP	test operations procedure
TSA	tryptic soy agar
USAMRIID	U.S. Army Medical Reserach Institute for Infectious Diseases
USPS	United States Postal Service
UV/VIS	ultraviolet/visible
VHP®	vaporous hydrogen peroxide
WR	Vaccinia virus strain Western Reserve, Strain 119
Yp	*Yersinia pestis*

APPENDIX E. REFERENCES.

1. U.S. Army Edgewood Chemical Biological Center (ECBC), Maryland, Surface Sampling-Based Decontamination Studies and Protocol for Determining Sporicidal Efficacy of Two Gaseous Fumigants on Four Military-Relevant Surfaces, Final Report on T&E Protocol for Sampling-Based Decontamination Efficacy, 27 March 2008.

2. U.S. Army Edgewood Chemical Biological Center (ECBC), Maryland, Detailed Test Plan for Decontamination Family of Systems (DFoS) General Purpose Decontaminant (GPD) Competitive Prototyping (CP) I Biological Efficacy Testing –Hardened Military Equipment (HME), 24 July 2012.

3. Naval Surface Warfare Center Dahlgren Division (NSWCDD), Dahlgren, Virginia, Single Tube Method (STM) for Testing Liquid Decontamination Efficacy on Large Coupons, Chemical, Biological, and Radiological (CBR) Concepts & Experimentation Branch, 31 March 2008.

4. Joint Research and Development, Inc., Stafford, Virginia, Selection of Biological Agent Surrogate, Chemical Agents and Environmental Conditions for Testing and Evaluation (T&E) of the Joint Service Sensitive Equipment Decontamination (JSSED) System, Contract: W911QY-08-F-0046, 9 September 2010.

5. Centers for Disease Control and Prevention (CDC), Biosafety in Microbiological and Biomedical Laboratories, 4th edition, April 1999.

6. TOP 08-2-500, Receipt Inspection of Chemical-Biological (CB) Materiel, 7 July 1984.

7. International Organization for Standardization (ISO), Geneva, Switzerland, www.iso.org, Laboratory Equipment: Piston-Operated Volumetric Apparatus, ISO 8655, 10 October 2002 (Corrigenda, 9 December 2008).

8. American Society for Testing and Materials (ASTM) International, West Conshohocken, Pennsylvania, www.astm.org, Laboratory Testing Standards: Standard Specification for Piston or Plunger Operated Volumetric Apparatus, ASTM Document Number E1154-89, revised 2008.

9. Headquarters, Department of the Army (DA), Washington, DC, Pamphlet (PAM) 385-69, Safety Standards for Microbiological and Biomedical Laboratories, 6 May 2009.

10. DeVries, T.A. and Hamilton, M.A, “Estimating the Antimicrobial Log Reduction: Part 1. Quantitative Assays,” Quantitative Microbiology, Volume 1, Number 1, October 1999, pp. 29-45.

11. DeVries, T.A. and Hamilton, M.A, “Estimating the Antimicrobial Log Reduction: Part 2. Presence/Absence Assays,” Quantitative Microbiology, Volume 1, Number 1, October 1999, pp. 47-62.

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APPENDIX F. APPROVAL AUTHORITY.

CAPABILITY AREA PROCESS ACTION TEAM (CAPAT) ENDORSEMENT

DEPARTMENT OF THE ARMY
OFFICE OF THE DEPUTY UNDER SECRETARY OF THE ARMY
102 ARMY PENTAGON
WASHINGTON, DC 20310-0102

MEMORANDUM FOR

Chemical, Biological, Radiological and Nuclear Defense Test and Evaluation Executive, Office of the Deputy Under Secretary of the Army, Taylor Building, Suite 8070, 2530 Crystal Drive, Arlington, VA 22202

SUBJECT: Test and Evaluation Capabilities and Methodologies Integrated Product Team (TECMIPT) Recommendation for Test Operations Procedure (TTOP) 08-2-065 Developmental Testing of Liquid and Gaseous/Vaporous Decontamination on Bacterial Spores and Other Biological Warfare Agents on Military-Relevant Surfaces

1. The Decontamination Capability Area Process Action Team (CAPAT) has completed its review of the subject TTOP in accordance with the Chemical and Biological Program Test and Evaluation Process, 18 April 2014. All signatory members of the CAPAT have provided their concurrences to the TTOP (enclosed).

2. Based on the concurrence of the CAPAT, I recommend the CBRN Defense T&E Executive endorse this TTOP as a Department of Defense Test and Evaluation Standard.

OBRIEN.SEAN.P.1230553501

Encl

SEAN P. O'BRIEN
TECMIPT Chair

APPENDIX F. APPROVAL AUTHORITY.

CAPABILITY AREA PROCESS ACTION TEAM (CAPAT) ENDORSEMENT

TECMIPT Test Operations Procedure (TTOP) 08-2-065 Developmental Testing of Liquid and Gaseous/Vaporous Decontamination on Bacterial Spores and Other Biological Warfare Agents on Military-Relevant Surfaces

Decontamination Capability Area Process Action Team (CAPAT):

William G. Davis, Allyssa A. Martinez, Deborah L. Beier,
Deborah L. Menking, Mohamed S. Ibrahim

CAPAT Review & Concurrence: February 2015

Test and Evaluation Capabilities and Methodologies Integrated Process Team (TECMIPT) Participants:

DISTRIBUTION A. Approved for public release: distribution unlimited.

REFERENCES:
(a) *Chemical and Biological Defense Program (CBDP) Test and Evaluation (T&E) Standards Development Plan*, dated 19 July 2010.
(b) *Memorandum of Understanding (MOU) Among the Department of National Defence of Canada the Secretary of State for Defense of the United Kingdom of Great Britain and Northern Ireland and the Secretary of Defense on Behalf of the Department of Defense of the United State of America concerning the Research, Development and Acquisition of Chemical, Biological and Radiological Defense Materiel*, dated June 2000. Amendment One, dated August 2006.

APPENDIX F. APPROVAL AUTHORITY.

CAPABILITY AREA PROCESS ACTION TEAM (CAPAT) ENDORSEMENT

Decontamination Capability Area Process Action Team (Decon CAPAT)
Test Operations Procedure (TOP) 08-2-065 Developmental Testing of Liquid and Gaseous/Vaporous Decontamination on Bacterial Spores and Other Biological Warfare Agents on Military-Relevant Surfaces Concurrence Sheet

The Decon CAPAT recommends approval of TOP 08-2-065. If a representative non-concurs, a dissenting position paper will be attached.

Organization	Signature	Date
Deputy Under Secretary of the Army Test and Evaluation (DUSA-TE)	OBRIEN.SEAN.P.1230553501 Digitally signed by OBRIEN.SEAN.P.1230553501 DN: c=US, o=U.S. Government, ou=DoD, ou=PKI, ou=USA, cn=OBRIEN.SEAN.P.1230553501 Date: 2015.03.18 09:21:30 -04'00' Sean P. O'Brien	
Joint Program Executive Office of Chemical Biological Defense (JPEO-CBD) Test & Evaluation	Mark F. Thomas	8/3/15
Joint Requirements Office for Chemical, Biological, Radiological and Nuclear Defense (JRO-CBRND)	Lt Col Christopher J. Leonard, USAF	18 Aug 15
Joint Science and Technology Office (JSTO)	Digitally signed by ROBERTS.MICHAEL.A.1228803371 DN: c=US, o=U.S. Government, ou=DoD, ou=PKI, ou=DTRA, cn=ROBERTS.MICHAEL.A.1228803371 Date: 2015.03.27 17:01:40 -04'00' Michael A. Roberts	3-27-2015
US Army Evaluation Command (AEC)	FISHER.TIMOTHY.WILLIAM.1166077830 Digitally signed by FISHER.TIMOTHY.WILLIAM.1166077830 DN: c=US, o=U.S. Government, ou=DoD, ou=PKI, ou=USA, cn=FISHER.TIMOTHY.WILLIAM.1166077830 Date: 2015.03.13 10:52:20 -04'00' LTC Timothy W. Fisher	
Operational Test and Evaluation Force (OPTEVFOR)	**Jeffrey L. Bobrow**	**7-15-15**
Air Force Operational Test and Evaluation Center (AFOTEC)	Grant D. Schaber, Civ, DAF Acting Director of Operations	26 Feb 15
Marine Corps Operational Test & Evaluation Activity (MCOTEA)	**LtCol Kevin P. Reilly**	27 MAY 2015
Decon CAPAT Co-Chair	MANTOOTH.BRENT.A.1290501691 Digitally signed by MANTOOTH.BRENT.A.1290501691 DN: c=US, o=U.S. Government, ou=DoD, ou=PKI, ou=USA, cn=MANTOOTH.BRENT.A.1290501691 Date: 2015.05.21 10:54:05 -04'00' Brent A. Mantooth	
Decon CAPAT Co-Chair	HALL.MONICIA.R.1251909063 Digitally signed by HALL.MONICIA.R.1251909063 DN: c=US, o=U.S. Government, ou=DoD, ou=PKI, ou=USA, cn=HALL.MONICIA.R.1251909063 Date: 2015.05.31 11:16:35 -04'00' Monicia R. Hall	

APPENDIX F. APPROVAL AUTHORITY.

CBRN DEFENSE T&E EXECUTIVE ENDORSEMENT

DEPARTMENT OF THE ARMY
OFFICE OF THE DEPUTY UNDER SECRETARY OF THE ARMY
102 ARMY PENTAGON
WASHINGTON, DC 20310-0102

DUSA-TE 6 Nov 2015

MEMORANDUM FOR DISTRIBUTION

SUBJECT: Endorsement of Test and Evaluation (T&E) Capabilities and Methodologies Integrated Process Team (TECMIPT) Test Operations Procedure (TTOP) 08-2-065 Developmental Testing of Liquid and Gaseous/Vaporous Decontamination on Bacterial Spores and Other Biological Warfare Agents on Military-Relevant Surfaces

1. Reference: Memorandum, DUSA-TE, and 19 July 10, subject: Chemical and Biological Defense Program (CBDP) Test and Evaluation (T&E) Standards Development Plan.

2. TTOP 08-2-065 was developed, coordinated, and approved by the members of the Decontamination Capability Area Process Action Team (CAPAT) in accordance with the reference. The U.S. Army Test and Evaluation Command (ATEC) approved according to their TOP approval process.

3. I endorse this TTOP as a DoD T&E Standard for decontamination testing and encourage its broad use across all test phases. All T&E Standards are for government associated program access and use. They are stored in Army Knowledge Online (AKO), located at (https://www.us.army.mil/suite/files/22142943), on the National Institute of Standards and Technology (NIST) website (http://gsi.nist.gov/global/index.cfm/L1-4/L2-19/A-664), and the TECMIPT (http://www.amsaa.army.mil/TECMIPT/Standards.html).

4. My point of contact for this action is Ms. Deborah Shuping, (703) 545-1119, deborah.f.shuping.civ@mail.mil.

Encl

PATRICK L. WALDEN
Colonel, IN
Acting CBRN Defense T&E Executive

APPENDIX F. APPROVAL AUTHORITY.

CSTE-TM 11 February 2016

MEMORANDUM FOR

Commanders, All Test Centers
Technical Directors, All Test Centers
Directors, U.S. Army Evaluation Center
Commander, U.S. Army Operational Test Command

SUBJECT: Test Operations Procedure (TOP) 08-2-065 Developmental Testing of Liquid and Gaseous/Vaporous Decontamination on Bacterial Spores and Other Biological Warfare Agents on Military-Relevant Surfaces, Approved for Publication

1. TOP 08-2-065 Developmental Testing of Liquid and Gaseous/Vaporous Decontamination on Bacterial Spores and Other Biological Warfare Agents on Military-Relevant Surfaces, has been reviewed by the U.S. Army Test and Evaluation Command (ATEC) Test Centers, the U.S. Army Operational Test Command, and the U.S. Army Evaluation Center. All comments received during the formal coordination period have been adjudicated by the preparing agency. The scope of the document is as follows:

This TOP provides a quantitative sampling-based biological decontamination protocol to analyze the efficacy of liquid and gaseous/vaporous decontaminants on military-relevant surfaces. The procedures in this TOP were developed for use in research and development and early developmental testing in the test and evaluation phase of an acquisition program, and are not intended for operational testing.

2. This document is approved for publication and will be posted to the Reference Library of the ATEC Vision Digital Library System (VDLS). The VDLS website can be accessed at https://vdls.atc.army.mil/.

3. Comments, suggestions, or questions on this document should be addressed to U.S. Army Test and Evaluation Command (CSTE-TM), 2202 Aberdeen Boulevard-Third Floor, Aberdeen Proving Ground, MD 21005-5001; or e-mailed to usarmy.apg.atec.mbx.atec-standards@mail.mil.

CHEW.JENNIFER.PM.1041816836

JENNIFER P. CHEW
Associate Director, Test Management Directorate (G9)

FOR

RAYMOND G. FONTAINE
Director, Test Management Directorate (G9)

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Forward comments, recommended changes, or any pertinent data which may be of use in improving this publication to the following address: Range Infrastructure Division (CSTE-TM), U.S. Army Test and Evaluation Command, 2202 Aberdeen Boulevard, Aberdeen Proving Ground, Maryland 21005-5001. Technical information may be obtained from the preparing activity: Commander, West Desert Test Center, U.S. Army Dugway Proving Ground, ATTN: TEDT-DPW, Dugway, UT 84022-5000. Additional copies can be requested through the following website: http://www.atec.army.mil/publications/topsindex.aspx, or through the Defense Technical Information Center, 8725 John J. Kingman Rd., STE 0944, Fort Belvoir, VA 22060-6218. This document is identified by the accession number (AD No.) printed on the first page.