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technical BRIEF

Bio-Response Operational Testing and Evaluation (BOTE) Project

Phase 1 – Decontamination Assessment

INTRODUCTION

The Bio-Response Operational Testing and Evaluation (BOTE) Project was a multi-agency effort designed to test and evaluate a complete response to a biological incident - from the initial public health and law enforcement reaction through environmental remediation. The scenario involved the intentional release of *Bacillus anthracis* (*Ba)* spores, the causative agent for anthrax, inside a building. In this study, *Bacillus atrophaeus spp. globigii* (*Bg*) spores were used as a non-pathogenic surrogate for *Ba* spores.

The BOTE Project was conducted in two distinct phases. Phase 1 was a field-level decontamination assessment. Phase 2 was an operational exercise involving key federal agencies that are responsible for the forensic investigation, public health assessment, and remediation following a biological incident. This summary is focused on Phase 1 of the project. Phase 1 was designed to assess three approaches to site remediation after the release of *Bg* spores within a building (figure 1). The assessment incorporated recent advances in biological sampling and decontamination that had previously been tested in small-scale applications.

BOTE Project Phase 1 Objectives

- Conduct and evaluate field-level application of three decontamination technologies/protocols for the cleanup of a building contaminated with *Bacillus anthracis* (*Ba*) spores, the causative agent for anthrax. Simulants of *Ba* spores were used.
- Utilize newly developed biological sampling and analysis methods for characterization of the anthrax simulant contamination (concentration and location) and determination of decontamination efficacy.
- Collect and analyze results and operational information from the decontamination operation.
- Perform a cost analysis of the complete remediation process.
- Determine the exposure to spores associated with reentry into the building following cleanup.

Figure 1. Two story building, without and with secondary containment (tenting), at Idaho National Laboratory.

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METHODS

The testing was conducted in a two story office building (4,025 ft^2/floor) that was tented to provide secondary containment of the spores in the building. The building was set up such that each floor included three rooms furnished with residential materials (e.g., sofa, bed), three rooms furnished with commercial materials (e.g., desk, file cabinet), one mailroom and one industrial-style workshop (Figure 2). Each floor had an independent heating, ventilation, and air conditioning (HVAC) system.

Phase 1 testing was conducted in three rounds, each utilizing a different decontamination method with all other test conditions being consistent across the rounds. Each round (Figure 3) consisted of preparing the facility, dissemination of *Bg* spores to achieve target surface loadings, characterization sampling, decontamination and waste management, post-decontamination sampling, and facility assessment. After each round, the facility was re-set to its initial configuration for the start of the next round.

Concentration of approximately 10^4-10^6 and 10^2 viable spores/ft^2 were released on the first and second floors, respectively, for each round in order to test the efficacy of each decontamination approach under two contamination challenge amounts.

Figure 3. Example rooms (top left to bottom right): mailroom, workshop, residential and commercial setting.

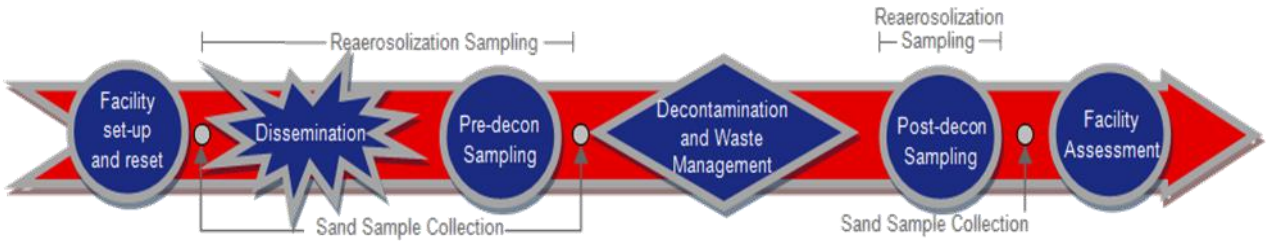


Figure 3. Timeline of major activities in each round.

Round 1: Fumigation with STERIS Corp. Vaporized Hydrogen Peroxide (VHP®) Technology

Figure 4 VHP generation system.

- Full-facility *in situ* decontamination, including contents (i.e., no materials were removed prior to fumigation)
- Fumigation of both floors and HVAC system utilizing two VHP® generation systems (Figure 4), one connected to each floor's air handling unit
- Target fumigation conditions: 250 ppmv of hydrogen peroxide vapor (H_2O_2) for 90 min and a cumulative concentration-time product (CT) of 400 ppmv-hrs at ≥ 65 ^{o}F (~18 ^{o}C)
- Portable fans were operating inside the facility to aid H_2O_2 distribution
- Temperature, relative humidity (RH) and H_2O_2 concentration were measured continuously at numerous locations
- Spor-Klenz® Ready to Use sterilant was sprayed on surfaces on which equipment was to be placed during fumigation
- The target H_2O_2 concentration was not achieved at all monitored locations
- Decontamination process was completed in 3 days from set-up through aeration

Round 2: Decontamination process incorporating pH-adjusted bleach spraying

Figure 5. Bagging porous materials (R) and spraying with pH-adjusted bleach (L).

- Preparation: Source reduction was conducted by teams in Level C PPE; all porous and difficult to decontaminate materials were removed from the facility (e.g., ceiling tiles, furniture, carpet, HVAC supply line). Materials were bagged, sprayed with pH-adjusted bleach (Figure 5), and removed for sampling and management as waste
- Facility was maintained under negative pressure and ambient temperature throughout decontamination
- Decontamination: Teams in Level B PPE (due to the decontaminant) sprayed all interior surfaces including HVAC return duct with pH-adjusted bleach using a gas-powered sprayer situated outside the facility; surfaces were maintained wetted for ≥10 min.
- Drying : Portable fans and heaters were run to the facility during drying phase
- The process was planned and implemented by EPA Region 10, based upon field experience from EPA Region 1 and recent EPA lab studies
- Decontamination was completed in 5 days from preparation through drying

Round 3: Fumigation with chlorine dioxide gas (ClO_2) (Sabre Technical Services, LLC.)

Figure 6. Truck-mounted gas-generation system.

- Full-facility *in situ* decontamination; only materials removed were mattresses and cushions due to the time required to aerate these materials following decontamination (i.e., preventing sampling due to the toxicity of ClO_2)
- A truck-mounted ClO_2 gas-generation system was used (Figure 6)
- Target fumigation conditions: 3000 ppmv of ClO_2 for 3 hrs and a cumulative CT of 9000 ppmv-hrs at ≥ 65 ^{o}F (~18 ^{o}C) and RH ≥ 65%
- Fans were added inside the facility to aid ClO_2 distribution, activated carbon was used to scrub ClO_2 during maintenance of negative pressure and during aeration
- Temperature, RH and ClO_2 concentrations were continuously measured at numerous locations
- Target ClO_2 concentration was achieved at all monitored locations; 2nd floor mean RH was below the target 65% (63.7±5.9%)
- Decontamination process was completed in 3 days, from set-up (~2 days) through aeration, with the exception of time required for staging the material used for tenting the facility (on the facility inside the secondary enclosure)

SAMPLE COLLECTION

Surface Sampling

The effectiveness of the three decontamination technologies was determined by measuring the surface concentrations of viable *Bg* spores in colony forming units ((CFU) per ft^2), before and after decontamination. Wipe sampling (Figure 7) using cellulose sponge-stick wipes and swabs, and vacuum sampling (using vacuum socks) were the primary collection methods. These sampling methods were consistent with current validated or Centers for Disease Control (CDC) recommended sampling for *Ba* spores. Additional surface samples using Versalon® wipes (gauze wipes) were also collected for use in an operational assessment of EPA's rapid-viability polymerase chain reaction (RV-PCR) analytical method. All sampling metadata (e.g. time, location, sample type) was collected using hand-held personal data acquisition (PDAs) devices and the Sandia National Laboratories' Building Restoration Operations Optimization Model (BROOM) software system.

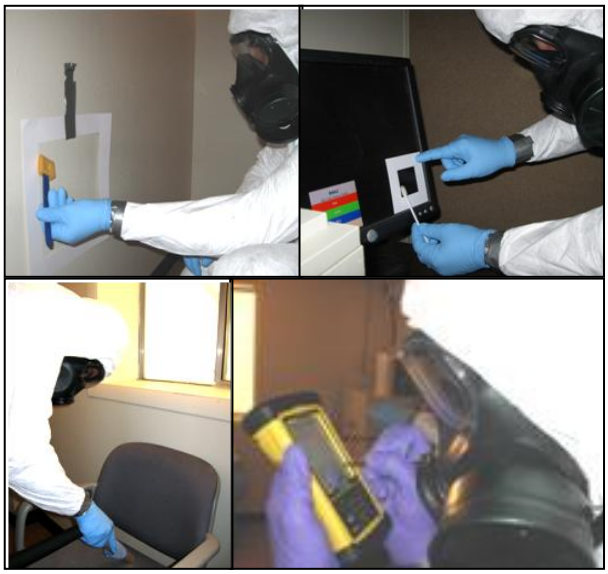


Figure 7. Above: Wipe sampling: sponge-stick and swab. Below: Vacuum sampling (left) and PDA with BROOM software (right).

Air Sampling

Aggressive air sampling offers the potential to reduce the post-decontamination sampling burden by collecting bulk air samples that could be used to determine if contamination remains. Following post-decontamination surface sampling, aggressive air sampling was conducted in two rooms as a secondary evaluation of decontamination effectiveness and to compare these results to surface sampling results. Air samples were collected during and after the agitation of potential surface contamination using a leaf blower (Figure 8); samples were collected using high volume samplers; and collection media were analyzed via culture methods. Aggressive air sampling was conducted successfully after all three decontamination rounds, and results were comparable to surface sample results. The air sampling results after Round 1 (fumigation with VHP®) showed the highest concentrations of spores detected in the air; the lowest spore concentrations were detected for Round 3 (fumigation with ClO_2).

Figure 8. Use of a leaf lower to agitate surface contamination.

Wastewater Treatment and Sampling

Figure 9. Personnel decontamination line.

Wash water was collected from the personnel decontamination line (Figure 9) in 55-gal drums and used to assess the effectiveness of an on-site bleach treatment procedure. An ultrafiltration concentrator was used to sample *Bg* spores in the wash water. The ultrafiltration device was intended to concentrate spores contained in a high volume of wastewater into a much smaller volume of water; thereby, increasing detection sensitivity. Unfortunately, the high turbidity of the wash water presented operational challenges for the ultrafiltration method and only a small number of viable spores were able to be detected in the wash water. The bleach treatment procedure was, therefore, alternatively assessed by spiking wash water with additional *Bg* spores prior to the addition of bleach to raise the concentration of spores to levels detectable without concentration. The bleach process was determined to provide greater than a 3-log reduction of viable spores (the upper limit that could be determined in this study). Results from the spiked wash water test were similar to those obtained from laboratory experiments using artificially generated wash water possessing similar water quality characteristics as field generated wash water. These findings suggest that the proposed inactivation procedure would be applicable for wash water derived from similar personnel decontamination activities.

Spore Transport and Reaerosolization

To examine the potential transportation of *Bg* spores from the initial area of dissemination inside the building to outside the building, Petri dishes containing sterilized sand (Figure 10) were placed directly outside the test facility, but within the secondary containment enclosure and around building entrances, exits and high traffic areas. The detection of *Bg* in some of these previously uncontaminated sand samples suggested that spores have the potential to migrate out of a contaminated building and settle into the surrounding environment. The study did not attempt to differentiate when exfiltration occurred from the facility (i.e., during dissemination or during subsequent remediation activities).

Figure 10. Tray of Petri dishes containing sterilized sand.

Reaerosolization was studied by measuring the concentration of *Bg* spores in the air within two rooms at five phases (background, after spore dissemination, prior to surface sampling, pre-decontamination and post-decontamination) throughout each round of decontamination. Air samples were collected using SKC BioSamplers®. Post-decontamination *Bg* spores were detected in the air following Round 1 (Fumigation with Vaporized Hydrogen Peroxide (VHP®)), but not Rounds 2 (process incorporating removal and spraying of pH-adjusted bleach) or 3 (chlorine dioxide fumigation). All samples collected after spore dissemination, before surface sampling and pre-decontamination contained measureable concentrations of spores indicating that airborne concentrations of the spores persist after dissemination and that spores may, potentially, be reaerosolized by typical remediation activities under certain conditions.

Exposure Assessment

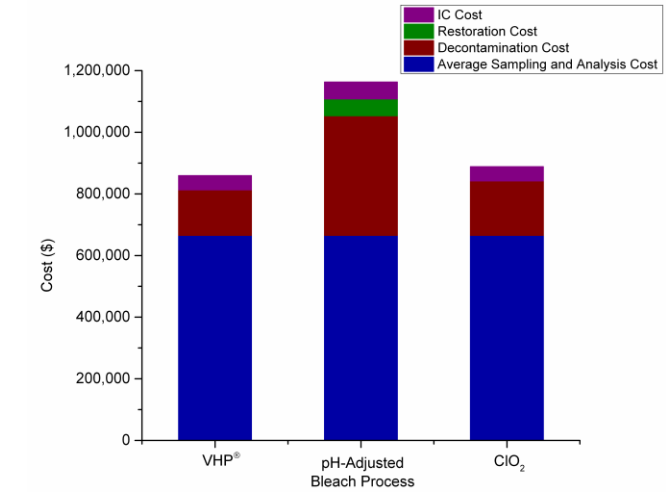
The surface and air samples collected, as well as the monitoring and assessment of decontamination operational parameters, provided measurements for the assessment of pre- and

post-decontamination exposure potential. However, limitations in the data and site-specific variables currently do not allow for accurate exposure predictions that can be extrapolated to other sites. Results and lessons learned from the BOTE Project will be used to develop a methodology for site-specific exposure assessment.

COST ANALYSIS

The BOTE Project cost analysis estimated the overall cost of the application of various decontamination technologies as a function of materials, time (including labor hours), waste disposal, and other resources.

- Sampling and analysis costs were roughly equivalent in all three rounds of decontamination due to the study design. As can be seen in Figure 11 (top), sampling and analysis costs were the largest contributors to the overall cost. However, since this was an operational assessment, considerably more samples were taken than anticipated for an actual incident in a building of this size. Regardless, sampling and analysis costs are anticipated to be a major cost factor, which should be considered in any cleanup of a biological incident.
- The Incident Command (IC) costs were also relatively independent of the decontamination method used in this project.
- The cumulative costs of the decontamination processes (e.g. materials, contracts, labor) were roughly equivalent for all three decontamination methods tested (Figure 11 bottom).
- Waste management costs were shown to be a significant cost component particularly for the pH-adjusted bleach decontamination process as used in this exercise. Waste characterization sampling was the largest single component of waste management costs. These costs are specific to the decontamination process**s**es as they were employed in the BOTE Project and based-upon documented assumptions made about waste management procedures and costs.



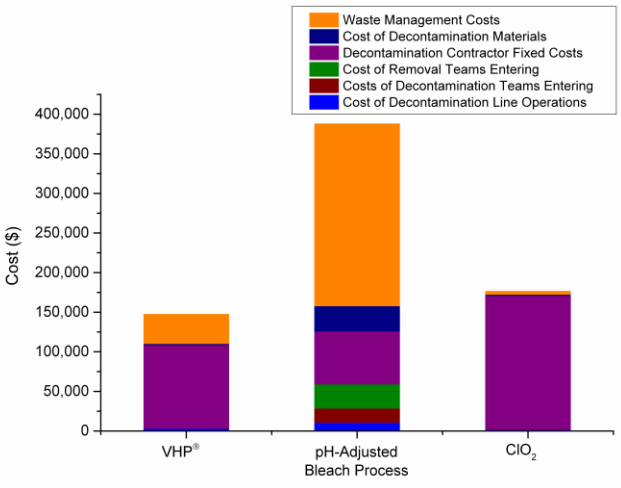


Figure 11. Overall all cost components (top) and waste management cost details (bottom)

CONCLUSIONS

Each decontamination method was performed a single time in the BOTE Project; the results and conclusions should be considered based upon the implementation as described above. Decontamination costs alone, not considering sampling and analysis or waste management, were roughly equivalent. Notable differences in waste generation and anticipated associated cost were documented. The decontamination efficacy findings for Rounds 1, 2, and 3 are summarized in Figure 12, below.

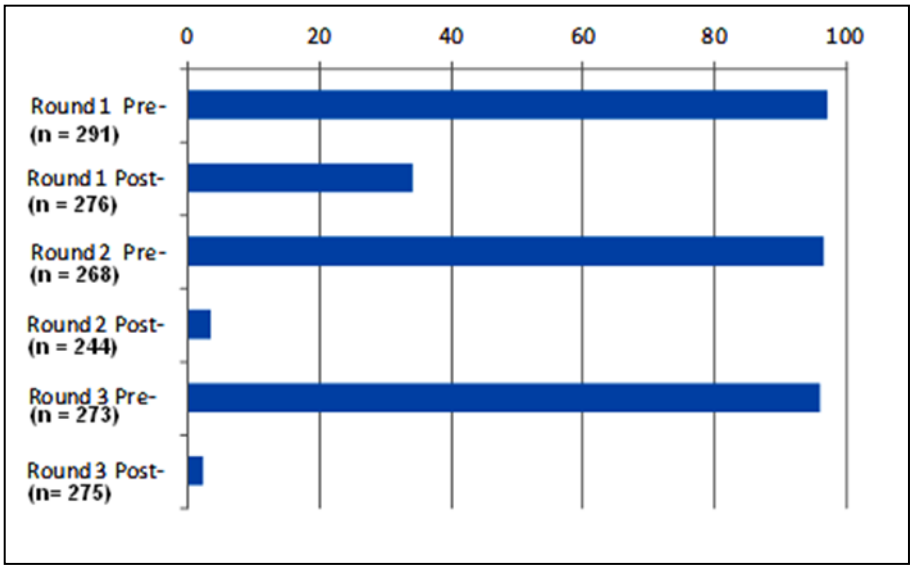


Figure 12. Percentage of surface samples (floors 1 and 2) with detected *Bg* for hydrogen peroxide vapor (Round 1), pH-adjusted bleach (Round 2), and chlorine dioxide (Round 3). (n = number of samples.)

Round 1: Full-facility fumigation using VHP® Technology

- Conditions not sufficient for effective decontamination
- No observed damage to facility or contents
- Low relative waste generation

Round 2: Decontamination process using removal and disposal of contaminated porous materials and pH-adjusted bleach spraying of non porous materials

- Effective process, few post-decontamination samples positive with very low CFU
- Damage to some surfaces, such as swelling of laminated floor
- High relative waste generation

Round 3: Full-facility fumigation with ClO_2

- Effective process, few post-decontamination samples positive with very low CFU
- No damage to surfaces; observed corrosion of equipment connections
- Low relative waste generation

FOR MORE INFORMATION

The complete report for Phase 1 of the BOTE Project can be found at: www.epa.gov/nhsrc
A video documentary is available at:
https://www.youtube.com/watch?v=BKIbONJfVn4&feature=youtu.be

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