

Appendix 1
Abbreviated Test Report for the Laboratory Validation of
Chlorine Dioxide Decontamination



Test Project No. 8-CO-210-000-084
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ABBREVIATED TEST REPORT
FOR THE
LABORATORY VALIDATION
OF
CHLORINE DIOXIDE DECONTAMINATION

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UNDER CONTRACT NO. DAAD-07-98-D-0105

WEST DESERT TEST CENTER
U.S. ARMY DUGWAY PROVING GROUND
DUGWAY, UT 84022-5000

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| 13. ABSTRACT (Maximum 200 words) The U.S. Environmental Protection Agency proposed to decontaminate the Hart Senate Office Building in Washington, DC, by using chlorine dioxide (ClO ₂) gas in a concentration of 500 parts per million (ppm) for 8 to 12 hours. The objective of this test was to determine how effective this concentration and time would be at killing <i>Bacillus anthracis</i> (BA) spores. A further objective was to determine the kill rate for different concentrations of ClO ₂ gas and different humidities. Spores from three strains of BA and three simulants were exposed to ClO ₂ gas for 1, 2, 4, 6, 8, and 12 hours. At the ClO ₂ concentrations tested (125 to 1050 ppm), high humidity (greater than 70 percent) was a more important factor than ClO ₂ concentration in the killing of BA spores. On a porous surface (filter paper), ClO ₂ was more effective at killing spores than on a nonporous surface (glass). | | | | |
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SECTION 1. EXECUTIVE DIGEST

1.1 SUMMARY

1.1.1 Background

a. The release of *Bacillus anthracis* (BA) spores from an envelope inside the Hart Senate Office Building (HSOB) in Washington, DC, raised legitimate concerns about the distribution of the spores within the building. Subsequent environmental testing found BA spores at various locations on different floors, in elevators, and other locations. The presence of BA spores at the levels detected in the HSOB was judged to be an unacceptable risk. Access to the building was restricted until the building and its infrastructure could be decontaminated and remediated. The U.S. Environmental Protection Agency (EPA) was given the responsibility to conduct the decontamination.

b. After reviewing several methods for decontaminating BA from the HSOB, the EPA decided that gaseous chlorine dioxide (ClO₂) would be the most effective and cause the least amount of disruption. Other methods considered were liquid-based or would leave messy residues, some of which were carcinogenic.

c. ClO₂ is not a chlorinator, as the name might suggest. It is a potent oxidizer, more closely related to hydrogen peroxide (H₂O₂) than any other compound. It is water-soluble and has accepted uses for water treatment and as a treatment on food surfaces, such as vegetables and meats (Reference 1). ClO₂ exists only as a gas and presents an explosive hazard at concentrations in the range of 10 percent by volume [100,000 parts per million (ppm)] and higher. ClO₂ is toxic to humans at 5 ppm.

d. Prior research [in-house tests conducted at the Life Sciences Test Facility (LSTF), West Desert Test Center (WDTC), U.S. Army Dugway Proving Ground (DPG), Utah, in August 2001; no documentation] indicated that ClO₂ solutions are effective in killing bacterial spores. The exposure of spores to the gaseous ClO₂ has received little documentation.

e. The decontamination procedure proposed by the EPA was a gaseous exposure of ClO₂ for 12 hours with a concentration of 500 ppm.

f. During the HSOB decontamination, spore strips (manufactured by Raven Biological Laboratories, Inc., Omaha, Nebraska) were used to verify that the decontamination was successful. Spore strips were also included in this test to determine the effect of ClO₂ on these strips in a controlled laboratory setting.

1.1.2 Test Findings

BA on the glass cover slips was much more resistant to the ClO₂ than BA on a more absorbent surface (filter paper). For both strains tested on filter paper [*Bacillus anthracis* var. *ames* (BAA) and *Bacillus anthracis* var. *vollum* (BAV)], significantly more spores were killed on filter paper than cover slips.

1.1.2.1 Cover Slips

a. In the samples with spores dried onto glass cover slips, relative humidity (RH) and time in the test chamber were significant factors for BAA, BAV, *Bacillus anthracis* var. *sterne* (BAS), *Bacillus thuringiensis* [American type culture collection (ATCC) number 29730 (BT)] and *Bacillus stearothermophilus* [ATCC number 74533 (BST)].

b. There was no indication that increasing the ClO₂ concentration in the range tested (125 to 1050 ppm) increased the number of spores killed.

c. Because of variability in the data, the *Bacillus subtilis* var. *niger* (BGN) data could not be analyzed and no conclusions as to significance could be drawn.

d. When the anthrax and simulant strains were compared with each other, BGN was the most susceptible to the ClO₂ treatment, followed by BAA and BAV, which were not significantly different from each other. BAS was less susceptible to the treatment; BT and BST were the least susceptible, but were not significantly different from each other. A relative ranking of the spores tested is in Table 1.

e. For a detailed discussion of the test findings, see Paragraph 2.2.4.1.

1.1.2.2 Filter Paper

a. For BAA and BAV, the amount of time that the samples were in the test chamber was a factor.

b. Because most measurements of colony forming units (cfus) went quickly (*i.e.*, 1 hour) to zero, it was not possible to distinguish if increasing RH and ClO₂ concentrations would increase the number or rate of spores being killed.

c. For a detailed discussion of the test findings, see Paragraph 2.2.4.2.

1.1.2.3 Spore Strips

a. When the spore strips were compared with the cover slips for the same strain of spores, there were very few false negatives (about 1 in 30 measurements). A false negative is when the cover slip data indicate that there are viable spores present, and the spore strip data indicate that all spores have been destroyed.

b. When the spore strips (BGN, BT, and BST) were compared with the anthrax cover slip data, BST had no false negatives, BT had 1 false negative, and BGN had 10 false negatives.

c. For a detailed discussion of the test findings, see Paragraph 2.2.4.4.

1.2 CONCLUSIONS

a. Higher RH was more important in the killing of BA spores than the concentration of the ClO₂ gas in the concentrations tested (125 to 1050 ppm).

Table 1. Relative Ranking of Susceptibility of Spores to the Chlorine Dioxide (ClO₂) Treatment; Laboratory Validation of ClO₂ Decontamination.

| Spore Type | Susceptibility Ranking ^a |
|--|-------------------------------------|
| <i>Bacillus subtilis</i> var. <i>niger</i> (BGN) | 1 |
| <i>Bacillus anthracis</i> var. <i>ames</i> (BAA) | 2 |
| <i>Bacillus anthracis</i> var. <i>vollum</i> (BAV) | 2 |
| <i>Bacillus anthracis</i> var. <i>sterne</i> (BAS) | 4 |
| <i>Bacillus thuringiensis</i> (BT) | 5 |
| <i>Bacillus stearothermophilus</i> (BST) | 5 |

^a1 = most susceptible to ClO₂ treatment and 5 = least susceptible to ClO₂ treatment.

b. The ClO₂ gas was much more effective at killing spores on absorbent surfaces, such as filter paper, than nonabsorbent surfaces, such as glass. However, the spores used in this study were in a slurry that was dried after application to the surface. The results could differ if a powdered form of the spores was used.

c. The proposed decontamination of the HSOB with a concentration of 500 ppm ClO₂ should be adequate, provided the RH is greater than 70 percent.

d. The simulants BT and BST are more resistant to the ClO₂ treatment than the anthrax strains.

e. When used to verify the anthrax data, the BT and BST spore strips had fewer false negatives than the BGN spore strips.

1.3 RECOMMENDATIONS

a. If ClO₂ gas is used to kill BA spores, it is recommended that the RH be as high as possible and at least 70 percent or greater.

b. Because the effectiveness of ClO₂ gas at concentrations lower than 125 ppm was not tested, decontamination at lower concentrations without further testing is not recommended.

c. If spore strips are used to verify that anthrax spores have been killed, it is recommended that spore strips containing BT or BST be used.

d. Further testing may be warranted to determine the effect of ClO₂ treatment on spores deposited as a powder, as opposed to wet slurry.

1.4 TEST OBJECTIVES

a. Determine how well the proposed decontamination level for the HSOB of 500 ppm ClO₂ and time of 12 hours will kill BA spores.

b. Determine the optimum combination of concentration of ClO₂ gas and RH for killing BA spores, as well as three simulants.

c. Determine how well various concentrations of ClO₂ gas (125 to 1100 ppm) at various humidities (30 to 92 percent) kill spores from three strains of BA (BAA, BAV, and BAS) and three simulants (BGN, BT, and BST).

d. Determine the effect of the ClO₂ treatment on spore strips, which will be used to determine the level of decontamination in the HSOB.

1.5 TESTING AUTHORITY

On 20 November 2001, U.S. Army Developmental Test Command (DTC), Aberdeen Proving Ground, Maryland, issued a test execution directive (Reference 2) tasking WDTC/DPG to support the EPA by conducting a laboratory validation of ClO₂ decontamination (Test Project No. 8-CO-210-000-084).

1.6 TEST CONCEPT

1.6.1 Overview

a. This test was designed to test the effectiveness of ClO₂ gas in killing BA spores.

b. Spores from three strains of BA (BAA, BAV and BAS) and three simulants (BGN, BT, and BST) were exposed to various concentrations of ClO₂ gas (125 to 1050 ppm) and humidities (30 to 92 percent) in a stainless steel test chamber.

c. Samples were removed from the chamber at 1, 2, 4, 6, 8, and 12 hours and were compared with the initial contamination density for each strain of spores to determine how effective the combination of ClO₂ and RH was at killing the spores.

d. Spore strips were removed from the chamber after 6 and 12 hours.

1.7 SYSTEM DESCRIPTION

a. The ClO₂ generator (Figure 1) was manufactured by PureLine[®] Treatment Systems, Irvine, California.

b. The ClO₂ generator produces chlorine free ClO₂ gas.

c. ClO₂ gas was generated electrochemically by the ClO₂ generator from sodium chlorite as described in Paragraph 2.2.3.1.



Figure 1. PureLine[®] Chlorine Dioxide (ClO₂) Generator; Laboratory Validation of ClO₂ Decontamination.

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SECTION 2. DETERMINATION OF FINDINGS

2.1 RECEIPT INSPECTION

No receipt inspection was necessary.

2.2 CLO₂ DECONTAMINATION LABORATORY VALIDATION

2.2.1 Objectives

a. Determine how well the proposed decontamination level for the HSOB of 500 ppm ClO₂ and time of 12 hours will kill BA spores.

b. Determine the optimum combination of concentration of ClO₂ gas and RH for killing selected BA spores and simulants.

c. Determine how well various concentrations of ClO₂ gas (125 to 1100 ppm) at various humidities (30 to 92 percent) kill spores from three strains of BA (BAA, BAV and BAS) and three simulants (BG, BT, and BST).

d. Determine the effect of the ClO₂ treatment on spore strips, which will be used to determine the level of decontamination in the HSOB.

2.2.2 Criteria

None.

2.2.3 Test Procedure

2.2.3.1 ClO₂ Generation

a. ClO₂ gas was generated electrochemically from sodium chlorite as shown in Figures 2 and 3.

b. The electrochemical generation was operated with excess sodium chlorite (20 to 30 grams per liter excess). Any possible chlorine produced during the generation of ClO₂ would react with the sodium chlorite.

2.2.3.2 ClO₂ Trials

a. Detailed test procedures are in Appendix C.

b. Spore samples were obtained from existing WDTC/DPG stock, or prepared fresh by harvesting from Tryptic[®] soy or blood agar plates.

c. The harvested spore crops were pasteurized (exposed to 70°C for 20 minutes) to kill any vegetative cells. Electron micrographs were taken of samples after pasteurization to verify that only spores remained (Figure 4).

Electrochemical Production of Chlorine Dioxide:

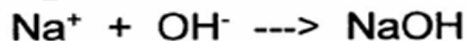
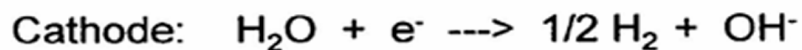
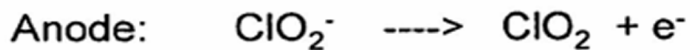


Figure 2. Electrochemical Production of Chlorine Dioxide (ClO_2); Laboratory Validation of ClO_2 Decontamination.

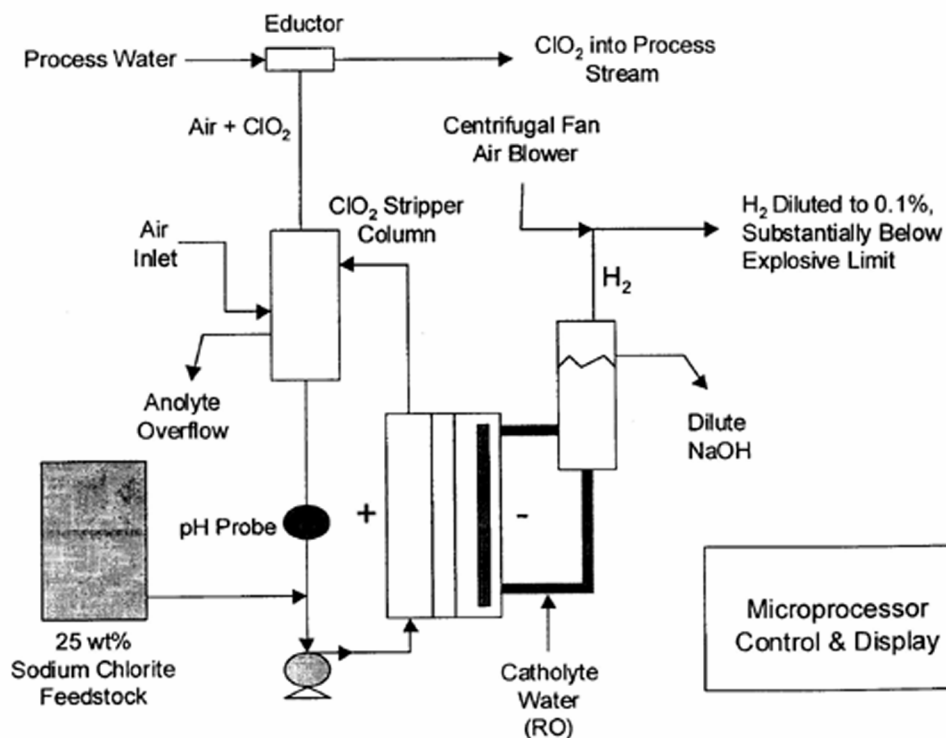


Figure 3. Flow Chart of the Production of Chlorine Dioxide (ClO_2) from Sodium Chlorite; Laboratory Validation of ClO_2 Decontamination.

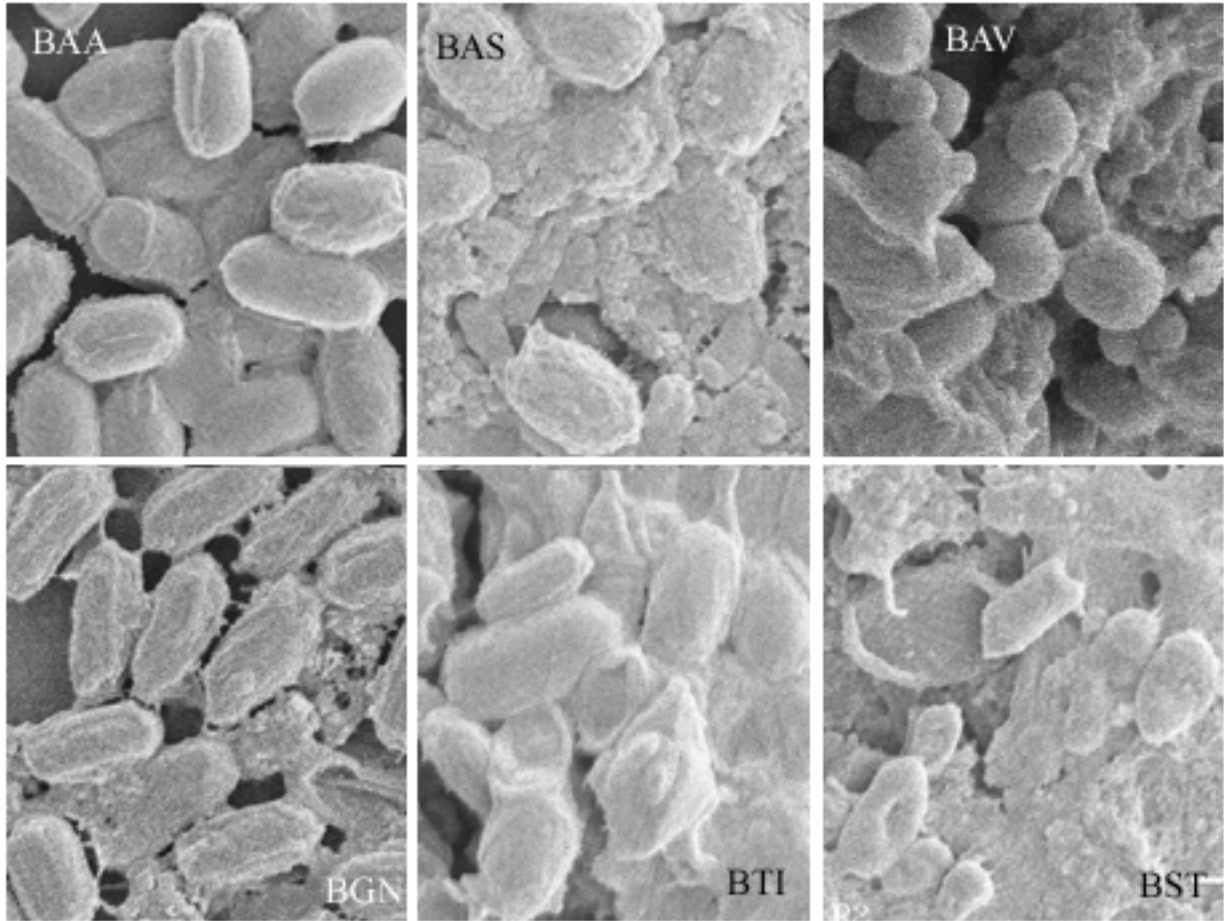


Figure 4. Electron Micrographs Showing *Bacillus* Spores; Laboratory Validation of Chlorine Dioxide Decontamination.

NOTE: *Bacillus anthracis* var. *ames* (BAA), *Bacillus anthracis* var. *sterne* (BAS), *Bacillus anthracis* var. *vollum* (BAV), *Bacillus subtilis* var. *niger* (BGN), *Bacillus thuringiensis* (BT), and *Bacillus stearothermophilus* (BST).

d. The samples, in triplicate, were exposed to the ClO₂ gas in a stainless steel test chamber (Figure 5) for 1 to 12 hours. The 0 hour sample, which never entered the chamber, was used to determine contamination density.

e. The ClO₂ gas concentration and the RH were varied from trial to trial (Table 2). There was some difficulty in measuring the ClO₂ concentrations in Trials 3 through 10 (Appendix D).

f. The concentration of ClO₂ was determined via collection of gas samples followed by titrations and/or measured using ultraviolet (UV) sensors mounted within the chamber.

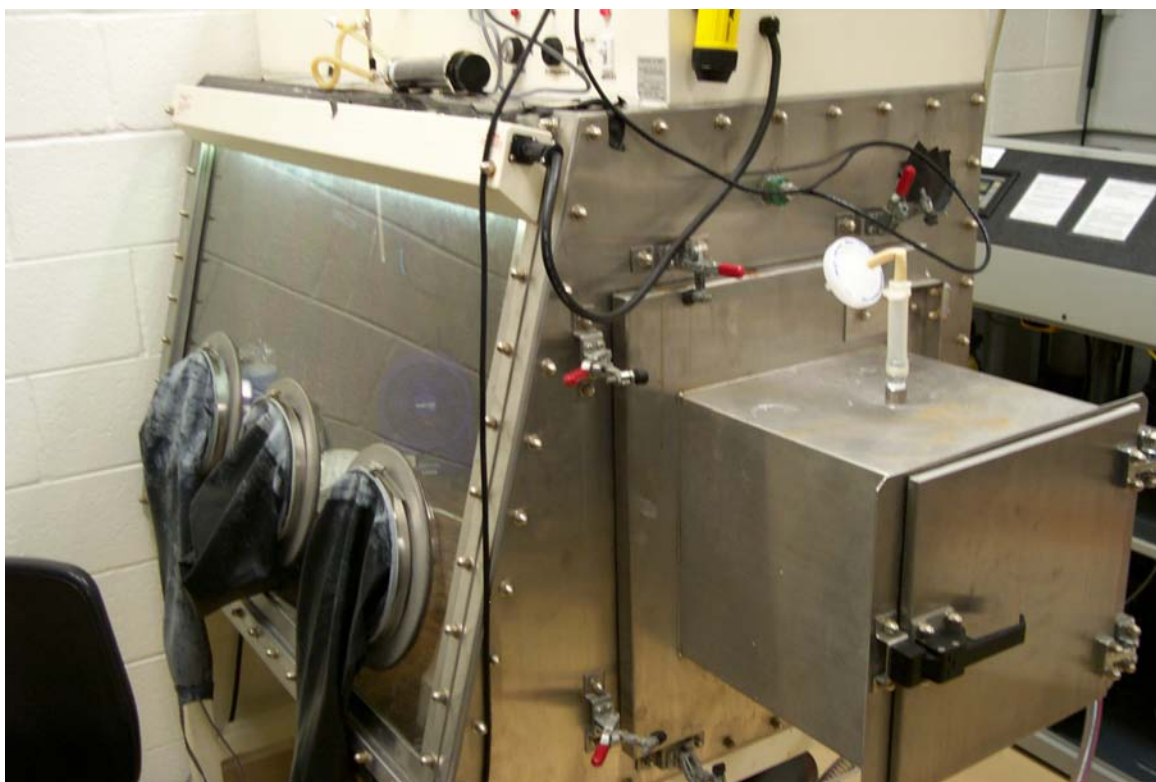


Figure 5. Testing Chamber; Laboratory Validation of Chlorine Dioxide Decontamination.

Table 2. Test Matrix Showing the Relative Humidity (RH) and Concentration of Chlorine Dioxide (ClO₂) Gas for Each Trial; Laboratory Validation of ClO₂ Decontamination.

| Trial Number | RH (%) | ClO ₂ Concentration (ppm) |
|--------------|----------------|--------------------------------------|
| T-01 | 75 | 650 |
| T-02 | 92 | 730 |
| T-03 | 70 | 620 |
| T-04 | 90 | 610 |
| T-05 | 60 | 613 |
| T-06 | Not Applicable | Hydrogen Peroxide |
| T-07 | Aborted | Aborted |
| T-08 | 40 | 800 |
| T-09 | 30 | 1050 |
| T-10 | 90 | 250 |
| T-11 | 30 | 250 |
| T-12 | 50 | 125 |
| T-13 | 70 | 250 |
| T-14 | 70 | 125 |
| T-15 | 90 | 125 |
| T-16 | 70 | 750 |

- g. At each time-point (1, 2, 4, 6, 8, and 12 hours), samples were removed from the chamber.
- h. The samples remained in the covered petri dishes until they were assayed the next day.
- i. There were two sets of samples at 12 hours. If the first set had no bacterial growth, the second set (retained sample) was incubated for 21 days to confirm that there were no viable spores remaining.

2.2.3.3 H₂O₂

- a. Trial 6 was a H₂O₂ trial.
- b. During Trial 6, the samples were prepared and handled as they were in the ClO₂ trials (Paragraph 2.2.3.1 and Appendix C), except that H₂O₂ was used in place of ClO₂.

c. H₂O₂ Generation

(1) Vaporized hydrogen peroxide (VHP) was used during Trial 6. VHP systems created a vaporous decontaminant by heating 30 to 35 percent liquid H₂O₂ to form a vapor.

(2) VHP was generated by a Steris VHP™1000 unit.

(a) VHP was generated in accordance with (IAW) the manufacturer's instructions (Steris Corporation, Erie, Pennsylvania) (Reference 3).

(b) The VHP™1000 measures a quantity of material, which is 30 percent H₂O₂ by mass, and heats it at a controlled rate to form VHP of known concentration.

(c) The VHP is then blown into the chamber with fans integral to the VHP™1000.

(d) The rate of addition must be controlled to match a given chamber's temperature, pressure, and humidity to prevent the VHP from undergoing a phase transition to liquid H₂O₂.

(e) Because any humidity in the test chamber lowers the maximum concentration of VHP that can be achieved, it is desirable to lower the humidity in the chamber as much as possible. The VHP™1000 has an integral dehumidifier that allows a dehumidification phase to be programmed into a VHP cycle.

(3) The optimal phase for this test consisted of a dehumidification phase where the integral VHP™1000 dehumidifier removed the water vapor from the chamber. Dehumidification was followed by a conditioning phase where elevated levels of VHP were introduced into the chamber. Conditioning allowed the sterilization concentration to be reached at an accelerated rate. After the conditioning phase, VHP addition was slowed to maintain a steady state concentration for 12 hours.

NOTE: The 12-hour contact time for H₂O₂ was a test parameter set by the customer and represented more exposure time than was calculated necessary for the test chamber.

(4) In order to introduce the elevated levels of VHP, it was necessary to refill the internal H₂O₂ reservoir of the VHP™1000, therefore, two separate cycle programs were written.

(a) The first included the dehumidification phase, the conditioning phase, and part of the steady state phase.

(b) The second program contained the remainder of the steady state phase and the aeration phase. The aeration phase was required to remove residual VHP so that the chamber could be opened without causing harm to personnel.

2.2.3.4 Spore Strips

a. Separate spore strips containing BGN, BT, and BST were treated as the other samples but were removed from the chamber at 6 and 12 hours only.

b. The spore strips were removed from their protective coverings and placed into a nutrient broth that changed color if bacterial growth was present.

c. The spore strips and broth were incubated at 35°C for up to 1 week, except the BST spore strips that were incubated at 55°C.

d. If a color change occurred, the number of days to the color change was noted.

2.2.4 Test Findings

The test findings are divided by substrate (cover slip or filter paper) and subdivided by spore strain (BAA, BAV, BAS, BGN, BT, and BGN). Each trial involved both substrates and all strains. Figures B.19 through B.33 are summary graphs showing the log of cfus remaining as a function of time for each trial.

2.2.4.1 Cover Slips

NOTE: In all cases where the 12-hour sample had no detectable levels of bacteria, the retained samples (which were incubated for 21 days) also had no detectable levels of bacteria.

a. When the anthrax and simulant strains were compared with each other, BGN was the most susceptible to the ClO₂ treatment, followed by BAA and BAV, which were not significantly different from each other. BAS was less susceptible to the treatment; BT and BST were the least susceptible, but were not significantly different from each other. Results for each strain are as follows:

b. BAA

(1) Figures B.1 through B.3 are summary graphs for the BAA trials.

(2) RH and time were factors in decreasing the number of cfus remaining.

(3) At 12 hours, none of the trials with 90 to 92 percent RH had detectable levels of BAA (Table B.8).

(4) At 12 hours, 40 percent of the trials with 70 to 75 percent RH had detectable levels of BAA (Table B.8).

(5) At 12 hours, 100 percent of the trials with 30 to 60 percent RH had detectable levels of BAA (Table B.8).

(6) There was no indication that increasing the level of ClO₂ between 125 and 1050 ppm decreased the number of BAA cfus remaining (Table B.14).

c. BAV

(1) Figures B.4 through B.6 are summary graphs for the BAV trials.

(2) RH and time were factors in decreasing the number of cfus remaining.

(3) At 12 hours, none of the trials with 90 to 92 percent RH had detectable levels of BAV (Table B.9).

(4) At 12 hours, 80 percent of the trials with 70 to 75 percent RH had detectable levels of BAV (Table B.9).

(5) At 12 hours, 100 percent of the trials with 30 to 60 percent RH had detectable levels of BAV (Table B.9).

(6) There was no indication that increasing the level of ClO₂ between 125 and 1050 ppm decreased the number of BAV cfus remaining (Table B.15).

d. BAS

(1) Figures B.7 through B.9 are summary graphs for the BAS trials.

(2) RH and time were factors in decreasing the number of cfus remaining.

(3) At 12 hours, none of the trials with 90 to 92 percent RH had detectable levels of BAS (Table B.10).

(4) At 12 hours, 20 percent of the trials with 70 to 75 percent RH had detectable levels of BAS (Table B.10).

(5) At 12 hours, 80 percent of the trials with 30 to 60 percent RH had detectable levels of BAS (Table B.10).

(6) There was no indication that increasing the level of ClO₂ between 125 and 1050 ppm decreased the number of BAS cfus remaining (Table B.16).

e. BGN

- (1) Figures B.10 through B.12 are summary graphs for the BGN trials.
- (2) At 12 hours, 25 percent of the trials with 90 to 92 percent RH had detectable levels of BGN (Table B.11).
- (3) At 12 hours, none of the trials with 70 to 75 percent RH had detectable levels of BGN (Table B.11).
- (4) At 12 hours point, 80 percent of the trials with 30 to 60 percent RH had detectable levels of BGN (Table B.11).
- (5) There was no indication that increasing the level of ClO₂ between 125 and 1050 ppm decreased the number of BGN cfus remaining (Table B.17).

f. BT

- (1) Figures B.13 through B.15 are summary graphs for the BT trials.
- (2) RH and time were factors in decreasing the number of cfus remaining.
- (3) At 12 hours, 25 percent of the trials with 90 to 92 percent RH had detectable levels of BT (Table B.12).
- (4) At 12 hours, 60 percent of the trials with 70 to 75 percent RH had detectable levels of BT (Table B.12).
- (5) At 12 hours, 100 percent of the trials with 30 to 60 percent RH had detectable levels of BT (Table B.12).
- (6) There was no indication that increasing the level of ClO₂ between 125 and 1050 ppm decreased the number of BT cfus remaining (Table B.18).

g. BST

- (1) Figures B.16 through B.18 are summary graphs for the BST trials.
- (2) RH and time were factors in decreasing the number of cfus remaining.
- (3) At 12 hours, 25 percent of the trials with 90 to 92 percent RH had detectable levels of BST (Table B.13).
- (4) At 12 hours, 60 percent of the trials with 70 to 75 percent RH had detectable levels of BST (Table B.13).
- (5) At 12 hours, 100 percent of the trials with 30 to 60 percent RH had detectable levels of BST (Table B.13).

(6) There was no indication that increasing the level of ClO₂ between 125 and 1050 ppm decreased the number of BST cfus remaining (Table B.19).

2.2.4.2 Filter Paper

a. BAA

(1) For BAA, there were no detectable cfus after 2 hours (Table B.26), regardless of RH or ClO₂ concentration.

(2) It was not possible to distinguish whether concentration of ClO₂ gas or RH was a factor in the reduction of cfus; however, time in the chamber was clearly a factor (Table B.27).

b. BAV

(1) For BAV, there were no detectable cfus after 8 hours (Table B.28), regardless of RH or ClO₂ concentration.

(2) It was not possible to distinguish whether concentration of ClO₂ gas or RH was a factor in the reduction of cfus; however, time in the chamber was clearly a factor (Table B.29).

2.2.4.3 Reduction Factors

a. The log reduction factors for each strain or simulant in response to each trial was calculated for each time-point.

b. The reduction factors for each anthrax strain or simulant are in Tables B.1 through B.6. Highlighted reduction factors indicate that there were no detectable spores and that the reduction factor was at the maximum.

2.2.4.4 Spore Strip Data

a. The results for the spore strips after 1-week incubation are in Tables B.30 through B.33. In the data reported in Table B.32, each of the replicates had a different initial contamination value (10⁴ through 10⁸) of BST spores. The data in Table B.32 are reported for informational purposes and was not used in data analysis, conclusions, or recommendations.

b. Simulant Spores

(1) BGN. There was one false negative (Table B.34) when comparing BGN cover slips with spore strips; this occurred during Trial 10 at 6 hours.

(2) BT. There was one false negative (Table B.35) when comparing BT cover slips with spore strips; this occurred during Trial 16 at 12 hours.

(3) BST. There were no false negatives found when comparing BST cover slip data with the spore strips (Table B.36).

c. BA Spores

(1) BAA (Table B.37)

(a) When compared with BGN, the spore strips had false negatives in Trials 14 and 16 at 6 hours and a false negative in Trial 14 at 12 hours.

(b) When compared with BT, the spore strips had no false negatives.

(c) When compared with BST, the spore strips had no false negatives.

(2) BAV (Table B.38)

(a) When compared with BGN, the spore strips had false negatives in Trials 14 and 16 at 6 hours and 12 hours.

(b) When compared with BT, the spore strips had a false negative in Trial 16 at 12 hours.

(c) When compared with BST, the spore strips had no false negatives.

(3) BAS (Table B.39)

(a) When compared with BGN, the spore strips had false negatives in Trials 14 and 16 at 6 hours and in Trial 16 at 12 hours.

(b) When compared with BT, the spore strips had no false negatives.

(c) When compared with BST, the spore strips had no false negatives.

2.2.4.5 H₂O₂

a. The effects of H₂O₂ on cfus remaining on cover slips are in Figure B.24.

b. Reduction factors were calculated for H₂O₂ and are in Table B.7.

c. No cfus were found on filter paper for BAA or BAV for any of the time-points sampled. The initial contamination levels were 2.2×10^6 and 8.1×10^5 cfus, respectively.

2.2.5 Technical Analysis

2.2.5.1 Cover Slip Data

a. In order to test for significant effects, data were required to pass certain assumptions. The assumptions were:

(1) The residuals from the model must have an error of zero and be normally distributed.

(2) The variance of the residuals must be constant.

b. When the data were combined for all agents, the above assumptions were met and an analysis of variance (ANOVA) was performed (Table 20). Multiple comparison analysis determined that BGN was significantly easier to kill than BAA and BAV, which were not different from each other. BAS was more resistant to the ClO_2 treatment than BAA and BAV; BT and BST were the most resistant, but were not significantly different from each other.

c. When the data were analyzed for each individual agent, the assumptions were not met. Because these data did not adhere to the above assumptions, specific transformations were performed. Some of the transformations included taking the square root, the fourth root, or adding 1 and then log-transforming the data. The data from each agent/simulant behaved differently and, therefore, required different transformations. After the transformations were made, an ANOVA was performed using the generalized linear model (GLM) method. The results are in Tables B.21 through B.25.

d. The data for BGN could not be transformed to fit the assumptions; therefore, no ANOVA was performed.

e. In all of the ANOVAs performed, except for BST, time was the only significant factor. Time and RH were both significant factors for BST (Table B.24).

f. In several of the ANOVAs, ClO_2 or the interaction between ClO_2 and RH showed up as significant factors (Tables B.21 through B.23). These were rejected as being significant for three reasons:

(1) The data indicated that if ClO_2 had an effect it would be in the middle range of 600 to 650 ppm. If any effect was observed, it would be expected to decrease the cfus remaining as the concentration of ClO_2 gas increased. This was not the case.

(2) It was determined that the range where the effect was observed (600 to 650 ppm) was the most variable of the ranges. Therefore, the 125 to 250 and the 730 to 1050 ppm ranges reflected the actual ppm values (see Appendix D).

(3) In all cases, the effect of the ClO_2 or the interaction was very small.

2.2.5.2 Filter Paper Data

a. The filter paper data differed from the glass cover slip data. Most of the data reported 100 percent removal and, therefore, the percent remaining was 0. Because there were so many zeros, any positive readings were reported as outliers.

b. Because of the nature of the data, GLM could not be used. In order to analyze the data, it was assumed that if 100 percent of the agent was removed, then the response was assigned a 1 (or success), and if any amount of agent remained, then a 0 was assigned (failure).

c. Changing the data into a pass/fail criterion allowed for a probit analysis to be performed. It was noticed that any amount remaining appeared in the earlier time periods. This made it impossible to distinguish between RH and ClO_2 as contributing factors. The probit analysis, therefore, included time as a factor and a 0/1 response. The results are in Tables B.27 and B.29. The amount of time in the chamber, combined with the RH and ClO_2 , is clearly a significant factor.

2.2.5.3 Log cfus and Reduction Factors

a. A log base 10 transformation was performed on the cfus to acquire log cfus for Figures B.19 through B.33.

b. To acquire the log reduction factor at each time point, the log cfu for each strain or simulant in a trial was subtracted from the log cfu for the zero time-point. The corresponding value was reported as the log reduction at that time-point (Tables B.1 through B.7).

2.2.5.4 Spore Strip Data

a. To compare the cover slip data with the spore strip data, the cover slip data at 6 and 12 hours were converted to positive (+ = bacterial growth present) or negative (– = no bacterial growth present) for each spore strain tested (Tables B.34 through B.39).

b. The cover slip data were compared with the spore strip data (Tables B.30, B.31 and B.33) to determine if the spore strip data had false negatives. The spore strip data had five replicates, while the cover slip data had three replicates. The spore strips were determined to have a false negative if one of the following conditions was met:

(1) All three replicates of the cover slip data were positive, and the spore strip data had two or fewer of the five replicates that were positive.

(2) Two out of the three replicates of the cover slip data were positive, and none of the spore strip data were positive.

(3) Because of the small sample size, possible false negatives were also reported. If all three replicates of the cover slip data were positive and three or four of the five spore strip replicates were positive, a possible false negative was reported. The difference at this level was not statistically significant, and the possible false negatives were not taken into account when making conclusions or recommendations.

c. Simulant Spores

(1) For the three simulant strains, the cover slip data were compared with the spore strip data containing the same simulant strain (Tables B.34 through B.36).

(2) In the case of BT and BST, the strains of bacteria were exactly the same. The cover slip bacteria were cultured from spore strips. In the case of BGN, the bacteria are the same genus and species; it is not known if they are exactly the same strain.

d. Anthrax Spores

(1) There were no anthrax spore strips.

(2) Each anthrax strain was compared with all the spore strips to determine if the simulant spore strips resulted in a false negative (Tables B.37 through B.39).

SECTION 3. APPENDICES

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None.

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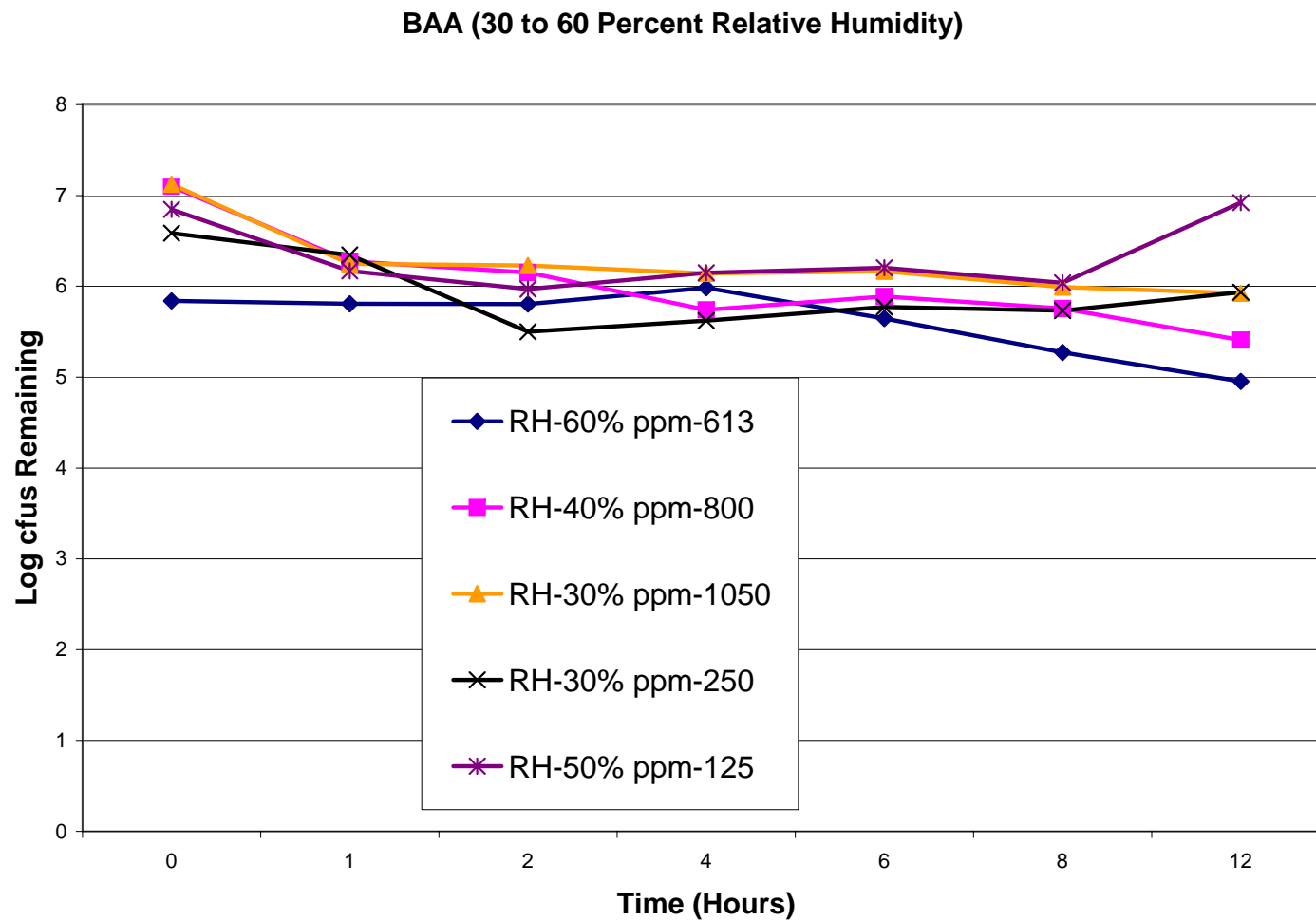


Figure B.1. Survivor Curve Showing the Colony Forming Units (cfus) Remaining Over Time for *Bacillus anthracis* var. *ames* (BAA) Trials, 30 to 60 Percent Relative Humidity (RH); Laboratory Validation of Chlorine Dioxide Decontamination.

BAA (70 to 75 Percent Relative Humidity)

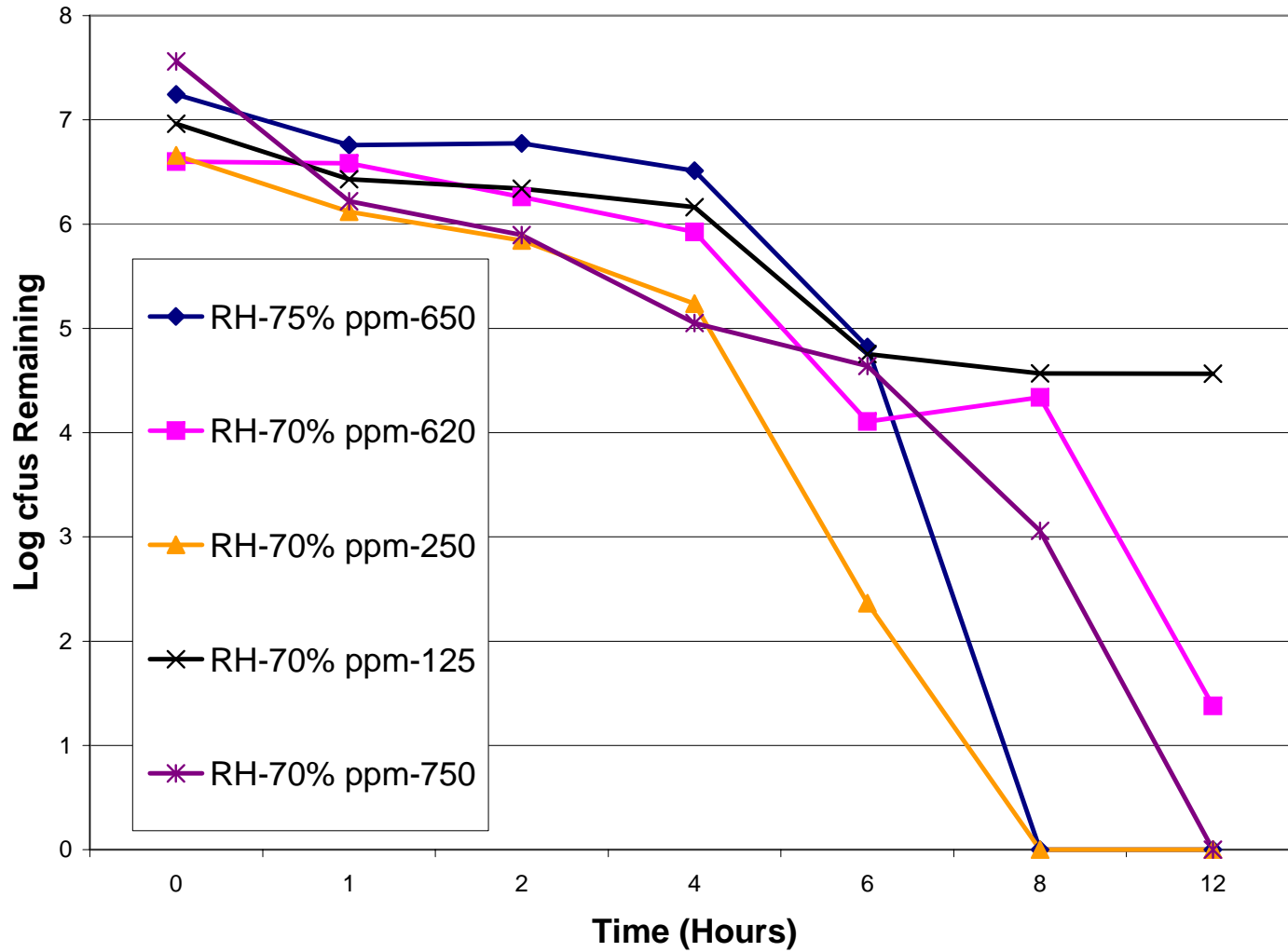


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BAA (90 to 92 Percent Relative Humidity)

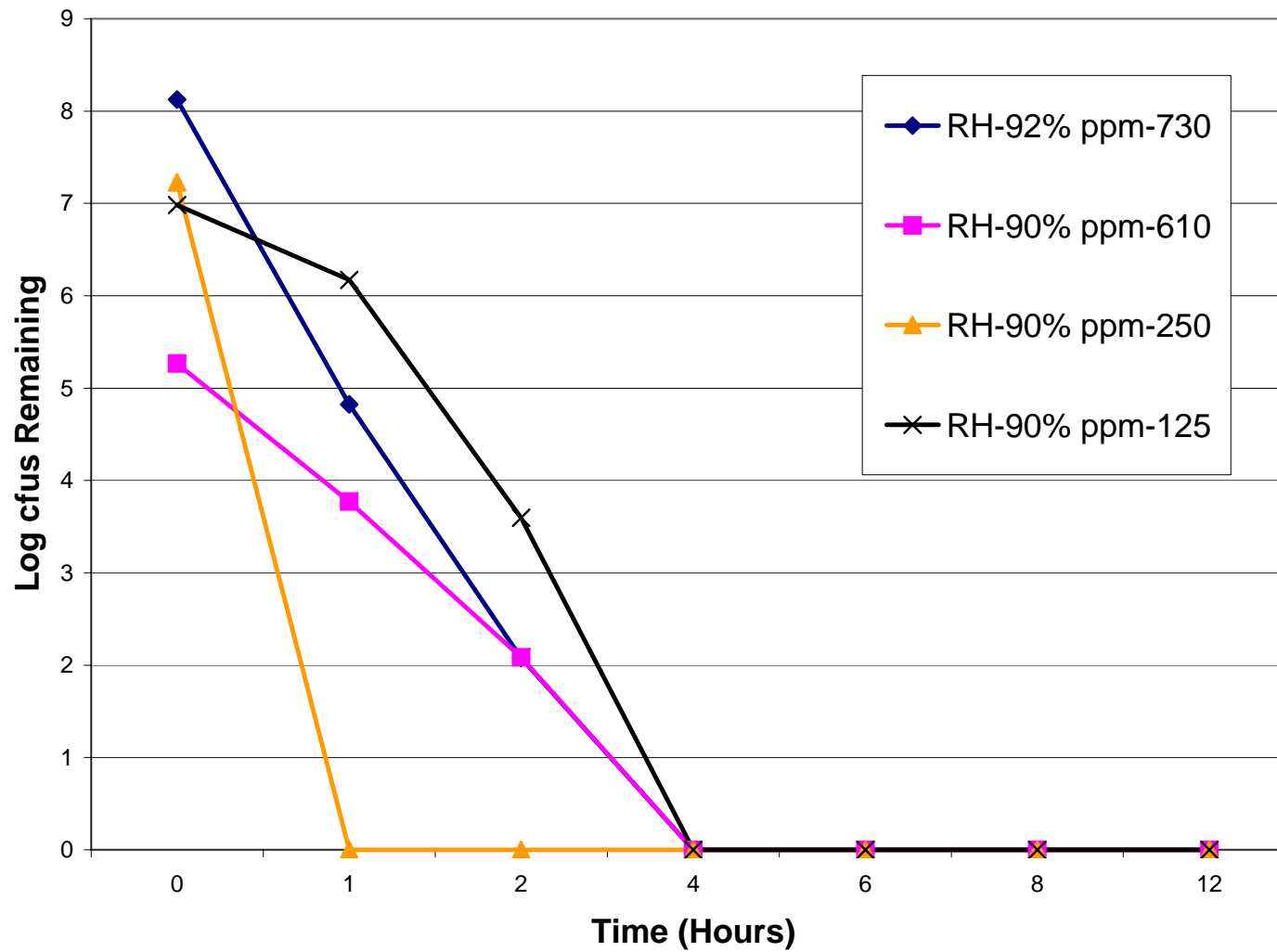


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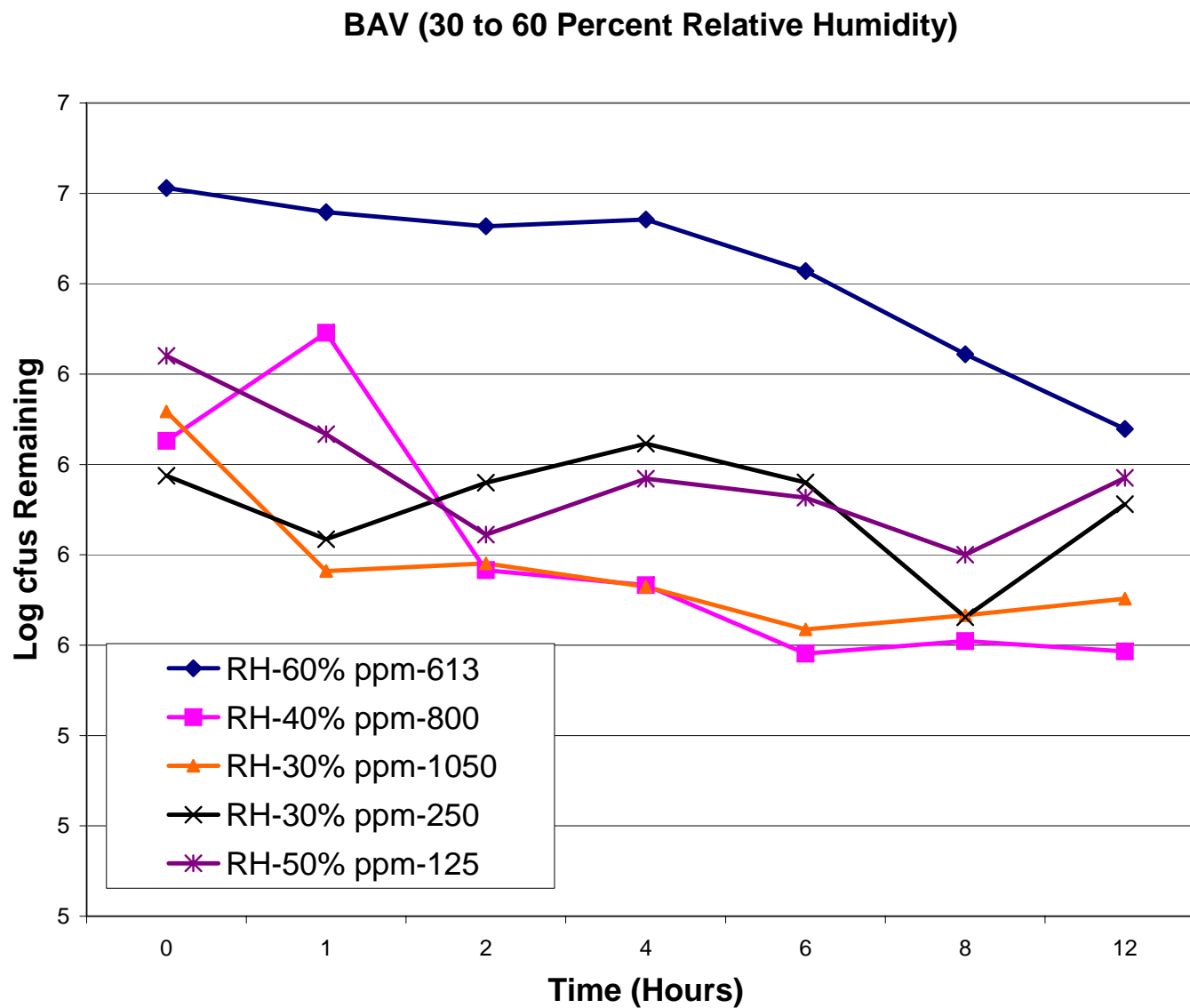


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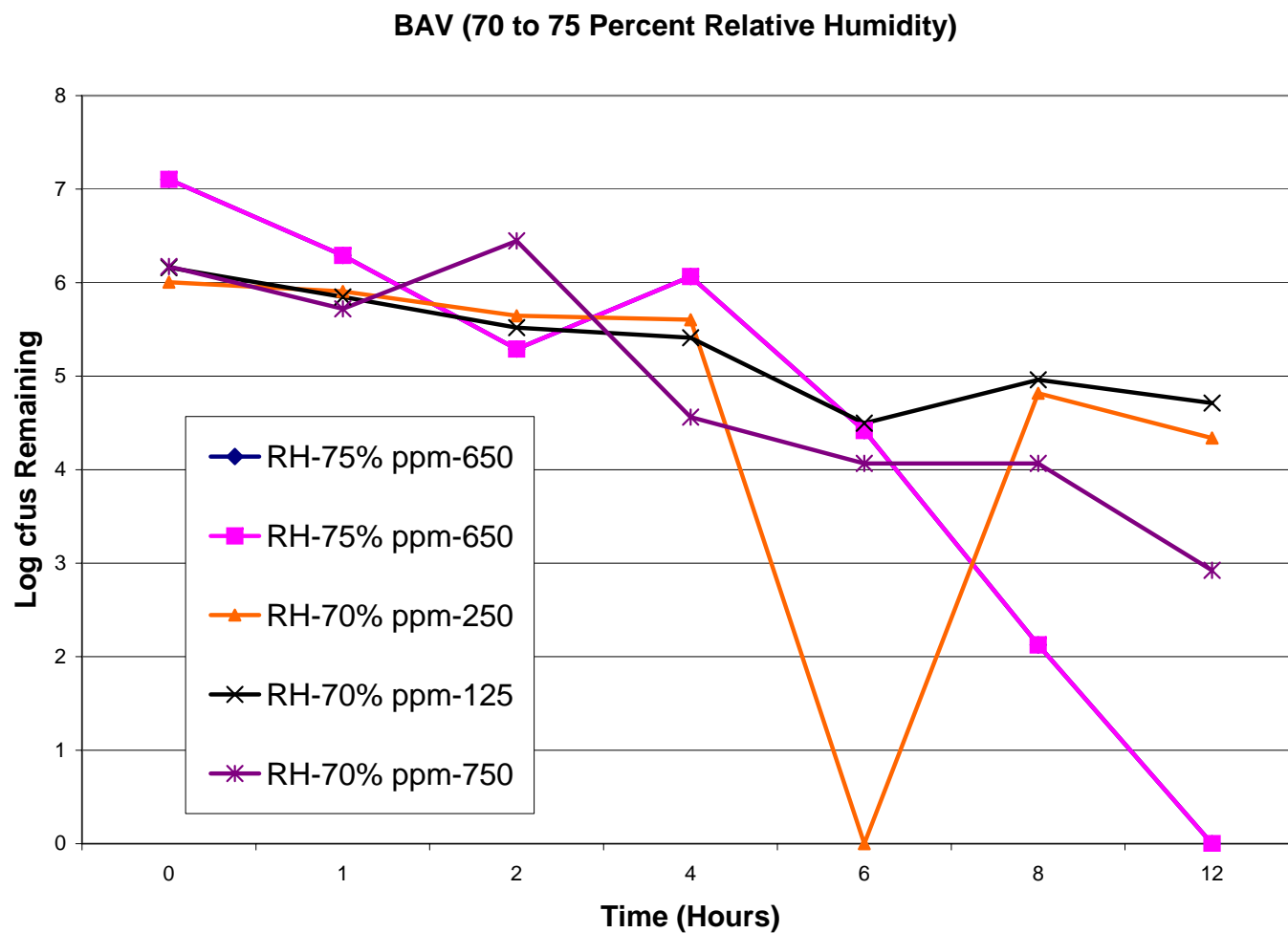


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BAV (90 to 92 Percent Relative Humidity)

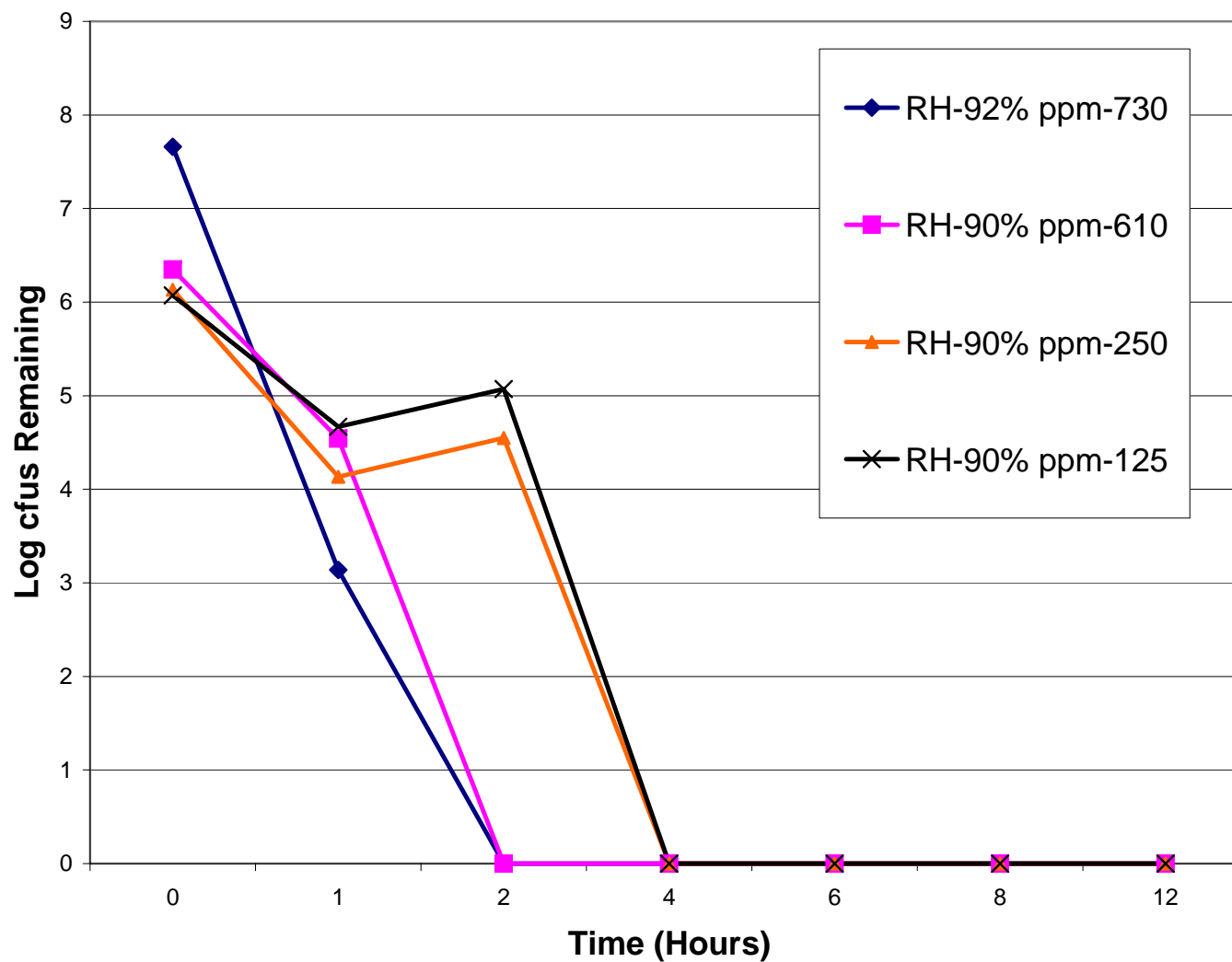


Figure B.6. Survivor Curve Showing the Colony Forming Units (cfus) Remaining Over Time for *Bacillus anthracis* var. *vollum* (BAV) Trials, 90 to 92 Percent Relative Humidity (RH); Laboratory Validation of Chlorine Dioxide Decontamination.

BAS (30 to 60 Percent Relative Humidity)

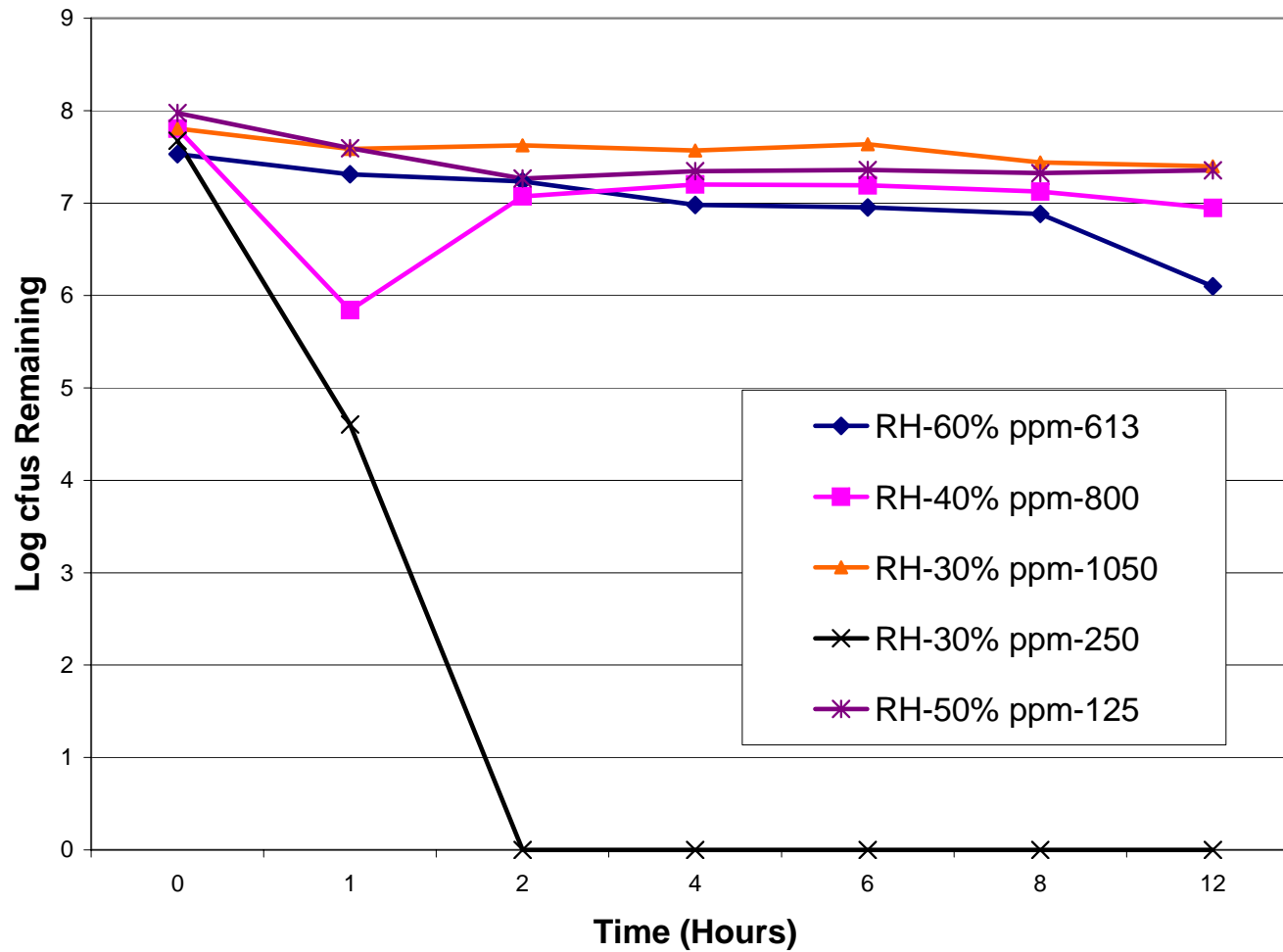


Figure B.7. Survivor Curve Showing the Colony Forming Units (cfus) Remaining Over Time for *Bacillus anthracis* var. *sterne* (BAS) Trials, 30 to 60 Percent Relative Humidity (RH); Laboratory Validation of Chlorine Dioxide Decontamination.

BAS (70 to 75 Percent Relative Humidity)

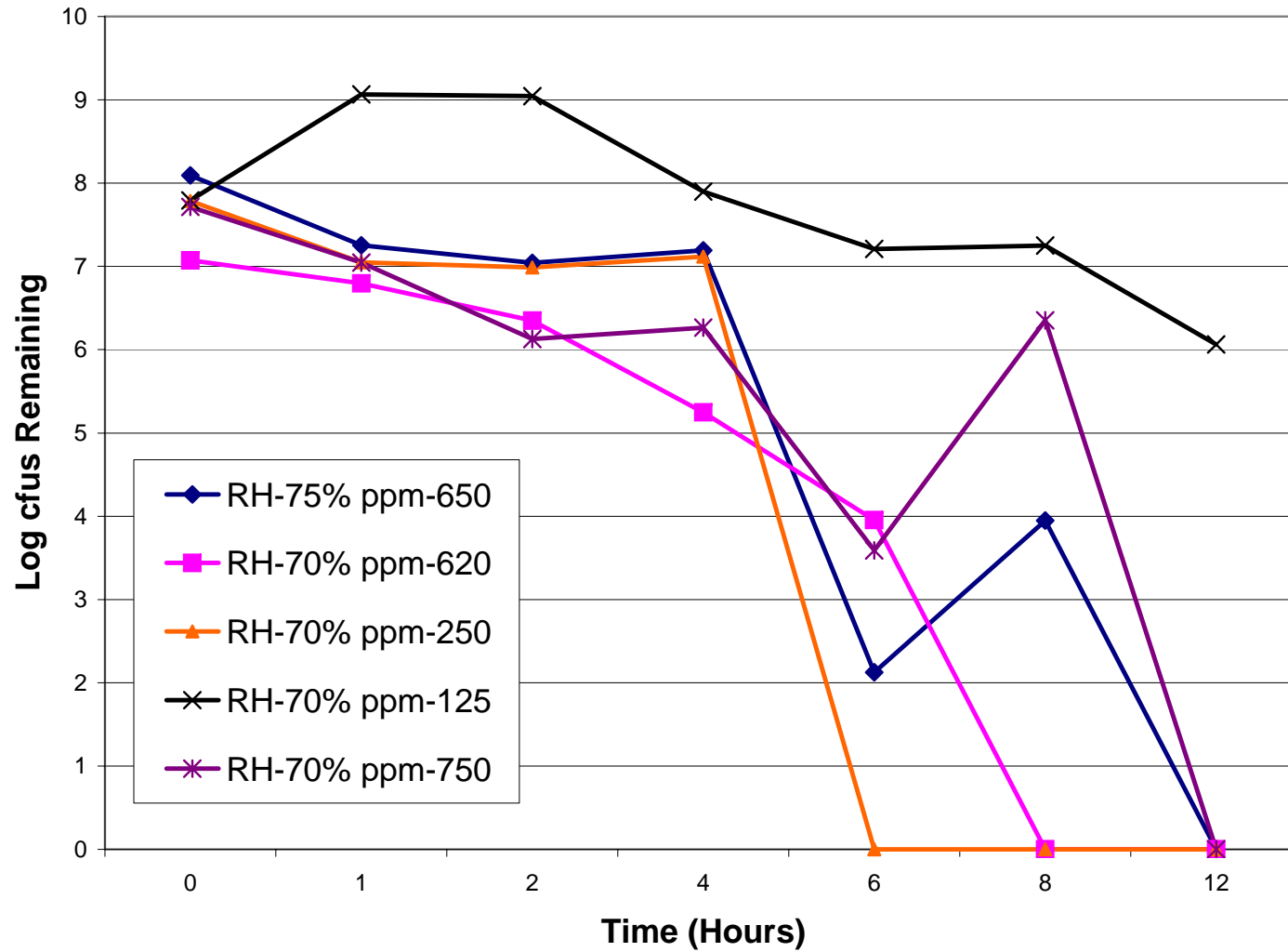


Figure B.8. Survivor Curve Showing the Colony Forming Units (cfus) Remaining Over Time for *Bacillus anthracis* var. *sterne* (BAS) Trials, 70 to 75 Percent Relative Humidity (RH); Laboratory Validation of Chlorine Dioxide Decontamination.

BAS (90 to 92 Percent Relative Humidity)

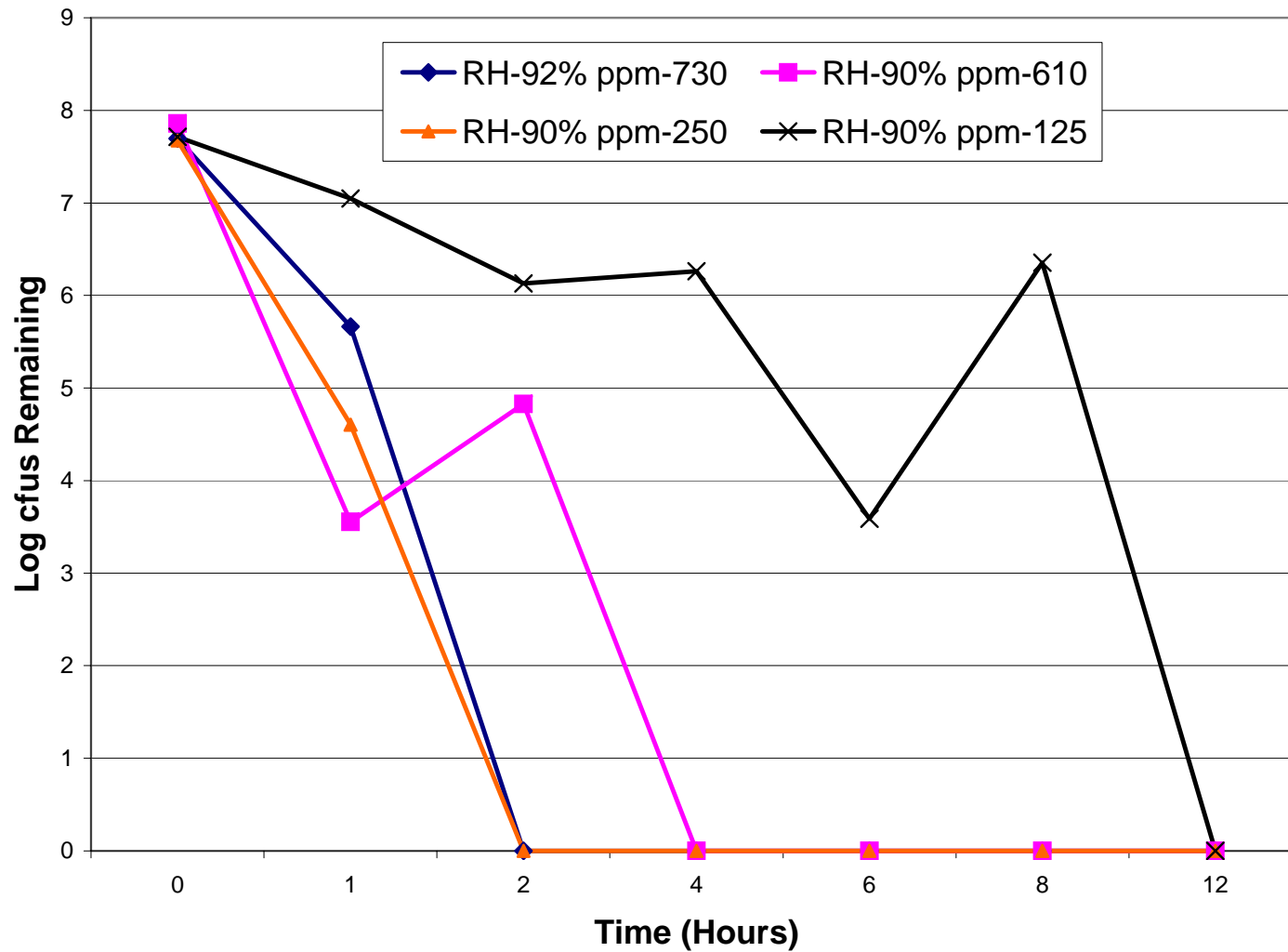


Figure B.9. Survivor Curve Showing the Colony Forming Units (cfus) Remaining Over Time for *Bacillus anthracis* var. *sterne* (BAS) Trials, 90 to 92 Percent Relative Humidity (RH); Laboratory Validation of Chlorine Dioxide Decontamination.

BGN (30 to 60 Percent Relative Humidity)

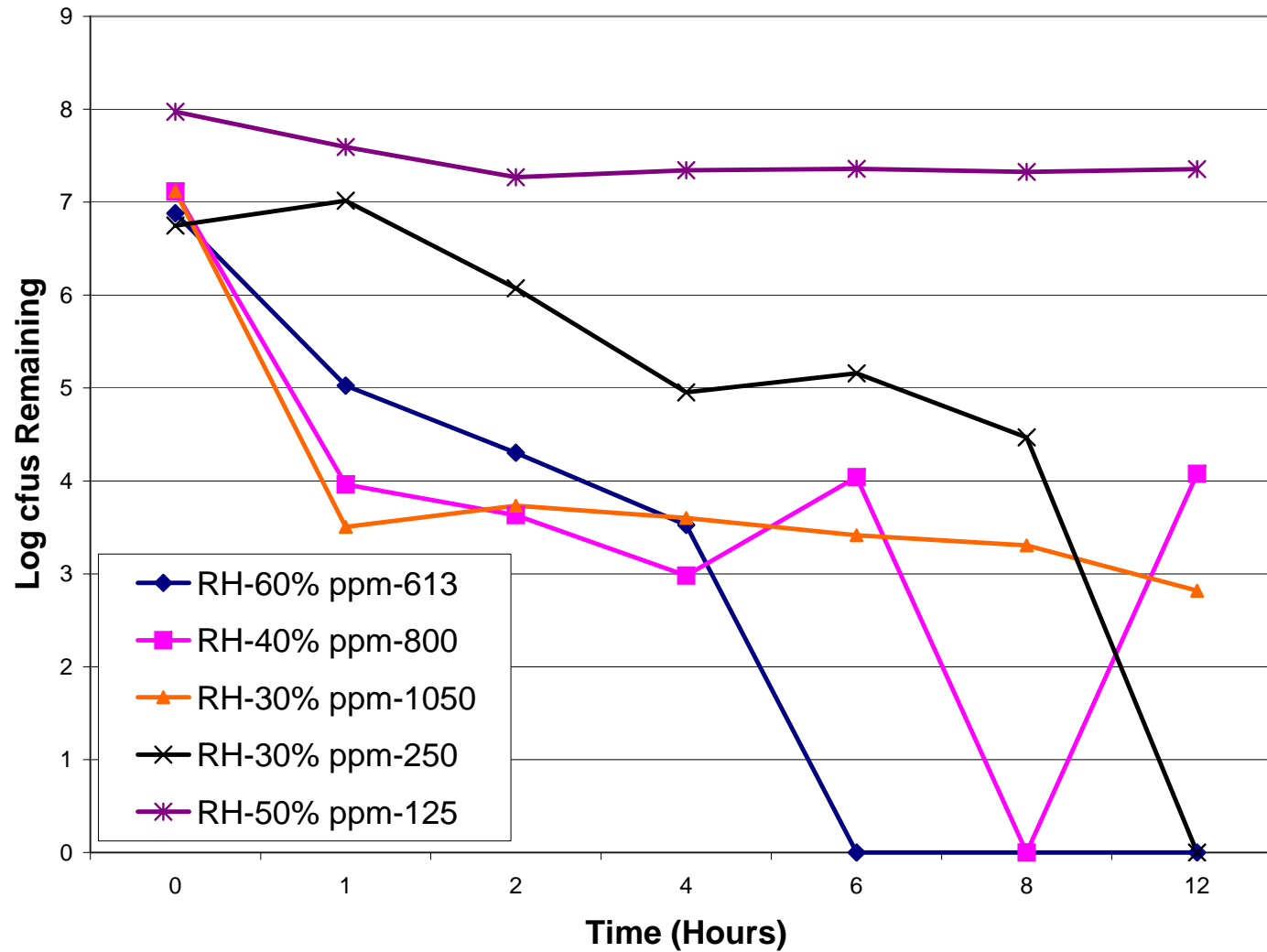


Figure B.10. Survivor Curve Showing the Colony Forming Units (cfus) Remaining Over Time for *Bacillus subtilis* var. *niger* (BGN) Trials, 30 to 60 Percent Relative Humidity (RH); Laboratory Validation of Chlorine Dioxide Decontamination.

BGN (70 to 75 Percent Relative Humidity)

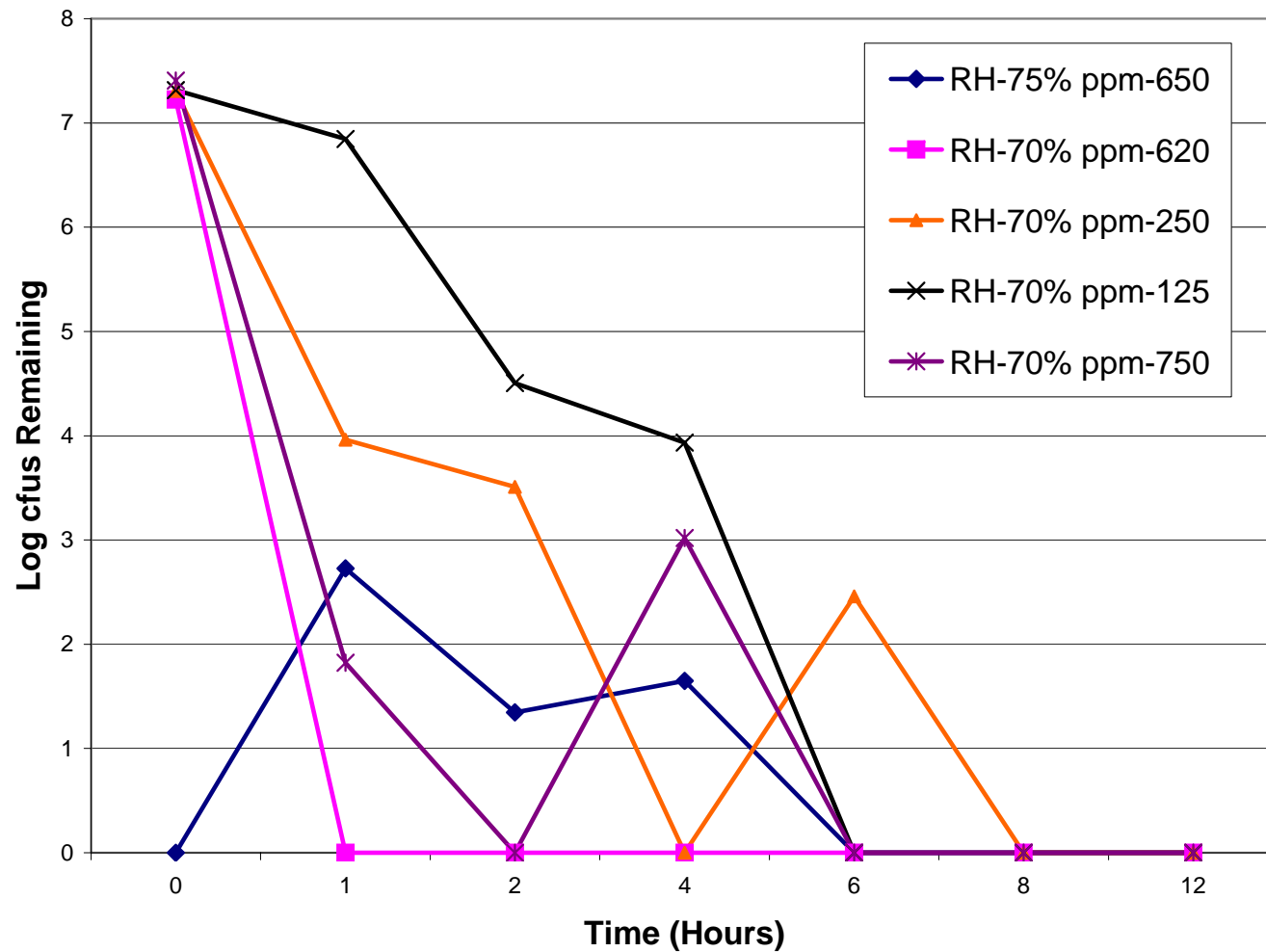


Figure B.11. Survivor Curve Showing the Colony Forming Units (cfus) Remaining Over Time for *Bacillus subtilis* var. *niger* (BGN) Trials, 70 to 75 Percent Relative Humidity (RH); Laboratory Validation of Chlorine Dioxide Decontamination.

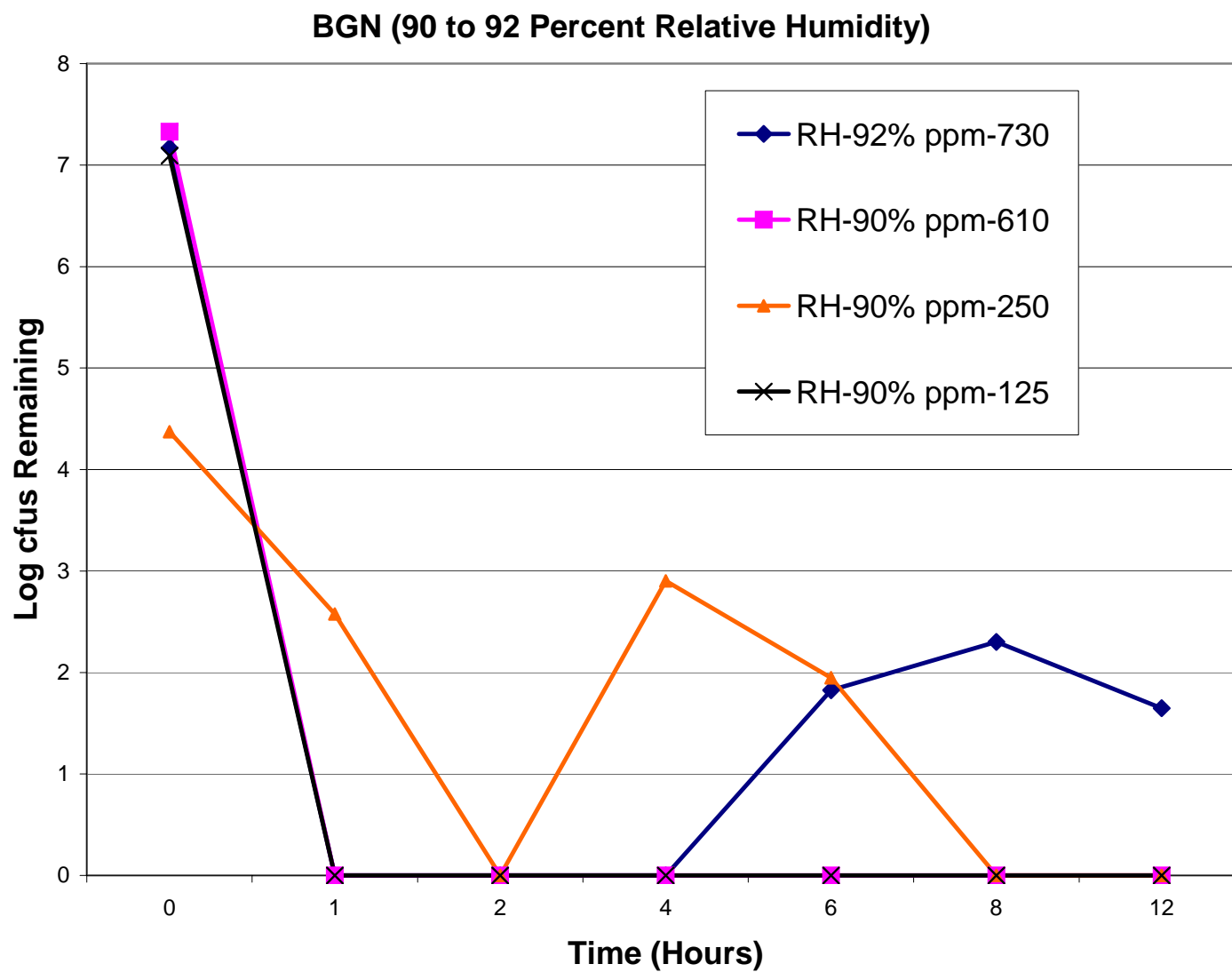


Figure B.12. Survivor Curve Showing the Colony Forming Units (cfus) Remaining Over Time for *Bacillus subtilis* var. *niger* (BGN) Trials, 90 to 92 Percent Relative Humidity (RH); Laboratory Validation of Chlorine Dioxide Decontamination.

BT (30 to 60 Percent Relative Humidity)

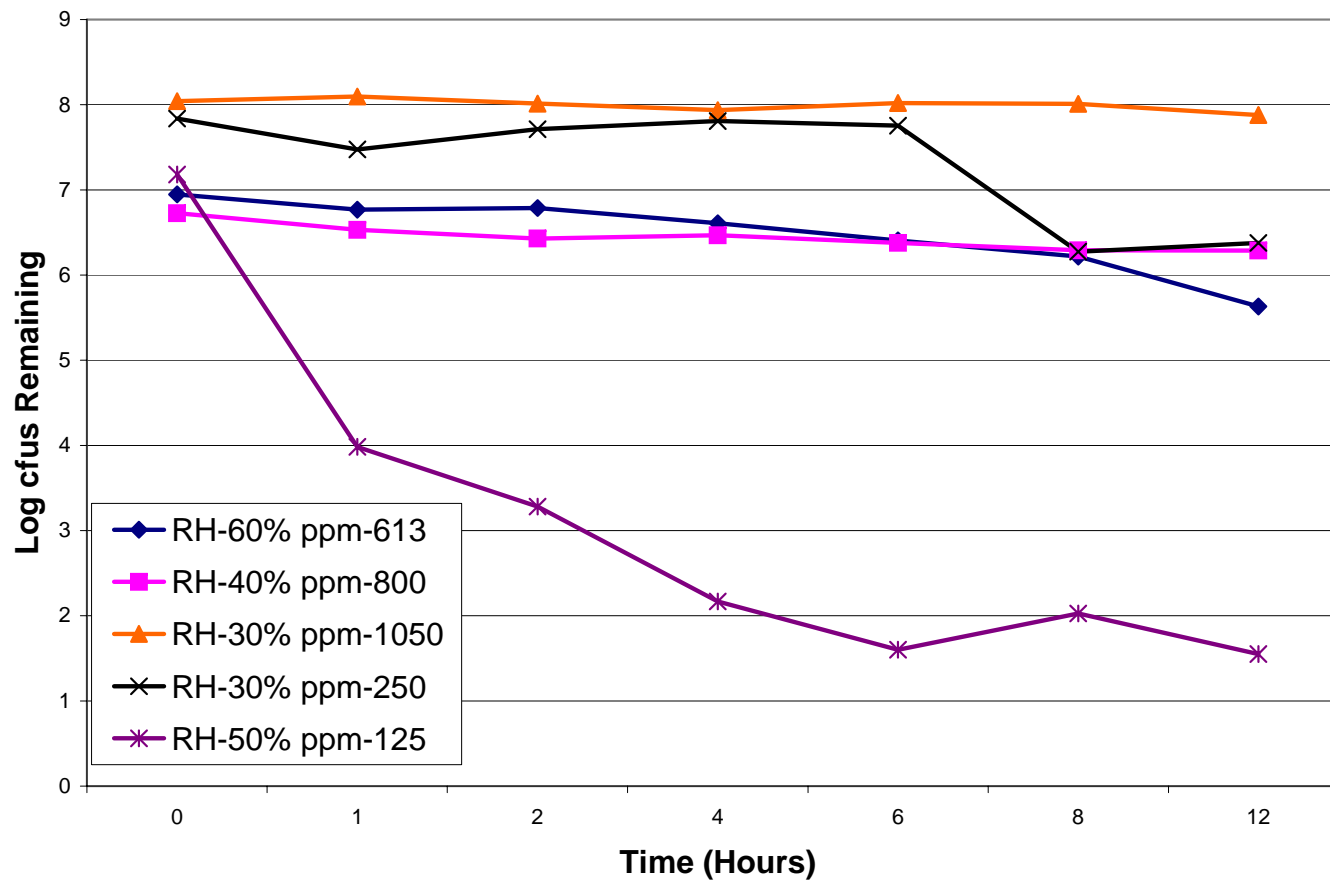


Figure B.13. Survivor Curve Showing the Colony Forming Units (cfus) Remaining Over Time for *Bacillus thuringiensis* (BT) Trials, 30 to 60 Percent Relative Humidity (RH); Laboratory Validation of Chlorine Dioxide Decontamination.

BT (70 to 75 Percent Relative Humidity)

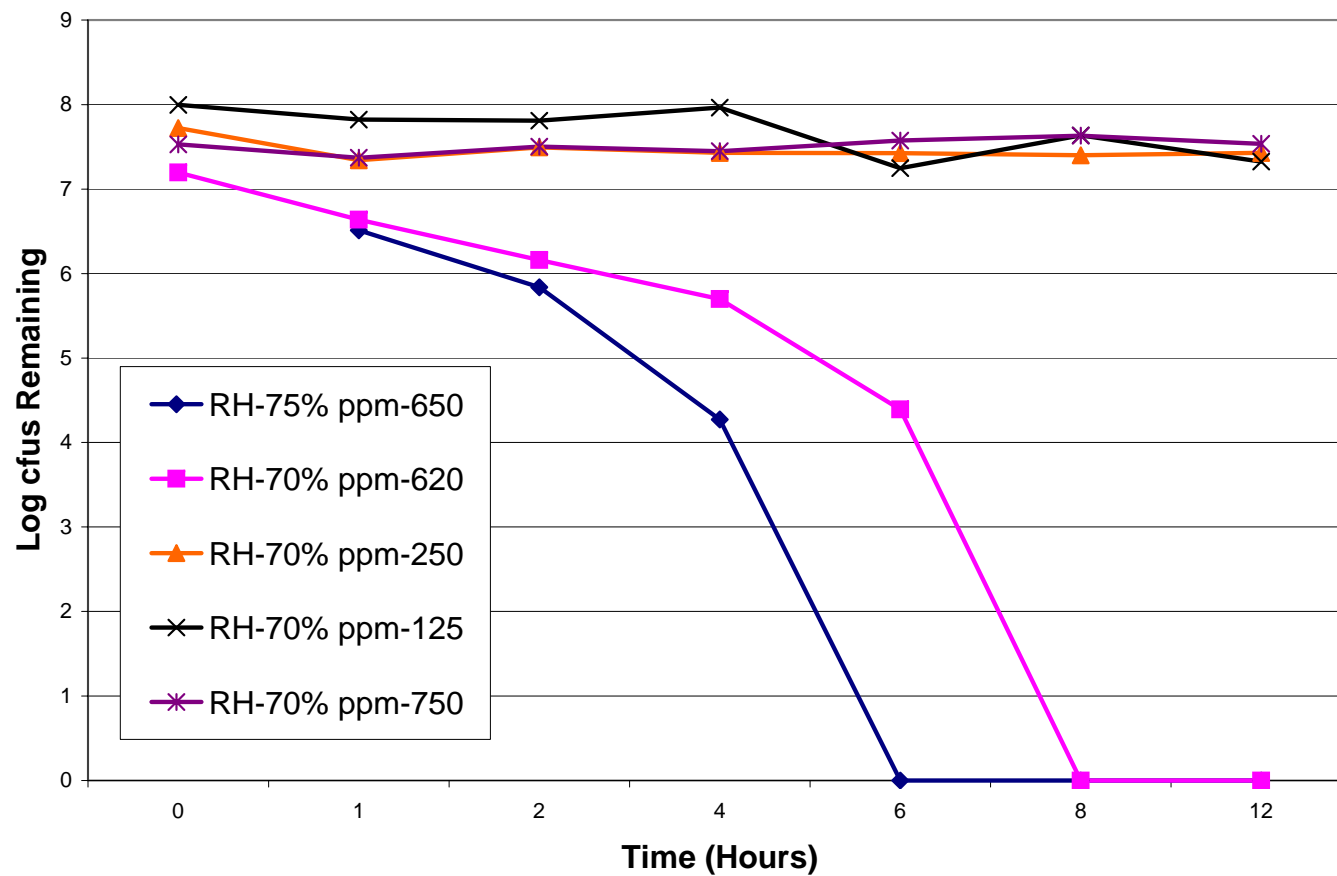


Figure B.14. Survivor Curve Showing the Colony Forming Units (cfus) Remaining Over Time for *Bacillus thuringiensis* (BT) Trials, 70 to 75 Percent Relative Humidity (RH); Laboratory Validation of Chlorine Dioxide Decontamination.

BT (90 to 92 Percent Relative Humidity)

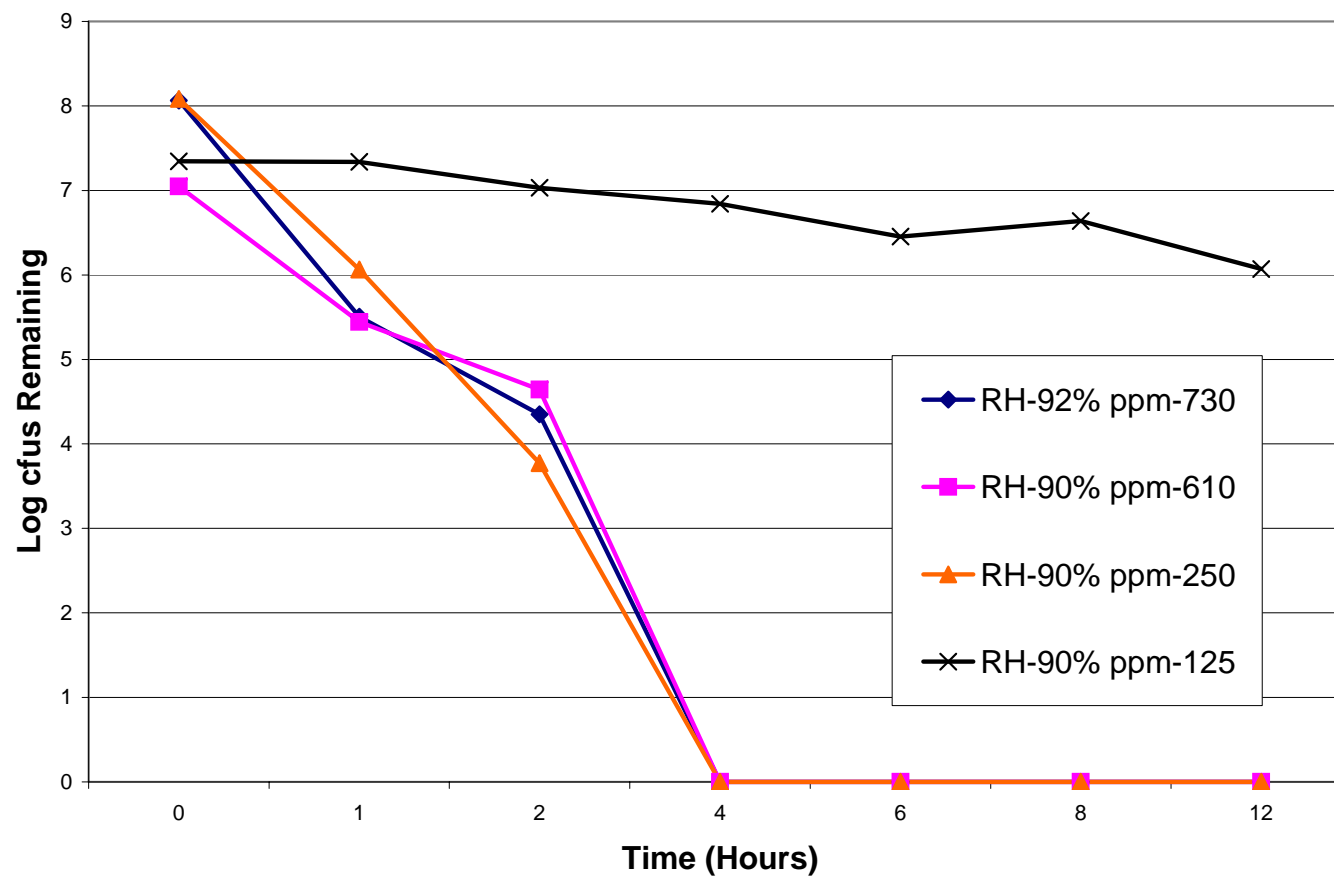


Figure B.15. Survivor Curve Showing the Colony Forming Units (cfus) Remaining Over Time for *Bacillus thuringiensis* (BT) Trials, 90 to 92 Percent Relative Humidity (RH); Laboratory Validation of Chlorine Dioxide Decontamination.

BST (30 to 60 Percent Relative Humidity)

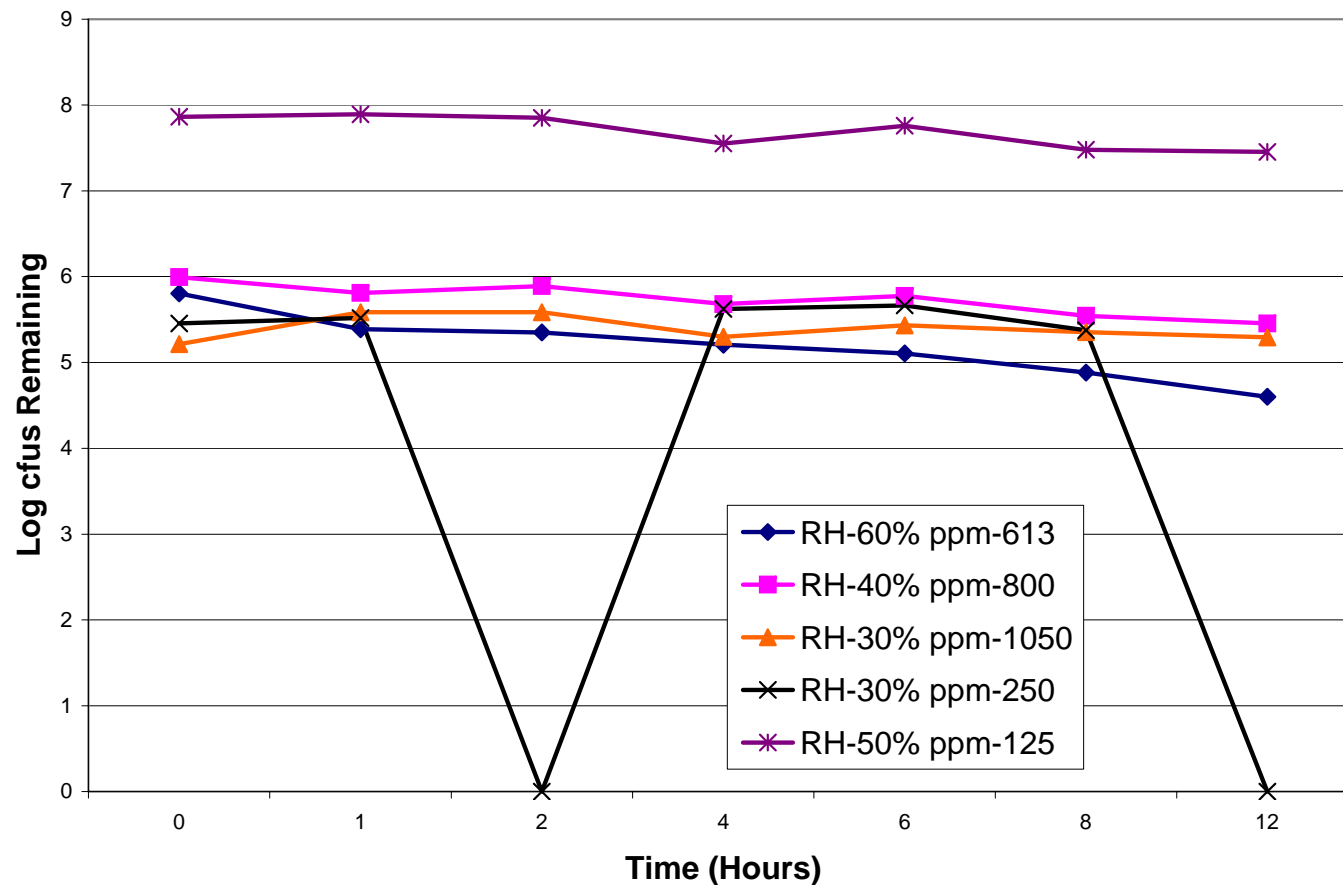


Figure B.16. Survivor Curve Showing the Colony Forming Units (cfus) Remaining Over Time for *Bacillus stearothermophilus* (BST) Trials, 30 to 60 Percent Relative Humidity (RH); Laboratory Validation of Chlorine Dioxide Decontamination.

BST (70 to 75 Percent Relative Humidity)

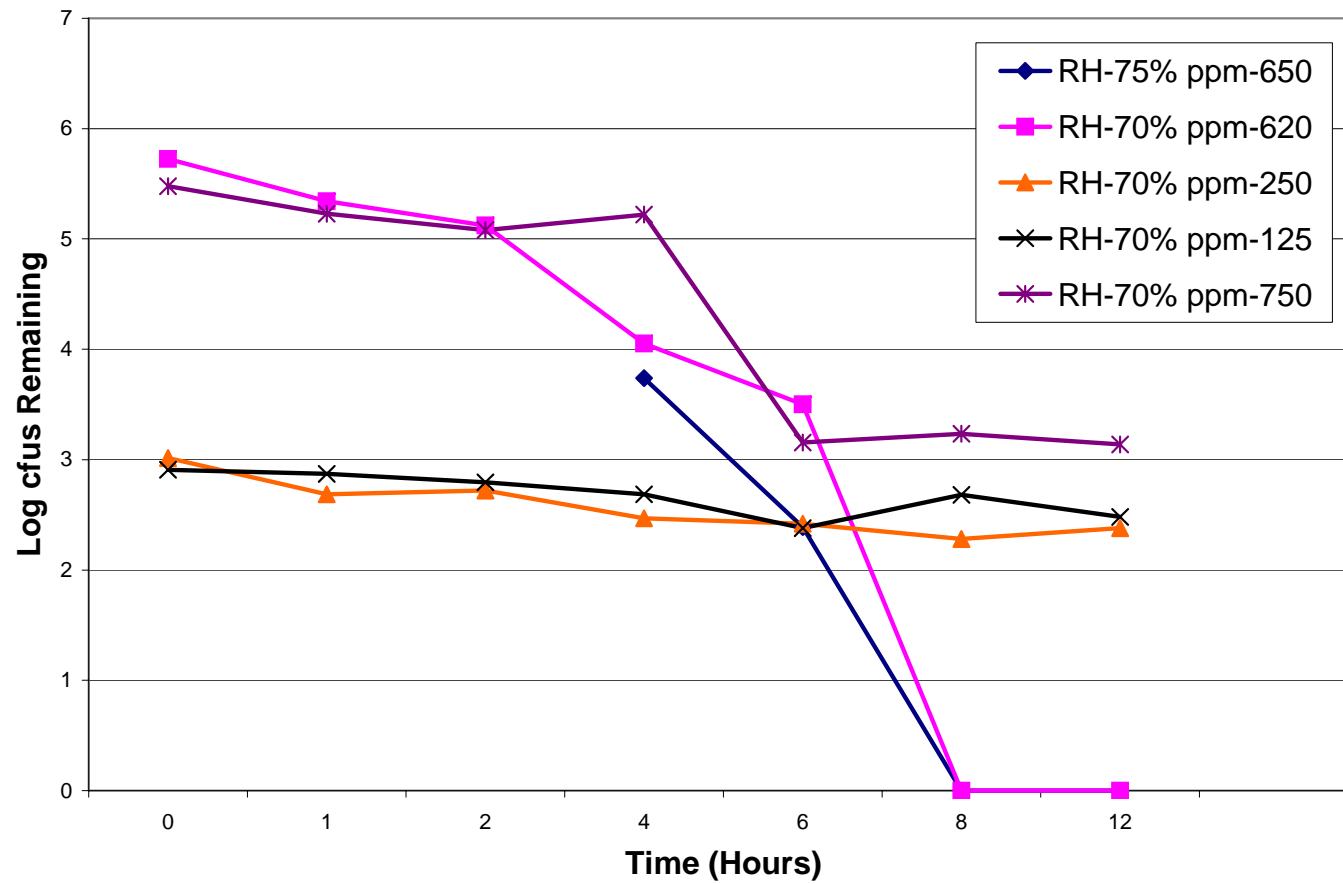


Figure B.17. Survivor Curve Showing the Colony Forming Units (cfus) Remaining Over Time for *Bacillus stearothermophilus* (BST) Trials, 70 to 75 Percent Relative Humidity (RH); Laboratory Validation of Chlorine Dioxide Decontamination.

BST (90 to 92 Percent Relative Humidity)

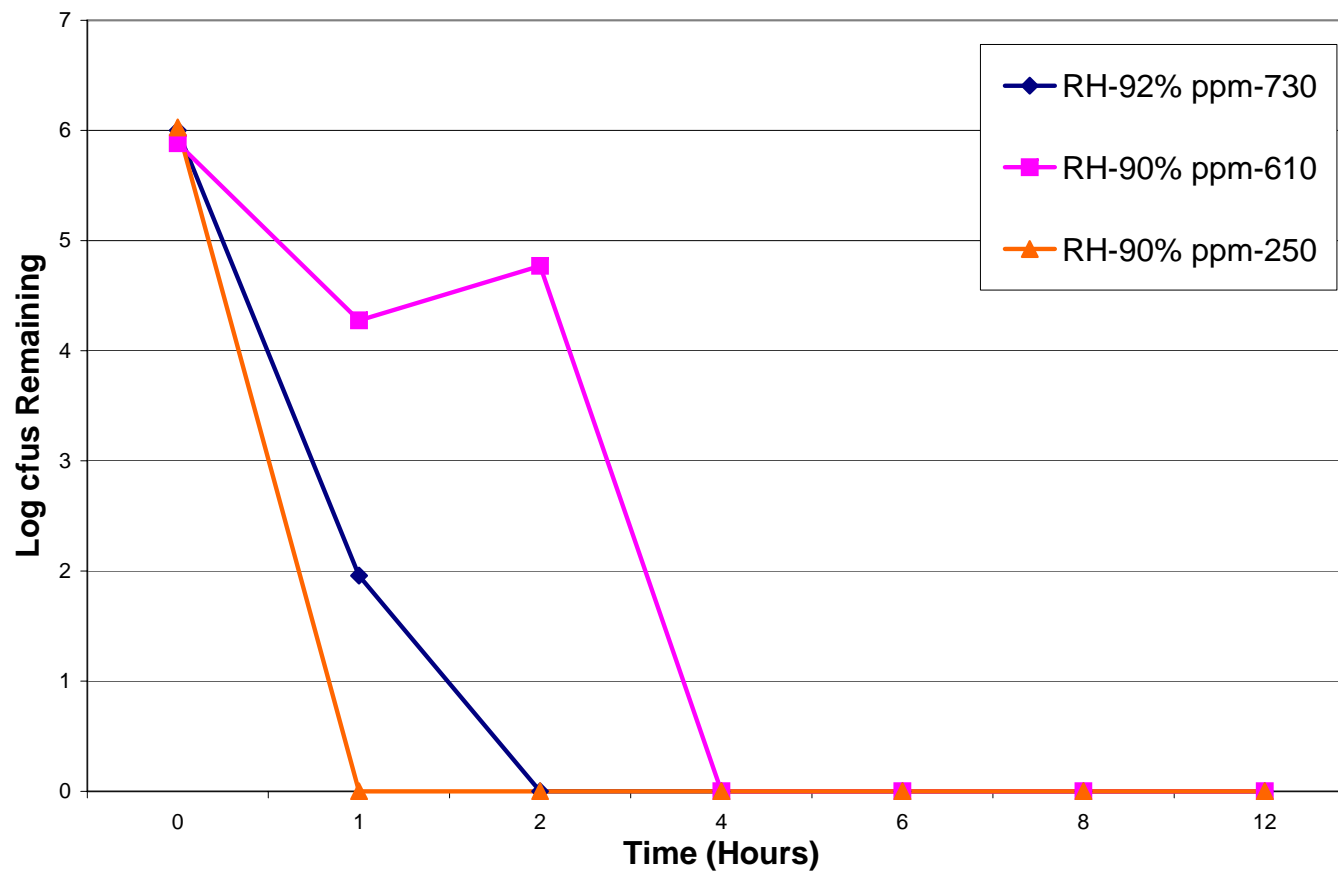


Figure B.18. Survivor Curve Showing the Colony Forming Units (cfus) Remaining Over Time for *Bacillus stearothermophilus* (BST) Trials, 90 to 92 Percent Relative Humidity (RH); Laboratory Validation of Chlorine Dioxide Decontamination.

Trial 1 - Relative Humidity 75% - Chlorine Dioxide 650 ppm

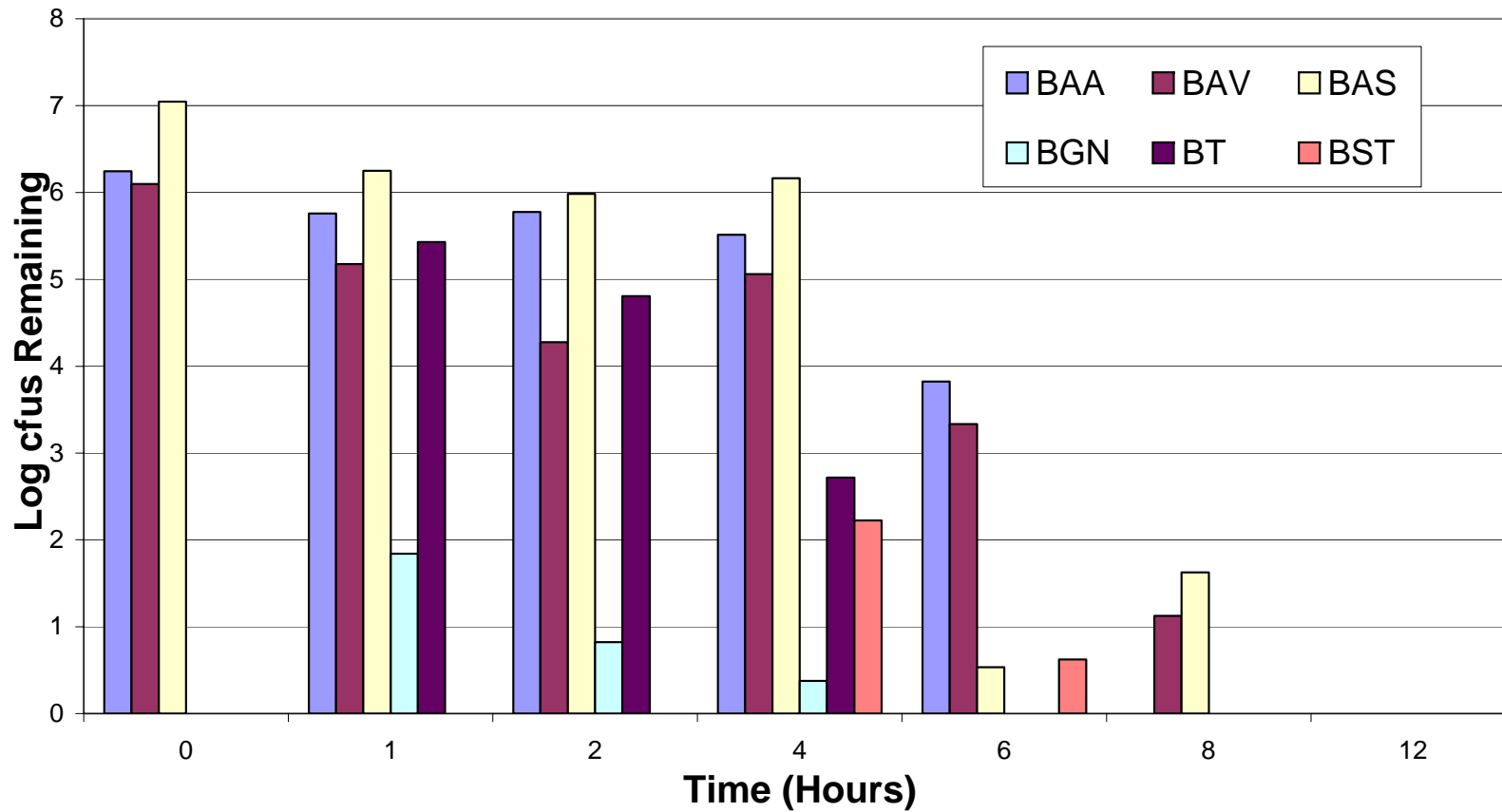


Figure B.19. Log of Colony Forming Units (cfus) Remaining at Each Time Point for Trial 1; Laboratory Validation of Chlorine Dioxide Decontamination.

NOTE: BAA – *Bacillus anthracis* var. *ames*, BAV – *Bacillus anthracis* var. *vollum*, BAS – *Bacillus anthracis* var. *sterne*, BGN – *Bacillus subtilis* var. *niger*, BT – *Bacillus thuringiensis*, BST – *Bacillus stearothermophilus*.

Trial 2 - Relative Humidity 92% - Chlorine Dioxide 730 ppm

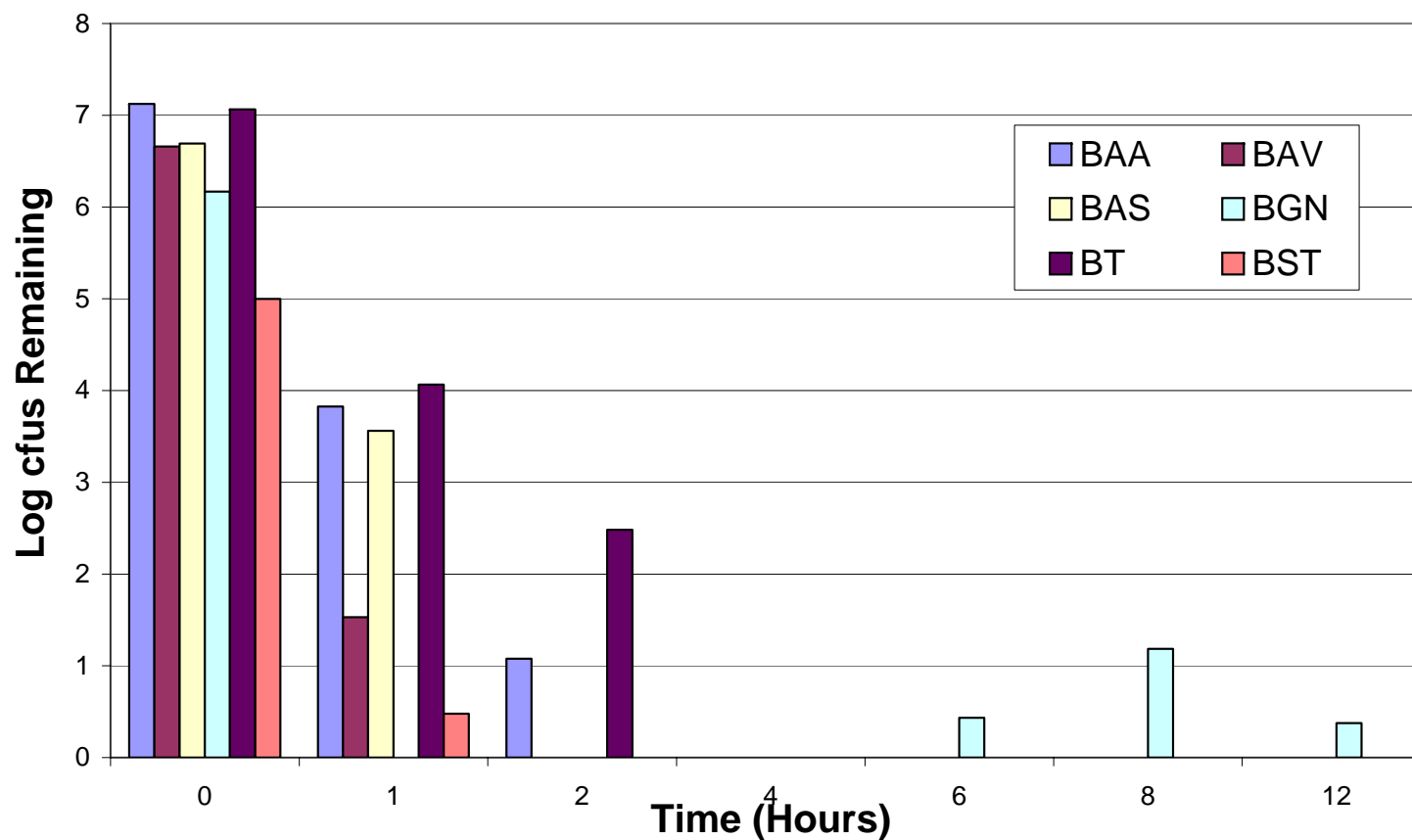


Figure B.20. Log of Colony Forming Units (cfus) Remaining at Each Time Point for Trial 2; Laboratory Validation of Chlorine Dioxide Decontamination.

NOTE: BAA – *Bacillus anthracis* var. *ames*, BAV – *Bacillus anthracis* var. *vollum*, BAS – *Bacillus anthracis* var. *sterne*, BGN – *Bacillus subtilis* var. *niger*, BT – *Bacillus thuringiensis*, BST – *Bacillus stearothermophilus*.

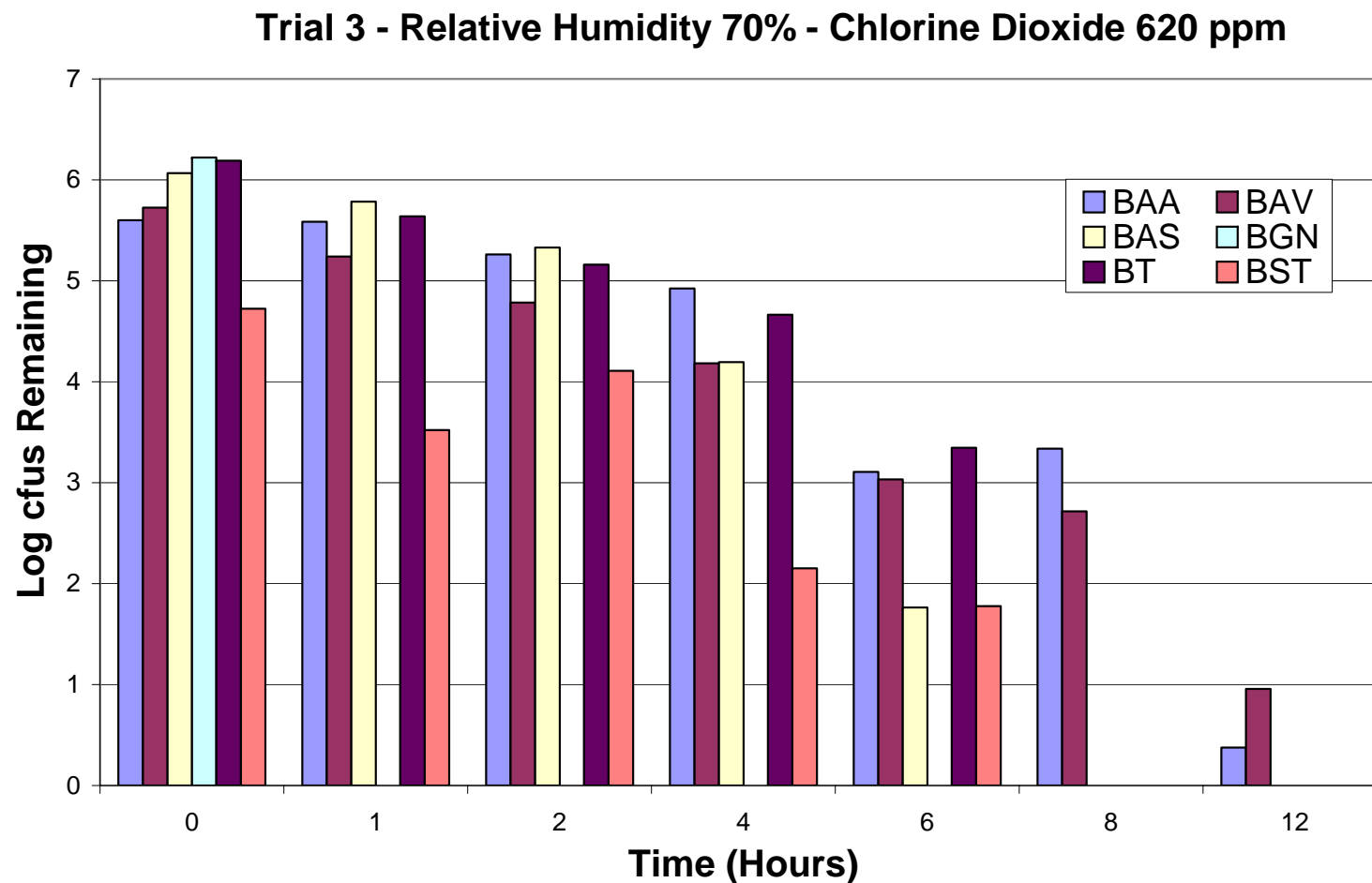


Figure B.21. Log of Colony Forming Units (cfus) Remaining at Each Time Point for Trial 3; Laboratory Validation of Chlorine Dioxide Decontamination.

NOTE: BAA – *Bacillus anthracis* var. *ames*, BAV – *Bacillus anthracis* var. *vollum*, BAS – *Bacillus anthracis* var. *sterne*, BGN – *Bacillus subtilis* var. *niger*, BT – *Bacillus thuringiensis*, BST – *Bacillus stearothermophilus*.

Trial 4 - Relative Humidity 90% - Chlorine Dioxide 610 ppm

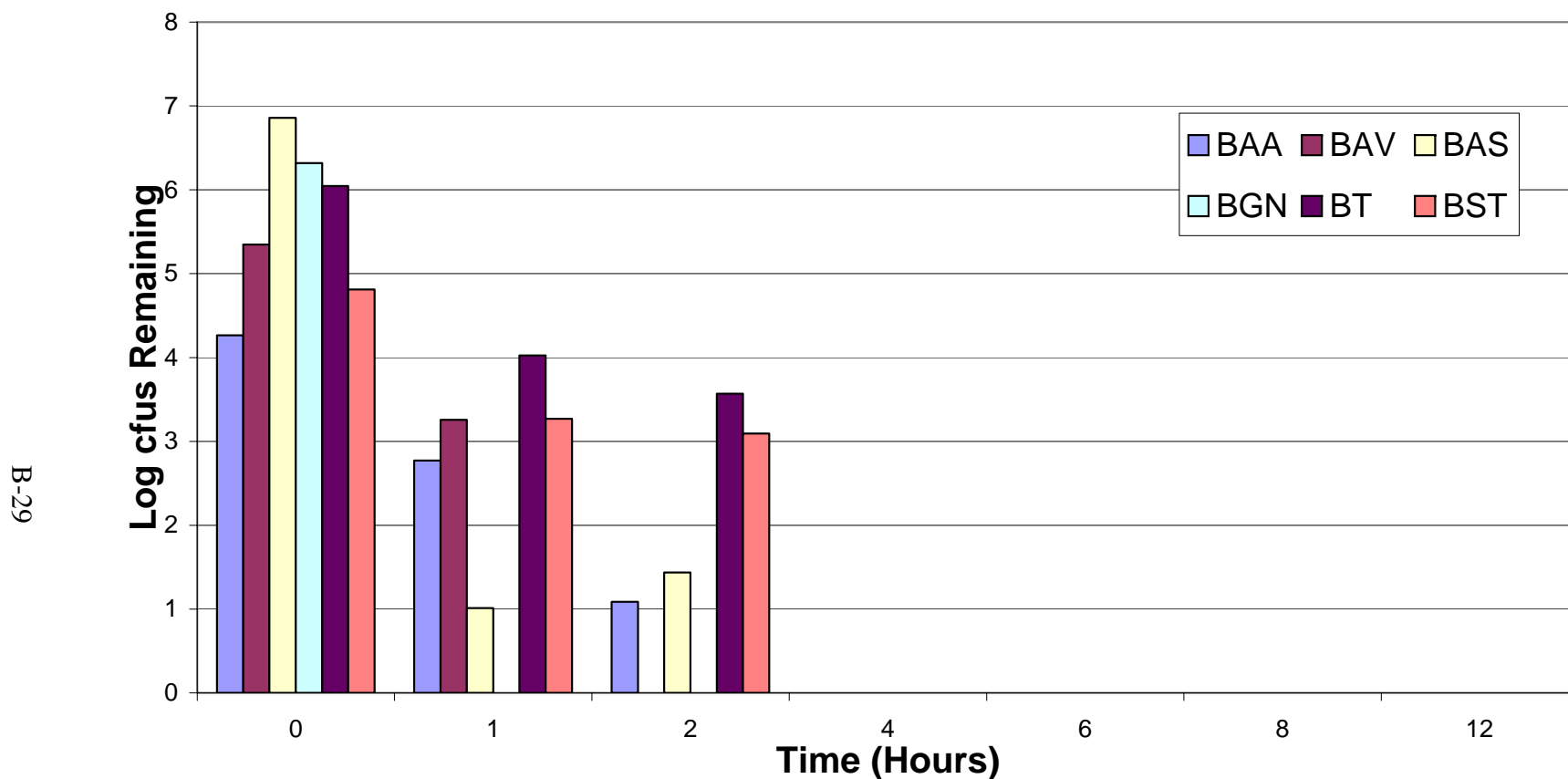


Figure B.22. Log of Colony Forming Units (cfus) Remaining at Each Time Point for Trial 4; Laboratory Validation of Chlorine Dioxide Decontamination.

NOTE: BAA – *Bacillus anthracis* var. *ames*, BAV – *Bacillus anthracis* var. *vollum*, BAS – *Bacillus anthracis* var. *sterne*, BGN – *Bacillus subtilis* var. *niger*, BT – *Bacillus thuringiensis*, BST – *Bacillus stearothermophilus*..

Trial 5 - Relative Humidity 60% - Chlorine Dioxide 613 ppm

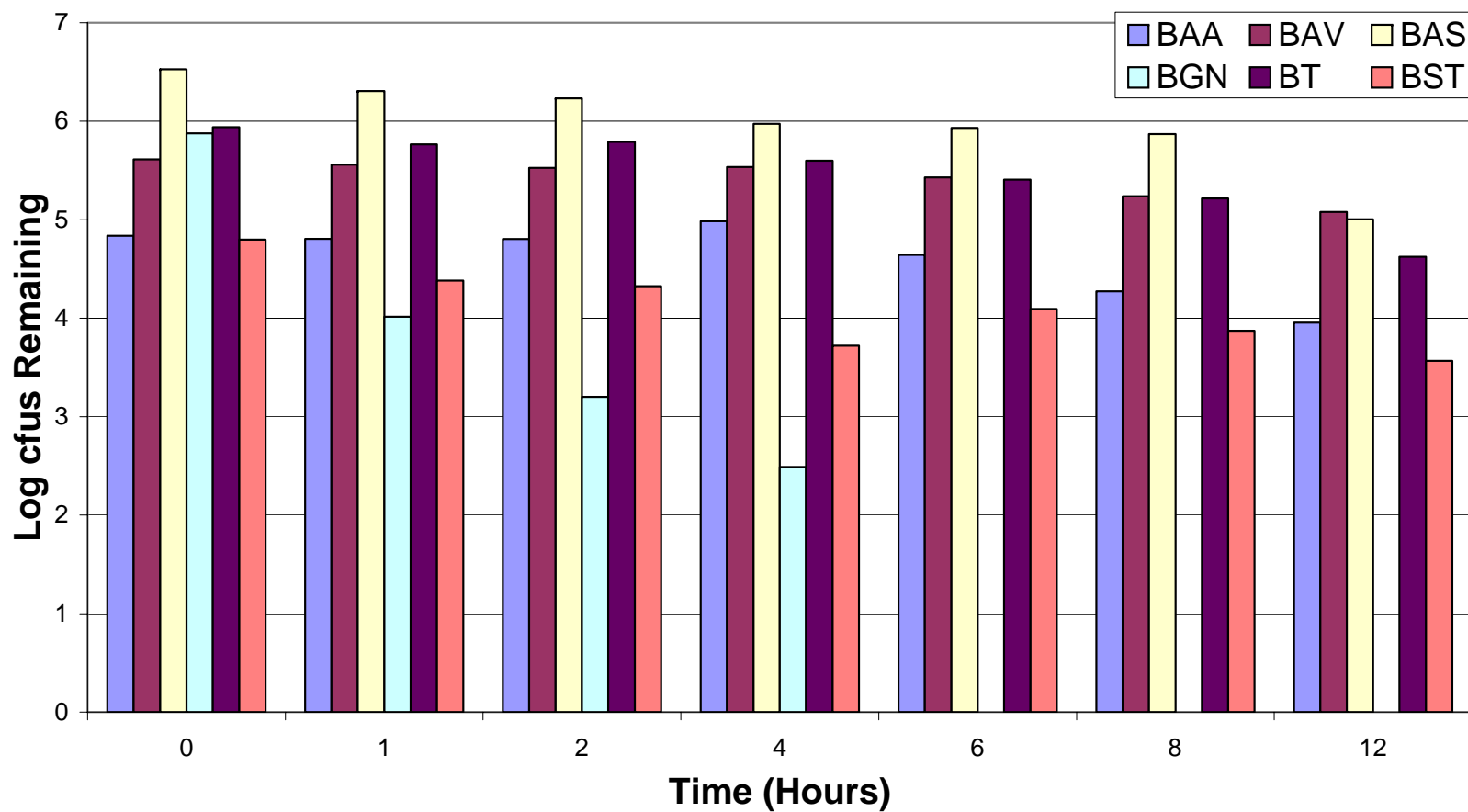


Figure B.23. Log of Colony Forming Units (cfus) Remaining at Each Time Point for Trial 5; Laboratory Validation of Chlorine Dioxide Decontamination.

NOTE: BAA – *Bacillus anthracis* var. *ames*, BAV – *Bacillus anthracis* var. *vollum*, BAS – *Bacillus anthracis* var. *sterne*, BGN – *Bacillus subtilis* var. *niger*, BT – *Bacillus thuringiensis*, BST – *Bacillus stearothermophilus*.

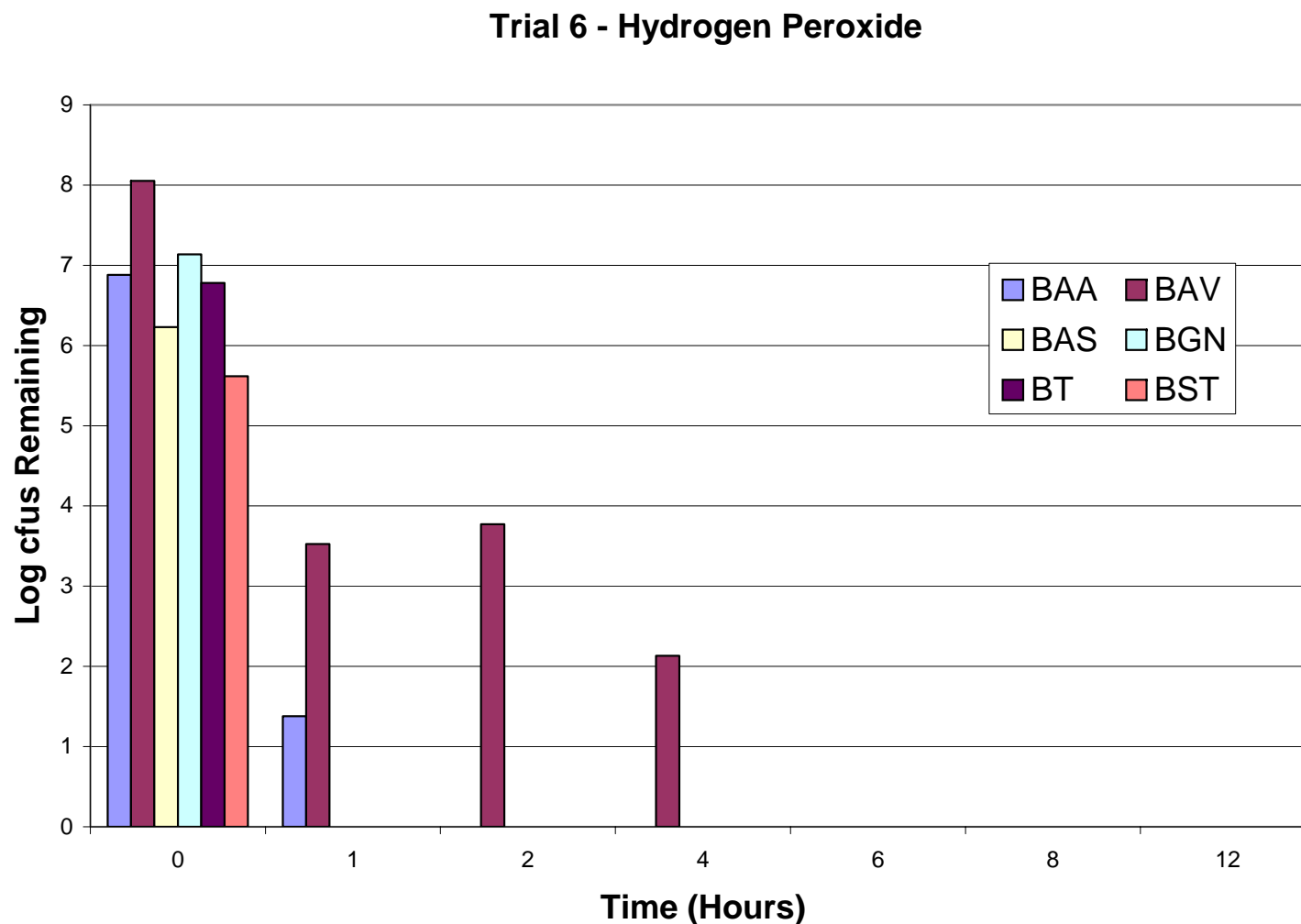


Figure B.24. Log of Colony Forming Units (cfus) Remaining at Each Time Point for Trial 6; Laboratory Validation of Chlorine Dioxide Decontamination.

NOTE: BAA – *Bacillus anthracis* var. *ames*, BAV – *Bacillus anthracis* var. *vollum*, BAS – *Bacillus anthracis* var. *sterne*, BGN – *Bacillus subtilis* var. *niger*, BT – *Bacillus thuringiensis*, BST – *Bacillus stearothermophilus*.

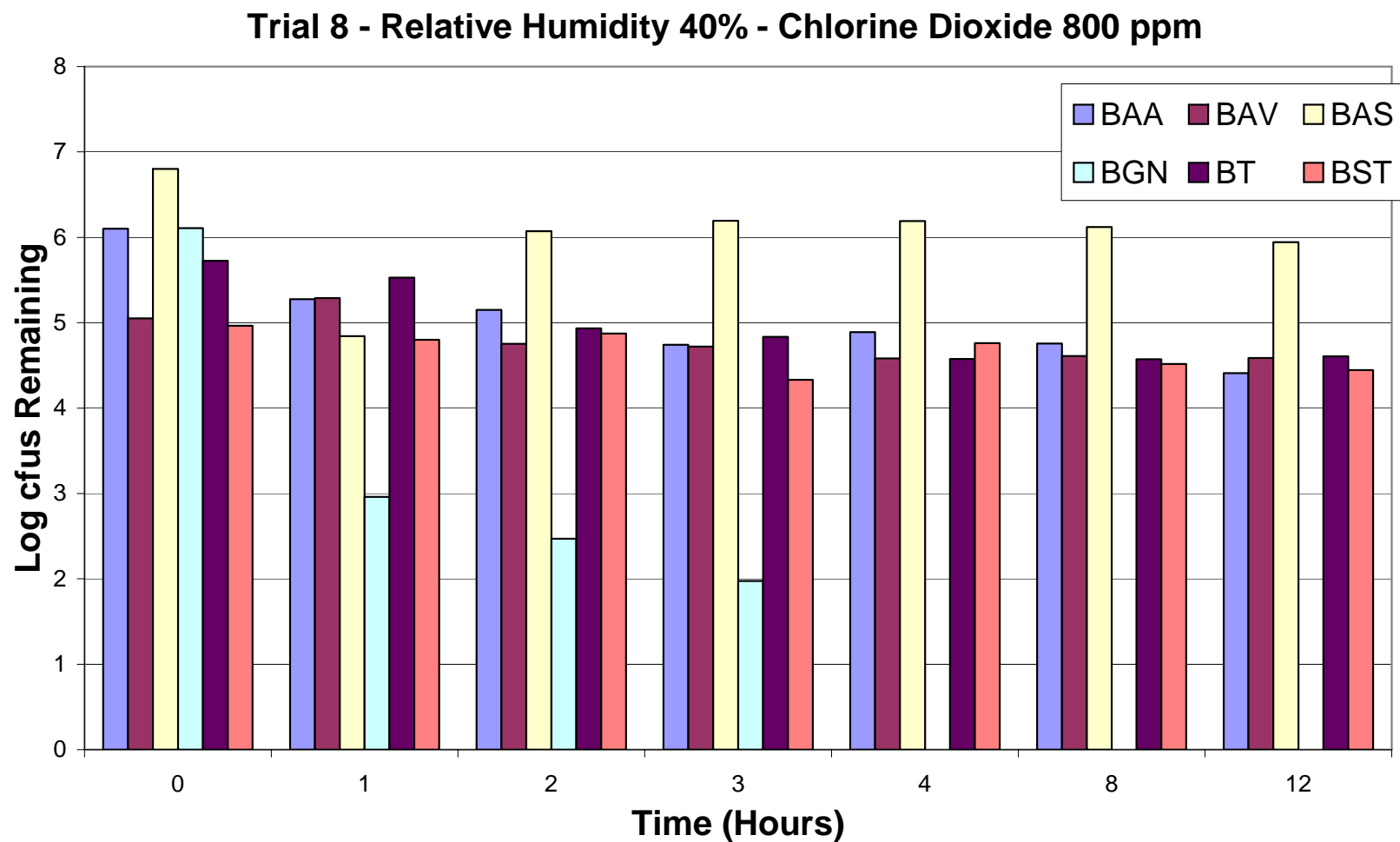


Figure B.25. Log of Colony Forming Units (cfus) Remaining at Each Time Point for Trial 8; Laboratory Validation of Chlorine Dioxide Decontamination.

NOTE: BAA – *Bacillus anthracis* var. *ames*, BAV – *Bacillus anthracis* var. *vollum*, BAS – *Bacillus anthracis* var. *sterne*, BGN – *Bacillus subtilis* var. *niger*, BT – *Bacillus thuringiensis*, BST – *Bacillus stearothermophilus*.

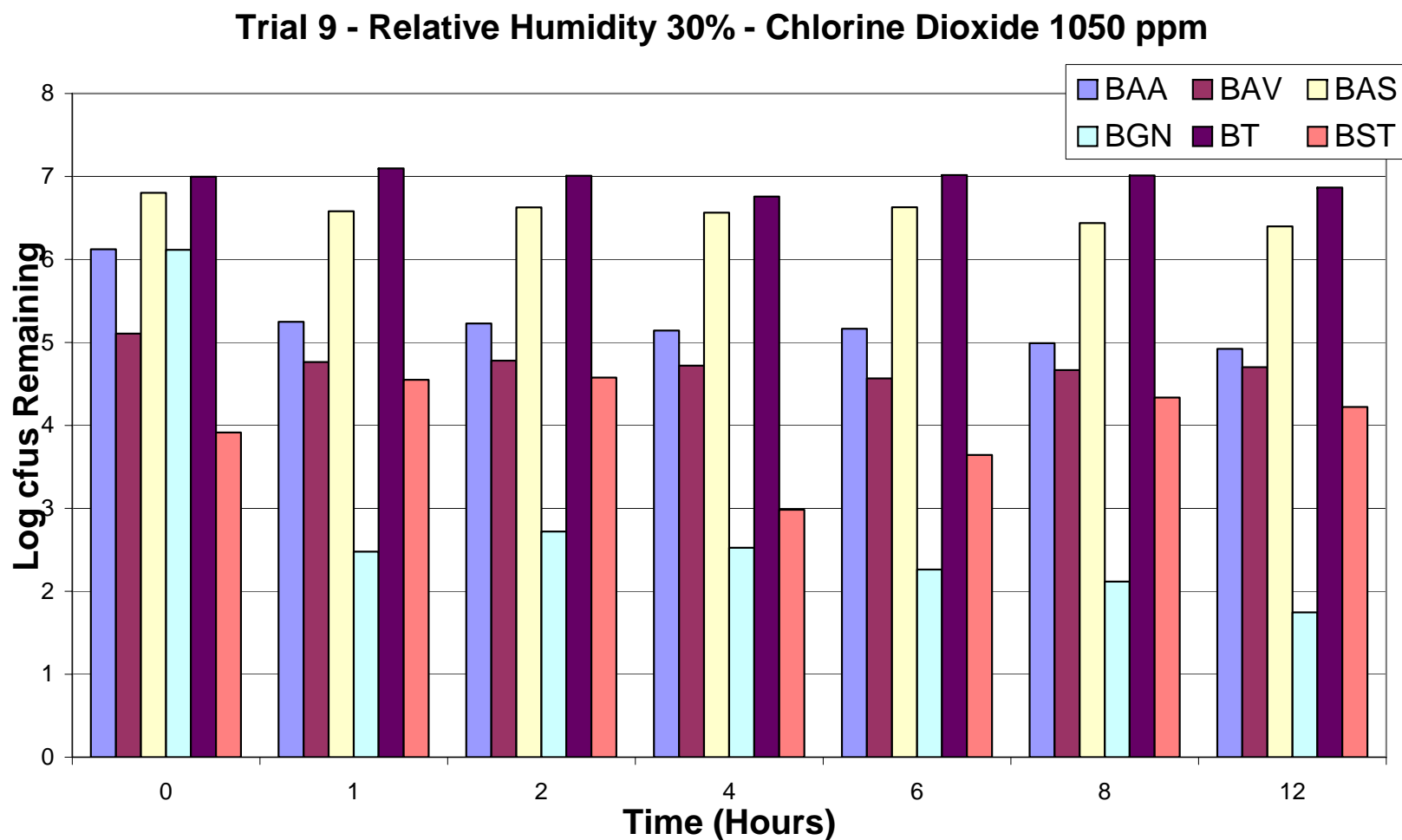


Figure B.26. Log of Colony Forming Units (cfus) Remaining at Each Time Point for Trial 9; Laboratory Validation of Chlorine Dioxide Decontamination.

NOTE: BAA – *Bacillus anthracis* var. *ames*, BAV – *Bacillus anthracis* var. *vollum*, BAS – *Bacillus anthracis* var. *sterne*, BGN – *Bacillus subtilis* var. *niger*, BT – *Bacillus thuringiensis*, BST – *Bacillus stearothermophilus*.

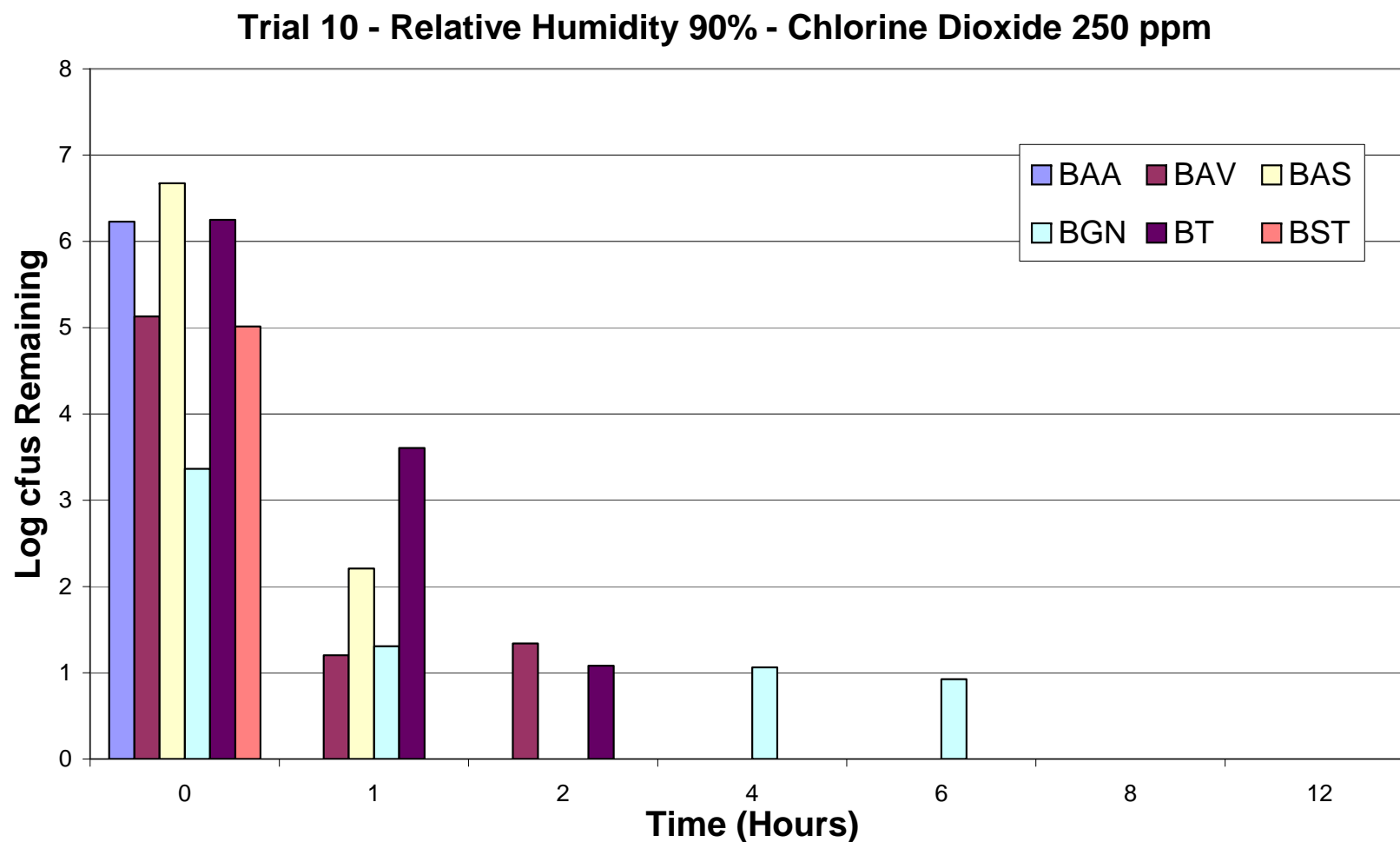


Figure B.27. Log of Colony Forming Units (cfus) Remaining at Each Time Point for Trial 10; Laboratory Validation of Chlorine Dioxide Decontamination.

NOTE: BAA – *Bacillus anthracis* var. *ames*, BAV – *Bacillus anthracis* var. *vollum*, BAS – *Bacillus anthracis* var. *sterne*, BGN – *Bacillus subtilis* var. *niger*, BT – *Bacillus thuringiensis*, BST – *Bacillus stearothermophilus*.

Trial 11 - Relative Humidity 30% - Chlorine Dioxide 250 ppm

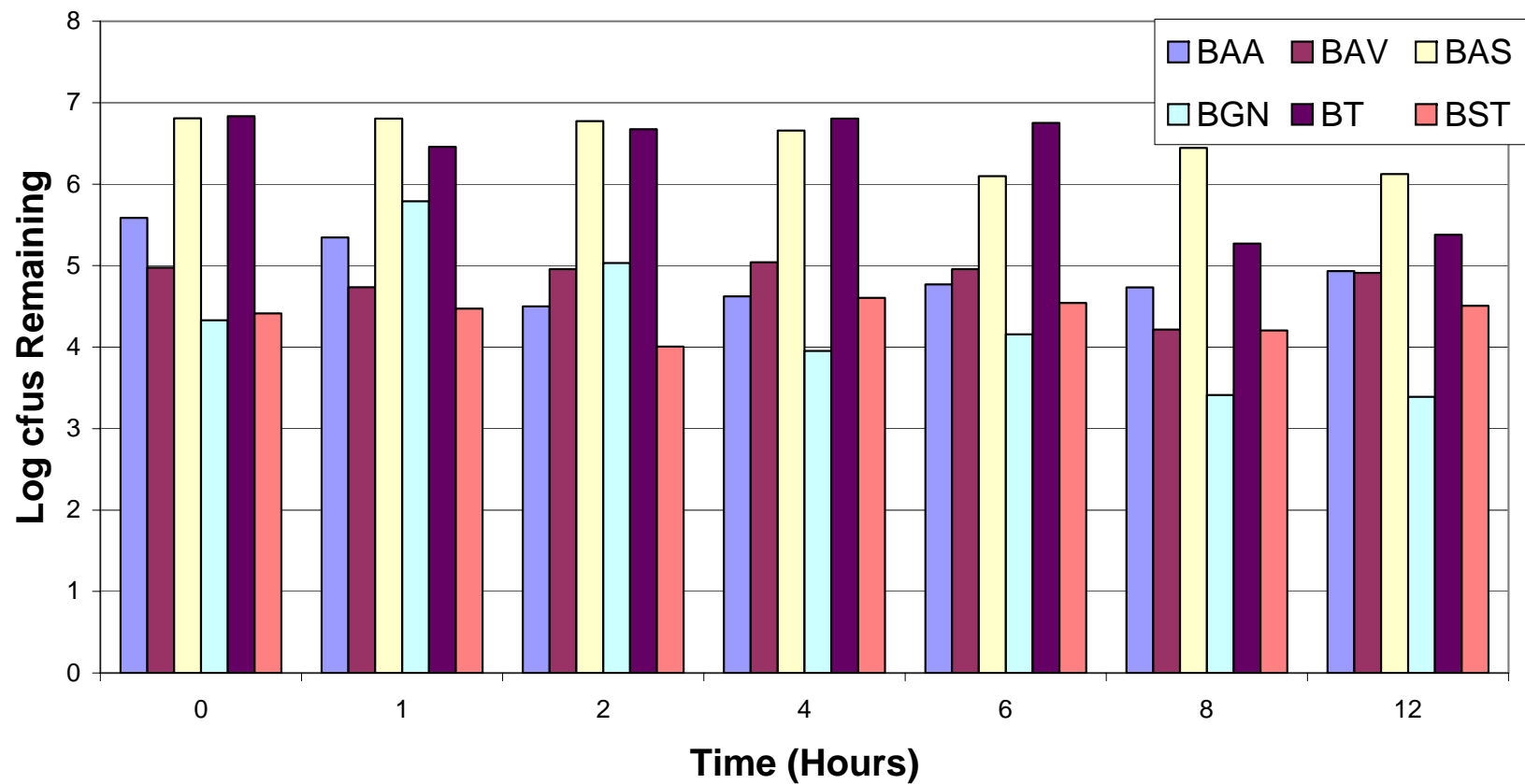


Figure B.28. Log of Colony Forming Units (cfus) Remaining at Each Time Point for Trial 11; Laboratory Validation of Chlorine Dioxide Decontamination.

NOTE: BAA – *Bacillus anthracis* var. *ames*, BAV – *Bacillus anthracis* var. *vollum*, BAS – *Bacillus anthracis* var. *sterne*, BGN – *Bacillus subtilis* var. *niger*, BT – *Bacillus thuringiensis*, BST – *Bacillus stearothermophilus*.

Trial 12 - Relative Humidity 50% - Chlorine Dioxide 250 ppm

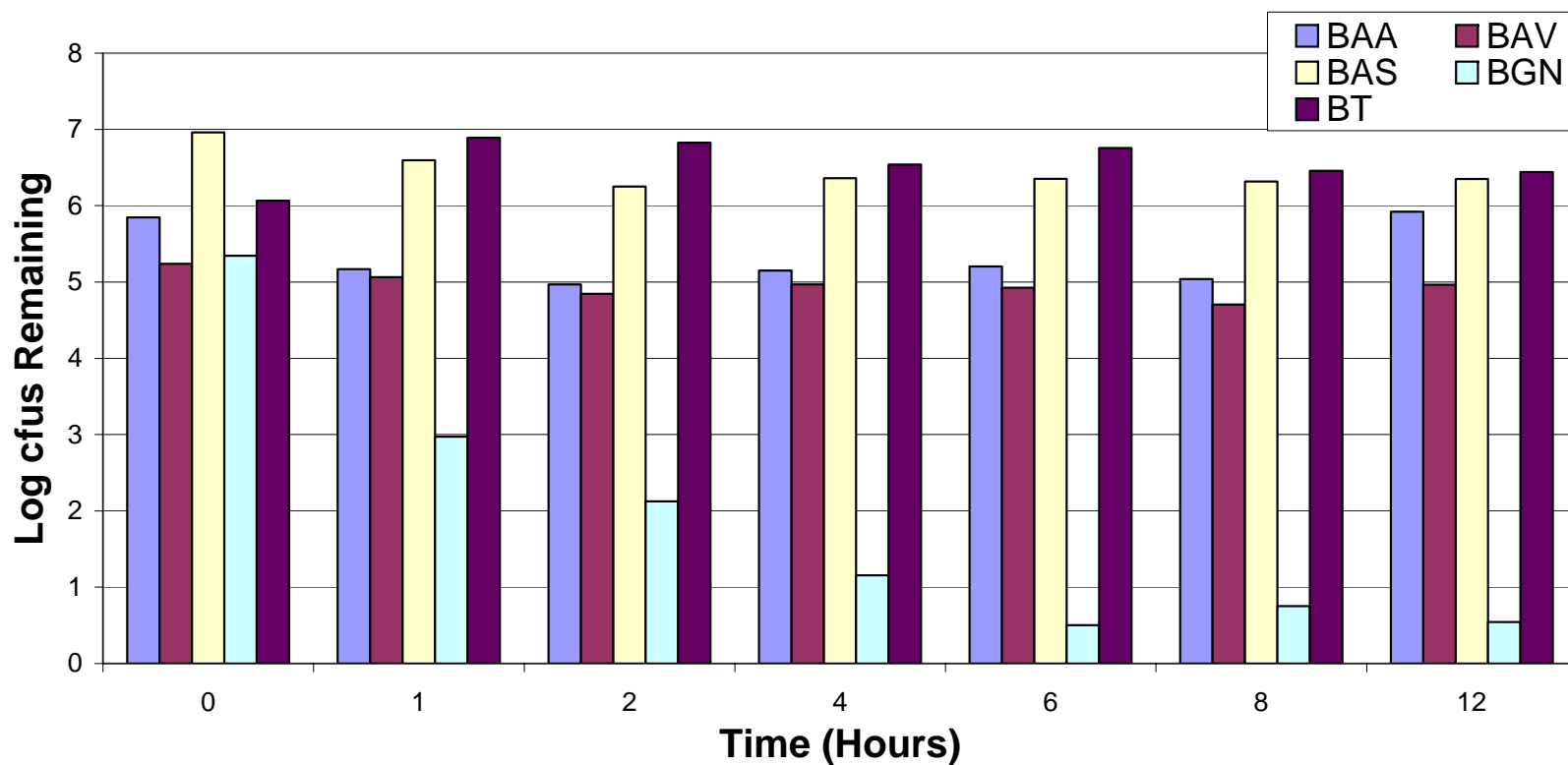


Figure B.29. Log of Colony Forming Units (cfus) Remaining at Each Time Point for Trial 12; Laboratory Validation of Chlorine Dioxide Decontamination.

NOTE: BAA – *Bacillus anthracis* var. *ames*, BAV – *Bacillus anthracis* var. *vollum*, BAS – *Bacillus anthracis* var. *sterne*, BGN – *Bacillus subtilis* var. *niger*, BT – *Bacillus thuringiensis*, BST – *Bacillus stearothermophilus*.

Trial 13 - Relative Humidity 70% - Chlorine Dioxide 250 ppm

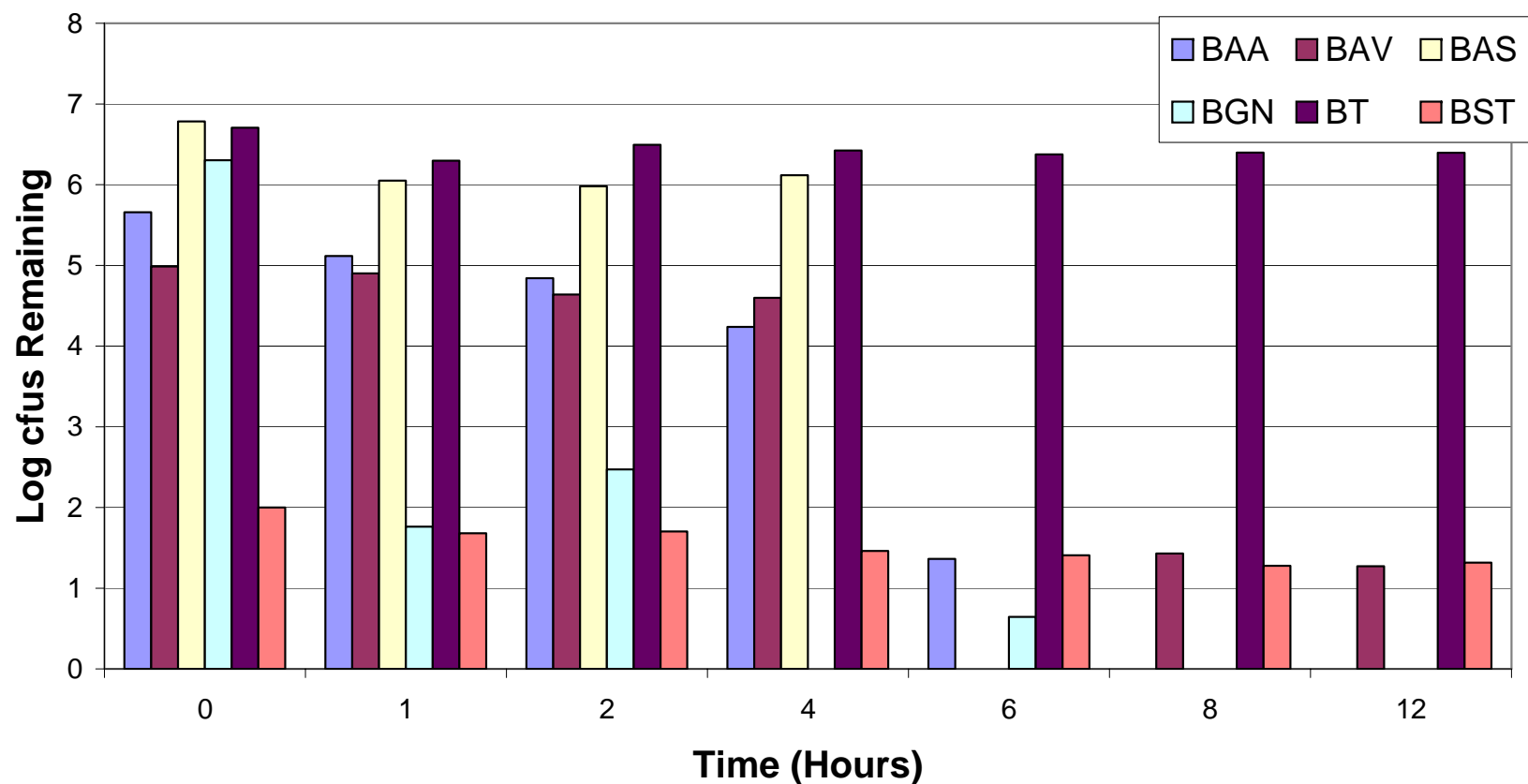


Figure B.30. Log of Colony Forming Units (cfus) Remaining at Each Time Point for Trial 13; Laboratory Validation of Chlorine Dioxide Decontamination.

NOTE: BAA – *Bacillus anthracis* var. *ames*, BAV – *Bacillus anthracis* var. *vollum*, BAS – *Bacillus anthracis* var. *sterne*, BGN – *Bacillus subtilis* var. *niger*, BT – *Bacillus thuringiensis*, BST – *Bacillus stearothermophilus*.

Trial 14 - Relative Humidity 70% - Chlorine Dioxide 125 ppm

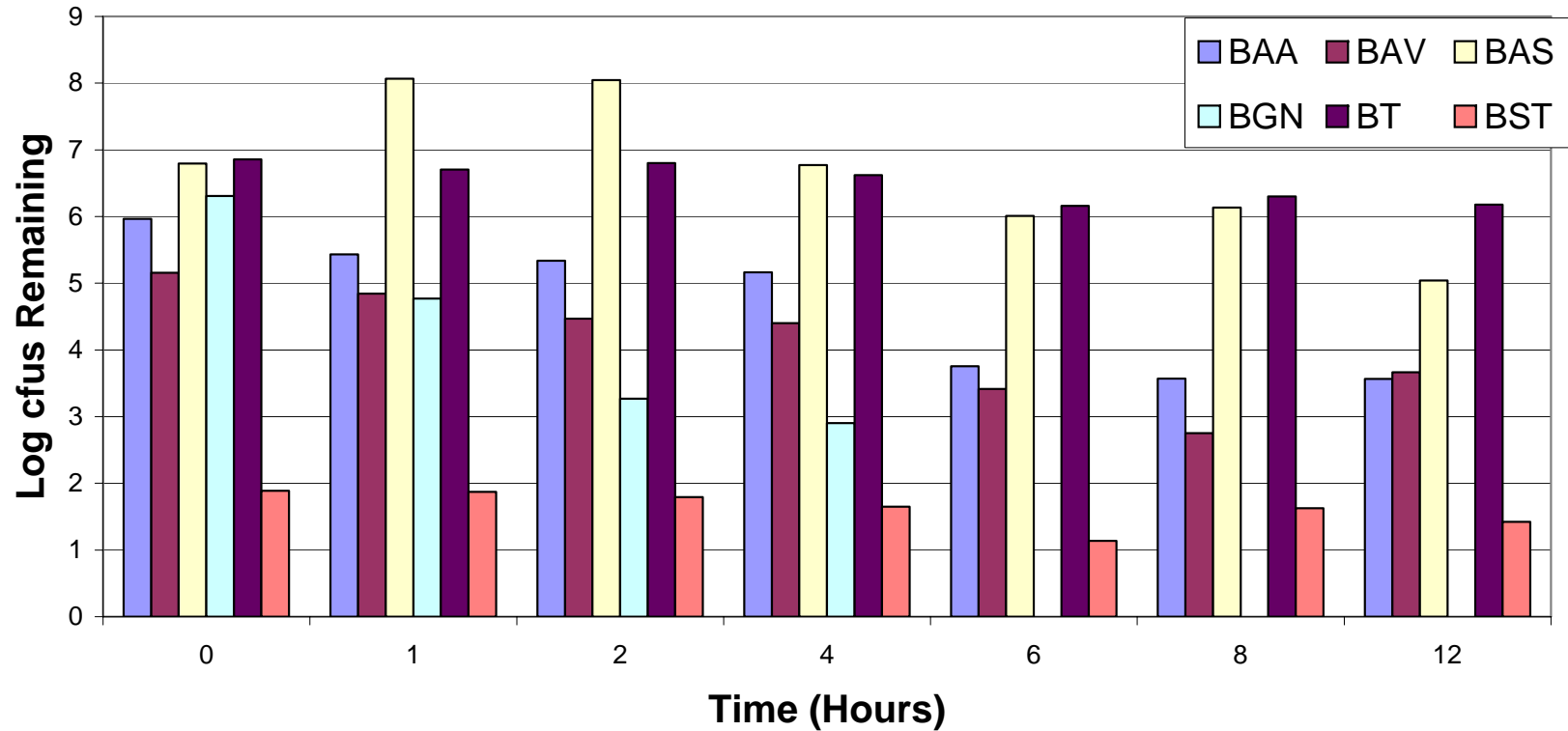


Figure B.31. Log of Colony Forming Units (cfus) Remaining at Each Time Point for Trial 14; Laboratory Validation of Chlorine Dioxide Decontamination.

NOTE: BAA – *Bacillus anthracis* var. *ames*, BAV – *Bacillus anthracis* var. *vollum*, BAS – *Bacillus anthracis* var. *sterne*, BGN – *Bacillus subtilis* var. *niger*, BT – *Bacillus thuringiensis*, BST – *Bacillus stearothermophilus*.

Trial 15 - Relative Humidity 90% - Chlorine Dioxide 125 ppm

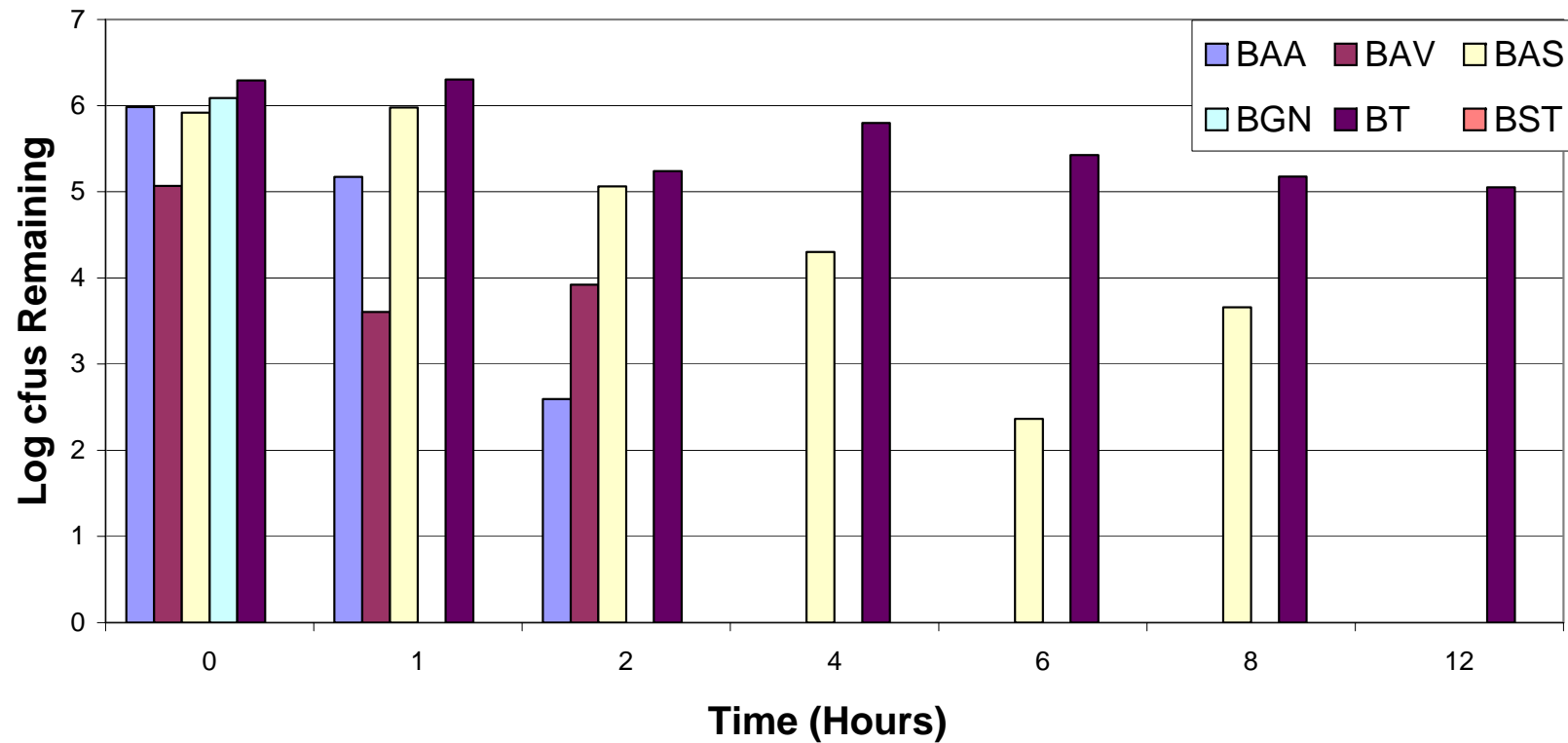


Figure B.32. Log of Colony Forming Units (cfus) Remaining at Each Time Point for Trial 15; Laboratory Validation of Chlorine Dioxide Decontamination.

NOTE: BAA – *Bacillus anthracis* var. *ames*, BAV – *Bacillus anthracis* var. *vollum*, BAS – *Bacillus anthracis* var. *sterne*, BGN – *Bacillus subtilis* var. *niger*, BT – *Bacillus thuringiensis*, BST – *Bacillus stearothermophilus*.

Trial 16 - Relative Humidity 70% - Chlorine Dioxide 750 ppm

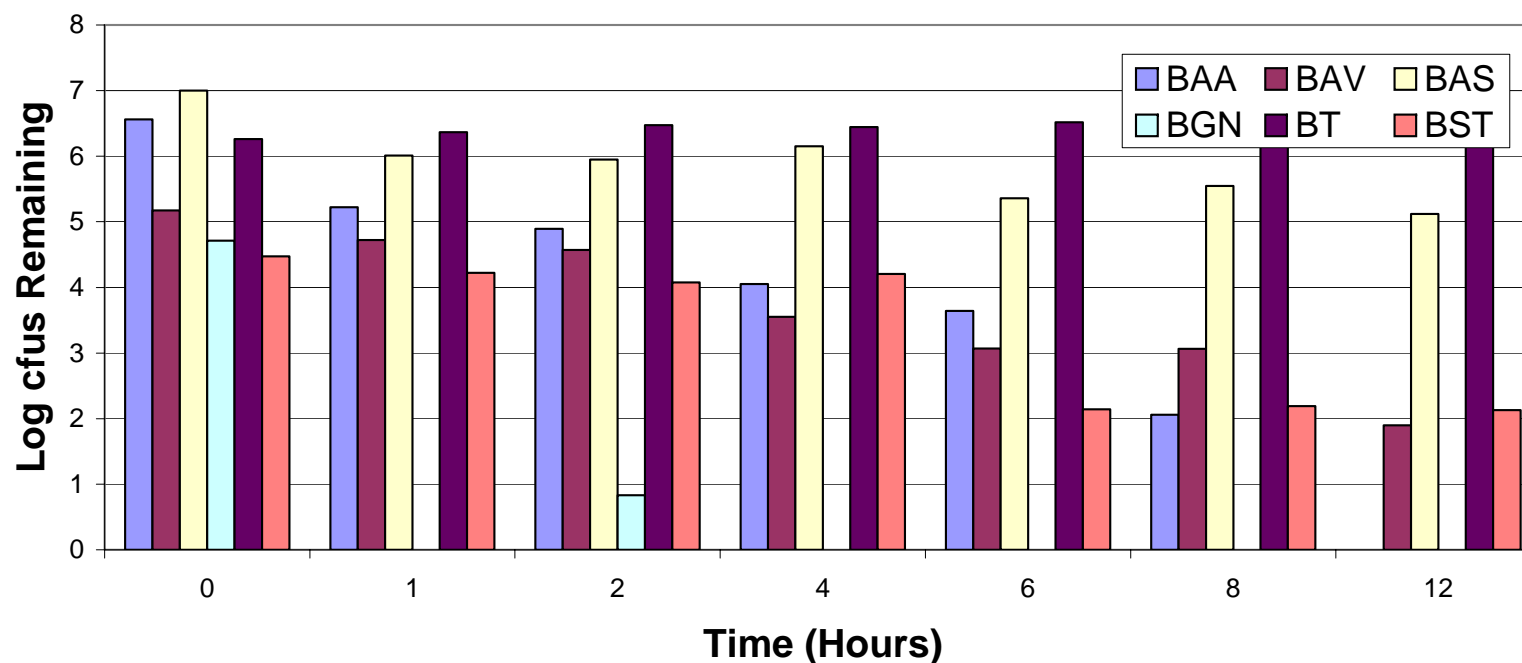


Figure B.33. Log of Colony Forming Units (cfus) Remaining at Each Time Point for Trial 16; Laboratory Validation of Chlorine Dioxide Decontamination.

NOTE: BAA – *Bacillus anthracis* var. *ames*, BAV – *Bacillus anthracis* var. *vollum*, BAS – *Bacillus anthracis* var. *sterne*, BGN – *Bacillus subtilis* var. *niger*, BT – *Bacillus thuringiensis*, BST – *Bacillus stearothermophilus*.

Table B.1. Log Reduction Factors by Trial for *Bacillus anthracis* var. *ames*; Laboratory Validation of Chlorine Dioxide (ClO₂) Decontamination.

| Trial | Relative Humidity (%) | ClO ₂ (ppm) | Log Initial Contamination | Log Reduction Factor | | | | | |
|-------|-----------------------|------------------------|---------------------------|----------------------|-------------|-------------|-------------|-------------|-------------|
| | | | | 1 Hour | 2 Hours | 4 Hours | 6 Hours | 8 Hours | 12 Hours |
| 1 | 75 | 650 | 7.24 | 0.49 | 0.47 | 0.73 | 2.42 | 7.24 | 7.24 |
| 2 | 92 | 730 | 8.12 | 3.30 | 6.05 | 8.12 | 8.12 | 8.12 | 8.12 |
| 3 | 70 | 620 | 6.60 | 0.02 | 0.34 | 0.68 | 2.49 | 2.26 | 5.22 |
| 4 | 90 | 610 | 5.27 | 1.50 | 3.18 | 5.27 | 5.27 | 5.27 | 5.27 |
| 5 | 60 | 613 | 5.84 | 0.03 | 0.03 | -0.15 | 0.19 | 0.56 | 0.88 |
| 8 | 40 | 800 | 7.10 | 0.83 | 0.95 | 1.36 | 1.21 | 1.35 | 1.69 |
| 9 | 30 | 1050 | 7.12 | 0.88 | 0.89 | 0.98 | 0.96 | 1.13 | 1.20 |
| 10 | 90 | 250 | 7.23 | 7.23 | 7.23 | 7.23 | 7.23 | 7.23 | 7.23 |
| 11 | 30 | 250 | 6.59 | 0.24 | 1.08 | 0.96 | 0.81 | 0.85 | 0.65 |
| 12 | 50 | 125 | 6.85 | 0.68 | 0.88 | 0.70 | 0.64 | 0.81 | -0.07 |
| 13 | 70 | 250 | 6.66 | 0.54 | 0.82 | 1.42 | 4.30 | 6.66 | 6.66 |
| 14 | 70 | 125 | 6.96 | 0.53 | 0.62 | 0.80 | 2.21 | 2.40 | 2.40 |
| 15 | 90 | 125 | 6.98 | 0.81 | 3.39 | 6.98 | 6.98 | 6.98 | 6.98 |
| 16 | 70 | 750 | 7.56 | 1.34 | 1.67 | 2.51 | 2.92 | 4.50 | 7.56 |

NOTE: Highlighted reduction factors indicate that there were no detectable spores and that the reduction factor was at the maximum.

Table B.2. Log Reduction Factors by Trial for *Bacillus anthracis* *vollum*; Laboratory Validation of Chlorine Dioxide (ClO₂) Decontamination.

| Trial | Relative Humidity (%) | ClO ₂ (ppm) | Log Initial Contamination | Log Reduction Factor | | | | | |
|-------|-----------------------|------------------------|---------------------------|----------------------|-------------|-------------|-------------|-------------|-------------|
| | | | | 1 Hour | 2 Hours | 4 Hours | 6 Hours | 8 Hours | 12 Hours |
| 1 | 75 | 650 | 7.10 | 0.92 | 1.82 | 1.04 | 2.76 | 4.97 | 7.10 |
| 2 | 92 | 730 | 7.66 | 5.13 | 7.66 | 7.66 | 7.66 | 7.66 | 7.66 |
| 3 | 70 | 620 | 6.72 | 0.48 | 0.94 | 1.54 | 2.69 | 3.01 | 4.77 |
| 4 | 90 | 610 | 6.35 | 2.09 | 6.35 | 6.35 | 6.35 | 6.35 | 6.35 |
| 5 | 60 | 613 | 6.61 | 0.05 | 0.09 | 0.08 | 0.18 | 0.37 | 0.53 |
| 8 | 40 | 800 | 6.05 | -0.24 | 0.30 | 0.33 | 0.47 | 0.44 | 0.46 |
| 9 | 30 | 1050 | 6.11 | 0.34 | 0.33 | 0.39 | 0.54 | 0.44 | 0.41 |
| 10 | 90 | 250 | 6.13 | 3.93 | 3.79 | 6.13 | 6.13 | 6.13 | 6.13 |
| 11 | 30 | 250 | 5.97 | 0.24 | 0.02 | -0.07 | 0.02 | 0.76 | 0.06 |
| 12 | 50 | 125 | 6.24 | 0.18 | 0.39 | 0.27 | 0.31 | 0.53 | 0.27 |
| 13 | 70 | 250 | 5.99 | 0.09 | 0.35 | 0.39 | 4.99 | 3.56 | 3.72 |
| 14 | 70 | 125 | 6.16 | 0.31 | 0.69 | 0.76 | 1.74 | 2.41 | 1.49 |
| 15 | 90 | 125 | 6.07 | 1.46 | 1.15 | 6.07 | 6.07 | 6.07 | 6.07 |
| 16 | 70 | 750 | 6.17 | 0.45 | 0.60 | 1.62 | 2.10 | 2.11 | 3.27 |

NOTE: Highlighted reduction factors indicate that there were no detectable spores and that the reduction factor was at the maximum.

Table B.3. Log Reduction Factors by Trial for *Bacillus anthracis* var. *sterne*; Laboratory Validation of Chlorine Dioxide (ClO₂) Decontamination.

| Trial | Relative Humidity (%) | ClO ₂ (ppm) | Log Initial Contamination | Log Reduction Factor | | | | | |
|-------|-----------------------|------------------------|---------------------------|----------------------|-------------|-------------|-------------|-------------|-------------|
| | | | | 1 Hour | 2 Hours | 4 Hours | 6 Hours | 8 Hours | 12 Hours |
| 1 | 75 | 650 | 8.04 | 0.80 | 1.06 | 0.88 | 6.51 | 5.42 | 8.04 |
| 2 | 92 | 730 | 7.69 | 3.13 | 7.69 | 7.69 | 7.69 | 7.69 | 7.69 |
| 3 | 70 | 620 | 7.06 | 0.28 | 0.73 | 1.87 | 4.30 | 7.06 | 7.06 |
| 4 | 90 | 610 | 7.86 | 5.85 | 5.42 | 7.86 | 7.86 | 7.86 | 7.86 |
| 5 | 60 | 613 | 7.53 | 0.22 | 0.30 | 0.55 | 0.60 | 0.66 | 1.52 |
| 8 | 40 | 800 | 7.80 | 1.96 | 0.73 | 0.61 | 0.61 | 0.68 | 0.86 |
| 9 | 30 | 1050 | 7.80 | 0.22 | 0.18 | 0.24 | 0.17 | 0.36 | 0.41 |
| 10 | 90 | 250 | 7.67 | 4.47 | 6.67 | 6.67 | 6.67 | 6.67 | 7.67 |
| 11 | 30 | 250 | 7.81 | 0.00 | 0.03 | 0.15 | 0.71 | 0.36 | 0.69 |
| 12 | 50 | 125 | 7.96 | 0.37 | 0.71 | 0.60 | 0.61 | 0.64 | 0.61 |
| 13 | 70 | 250 | 7.78 | 0.73 | 0.80 | 0.67 | 7.78 | 7.78 | 7.78 |
| 14 | 70 | 125 | 7.79 | -1.27 | -1.25 | 0.02 | 0.79 | 0.66 | 1.75 |
| 15 | 90 | 125 | 6.92 | -0.06 | 0.85 | 1.62 | 3.55 | 2.26 | 6.92 |
| 16 | 70 | 750 | 8.00 | 0.99 | 1.05 | 0.85 | 1.64 | 1.45 | 1.88 |

NOTE: Highlighted reduction factors indicate that there were no detectable spores and that the reduction factor was at the maximum.

Table B.4. Log Reduction Factors by Trial for *Bacillus subtilis* var. *niger*; Laboratory Validation of Chlorine Dioxide (ClO₂) Decontamination.

| Trial | Relative Humidity (%) | ClO ₂ (ppm) | Log Initial Contamination | Log Reduction Factor | | | | | |
|-------|-----------------------|------------------------|---------------------------|----------------------|---------|---------|---------|---------|----------|
| | | | | 1 Hour | 2 Hours | 4 Hours | 6 Hours | 8 Hours | 12 Hours |
| 1 | 75 | 650 | No Data | No Data | No Data | No Data | No Data | No Data | No Data |
| 2 | 92 | 730 | 7.17 | 7.17 | 7.17 | 7.17 | 5.73 | 4.98 | 5.79 |
| 3 | 70 | 620 | 7.22 | 7.22 | 7.22 | 7.22 | 7.22 | 7.22 | 7.22 |
| 4 | 90 | 610 | 7.32 | 7.32 | 7.32 | 7.32 | 7.32 | 7.32 | 7.32 |
| 5 | 60 | 613 | 6.88 | 1.86 | 2.67 | 3.39 | 6.88 | 6.88 | 6.88 |
| 8 | 40 | 800 | 7.11 | 3.15 | 3.64 | 4.13 | 7.11 | 7.11 | 7.11 |
| 9 | 30 | 1050 | 7.12 | 3.64 | 3.40 | 3.59 | 3.86 | 4.00 | 4.37 |
| 10 | 90 | 250 | 4.36 | 2.06 | 4.36 | 2.30 | 2.44 | 4.36 | 4.36 |
| 11 | 30 | 250 | 5.33 | -1.46 | -0.70 | 0.38 | 0.17 | 0.92 | 0.94 |
| 12 | 50 | 125 | 6.34 | 2.37 | 3.22 | 4.19 | 4.84 | 4.59 | 4.80 |
| 13 | 70 | 250 | 7.31 | 4.54 | 3.83 | 7.31 | 5.66 | 7.31 | 7.31 |
| 14 | 70 | 125 | 7.31 | 1.54 | 3.04 | 3.41 | 7.31 | 7.31 | 7.31 |
| 15 | 90 | 125 | 7.09 | 7.09 | 7.09 | 7.09 | 7.09 | 7.09 | 7.09 |
| 16 | 70 | 750 | 5.71 | 5.71 | 3.88 | 5.71 | 5.71 | 5.71 | 5.71 |

NOTE: Highlighted reduction factors indicate that there were no detectable spores and that the reduction factor was at the maximum.

Table B.5. Log Reduction Factors by Trial for *Bacillus thuringiensis*; Laboratory Validation of Chlorine Dioxide (ClO₂) Decontamination.

| Trial | Relative Humidity (%) | ClO ₂ (ppm) | Log Initial Contamination | Log Reduction Factor | | | | | |
|-------|-----------------------|------------------------|---------------------------|----------------------|---------|-------------|-------------|-------------|-------------|
| | | | | 1 Hour | 2 Hours | 4 Hours | 6 Hours | 8 Hours | 12 Hours |
| 1 | 75 | 650 | No Data | No Data | No Data | No Data | No Data | No Data | No Data |
| 2 | 92 | 730 | 8.06 | 3.00 | 4.58 | 8.06 | 8.06 | 8.06 | 8.06 |
| 3 | 70 | 620 | 7.19 | 0.55 | 1.03 | 1.53 | 2.84 | 7.19 | 7.19 |
| 4 | 90 | 610 | 7.05 | 2.02 | 2.48 | 7.05 | 7.05 | 7.05 | 7.05 |
| 5 | 60 | 613 | 6.94 | 0.17 | 0.15 | 0.34 | 0.53 | 0.73 | 1.32 |
| 8 | 40 | 800 | 6.72 | 0.20 | 0.79 | 0.89 | 1.15 | 1.15 | 1.12 |
| 9 | 30 | 1050 | 8.00 | -0.10 | -0.01 | 0.24 | -0.02 | -0.01 | 0.13 |
| 10 | 90 | 250 | 7.25 | 2.64 | 5.17 | 7.25 | 7.25 | 7.25 | 7.25 |
| 11 | 30 | 250 | 7.83 | 0.38 | 0.16 | 0.03 | 0.08 | 1.56 | 1.46 |
| 12 | 50 | 125 | 7.06 | -0.83 | -0.76 | -0.47 | -0.69 | -0.39 | -0.38 |
| 13 | 70 | 250 | 7.71 | 0.41 | 0.21 | 0.28 | 0.33 | 0.31 | 0.31 |
| 14 | 70 | 125 | 7.86 | 0.15 | 0.06 | 0.24 | 0.70 | 0.56 | 0.68 |
| 15 | 90 | 125 | 7.29 | -0.01 | 1.05 | 0.49 | 0.86 | 1.11 | 1.24 |
| 16 | 70 | 750 | 7.26 | -0.11 | -0.21 | -0.18 | -0.26 | -0.36 | -0.27 |

NOTE: Highlighted reduction factors indicate that there were no detectable spores and that the reduction factor was at the maximum.

Table B.6. Log Reduction Factors by Trial for *Bacillus stearothermophilus*; Laboratory Validation of Chlorine Dioxide (ClO₂) Decontamination.

| Trial | Relative Humidity (%) | ClO ₂ (ppm) | Log Initial Contamination | Log Reduction Factor | | | | | |
|-------|-----------------------|------------------------|---------------------------|----------------------|-------------|-------------|-------------|-------------|-------------|
| | | | | 1 Hour | 2 Hours | 4 Hours | 6 Hours | 8 Hours | 12 Hours |
| 1 | 75 | 650 | No Data | No Data | No Data | No Data | No Data | No Data | No Data |
| 2 | 92 | 730 | 6.00 | 4.52 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 |
| 3 | 70 | 620 | 5.72 | 1.20 | 0.62 | 2.57 | 2.95 | 5.72 | 5.72 |
| 4 | 90 | 610 | 5.81 | 1.54 | 1.72 | 5.81 | 5.81 | 5.81 | 5.81 |
| 5 | 60 | 613 | 5.80 | 0.42 | 0.47 | 1.08 | 0.71 | 0.93 | 1.23 |
| 8 | 40 | 800 | 5.96 | 0.16 | 0.09 | 0.63 | 0.20 | 0.45 | 0.52 |
| 9 | 30 | 1050 | 4.91 | -0.64 | -0.66 | 0.93 | 0.27 | -0.42 | -0.31 |
| 10 | 90 | 250 | 6.01 | 6.01 | 6.01 | 6.01 | 6.01 | 6.01 | 6.01 |
| 11 | 30 | 250 | 5.41 | -0.06 | 0.41 | -0.19 | -0.13 | 0.21 | -0.09 |
| 12 | 50 | 125 | No Data | No Data | No Data | No Data | No Data | No Data | No Data |
| 13 | 70 | 250 | 3.00 | 0.32 | 0.30 | 0.54 | 0.59 | 0.72 | 0.68 |
| 14 | 70 | 125 | 2.89 | 0.02 | 0.09 | 0.24 | 0.75 | 0.26 | 0.46 |
| 15 | 90 | 125 | No Data | No Data | No Data | No Data | No Data | No Data | No Data |
| 16 | 70 | 750 | 5.48 | 0.25 | 0.40 | 0.27 | 2.33 | 2.28 | 2.34 |

NOTE: Highlighted reduction factors indicate that there were no detectable spores and that the reduction factor was at the maximum.

Table B.7. Log Reduction Factors by Agent/Simulant for Hydrogen Peroxide; Laboratory Validation of Chlorine Dioxide (ClO₂) Decontamination.

| Agent/Simulant | Log Initial Contamination | Log Reduction Factor | | | | | |
|--|---------------------------|----------------------|-------------|-------------|-------------|-------------|-------------|
| | | 1 Hour | 2 Hours | 4 Hours | 6 Hours | 8 Hours | 12 Hours |
| <i>Bacillus anthracis</i> var. <i>ames</i> (BAA) | 6.88 | 5.50 | 6.88 | 6.88 | 6.88 | 6.88 | 6.88 |
| <i>Bacillus anthracis</i> var. <i>vollum</i> (BAV) | 8.05 | 4.53 | 5.62 | 5.92 | 8.05 | 8.05 | 8.05 |
| <i>Bacillus anthracis</i> var. <i>sterne</i> (BAS) | 6.23 | 6.23 | 6.23 | 6.23 | 6.23 | 6.23 | 6.23 |
| <i>Bacillus subtilis</i> var. <i>niger</i> (BGN) | 7.14 | 7.14 | 7.14 | 7.14 | 7.14 | 7.14 | 7.14 |
| <i>Bacillus thuringiensis</i> (BT) | 6.78 | 6.78 | 6.78 | 6.78 | 6.78 | 6.78 | 6.78 |
| <i>Bacillus stearothermophilus</i> (BST) | 5.62 | 5.62 | 5.62 | 5.62 | 5.62 | 5.62 | 5.62 |

NOTE: Highlighted reduction factors indicate that there were no detectable spores and that the reduction factor was at the maximum.

Table B.8. Detectable *Bacillus anthracis* var. *ames* (BAA) After 12 Hours by Relative Humidity (RH); Laboratory Validation of Chlorine Dioxide Decontamination.

| RH Range (%) | Chlorine Dioxide Range (ppm) | Number of Trials | Percentage with Detectable BAA (%) |
|--------------|------------------------------|------------------|------------------------------------|
| 30-60 | 250-1050 | 5 | 100 |
| 70-75 | 125-750 | 5 | 40 |
| 90-92 | 125-730 | 4 | 0 |

Table B.9. Detectable *Bacillus anthracis* var. *vollum* (BAV) After 12 Hours by Relative Humidity (RH); Laboratory Validation of Chlorine Dioxide Decontamination.

| RH Range (%) | Chlorine Dioxide Range (ppm) | Number of Trials | Percentage with Detectable BAV (%) |
|--------------|------------------------------|------------------|------------------------------------|
| 30-60 | 250-1050 | 5 | 100 |
| 70-75 | 125-750 | 5 | 80 |
| 90-92 | 125-730 | 4 | 0 |

Table B.10. Detectable *Bacillus anthracis* var. *sterne* (BAS) After 12 Hours by Relative Humidity (RH); Laboratory Validation of Chlorine Dioxide Decontamination.

| RH Range (%) | Chlorine Dioxide Range (ppm) | Number of Trials | Percentage with Detectable BAS (%) |
|--------------|------------------------------|------------------|------------------------------------|
| 30-60 | 250-1050 | 5 | 80 |
| 70-75 | 125-750 | 5 | 20 |
| 90-92 | 125-730 | 4 | 0 |

Table B.11. Detectable *Bacillus subtilis* var. *niger* (BGN) After 12 Hours by Relative Humidity (RH); Laboratory Validation of Chlorine Dioxide Decontamination.

| RH Range (%) | Chlorine Dioxide Range (ppm) | Number of Trials | Percentage with Detectable BGN (%) |
|--------------|------------------------------|------------------|------------------------------------|
| 30-60 | 250-1050 | 5 | 80 |
| 70-75 | 125-750 | 5 | 0 |
| 90-92 | 125-730 | 4 | 25 |

Table B.12. Detectable *Bacillus thuringiensis* (BT) After 12 Hours by Relative Humidity (RH); Laboratory Validation of Chlorine Dioxide Decontamination.

| RH Range (%) | Chlorine Dioxide Range (ppm) | Number of Trials | Percentage with Detectable BT (%) |
|--------------|------------------------------|------------------|-----------------------------------|
| 30-60 | 250-1050 | 5 | 100 |
| 70-75 | 125-750 | 5 | 60 |
| 90-92 | 125-730 | 4 | 25 |

Table B.13. Detectable *Bacillus stearothermophilus* (BST) After 12 Hours by Relative Humidity (RH); Laboratory Validation of Chlorine Dioxide Decontamination.

| RH Range (%) | Chlorine Dioxide Range (ppm) | Number of Trials | Percentage with Detectable BST (%) |
|--------------|------------------------------|------------------|------------------------------------|
| 30-60 | 250-1050 | 5 | 100 |
| 70-75 | 125-750 | 5 | 60 |
| 90-92 | 125-730 | 4 | 25 |

Table B.14. Detectable *Bacillus anthracis* var. *ames* (BAA) After 12 Hours by Chlorine Dioxide Concentration (ppm); Laboratory Validation of Chlorine Dioxide Decontamination.

| Chlorine Dioxide Range (ppm) | Relative Humidity Range (%) | Number of Trials | Percentage with Detectable BAA (%) |
|------------------------------|-----------------------------|------------------|------------------------------------|
| 125-250 | 70-90 | 6 | 50 |
| 610-650 | 60-90 | 4 | 50 |
| 730-1050 | 30-92 | 4 | 50 |

Table B.15. Detectable *Bacillus anthracis* var. *vollum* (BAV) After 12 Hours by Chlorine Dioxide Concentration (ppm); Laboratory Validation of Chlorine Dioxide Decontamination.

| Chlorine Dioxide Range (ppm) | Relative Humidity Range (%) | Number of Trials | Percentage with Detectable BAV (%) |
|------------------------------|-----------------------------|------------------|------------------------------------|
| 125-250 | 70-90 | 6 | 80 |
| 610-650 | 60-90 | 4 | 50 |
| 730-1050 | 30-92 | 4 | 75 |

Table B.16. Detectable *Bacillus anthracis* var. *sterne* (BAS) After 12 Hours by Chlorine Dioxide Concentration (ppm); Laboratory Validation of Chlorine Dioxide Decontamination.

| Chlorine Dioxide Range (ppm) | Relative Humidity Range (%) | Number of Trials | Percentage with Detectable BAS (%) |
|------------------------------|-----------------------------|------------------|------------------------------------|
| 125-250 | 70-90 | 6 | 40 |
| 610-650 | 60-90 | 4 | 25 |
| 730-1050 | 30-92 | 4 | 50 |

Table B.17. Detectable *Bacillus subtilis* var. *niger* (BGN) After 12 Hours by Chlorine Dioxide Concentration (ppm); Laboratory Validation of Chlorine Dioxide Decontamination.

| Chlorine Dioxide Range (ppm) | Relative Humidity Range (%) | Number of Trials | Percentage with Detectable BGN (%) |
|------------------------------|-----------------------------|------------------|------------------------------------|
| 125-250 | 70-90 | 6 | 40 |
| 610-650 | 60-90 | 4 | 0 |
| 730-1050 | 30-92 | 4 | 75 |

Table B.18. Detectable *Bacillus thuringiensis* (BT) After 12 Hours by Chlorine Dioxide Concentration (ppm); Laboratory Validation of Chlorine Dioxide Decontamination.

| Chlorine Dioxide Range (ppm) | Relative Humidity Range (%) | Number of Trials | Percentage with Detectable BT (%) |
|------------------------------|-----------------------------|------------------|-----------------------------------|
| 125-250 | 70-90 | 6 | 80 |
| 610-650 | 60-90 | 4 | 25 |
| 730-1050 | 30-92 | 4 | 75 |

Table B.19. Detectable *Bacillus stearothermophilus* (BST) After 12 Hours by Chlorine Dioxide Concentration (ppm); Laboratory Validation of Chlorine Dioxide Decontamination.

| Chlorine Dioxide Range (ppm) | Relative Humidity Range (%) | Number of Trials | Percentage with Detectable BST (%) |
|------------------------------|-----------------------------|------------------|------------------------------------|
| 125-250 | 70-90 | 6 | 80 |
| 610-650 | 60-90 | 4 | 25 |
| 730-1050 | 30-92 | 4 | 75 |

Table B.20. Analysis of Variance Table Including Agent; Laboratory Validation of Chlorine Dioxide Decontamination.

| Source | DF ^a | Sum of Squares | Mean Square Error | F-value | P-value |
|-----------------|-----------------|----------------|-------------------|---------|---------|
| Agent | 5 | 170055 | 34011 | 58.43 | 0.000 |
| Time | 1 | 43303 | 43303 | 10.39 | 0.000 |
| RH ^a | 1 | 268181 | 268181 | 32.49 | 0.000 |
| Error | 1422 | 827742 | 582 | | |
| Total | 1429 | 1309281 | | | |

^aDegrees of freedom.

^bRelative humidity.

Table B.21. Analysis of Variance Table for *Bacillus anthracis* var. *ames* Results; Laboratory Validation of Chlorine Dioxide Decontamination.

| Source | DF ^a | Sum of Squares | Mean Square Error | F-value | P-value |
|-------------------|-----------------|----------------|-------------------|---------|---------|
| Time | 1 | 4.463 | 4.463 | 30.51 | 0.000 |
| Time ² | 1 | 1.520 | 1.520 | 10.39 | 0.001 |
| RH ^c | 1 | 4.752 | 4.752 | 32.49 | 0.000 |
| RH ² | 1 | 11.038 | 11.038 | 75.46 | 0.000 |
| Error | 242 | 0.146 | 0.146 | | |
| Total | 245 | 21.919 | | | |

^aDegrees of freedom.

^bRelative humidity.

Table B.22. Analysis of Variance Table for *Bacillus anthracis* var. *vollum* Results; Laboratory Validation of Chlorine Dioxide (ClO₂) Decontamination.

| Source | DF ^a | Sum of Squares | Mean Square Error | F-value | P-value |
|----------------------|-----------------|----------------|-------------------|---------|---------|
| Time | 1 | 93.43 | 93.43 | 30.60 | 0.000 |
| Time ² | 1 | 49.16 | 49.16 | 16.10 | 0.000 |
| RH ^b | 1 | 27.09 | 27.09 | 8.87 | 0.003 |
| RH ² | 1 | 10.55 | 10.55 | 3.456 | 0.064 |
| ClO ₂ ppm | 1 | 96.33 | 96.33 | 31.55 | 0.000 |
| RH·ClO ₂ | 1 | 39.77 | 39.77 | 13.02 | 0.000 |
| Error | 240 | 73.277 | 3.05 | | |
| Total | 246 | 389.607 | | | |

^aDegrees of freedom.

^bRelative humidity.

Table B.23. Analysis of Variance Table for *Bacillus anthracis* var. *sterne* Results; Laboratory Validation of Chlorine Dioxide (ClO₂) Decontamination.

| Source | DF ^a | Sum of Squares | Mean Square Error | F-value | P-value |
|----------------------|-----------------|----------------|-------------------|---------|---------|
| Time | 1 | 8.765 | 8.765 | 6.72 | 0.010 |
| Time ² | 1 | 102.171 | 102.171 | 78.34 | 0.000 |
| RH ^b | 1 | 99.562 | 99.562 | 76.34 | 0.000 |
| RH ² | 1 | 1.696 | 1.696 | 1.3 | 0.255 |
| ClO ₂ ppm | 1 | 138.965 | 138.965 | 106.55 | 0.000 |
| RH ClO ₂ | 1 | 141.301 | 141.301 | 108.34 | 0.000 |
| Error | 242 | 315.612 | 1.304 | | |
| Total | 248 | 808.072 | | | |

^aDegrees of freedom.

^bRelative humidity.

Table B.24. Analysis of Variance Table for *Bacillus stearothermophilus* Results; Laboratory Validation of Chlorine Dioxide (ClO₂) Decontamination.

| Source | DF ^a | Sum of Squares | Mean Square Error | F-value | P-value |
|----------------------|-----------------|----------------|-------------------|---------|---------|
| Time | 1 | 30.24 | 30.24 | 5.89 | 0.016 |
| Time ² | 1 | 11.01 | 11.01 | 2.15 | 0.145 |
| RH ^b | 1 | 3.74 | 3.74 | 0.73 | 0.394 |
| RH ² | 1 | 9.00 | 9.00 | 1.75 | 0.187 |
| ClO ₂ ppm | 1 | 0.52 | 0.52 | 0.10 | 0.751 |
| RH ClO ₂ | 1 | 25.09 | 25.09 | 4.89 | 0.028 |
| Error | 242 | 1052 | 1052 | | |
| Total | 248 | 1131.6 | | | |

^aDegrees of freedom.

^bRelative humidity.

Table B.25. Analysis of Variance Table for *Bacillus thuringiensis* Results; Laboratory Validation of Chlorine Dioxide (ClO₂) Decontamination.

| Source | DF ^a | Sum of Squares | Mean Square Error | F-value | P-value |
|----------------------|-----------------|----------------|-------------------|---------|---------|
| Time | 1 | 9.98 | 9.98 | 1.00 | 0.317 |
| Time ² | 1 | 0.51 | 0.51 | 0.05 | 0.822 |
| RH ^b | 1 | 69.45 | 69.45 | 6.99 | 0.009 |
| RH ² | 1 | 68.21 | 68.21 | 6.86 | 0.009 |
| ClO ₂ ppm | 1 | 326.97 | 326.97 | 32.89 | 0.00 |
| RH ClO ₂ | 1 | 309.88 | 309.88 | 31.17 | 0.00 |
| Error | 242 | 2256.98 | 9.94 | | |
| Total | 248 | 3041.98 | | | |

^aDegrees of freedom.

^bRelative humidity.

Table B.26. Colony Forming Units (cfus) Remaining on Filter Paper by Trial for *Bacillus anthracis* var. *ames*; Laboratory Validation of Chlorine Dioxide (ClO₂) Decontamination.

| Trial | RH ^a | ClO ₂ | cfus Remaining at Hour | | | | | | |
|-------|-----------------|------------------|------------------------|---------------------|---------------------|----|---|---|----|
| | | | 0 | 1 | 2 | 4 | 6 | 8 | 12 |
| 1 | 75 | 650 | ND ^b | ND | 4.7x10 ³ | ND | 0 | 0 | 0 |
| 2 | 92 | 730 | 6.4x10 ⁴ | ND | ND | 0 | 0 | 0 | 0 |
| 3 | 70 | 620 | 1.6x10 ⁵ | 6.8 | 0 | 0 | 0 | 0 | 0 |
| 4 | 90 | 610 | 1.6x10 ⁴ | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 60 | 613 | 7.8x10 ³ | 1.6x10 ³ | 0 | 0 | 0 | 0 | 0 |
| 8 | 40 | 800 | 6.9x10 ⁴ | 2.5x10 ² | 0 | 0 | 0 | 0 | 0 |
| 9 | 30 | 1050 | 4.6x10 ⁴ | 3.0x10 ³ | 0 | 0 | 0 | 0 | 0 |
| 10 | 90 | 250 | 1.9x10 ⁶ | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 | 30 | 250 | 1.9x10 ⁵ | 9.0x10 ⁴ | 1.2x10 ⁴ | 0 | 0 | 0 | 0 |
| 12 | 50 | 250 | 5.1x10 ⁵ | 4.3x10 ¹ | 0 | 0 | 0 | 0 | 0 |
| 13 | 70 | 250 | 7.6x10 ⁵ | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 70 | 125 | 2.6x10 ⁶ | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 90 | 125 | 2.4x10 ⁶ | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | 70 | 750 | 1.1x10 ⁶ | 0 | 0 | 0 | 0 | 0 | 0 |

^aRelative humidity.

^bNo data.

Table B.27. Regression Table, Probit Analysis for *Bacillus anthracis* var. *Ames* on Filter Paper; Laboratory Validation of Chlorine Dioxide Decontamination.

| Variable | Coefficient | Standard Error | Z-value | P-value |
|------------------|-------------|----------------|---------|---------|
| Constant | 0.2708 | 0.1691 | 1.6 | 0.109 |
| Time | 0.7162 | 0.1555 | 4.61 | 0.000 |
| Natural Response | 0.000 | | | |

Table B.28. Colony Forming Units (cfus) Remaining on Filter Paper by Trial for *Bacillus anthracis* var. *vollum*; Laboratory Validation of Chlorine Dioxide (ClO₂) Decontamination.

| Trial | RH ^a | ClO ₂ | cfus Remaining at Hour | | | | | | |
|-------|-----------------|------------------|------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|----|
| | | | 0 | 1 | 2 | 4 | 6 | 8 | 12 |
| 1 | 75 | 650 | ND ^b | ND | 0 | ND | 2.2x10 ² | 0 | 0 |
| 2 | 92 | 730 | 5.1x10 ³ | ND | ND | 0 | 0 | 0 | 0 |
| 3 | 70 | 620 | 1.4x10 ⁵ | 4.7x10 ² | 3.2x10 ¹ | 6.4x10 ¹ | 0 | 2.0x10 ¹ | 0 |
| 4 | 90 | 610 | 2.9x10 ⁵ | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 60 | 613 | 1.2x10 ⁵ | 3.7x10 ³ | 1.8x10 ³ | 2.3x10 ² | 0 | 3.4x10 ¹ | 0 |
| 8 | 40 | 800 | 2.6x10 ⁶ | 3.9x10 ¹ | 4.5 | 2.3 | 0 | 0 | 0 |
| 9 | 30 | 1050 | 5.3x10 ⁶ | 3.0x10 ³ | 0 | 0 | 0 | 0 | 0 |
| 10 | 90 | 250 | 1.0x10 ⁵ | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 | 30 | 250 | 1.1x10 ⁵ | 9.6x10 ⁴ | 1.9x10 ² | 0 | 0 | 0 | 0 |
| 12 | 50 | 250 | 5.1x10 ⁵ | 4.3x10 ¹ | 0 | 0 | 0 | 0 | 0 |
| 13 | 70 | 250 | 2.3x10 ⁵ | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 70 | 125 | 9.6x10 ⁴ | 1.7x10 ² | 0 | 0 | 0 | 0 | 0 |
| 15 | 90 | 125 | 3.0x10 ⁴ | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | 70 | 750 | 6.5x10 ⁴ | 0 | 0 | 0 | 0 | 0 | 0 |

^aRelative humidity.

^bNo data.

Table B.29. Regression Table, Probit Analysis for *Bacillus anthracis* var. *vollum* on Filter Paper; Laboratory Validation of Chlorine Dioxide (ClO₂) Decontamination.

| Variable | Coefficient | Standard Error | Z-value | P-value |
|------------------|-------------|----------------|---------|---------|
| Constant | 0.0360 | 0.1640 | 0.22 | 0.827 |
| Time | 0.6342 | 0.1180 | 5.37 | 0.000 |
| Natural Response | 0.000 | | | |

Table B.30. *Bacillus subtilis* var. *niger*, Spore Strip Results After Incubation for 1 Week; Laboratory Validation of Chlorine Dioxide (ClO₂) Decontamination.

| Trial Number | Relative Humidity (%) | ClO ₂ (ppm) | Time in Chamber | | | | | | | | | |
|--------------|-----------------------|------------------------|-----------------|---|---|----|----------------|-----------------|----|----|----|----|
| | | | 6 Hours | | | | | 12 Hours | | | | |
| 1 | 75 | 650 | 3 ^a | 3 | 3 | 3 | - ^b | 3 | - | - | - | - |
| 2 | 92 | 730 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 3 | 70 | 620 | 1 | 1 | 1 | 1 | 1 | 3 | - | 3 | 3 | - |
| 4 | 90 | 610 | 4 | 4 | 4 | 4 | 4 | - | - | - | - | - |
| 5 | 60 | 613 | 4 | 4 | 4 | 4 | - | 1 | 4 | 4 | 4 | 4 |
| 6 | Hydrogen Peroxide | | - | - | - | - | - | ND ^c | ND | ND | ND | ND |
| 8 | 40 | 800 | 3 | 3 | 3 | 3 | 3 | - | 3 | 3 | 3 | 3 |
| 9 | 30 | 1050 | 3 | 3 | 3 | 3 | 3 | ND | ND | ND | ND | ND |
| 10 | 90 | 250 | 2 | - | - | - | - | 2 | 3 | 3 | - | - |
| 11 | 30 | 250 | 1 | 2 | 3 | 1 | 1 | ND | ND | ND | ND | ND |
| 12 | 50 | 250 | 1 | 2 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | - |
| 13 | 70 | 250 | 2 | 3 | 3 | 1 | 1 | 3 | 3 | 1 | 3 | - |
| 14 | 70 | 125 | - | - | - | - | - | - | - | - | - | ND |
| 15 | 90 | 125 | 1 | 1 | 1 | ND | 1 | 1 | 1 | 1 | 2 | 1 |
| 16 | 70 | 750 | - | - | - | 3 | 3 | - | - | - | - | - |

^aNumber of days before growth was observed.

^bNo bacteria had grown.

^cNo data; no strips in the chamber.

Table B.31. *Bacillus thuringiensis*, Spore Strip Results After Incubation for 1 Week; Laboratory Validation of Chlorine Dioxide (ClO₂) Decontamination.

| Trial Number | Relative Humidity (%) | ClO ₂ (ppm) | Time in Chamber | | | | | | | | | |
|--------------|-----------------------|------------------------|-----------------|----|----|----|----|-----------------|----------------|----|----|----|
| | | | 6 Hours | | | | | 12 Hours | | | | |
| 1 | 75 | 650 | 1 ^a | 1 | 1 | 1 | 1 | 3 | - ^b | - | - | - |
| 2 | 92 | 730 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - |
| 3 | 70 | 620 | 1 | 1 | 1 | 1 | 1 | 4 | - | 4 | 4 | - |
| 4 | 90 | 610 | 1 | 1 | 1 | 1 | 1 | - | - | - | - | 4 |
| 5 | 60 | 613 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 6 | Hydrogen Peroxide | | - | - | - | - | - | ND ^c | ND | ND | ND | ND |
| 8 | 40 | 800 | 1 | 1 | 1 | 1 | 1 | 3 | - | - | 1 | 1 |
| 9 | 30 | 1050 | ND | ND | ND | ND | ND | - | 2 | 1 | 1 | 1 |
| 10 | 90 | 250 | 1 | 2 | - | - | - | 3 | - | - | - | - |
| 11 | 30 | 250 | 1 | 1 | 1 | 1 | 1 | ND | ND | ND | ND | ND |
| 12 | 50 | 250 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 13 | 70 | 250 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 14 | 70 | 125 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | ND |
| 15 | 90 | 125 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 16 | 70 | 750 | 1 | 1 | 1 | 1 | 1 | 2 | - | - | 2 | - |

^aNumber of days before growth was observed.

^bNo bacteria had grown.

^cNo data; no strips in the chamber.

Table B.32. *Bacillus stearothermophilus*, Spore Strip Results After Incubation for 1 Week; Laboratory Validation of Chlorine Dioxide (ClO₂) Decontamination.

| Trial Number | Relative Humidity (%) | ClO ₂ (ppm) | Time in Chamber/Initial Concentration | | | | | | | | | |
|--------------|-----------------------|------------------------|---------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | | | 6 Hours | | | | | 12 Hours | | | | |
| | | | 10 ⁴ | 10 ⁵ | 10 ⁶ | 10 ⁷ | 10 ⁸ | 10 ⁴ | 10 ⁵ | 10 ⁶ | 10 ⁷ | 10 ⁸ |
| 1 | 75 | 650 | ND ^a | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| 2 | 92 | 730 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| 3 | 70 | 620 | 4 ^b | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 4 | 90 | 610 | - ^c | 4 | 4 | 4 | 4 | - | - | - | - | - |
| 5 | 60 | 613 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 6 | Hydrogen Peroxide | | - | - | - | - | 7 | ND | ND | ND | ND | ND |
| 8 | 40 | 800 | 5 | 3 | 3 | 3 | 3 | 2 | 1 | 5 | 3 | 3 |
| 9 | 30 | 1050 | ND | ND | ND | ND | ND | 4 | - | - | 3 | 3 |
| 10 | 90 | 250 | - | 2 | 2 | 3 | - | 2 | - | 2 | - | 3 |
| 11 | 30 | 250 | 2 | 3 | 3 | 3 | 2 | 2 | 3 | 3 | 3 | 3 |
| 12 | 50 | 250 | 3 | 3 | 3 | 3 | 1 | 3 | 3 | 3 | 3 | 3 |
| 13 | 70 | 250 | 3 | 3 | 3 | 3 | 2 | 2 | 3 | 3 | 3 | - |
| 14 | 70 | 125 | 3 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 |
| 15 | 90 | 125 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 16 | 70 | 750 | - | - | | 2 | 2 | 3 | - | 3 | 3 | 3 |

^aNo data; no strips in the chamber.

^bNumber of days before growth was observed.

^cNo bacteria had grown.

Table B.33. *Bacillus stearothermophilus*, Spore strip Results After Incubation for One Week;
Laboratory Validation of Chlorine Dioxide (ClO₂) Decontamination.

| Trial Number | Relative Humidity (%) | ClO ₂ (ppm) | Time in Chamber | | | | | | | | | |
|--------------|-----------------------|------------------------|-----------------|---|---|---|---|-----------------|----|----------------|----|----|
| | | | 6 Hours | | | | | 12 Hours | | | | |
| 1 | 75 | 650 | 1 ^a | 1 | 1 | 1 | 1 | 3 | 3 | - ^b | - | - |
| 2 | 92 | 730 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - |
| 3 | 70 | 620 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 4 | 90 | 610 | - | - | 1 | 4 | - | - | - | - | - | - |
| 5 | 60 | 613 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 6 | Hydrogen Peroxide | | 6 | - | 6 | 6 | 1 | ND ^c | ND | ND | ND | ND |
| 8 | 40 | 800 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - |
| 9 | 30 | 1050 | 1 | 1 | 1 | 1 | 1 | - | 1 | 1 | 1 | - |
| 10 | 90 | 250 | - | - | - | - | - | - | - | - | - | - |
| 11 | 30 | 250 | 1 | 1 | 1 | 1 | 1 | ND | ND | ND | ND | ND |
| 12 | 50 | 250 | 1 | 1 | 1 | 1 | - | 1 | 1 | 1 | 1 | 1 |
| 13 | 70 | 250 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 14 | 70 | 125 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 15 | 90 | 125 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 16 | 70 | 750 | 1 | 1 | 1 | 1 | 1 | 3 | - | 3 | - | 2 |

^aNumber of days before growth was observed.

^bNo bacteria had grown.

^cNo strips in the chamber.

Table B.34. *Bacillus subtilis* var. *niger* (BGN) Cover Slip Data at 6 and 12 Hours Compared with BGN Spore Strips for False Negatives; Laboratory Validation of Chlorine Dioxide Decontamination.

| Trial | 6 Hours | | | False Negative ^a | 12 Hours | | | False Negative |
|-------|----------------|---|----------------|-----------------------------|-----------------|----|----|----------------|
| 1 | - ^b | - | - | No | - | - | - | No |
| 2 | - | - | + ^c | No | - | + | - | No |
| 3 | - | - | - | No | - | - | - | No |
| 4 | - | - | - | No | - | - | - | No |
| 5 | - | - | - | No | - | - | - | No |
| 6 | - | - | - | No | ND ^d | ND | ND | No |
| 8 | + | - | + | No | + | + | + | Possible |
| 9 | + | + | + | No | + | + | + | ND |
| 10 | + | + | + | Yes | - | - | - | No |
| 11 | + | + | + | No | + | + | ND | ND |
| 12 | + | + | + | No | + | + | + | Possible |
| 13 | + | - | - | No | - | - | - | No |
| 14 | - | - | - | No | - | - | - | No |
| 15 | - | - | - | No | - | - | - | No |
| 16 | - | - | - | No | - | - | - | No |

^aSpore strip data are negative while cover slip data are positive.

^bNo bacterial growth.

^cBacterial growth.

^dNo data collected.

Table B.35. *Bacillus thuringiensis* Cover Slip Data at 6 and 12 Hours Compared with Simulant Spore Strips for False Negatives; Laboratory Validation of Chlorine Dioxide Decontamination.

| Trial | 6 Hours | | | False Negative ^a | 12 Hours | | | False Negative |
|-------|----------------|---|---|-----------------------------|-----------------|----|----|----------------|
| 1 | - ^b | - | - | No | - | - | - | No |
| 2 | - | - | - | No | - | - | - | No |
| 3 | + ^c | + | + | No | - | - | - | No |
| 4 | - | - | - | No | - | - | - | No |
| 5 | + | + | + | No | + | + | + | No |
| 6 | - | - | - | No | ND ^d | ND | ND | No |
| 8 | + | + | + | No | + | + | + | Possible |
| 9 | + | + | + | ND | + | + | + | Possible |
| 10 | - | - | - | No | - | - | - | No |
| 11 | + | + | + | No | + | + | + | ND |
| 12 | - | + | + | No | + | + | + | No |
| 13 | + | + | + | No | + | + | + | No |
| 14 | + | + | + | No | + | + | + | No |
| 15 | + | + | + | No | + | + | + | No |
| 16 | + | + | + | No | + | + | + | Yes |

^aSpore strip data are negative while cover slip data are positive.

^bNo bacterial growth.

^cBacterial growth.

^dNo data collected.

Table B.36. *Bacillus stearothermophilus* (BST) Cover Slip Data at 6 and 12 Hours Compared with BST Spore Strips for False Negatives; Laboratory Validation of Chlorine Dioxide Decontamination.

| Trial | 6 Hours | | | False Negative ^a | 12 Hours | | | False Negative |
|-------|----------------|----------------|---|-----------------------------|-----------------|----|----|----------------|
| 1 | - ^b | + ^c | - | No | - | - | - | No |
| 2 | - | - | - | No | - | - | - | No |
| 3 | + ^d | + | - | No | - | - | - | No |
| 4 | - | - | - | No | - | - | - | No |
| 5 | + | + | + | No | + | + | + | No |
| 6 | - | - | - | No | ND ^e | ND | ND | No |
| 8 | + | + | + | No | + | + | + | No |
| 9 | + | + | + | No | + | + | + | Possible |
| 10 | - | - | - | No | - | - | - | No |
| 11 | + | + | + | No | + | + | ND | ND |
| 12 | + | + | + | Possible | + | + | + | No |
| 13 | + | + | + | No | + | + | + | No |
| 14 | + | + | + | No | + | + | + | No |
| 15 | - | - | - | No | - | - | - | No |
| 16 | + | + | + | No | + | + | + | Possible |

^aSpore strip data are negative while cover slip data are positive.

^bNo bacterial growth.

^cBacterial growth.

^dNo data collected.

Table B.37. *Bacillus anthracis* var. *ames* Cover Slip Data at 6 and 12 Hours Compared with Simulant Spore Strips for False Negatives; Laboratory Validation of Chlorine Dioxide Decontamination.

| Trial | 6 Hours | | | False Negatives ^a | | | 12 Hours | | | False Negatives ^a | | |
|-------|---------|---|---|------------------------------|----|-----------------|----------------|----|----|------------------------------|----------|----------|
| | | | | BGN | BT | BST | | | | BGN | BT | BST |
| 1 | + | + | + | Possible | No | ND ^c | - ^d | - | - | No | No | ND |
| 2 | - | - | - | No | No | ND | - | - | - | No | No | ND |
| 3 | + | + | + | No | No | No | + | - | - | No | No | No |
| 4 | - | - | - | No | No | No | - | - | - | No | No | No |
| 5 | + | + | + | Possible | No | No | + | + | + | No | No | No |
| 6 | - | - | - | No | No | No | ND | ND | ND | ND | ND | ND |
| 8 | + | + | + | No | No | No | + | + | + | Possible | Possible | Possible |
| 9 | + | + | + | No | No | No | + | + | + | No | Possible | Possible |
| 10 | - | - | - | No | No | No | - | - | - | No | No | No |
| 11 | + | + | + | No | No | No | + | + | + | No | No | ND |
| 12 | + | + | + | No | No | Possible | + | + | + | Possible | No | No |
| 13 | - | + | - | No | No | No | - | - | - | No | No | No |
| 14 | + | + | + | Yes | No | No | + | + | + | Yes | No | No |
| 15 | - | - | - | No | No | No | - | - | - | No | No | No |
| 16 | + | + | + | Yes | No | No | - | - | - | No | No | No |

^aSpore strip data are negative while cover slip data are positive. BGN – *Bacillus subtilis* var. *niger*; BT – *Bacillus thuringiensis*; BST – *Bacillus stearothermophilus*.

^bBacterial growth.

^cNo data collected.

^dNo bacterial growth.

Table B.38. *Bacillus anthracis* var. *vollum* Cover Slip Data at 6 and 12 Hours Compared with Simulant Spore Strips for False Negatives; Laboratory Validation of Chlorine Dioxide Decontamination.

| Trial | 6 Hours | | | False Negatives ^a | | | 12 Hours | | | False Negatives ^a | | |
|-------|---------|---|---|------------------------------|----|-----------------|----------------|----|----|------------------------------|----------|----------|
| | | | | BGN | BT | BST | | | | BGN | BT | BST |
| 1 | + | + | + | No | No | ND ^c | - ^d | - | - | No | No | ND |
| 2 | - | - | - | No | No | ND | - | - | - | No | No | ND |
| 3 | + | + | + | No | No | No | + | - | + | No | No | No |
| 4 | - | - | - | No | No | No | - | - | - | No | No | No |
| 5 | + | + | + | Possible | No | No | + | + | + | No | No | No |
| 6 | - | - | - | No | No | No | ND | ND | ND | ND | ND | ND |
| 8 | + | + | + | No | No | No | + | + | + | Possible | Possible | No |
| 9 | + | + | + | No | No | No | + | + | + | ND | Possible | Possible |
| 10 | - | - | - | No | No | No | - | - | - | No | No | No |
| 11 | + | + | + | No | No | No | + | + | + | ND | No | ND |
| 12 | + | + | + | No | No | Possible | + | + | + | Possible | No | No |
| 13 | - | - | - | No | No | No | - | - | + | No | No | No |
| 14 | + | + | + | Yes | No | No | + | + | + | Yes | No | No |
| 15 | - | - | - | No | No | No | - | - | - | No | No | No |
| 16 | + | + | + | Yes | No | No | + | + | + | Yes | Yes | Possible |

^aSpore strip data are negative while cover slip data are positive. BGN – *Bacillus subtilis* var. *niger*; BT – *Bacillus thuringiensis*; BST – *Bacillus stearothermophilus*.

^bBacterial growth.

^cNo data collected.

^dNo bacterial growth.

Table B.39. *Bacillus anthracis* var. *sterne* Cover Slip Data at 6 and 12 Hours Compared with Simulant Spore Strips for False Negatives; Laboratory Validation of Chlorine Dioxide Decontamination.

| Trial | 6 Hours | | | False Negatives ^a | | | 12 Hours | | | False Negatives ^a | | |
|-------|----------------|----------------|---|------------------------------|----|-----------------|----------|----|----|------------------------------|----------|----------|
| | | | | BGN | BT | BST | | | | BGN | BT | BST |
| 1 | - ^b | + ^c | - | No | No | ND ^d | - | - | - | No | No | ND |
| 2 | - | - | - | No | No | ND | - | - | - | No | No | ND |
| 3 | + | - | + | No | No | No | - | - | - | No | No | No |
| 4 | - | - | - | No | No | No | - | - | - | No | No | No |
| 5 | + | + | + | Possible | No | No | + | + | + | No | No | No |
| 6 | - | - | - | No | No | No | ND | ND | ND | ND | ND | ND |
| 8 | + | + | + | No | No | No | + | + | + | Possible | Possible | Possible |
| 9 | + | + | + | No | ND | No | + | + | + | No | Possible | Possible |
| 10 | - | - | - | No | No | No | - | - | - | No | No | No |
| 11 | - | - | - | No | No | No | - | - | - | No | No | ND |
| 12 | + | + | + | No | No | Possible | + | + | + | Possible | No | No |
| 13 | - | - | - | No | No | No | - | - | - | No | No | No |
| 14 | + | + | + | Yes | No | No | + | + | + | Yes | No | No |
| 15 | + | + | + | No | No | No | - | - | - | No | No | No |
| 16 | + | + | + | Yes | No | No | - | - | - | No | No | No |

^aSpore strip data are negative while cover slip data are positive. BGN – *Bacillus subtilis* var. *niger*; BT – *Bacillus thuringiensis*; BST – *Bacillus stearothermophilus*.

^bNo bacterial growth.

^cBacterial growth.

^dNo data collected.

APPENDIX C. DETAILED TEST PROCEDURES

a. All testing was performed at the LSTF at WDTC/DPG. The BA preparation was performed in a biosafety level 3 (BSL3) laboratory. The preparation of the simulant bacteria and subsequent testing was performed in a BSL2 laboratory.

b. The ClO₂ testing was performed in a stainless steel glove box of approximately 0.62 m³ (22 ft³) [about 0.76 by 1.21 by 0.79 m (about 30 by 48 by 31 in)] (Figure 5), which was attached to a ClO₂ gas generator provided by PureLine[®] Treatment Systems (Figure 1). The chamber had glove ports and a transfer chamber to facilitate sample removal. It also had a humidifier to help regulate RH.

c. ClO₂ gas levels were monitored in two ways.

(1) Wet chemistry performed on gas samples.

(a) This was the primary method of determining the concentration of ClO₂ gas in the chamber.

(b) A measured volume of gas was collected using one of these methods: plastic syringe, Buck[®] pump (A.P. Buck[®], Inc., Orlando, Florida) with impingers, house vacuum plus a critical orifice, and impingers or a gas-tight glass and Teflon[®] syringe.

(c) The gas was bubbled through a buffered [10X phosphate-buffered saline (PBS)] aqueous potassium iodide solution (either in a beaker when using a syringe, or directly into the impinger) and titrated with 0.01N sodium thiosulfate using a Fischer-Porter titrator with a platinum electrode.

(d) This was converted to ppm ClO₂ using the following equation:

$$\frac{(\text{mL titrant}) \times (\text{Normality of thiosulfate}) \times (1000 \text{ mL/1 L}) \times (24.45)}{\text{Sample Volume in Liters}} = \text{ppm}$$

(2) UV sensor from Custom Sensors and Technology, St. Louis, Missouri.

(a) A secondary method of tracking ClO₂ concentration was used within the chamber and consisted of a UV sensor.

(b) This system consisted of two optical devices, separated by a measured pathlength within the chamber. One sent and the other received the UV signal at a wavelength of 350 nanometers. Each optical sensor was connected to an external generator/analyzer via fiber optic lines.

(c) UV absorption values were correlated with the ClO₂ concentrations as determined via gas sampling and titrating.

(3) Use of these methods introduced some shortcomings, discussed in Appendix D.

d. One requirement was that the test be conducted on spores. Vegetative cells were killed by pasteurization (exposed to 70°C for 20 minutes) before sample preparation. Electron microscopy demonstrated the presence of spores in each microbial slurry used (Figure 4).

e. The samples were prepared by placing 100 µL of slurry onto a glass cover slip or onto filter paper. BAA, BAV, BAS, BG, BT, and BST were used with the cover slips, while only the anthrax strains were placed on filter paper. The samples were allowed to dry completely and were placed in petri dishes (Figures C.1 and C.2) before testing began. Gauze was placed in the petri dishes under the samples to facilitate removal of the samples with forceps.

f. The samples were exposed to the ClO₂ in the following way.

(1) The petri dishes containing the samples were placed on racks and placed inside the chamber.

(2) The PureLine[®] ClO₂ generator was turned on.

(3) The UV readings were monitored (custom sensors).

(4) When the UV reading was close to the desired reading, a gas sample was collected.

(5) Wet chemistry was performed to determine the concentration of ClO₂ gas in the chamber.

(6) When the target ppm of ClO₂ was reached, the ClO₂ generator was turned off manually or automatically.

(7) As the ClO₂ was being generated, the RH was adjusted until the target RH was met.

(8) The timer was started.

(9) At any time after the start of the timer, the ClO₂ generator was turned on manually or automatically when the ClO₂ level fell below the target range.

(10) The first sample was removed at 1 hour after the start of the timer. The removal process is described in Paragraphs f(10)(a) through (g) below.

(a) The lid was placed on the petri dishes containing the 1-hour samples.

(b) The inner sample chamber door was opened and the dishes were moved into the transfer chamber, using the glove ports.

(c) The inner transfer chamber door was closed.

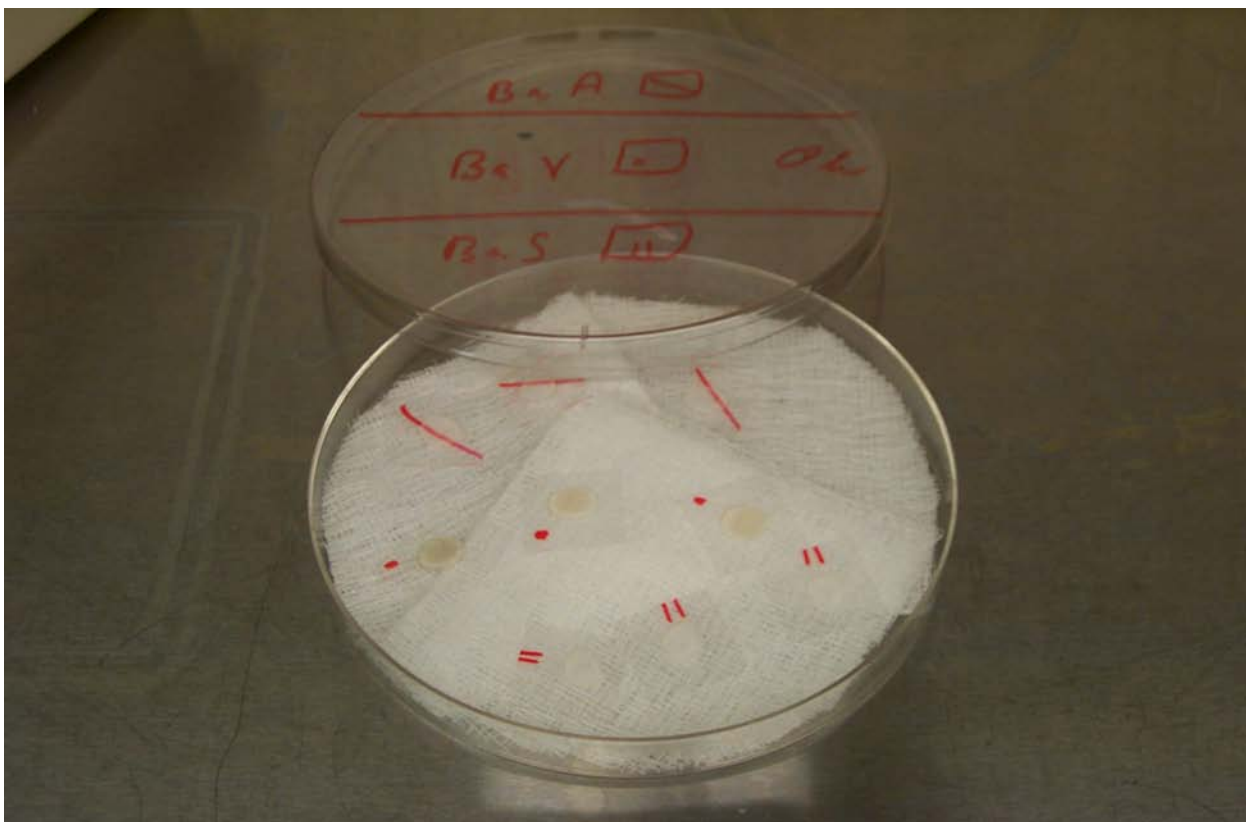


Figure C.1. Petri Dish Containing Samples on Glass Cover Slips; Laboratory Validation of Chlorine Dioxide Decontamination.

- (d) The ClO_2 gas was evacuated from the transfer chamber.
 - (e) The outer transfer chamber door was opened.
 - (f) The samples were removed from the transfer chamber.
 - (g) The outer transfer chamber door was closed.
- (11) Steps (10)(a) through (g) were repeated to remove samples at 2, 4, 6, and 8 hours.
- (12) The 12-hour and retained samples were removed after the chamber was shut down and the ClO_2 gas was evacuated from the chamber.
- (13) All samples remained in the covered petri dishes until they were assayed the next day.
- g. All samples were assayed IAW Standing Operating Procedure (SOP) WD-L-BIO135 (Reference 4), specifically:



Figure C.2. A Petri Dish Containing Samples on Filter Paper; Laboratory Validation of Chlorine Dioxide (ClO_2) Decontamination.

- (1) Cover slips, or filter paper, were transferred into 10 mL of buffer contained in conical 50-mL centrifuge tubes and vortexed for 20 seconds.
- (2) A 2-mL solution was retained and refrigerated.
- (3) An aliquot was serially diluted and plated by a spiral plater.
- (4) The retained samples were assayed at least 1 week later when the 12-hour sample that was plated from the automatic spiral plater showed that all bacteria had been killed.
- (5) One mL from a retained sample was spread onto 150-mm plates to confirm negative results from the initial spiral plating.

APPENDIX D. DIFFICULTIES MEASURING ClO₂ CONCENTRATION

a. The requirement to acquire data as soon as possible to support decontamination activities at the HSOB caused testing to proceed before all factors influencing ClO₂ measurement were established.

b. There were two methods of determining ClO₂ concentrations with the chamber. Difficulties in measuring the exact concentration of ClO₂ gas were encountered with both methods and are described below.

(1) Wet chemistry.

(a) Initially, 50-mL gas volumes were collected by using a plastic-type syringe. After 3 days of use, the syringe's rubber plunger appeared to be leaking gas, as noted by changes in the hand pressure needed to move the plunger through the piston.

(b) Investigators at the HSOB were using personal air monitors made by Buck[®] (A.P. Buck[®], Inc. Orlando, Florida) to collect volumetric samples of gas into small glass impingers; it was strongly urged that this system be used after recognition of the shortcomings with the plastic syringes. At WDTC/DPG, the provided Buck[®] pumps performed poorly and inconsistently.

(c) Therefore, the house vacuum and calibrated critical orifices were used in place of the pumps and in conjunction with two impingers linked in series. The volume collected using this setup was verified by a Gilibrator[™]. Variation in these volume measurements occurred with simple routine movements of the sample equipment from bench top to the exposure hood, as well as between one setup of sample collection train to the next.

(d) Then a gas-tight glass syringe with a Teflon[®] plunger was used. No problems were observed when this system was used.

(2) UV absorption.

(a) UV sensor cells were placed inside of the exposure chamber on a self-centering soft iron bar (rail) and linked via fiber optics to a UV analyzer from Custom Sensors and Technology.

(b) This allowed constant monitoring of UV absorbance at 350 nm, which correlates to the concentrations of ClO₂. However, the correlation of UV absorbance to the ClO₂ concentration must be derived from determinations of ClO₂ concentrations from gas collections and titrations.

(c) The concerns about the inability to capture precise volumes of gas were set aside because it was believed that the constant UV readings would provide firm measurements. However, the pathlength UV light had to be increased for low (or decreased for high) concentrations of ClO₂. As a result, the absolute readings of UV absorbance were not always the same when the sensors were returned to a previously used pathlength. Whether this was due to deviations in

sensor alignment, because of the badly corroded soft iron rail on which they were placed, or to other alignment problems is not known.

(d) There were no absolute values or standards to return to and to ensure that the sensors were aligned with 100 percent efficiency or were properly returned to their previous positions. Thus, it was believed that, within a given trial, the UV readings were very useful in maintaining a constant concentration of ClO_2 . However, because of undocumented movements of the sensors between tests and the fact that the self-centering alignments relied upon were eventually discovered to be unsatisfactory, the UV data could not be used to compare separate tests.

c. Even with the above problems, it was believed that, in most cases, there was an accurate measurement of the concentration of ClO_2 gas.

(1) During Trials 1 and 2, the representatives from Custom Sensors were still on site verifying that the UV data were correct and that they correlated with the wet chemistry. Because the wet chemistry had very consistent values, as did the UV data, it was concluded that the plastic syringe had not experienced considerable breakdown during these trials.

(2) After Trial 10, use of the GilibratorTM solved most of the problems with the impingers. The wet chemistry gave consistent results and, although the UV sensors could not verify the concentrations exactly, because there was no way to calibrate the alignment, the UV values had almost no variation, indicating that ClO_2 concentrations were consistent throughout the trial.

(3) H_2O_2 was used for Trial 6. The H_2O_2 concentrations were not measured.

(4) In Trials 3 through 5 and 8 through 10, the wet chemistry values were highly variable due to the problems in measuring a correct gas volume. The UV data were more consistent, indicating that the problem was with the measurement and not with the ClO_2 generator. In addition, the UV data were consistent with the 600 to 700 ppm range, with the exception of Trials 8 and 9, which had higher UV readings, as expected because those trials had a higher target ClO_2 concentration. The best estimate of ClO_2 concentration, based on wet chemistry and UV data, was used in these trials for comparison purposes (Table 2). Although there is higher variability in these trials, there is no reason to believe that the values were outside the target range of 125 to 1050 ppm.

APPENDIX E. REFERENCES

1. Han, Y., A.M. Guentert, R.S. Smith, R.H. Linton, and P.E. Nelson, Food Microbiology, Efficacy of Chlorine Dioxide Gas as a Sanitizer for Tanks Used in Aseptic Juice Storage, 1999.
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3. Steris Corporation, Erie, Pennsylvania, Cycle Development Guide for VHP™ 1000 Biodecontamination System, P-129372-711, 23 February 1996.
4. U.S. Army Dugway Proving Ground (DPG), Utah, Standing Operating Procedure (SOP) WD-L-WI-BIO-135, Assay of Biological Simulants, 2 August 2001.

APPENDIX F. ABBREVIATIONS

ANOVA – analysis of variance

ATCC – American Test Culture Collection

BA – *Bacillus anthracis*

BAA – *Bacillus anthracis* var. *ames*

BAS – *Bacillus anthracis* var. *sterne*

BAV – *Bacillus anthracis* var. *vollum*

BGN – *Bacillus subtilis* var. *niger*

BSL – biosafety level

BST – *Bacillus stearothermophilus*

BT – *Bacillus thuringiensis*

cfu – colony forming unit

ClO₂ – chlorine dioxide

DPG – U.S. Army Dugway Proving Ground

DTC – U.S. Army Developmental Test Command

EPA – U.S. Environmental Protection Agency

GLM – generalized linear model

H₂O₂ – hydrogen peroxide

HSOB – Hart Senate Office Building

IAW – in accordance with

LSTF – Life Sciences Test Facility

PBS – phosphate-buffered saline

ppm – parts per million

RH – relative humidity

SOP – standing operating procedure

UV – ultraviolet

VHP – vaporized hydrogen peroxide

WDTC – West Desert Test Center

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