THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

U.S. Environmental Protection Agency

Battelle
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ETV Joint Verification Statement

TECHNOLOGY TYPE:	**Hydrogen Peroxide Gas Generator**		
APPLICATION:	**BIOLOGICAL AGENT DECONTAMINATION**		
TECHNOLOGY NAME:	**CLARUS™ C Hydrogen Peroxide Gas Generator**		
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The U.S. Environmental Protection Agency (EPA) has established the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative or improved environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies. Information and ETV documents are available at www.epa.gov/etv.

ETV works in partnership with recognized standards and testing organizations; with stakeholder groups that consist of buyers, vendor organizations, and permitters; and with the full participation of individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field or laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The Building Decontamination Technology (BDT) Center, under ETV, is operated by Battelle in cooperation with EPA's Office of Research and Development. The BDT Center has recently evaluated the performance of hydrogen peroxide vapor technologies for decontaminating indoor surfaces contaminated with biological agents. This verification statement provides a summary of the test results for the BIOQUELL, Inc., CLARUS™ C hydrogen peroxide gas generator.

VERIFICATION TEST DESCRIPTION

The CLARUS C unit was verified in terms of its ability to achieve a reduction in biological agents/surrogates on representative indoor surfaces. Qualitative factors were also evaluated, including ease of use and physical degradation of the indoor materials used as test materials.

The verification test consisted of using the CLARUS C unit to decontaminate seven types of surfaces contaminated with biological agent (or surrogate) spores. The surfaces included industrial-grade carpet, bare wood (pine), glass, decorative laminate, galvanized metal ductwork, painted wallboard paper, and painted concrete. The condition of test surfaces was determined by visual examination.

Test surfaces, 0.75 in x 3 in (1.9 cm x 7.6 cm), were wiped with 70% isopropanol and subsequently contaminated at challenge levels of approximately 1 x 10^8 viable biological spores per coupon. Spore suspensions were enumerated each day of use to confirm application density. Efficacy was evaluated using spores from *Bacillus anthracis* Ames strain, as well as the surrogates, *Bacillus subtilis* (ATCC 19659) and *Geobacillus stearothermophilus* (ATCC 12980). In addition, surrogate biological indicators (*Bacillus subtilis* and *Geobacillus stearothermophilus*) and biological spore strips (*Bacillus atrophaeus*) were used to further evaluate decontamination efficacy.

The CLARUS C unit was operated using cycle parameters specified by the vendor to introduce the hydrogen peroxide into a test chamber. The cycle parameters were as follows:

- Cycle pressure: 20 Pascals
- Conditioning time: 10 minutes
- Gassing time: 20 minutes
- Gassing dwell: 20 minutes
- H_2O_2 injection rate: 2.0 grams per minute
- H_2O_2 dwell rate: 0.5 grams/minute
- Aeration time: set for 9,999 minutes

The test chamber, containing the contaminated test samples, consisted of a Compact Glove Box modified to the vendor's specifications. Subsequent to the treatment, the samples were visually examined for surface damage. Spores were extracted from the surfaces and, after appropriate serial dilutions, plated onto tryptic soy agar and incubated at appropriate growth conditions. Colonies were enumerated the following day. Efficacy of the decontamination procedure was evaluated by comparing the number of viable spores after decontamination to the number of viable spores from a control surface (of the same material, size, and challenge) that was not subjected to the decontamination. Efficacy was expressed in terms of a log reduction.

The extraction procedure did not remove 100% of the spores on the surface due to material-dependent characteristics, such as texture and/or porosity. To determine whether viable organisms remained on the test surface, the test coupon was placed in a liquid tryptic soy broth culture medium. The broth was checked after one and seven days for cloudiness, which indicated growth of residual viable organisms on the coupon. Growth may result from the microorganisms in the sample not killed by the 70% isopropanol wipe or by the subsequent hydrogen peroxide treatment.

QA oversight of verification testing was provided by both EPA and Battelle. The EPA and Battelle each performed a technical systems audit. Battelle QA staff conducted a data quality audit (minimum 10%) of the test data. This verification statement, the full report on which it is based, and the test/QA plan for this verification are all available at www.epa.gov/etv/centers/center9.html.

TECHNOLOGY DESCRIPTION

The following is a description of the CLARUS C unit, based on information provided by the vendor. The technology description was not verified in this test.

The CLARUS C unit injects hydrogen peroxide into air recirculating through the unit until the chamber reaches saturation conditions for a hydrogen peroxide/air mixture, leading to the deposition of a microscopic film of hydrogen peroxide (microcondensation) on all surfaces. After a pre-determined exposure time, the CLARUS C unit recirculates the air through a separate loop that filters particles, catalytically decomposes the hydrogen peroxide, and dehumidifies the air. This returns the chamber to a safe condition. Critical parameters are monitored and recorded by the system.

The CLARUS C unit was designed to decontaminate enclosures up to 7,000 cubic feet (200 cubic meters). It weighs 300 pounds (128 kilograms), and is 26 in (68 cm) wide by 35 in (90 cm) in depth by 45 in (106 cm) in height. It operates from normal domestic power supply. The CLARUS C unit is controlled by a Siemens programmable logic controller complemented by optional sensors.

VERIFICATION OF PERFORMANCE

By following the user manual, the CLARUS C unit can be set up and programmed for operation within minutes. The CLARUS C unit program contains defined test parameters that can be stored, retrieved, and executed within seconds. The only maintenance required for the CLARUS C unit during this verification test was the addition of new hydrogen peroxide at the beginning of each run and disposal of unused hydrogen peroxide and waste by-product (i.e., water) at the end of each run. The printer paper had to be refilled once during testing. The automation of the CLARUS C unit left little room for operator error.

Subsequent to decontamination, the test coupons were evaluated qualitatively for visible surface damage. No damage (e.g., change in surface texture, color, etc.) to any of the test materials was observed.

For biological agents and surrogates, a quantitative analysis of efficacy was performed by comparing the number of spores extracted from a control coupon to the number of spores from the decontaminated test coupons. Because of the magnitudes of difference, efficacy is reported as the log of the ratio. Thus, a 1,000-fold reduction in spores after treatment is reported as 3 (the log of 1,000). Quantitative performance results for efficacy, based on extraction of spores in triplicate from the test materials, are summarized in Table 1.

Table 1. Mean Efficacy (Log Reduction) for Spores

Material[a]		*B. anthracis*[b]	*B. subtilis*[b]	*G. stearothermophilus*[b]
Porous	**Industrial-Grade Carpet**	3.01 (2.62-3.55)[c]	1.63 (1.46-1.76)[c, d]	0.81 (0.69-0.89)[d]
	Painted Concrete	6.36 (3.92-7.58)[c]	6.09 (5.58-7.10)[c]	4.09 (3.09-5.15)[c, d]
	Bare Wood	3.70 (3.20-4.46)[c]	2.18 (1.81-2.75)[c, d]	4.09 (3.80-4.61)[c]
Non-porous	**Glass**	7.92 (7.92)[c]	7.57 (7.57)[c]	4.68 (4.27-5.11)[c, d]
	Decorative Laminate	7.85 (7.85)[c]	7.66 (7.66)[c]	3.75 (2.20-4.77)[c, d]
	Painted Wallboard Paper	6.92 (6.92)[c]	7.52 (7.52)[c]	5.98 (5.47-6.99)[c]
	Galvanized Metal Ductwork	7.54 (7.54)[c]	6.44 (5.73-7.56)[c]	1.97 (1.90-2.04)[c, d]

[a] Three replicates were used for each test material for each organism.
[b] Log reduction in spores with range in parentheses.
[c] Mean significantly different from 0 ($P \leq 0.05$).
[d] Surrogate significantly different from *B. anthracis* for specified material ($P \leq 0.05$).

The results from the qualitative analysis of residual test spores or other surviving microorganisms following decontamination are summarized in Table 2.

Table 2. Growth (After Seven Days) of Residual Organisms on the Materials

Material[a]		*B. anthracis*[c]	*B. subtilis*[c]	*G. stearothermophilus*[c]
Porous	**Industrial-Grade Carpet**[b]	0	0	0
	Painted Concrete	++	0	0
	Bare Wood	+	+++	+++
Non-Porous	**Glass**	0	0	0
	Decorative Laminate	+++	0	0
	Painted Wallboard Paper	0	0	0
	Galvanized Metal Ductwork	0	0	+++

[a] Three replicates were used for each test material for each organism.
[b] The carpet, as manufactured, contains a broad-spectrum antimicrobial chemical. Although no bacterial growth was observed for these samples, no conclusions can be drawn as to residual organisms on the carpet.
[c] 0 indicates no growth in media for any of the samples after 7 days. + indicates growth in media for one of the samples. ++ indicates growth in media from two samples. +++ indicates growth in media in all three samples.

Surrogate biological indicators (*Bacillus subtilis* and *Geobacillus stearothermophilus*) and biological spore strips (*Bacillus atrophaeus*) showed no growth after decontamination (Table 3). These results are consistent with the high log reductions noted for non-porous surfaces, but do not reflect the lower decontamination observed for porous materials.

Table 3. Post-Decontamination Growth of Surrogate Indicators and Spore Strips

Biological Indicators/ Spore Strips[a]	Growth[b]
Biological Indicator (*B. subtilis*)	0
Biological Indicator (*G. stearothermophilus*)	0
Spore Strip (*B. atrophaeus*)	0

[a] For each testing day, 2 to 3 replicates were evaluated for each of the biological indicators and spore strips.
[b] 0 indicates no growth in media for samples after 7 days.

In summary, the CLARUS C unit did not change or damage any of the materials evaluated in the test. ETV testing of the CLARUS C unit provided a wide range of results, depending on the material being decontaminated. The CLARUS C unit demonstrated a log reduction of ≥ 6.9 for *B. anthracis* spores on non-porous surfaces and as low as 3 in *B. anthracis* spores on porous materials. In general, significant differences in efficacy between *B. anthracis* and both surrogate organisms were observed for porous materials. For non-porous materials, significant differences in efficacy between *B. anthracis* and *G. stearothermophilus* were observed in most cases. Surrogate biological indicators (*B. subtilis* and *G. stearothermophilus*) and biological spore strips (*B. atrophaeus*) showed results consistent with the high log reductions.

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