Autoclave Processing of Medical Waste

New Testing Reveals that Traditional Autoclave Processing of Medical Waste is Not Effective

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Introduction

Autoclave devices have traditionally been used for the treatment of infectious medical waste ……. or more *accuractely have been believed to be effectively treating medical waste for decades.*

Autoclaves used for the sterilization of medical instruments have been proven to be very effective for that purpose. Manufacturers designed these steam sterilizers with precise criteria that ensure that all surfaces of each item placed in the device would be uniformly exposed to the proper temperature and humidity for the proper time. Often, trays or other such positioning features would be used to ensure that instruments would be thoroughly in contact with steam to achieve sterility. A treatment parameter of 250^0F (121^0C) for around 60 minutes for this application was determined to be necessary to ensure sterility.

The medical waste industry made the assumption that the same time and temperature parameters could be applied to medical waste in devices that did not make any special design consideration for the density and random packing of the waste. In essence, the industry made a leap of faith that an autoclave stuffed with bags of medical waste forming a relatively huge mass of material would be thoroughly treated, i.e. "sterilized" using the same parameter.

For years, engineers familiar with heat transfer and thermodynamic design principles found it somewhat baffling that medical waste could be stuffed into large pressure vessels, under the same conditions as instrument sterilization procedures, and achieve the same result. However, the industry never conducted any serious testing on autoclaves to evaluate the ability of the device to treat medical waste thoroughly using test protocols developed to challenge design principals claimed by manufacturers.

On the other hand, alternative non-burn technologies introduced since the 1990's, were routinely challenged by regulators and the scientific community to verify the efficacy and environmental performance of their devices with specific test challenges tailored to test confirm their product claims. Such rigorous testing approaches have never been applied to traditional medical waste autoclaves until recently.

New Testing on Autoclave Efficacy Performance

In 2005, the California Department of Health Services (CADHS) announced that it had conducted tests on autoclave devices processing medical waste to evaluate the efficacy of their performance on suction canisters …… ***"The Department of Health Services (Department) has recently been involved in an evaluation of the efficacy of the treatment of suction canister waste by steam sterilization. The results of that evaluation suggest that in certain circumstances, suction canister waste may not be adequately treated by steam sterilization"***…… The CADHS directives also stated ***"Treatment systems that include a grinding component that destroys the suction canister and exposes a greater waste surface area for treatment appear to adequately sterilize the waste even if solidified …… For onsite treatment facilities this***

demonstration may include testing using proper spore media or putting procedures into place such as; removing the canisters from autoclaving, using canisters that do not withstand autoclave temperature conditions, installing an engineered system that eliminates the need to dispose of filled canisters in the medical waste stream, etc. For offsite treatment facilities using steam sterilization without a grinding function, testing using proper spore media will be required unless procedural changes are put into place to eliminate all suction canisters from the autoclave waste stream." *……. Steam Sterilization of Suction Canisters, Darice G. Bailey, Chief, Waste Management Section, letter dated February 6, 2005 (See attached document).*

The CADHS was one of the earliest regulatory agencies to publicly apply a test challenge to evaluate autoclave performance using traditional operational parameters. As stated in the testing report prepared by OnSite Sterilization, LLC, ***"These required operating parameters are based on the historical application of autoclaves in the treatment of medical devices. It was thought that if autoclaves could effectively treat such "clean" medical equipment, they would be equally acceptable in the processing of "dirty" medical waste. "Regulatory" operating parameters are based on temperature, pressure, and residence time at temperature. The controlling factor, temperature, is measured in the space between the autoclave shell and the RMW load. It is not a measure of actual temperature WITHIN the waste load."*** The CADHS testing was one of the first indications that medical waste autoclaves could not achieve efficacy performance claims as previously thought.

In fact, an autoclave test report released by OnSite Sterilization, LLC, an autoclave supplier, called **"NEW INSIGHTS and BETA TESTING"** (attached hereto) reveals test data that demonstrate the inadequacy of autoclave treatment under the operational parameters currently accepted by many regulatory agencies. For example, the following test results, documented by OnSite Sterilization, illustrate this point very clearly:

Cycle Selection		Phase One				Phase Two			Phase Three	
Type	Weight	Pre-Heat Time	Steam/Vacuum Time‡ (including repeats)	Repeat	Total Time	Waste Heating Time	Waste at Temperature (6 Log $_{10}$ Kill Time 18 min)†	Total Residence Time	Post Vacuum and Cool Down Time	Total Cycle Time
Red Bag										
Chart #1	73 LBS	16 min	21 min	1	37 min	39 min	21 min	60 min	10 min	107 min
Chart #2	243 LBS	20 min	21 min	1	41 min	72 min	18 min	90 min	15 min	146 min
Red Bag & Sharps										
Chart #3	98 LBS	18 min	39 min	2	57 min	102 min	23 min	125 min	15 min	197 min
Chart #4	240 LBS	14 min	40 min	2	54 min	127 min	37 min	164 min	18 min	237 min
Red Bag & Canisters										
Chart #5	91 LBS	25 min	54 min	3	79 min	324 min	36 min	360 min	16 min	455 min
Chart #6	254 LBS	13 min	59 min	3	72 min	529 min	19 min	548 min	16 min	636 min

‡ Steam/Vacuum Time begins when the chamber first reaches 250°F (121°C) and ends after programmed repeats of steam and vacuum.
† Kill Time begins when internal waste temperature has reached 250°F (121°C)

These test show that autoclaves cannot treat all categories of waste with the same cycle time parameters. Essentially, sharps container and suctions canisters could not be treated properly using traditional operational parameters. In addition, the autoclave cycle had to use multiple vacuum cycles to ensure that the steam could effectively penetrate the dense load of waste. Again, traditional autoclave practice typically uses only one vacuum step. The OnSite testing also reveals that load residence times at 250^0F (121^0C) would need to be 6-9 hours if the waste loads did not segregate sharps containers and suction canisters out of the load. The test loads used by OnSite were relatively small 91-254 lbs (41-115 kg) in a small diameter device. Therefore, one could predict that larger autoclaves using the same conditions would need even more time at temperature to properly treat the waste. These tests were conducted by using digital temperature data logging instruments placed in different points in the loads to accurately assess whether there was uniform heating of the load. These tests also were correlated with microbiological testing to achieve a target log 6 reduction of Geobacillis stearothermophilis spores.

A 2006 published report by the U.S. Environmental Protection Agency, National Homeland Security Research Center, Eastern Research Group, Inc and New York State Department of Environmental Conservation entitled ***"Destruction of Spores on Building Decontamination Residue in a Commercial Autoclave"*** confirmed the results as found in the OnSite testing mentioned above. The density of the material affected efficacy performance and multiple treatment cycles were needed to ensure proper treatment. The report concluded**, "*Autoclave cycles consisting of 120 min at 31.5 lb/in2 and 275°F and 75 min at 45 lb/in2 and 292°F effectively decontaminated the Building Decontamination Residue (BDR) material. Two sequential standard autoclave cycles consisting of 40 min at 31.5 lb/in2 and 275°F proved to be particularly effective, probably because the second cycle's evacuation step pulled the condensed water out of the pores of the materials, allowing better steam penetration. The results also indicated that the packing density and material type of the BDR in the autoclave could have a significant impact on the effectiveness of the decontamination process** *(see paper attached herein) published in Applied and Environmental Microbiology, Dec. 2006.*

Future Changing in Regulations

Regulations for the treatment of medical waste have been greatly influenced by the State and Territorial Association on Alternative Treatment Technologies (STAATT) which first published guidelines in the 1990's (STAATT I&II). These guidelines have become the accepted standard for treatment technologies throughout the US and in many countries.

In December 2005, the State and Territorial Association on Alternative Treatment Technologies (STAATT) held a conference to update their guidelines based on the experiences and data that have been made available from close scrutiny of medical waste treatment technologies in the intervening years. Conference participants included recognized experts in the evaluation and testing of medical waste treatment technologies from state and federal agencies, as well as representatives of governmental organizations within the United Kingdom and of technology vendors.

Several key issues were reviewed and discussed including new information on potential treatment limitations of steam autoclaves and other topics. During the STAATT I and II conferences autoclaves were not considered "emerging" or "alternative" technologies.

However, the current consensus is that autoclaves be included under the broad umbrella of medical waste treatment technologies. As such, they must meet the same standards in efficacy/validation testing as any other treatment systems, especially if used for the treatment of suction canisters, human pathological waste, animal carcasses, and/or other thermally resistant waste materials, e.g. items within sharps containers or material wrapped in tyvek plastic. Operational parameters should continue to be determined through discussions between vendors (or on rare occasions, the operator) and regulators, but the parameters should never be below those established in efficacy testing by vendors/operators of treatment systems.

In the majority of US states, the operating standards are based on the century old practices employed in the sterilization of medical devices, i.e., those that are employed within the sterile environment of the human body. It was the general consensus that effective treatment of medical waste creates a different set of challenges for autoclaves than do medical devices. Presentations by several participants of their own investigations indicated that the efficacy of autoclaves was dependent upon many variables including, but not limited to, the composition, density, liquid content, weight, and types of containers of the loads as they all affect the physics of heat transfer and steam penetration. In certain instances, the efficacy of autoclaves was found to be less than the minimum standards recommended by STAATT. These investigations have found that autoclave systems (without integral shredding before treatment) are failing to achieve minimum treatment levels of medical waste as illustrated in the above mentioned reports.

During the December 2005 STAATT meeting, the United Kingdom Environmental Agency representatives presented its findings on autoclave testing on various autoclave installations. As a result of the UK's testing on autoclaves, their new proposed guidance document published 2006 stated***, "These technologies may have severe limitations and may lack the technical ability to treat a worst case scenario challenge load of clinical waste. Where there is no physical action to enable sealed waste containers, and sealed voids in the waste to be punctured, then the treatment is unlikely to penetrate the waste fully."*** The UK also added that ***"Static autoclaves, including those with vacuum cycles, are particularly affected by this issue and the waste will require some form of physical pre-treatment (e.g.maceration) to enable effective treatment to take place."****(See attached UK-Environmental Agency CONSULTATION DRAFT V.1.1 - 24/02/06)*

Other experts attending the STAATT meeting shared similar discoveries on the limitations of autoclave, where testing resulted in performance to be less than traditionally accepted operating parameters.

Summary

In view of the growing body of test evidence, there has been growing recognition by industry experts and regulatory bodies, that traditional autoclave treatment parameters are often ineffective. However, under certain circumstances and proper management of treatment variables autoclaves can be made to work. Available testing shows that dramatically increasing residence time, employing multiple vacuum / steaming steps, is often necessary to achieve minimum acceptable treatment levels required by regulatory agencies in the US and many countries.

State of California—Health and Human Services Agency
Department of Health Services

SANDRA SHEWRY
Director

ARNOLD SCHWARZENEGGER
Governor

February 6, 2005

To: All Medical Waste Generators and Offsite Treatment Facilities

Subject: Steam Sterilization of Suction Canisters

The Department of Health Services (Department) has recently been involved in an evaluation of the efficacy of the treatment of suction canister waste by steam sterilization. The results of that evaluation suggest that in certain circumstances, suction canister waste may not be adequately treated by steam sterilization. Per the Medical Waste Management Act, Chapter 8, Section 118215 (a)(2)(D) treatment of waste must be demonstrated using a biological indicator. The law states that the indicator must be placed in the center of the waste load. Due to the density of filled waste canisters, the center of the load should be considered the center of a filled canister surrounded by waste if such containers are routinely part of waste loads.

The Department views the results from this initial evaluation as preliminary and further investigation, including testing, is necessary to allow for any definitive conclusions regarding steam sterilization treatment of this waste stream. Treatment systems that include a grinding component that destroys the suction canister and exposes a greater waste surface area for treatment appear to adequately sterilize the waste even if solidified. Sterilization also appears to be complete when un-solidified waste is in a suction canister made of a material that does not withstand the temperature conditions under which an autoclave is typically operated.

Based on the preliminary testing performed on surrogate suction canister waste, it appears that the addition of solidifiers to the liquid waste present in the suction canister prevents or inhibits the resulting solidified waste from reaching optimum temperatures that ensure adequate sterilization. Therefore it is inappropriate for solidified suction canisters to be treated by steam sterilization. The options for this waste stream would include treatment by incineration, extreme heat treatment systems such as pyrolysis and plasma arc technologies or systems than include grinding of the waste as part of the treatment technology.

Due to waste management controls in place, the Department believes that, even in those cases where the treatment of the contents of the suction canisters may be incomplete, the risk to the general public is minimal. However, facilities must review their waste treatment and quality control procedures, including the proper use of spore

testing and the placement of such controls in the waste stream, to ensure that all wastes are treated adequately. Information must be provided to the Department to demonstrate that suction canister waste is being properly treated. Any entity performing treatment of medical waste must submit proof that the waste is being properly treated. For onsite treatment facilities this demonstration may include testing using proper spore media or putting procedures into place such as; removing the canisters from autoclaving, using canisters that do not withstand autoclave temperature conditions, installing an engineered system that eliminates the need to dispose of filled canisters in the medical waste stream, etc. For offsite treatment facilities using steam sterilization without a grinding function, testing using proper spore media will be required unless procedural changes are put into place to eliminate all suction canisters from the autoclave waste stream. A testing procedure is attached as an example.

Information demonstrating adequate treatment of suction canister waste must be submitted to the Medical Waste Management Program within forty-five (45) days of the date of this letter.

Should you have any questions, you may contact Ron Pilorin at 449-5689 or via e-mail at rpilorin@dhs.ca.gov.

Sincerely,

Darice G. Bailey, Chief
Waste Management Section

Attachment

OnSite STERILIZATION, LLC

VariClave®

New Insights and Beta Testing

"Providing Safe, Efficient and Economical Solutions"

OnSite
STERILIZATION, LLC

TABLE OF CONTENTS

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NEW INSIGHTS

INTO THE APPLICATION OF AUTOCLAVES IN THE TREATMENT OF MEDICAL WASTE

Healthcare and commercial facilities that employ autoclaves to treat regulated medical waste (RMW) generally operate these devices at the minimum standards set by state regulations. These required operating parameters are based on the historical application of autoclaves in the treatment of medical devices. It was thought that if autoclaves could effectively treat such "clean" medical equipment, they would be equally acceptable in the processing of "dirty" medical waste. "Regulatory" operating parameters are based on temperature, pressure, and residence time at temperature. The controlling factor, temperature, is measured in the space between the autoclave shell and the RMW load. It is not a measure of actual temperature WITHIN the waste load. The associated residence times must be viewed as a surrogate for the time required for heat transfer sufficient to achieve the required log reduction of biological indicators within the load. Current "regulatory" minimum operating parameters do not provide for variations in the size of the loads in terms of weight or density, inherent difficulties of heat transfer, fluid content, types of containerization and/or the capabilities of the containers to withstand the environment created within the autoclave. OnSite's VariClave represents the first and only autoclave specifically designed and thoroughly tested for the treatment of the variety of components that now comprise the medical waste stream.

As an example, listed below is a portion of New York State Department of Environmental Conservation Rules and Regulations, Subpart 360-17, Regulated Medical Waste Treatment Facilities.

> Section 360-17.5 Requirements for treatment of regulated medical waste.
>
> (a) Operating parameters for autoclaves. An autoclave used to treat RMW shall be operated in accordance with the following minimum requirements:
>
> (1) When operating a gravity flow autoclave, RMW shall be subjected to:
>
> (i) a temperature of not less than 250 F and a pressure of 15 pounds per square inch gauge (psig) for an autoclave residence time of not less than 60 minutes;
>
> (ii) a temperature of not less than 275 F and a pressure of 31 psig for an autoclave residence time of not less than 45 minutes; or

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(iii) a temperature of not less than 300 F and a pressure of 52 psig for an autoclave residence time of not less than 30 minutes;

(2) When operating a vacuum autoclave, RMW shall be subjected to a minimum of one pre-vacuum pulse to purge the autoclave of all air, and the following:

(i) a temperature of not less than 250 F and a pressure of 15 psig for an autoclave residence time of not less than 45 minutes; or

(ii) a temperature of not less than 275 F and a pressure of 31 psig for an autoclave residence time of not less than 30 minutes;

(3) the minimum operating parameters for temperature, pressure, and residence time proposed for each autoclave unit shall be determined during start-up of the facility utilizing the approved validation testing program and standardized loads;

As previously noted, the regulatory requirements were based on century old practices employed in the sterilization of medical devices, i.e., those that are employed within the sterile environment of the human body. In addition, autoclaves were not considered "emerging" or "alternative" technologies in the 1994 and 1998 guidance reports of the State and Territorial Association on Alternative Treatment Technologies (STAATT) [1,2] Therefore, the stipulated operating parameters have essentially remained unchanged or have undergone only minimum revisions since their introduction into state regulations during the early 1990's. In fact, many states accept autoclaves by "rule", requiring that they be validated only when they are sited at a facility through tests that utilize a single biological indicator. It is interesting to note issues regarding the loading/conditioning of the waste relative to treatment in small countertop autoclave vessels had been raised by Vesley et al and Rutala et al in 1982 [3,4], and Palenik et al in 1993 [5]. These are now referenced in the 2003 CDC Guidelines for Environmental Infection Control in Health-Care Facilities [6]

The application of autoclaves in the treatment of medical waste was one of the items discussed at a meeting of state and federal regulators and treatment technology vendors in December 2005. The consensus was that effective treatment of medical waste creates a different set of challenges for autoclaves than do medical devices. The attendees recommended that autoclaves be included under the broad umbrella of medical waste treatment technologies. As such, they must meet the same standards in efficacy/validation testing as any other treatment systems. This is especially needed when autoclaves are used in the treatment of suction canisters, human pathological waste, animal carcasses and/or other thermally resistant materials. For example, Lemieux and co-workers recently described their evaluation of the effectiveness of a commercial autoclave for treating simulated building decontamination residue (BDR) [7]. The latter are the materials removed from a building that has been deliberately exposed to biological agents such as the spores of *Bacillus anthracis* (anthrax). They found that a single standard autoclave cycle, i.e., 275^{o}F for 40 minutes at 31.5 lb/in^{2} did not effectively decontaminate *Geobacillus stearothermophilus* spores contained within the simulated BDR. They reported that inactivation of the biological indicator required exposure for upwards of 120 minutes at the indicated standard parameters. In addition, they

noted that packing density and variations in the components of the stimulated loads could significantly influence the effectiveness of treatment in the autoclave.

Lemieux and his colleagues undertook their investigations because it was "not known whether the standard operating procedure for a commercial autoclave provides sufficient time, temperature, and pressure to adequately destroy residual bacterial spores bound to SDR." Similarly, given the historical use of autoclaves in the treatment of medical devices and the acceptance by state regulatory agencies of the operating parameters based on such use, there has been little motivation and consequently few controlled scientific investigations of the effective operating parameters for the treatment of medical waste by autoclaves.

It is for this reason that OnSite conducted exhaustive investigations to establish the appropriate operating parameters for medical waste using the VariClave. A review of literature regarding the application of autoclaves in the treatment of other forms of waste indicated that efficacy was dependent upon many variables, including, but not limited to, the composition, density, liquid content, weight, and types of containers within the test loads, as they all affect the physics of heat transfer and steam penetration. In addition, types of biological indicators, e.g., genus and species of bacterial spores, the spores "D" values, the placement of the indicators in the load, the use of strips or ampoules, as well as the methods used to determine the temperatures both in the autoclave and within the test loads could affect the selection of the operating parameters [8-11].

Due to these variables in the waste stream, it became apparent that it was necessary to define loads based on the composition of the waste generated in the routine operations of healthcare and research facilities. Based upon surveys and interviews, the OnSite staff determined that there were three types of waste loads, each with its own challenges to effective treatment by autoclaving, i.e., red bags, sharps containers and suction canisters. Therefore, tests were conducted employing variable densities and weights of each of these types of loads, with biological indicators from different sources, containing different spore concentrations and "D" values, on spore strips and in ampoules, and employing data tracers to establish the temperatures within the autoclave and test loads.

The results of these tests established the operating parameters indicated in the attached table and graphs (see Appendix A). The "red bag" cycle could be used to effectively treat bags containing a mixed load of waste components, as well as the most commonly used sharps containers, i.e., up to 3.5 gals. For larger sharps containers, up to 17 gal, as frequently employed in microbiology or clinical chemistry laboratories, treatment could be achieved with the "red bag + sharps container" cycle. However, suction canisters, with or without solidifying agents, as well as highly heat resistant materials require the extended exposure at the "suction canister" cycle.

Other general observations that were found through these tests:

- While other autoclaves used to process medical waste operate at steam temperatures of 272°F, 292 °F or even 304 °F, the VariClave uses saturated steam at approximately 258 °F with a set point of 250 °F. The fact of the matter is that there is little

thermodynamic advantage to operate at higher temperature. According to the steam tables, for every 2°F increase in temperature there is only an increase of 0.5 to 0.7 BTU/lb of available energy to heat the waste components. Put another way, significantly increasing the steam temperature does not materially reduce the time required to heat the waste load to the critical 250°F needed to inactivate potential pathogens. Once at this temperature, the VariClave can inactivate 10^6 bacterial spores in as little as 18 minutes;

- With the variables of the waste loads and the application of biological indicators controlled, it became apparent that larger loads require a longer residence time to reach the critical 250°F to effect treatment. A one-half ton load of medical waste cannot be treated at the same operating parameters as a 250 lb load. However, current regulations in many states make no distinction in the mandated operating requirements relative to the size of the load;

- Treatment of mixed loads containing components of different densities and/or different size and types of containers, e.g., red bags with culture plates and/or vacutainer tubes and sharps containers require a residence time suitable for the most thermodynamically challenging of these components. Treatment parameters for a load of culture plates in red bags is quite different from that containing 17 gal sharps containers;

- Multiple vacuum cycles have a positive impact on autoclave operation as they reduce the residence time required to attain 250°F within the load to effect treatment. Lemieux and co-workers noted in their studies that two vacuum cycles could reduce the residence time and were critical "for ensuring effective decontamination of porous materials in an autoclave" [3]. The multiple vacuum cycles compromise the structure of the containers, e.g., sharps containers, to allow easier steam penetration (for further information on the application of multiple vacuum cycles in the operation of the VariClave see Appendix C);

- These observations call into question the operating parameters used by other autoclaves in the treatment of medical waste, as well as those defined in the regulations of many states. The operating parameters should be determined by each vendor or user only after tests have been conducted that incorporate the multiple variables that present significant challenges to the autoclave's capability to effect treatment. The VariClave is one of only a few autoclaves, if not the only one, for which such tests have been conducted. The results from the studies have been used to define the three distinct operating cycles.

OnSite's responses to the test data and survey interviews have been:

- Never to compromise the efficacy of the VariClave in the treatment of medical waste to achieve higher throughput, i.e., pounds of waste treated per hour. Rather, the design of the VariClave's three treatment cycles achieves the maximum throughout within the bounds of occupational and public health safety;

- To establish a preprogrammed control panel to allow the operator to select which of the three cycles, i.e., red bag, red bag + sharps containers, or red bag + suction canisters, is most appropriate for the load to be treated with the VariClave;

- The development of variable cycles based upon extensive controlled scientific studies such that the VariClave's preset cycles automatically compensate for the variations in the waste loads generated within healthcare and research facilities;

- To employ an integral scale which weighs each waste cart to establish the total weight of the load and then modifies the residence time in accordance with an algorithm contained in its computer program;

- The introduction of a "turnable" cart and bin assembly to minimize waste handling by employees and consequently limit their exposure to the waste;

- To provide a comprehensive data collection, display, and retention control system which is operator "friendly" while providing detailed electronic and hard copy management of data for regulatory purposes and securing the data from each run for 7 years;

- To respond to the concerns of many regulators relative to the presence of radioactive materials in the waste loads by having a full bin radioactivity detector which gives an alarm upon the detection of radioactive substances in the load and locks out the autoclave cycle and requires the intervention of the operator; and

- The incorporation of an integral bin washing system to enhance the versatility of the VariClave.

In summary, the VariClave is the first and only autoclave that has been specifically designed and thoroughly tested for the treatment of medical waste. Responding to requests from healthcare and research facilities' personnel who are responsible for the processing of the waste, OnSite has incorporated numerous new features, which enhance the ease of operating the VariClave, and ensure worker, public, and environmental safety. Treatment of medical waste at each of the VariClave's three operating cycles meets and exceeds all current state and federal requirements. OnSite's VariClave is the new "gold standard" to which all other autoclaves used in the treatment of medical waste will be compared.

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I/S

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January 29, 2007

To Whom It May Concern:

This is to verify that we designed the efficacy test protocols and were directly responsible for conducting Round One and Two Beta tests of the On-Site VariClave autoclave. The investigations were conducted during November and December, 2006 at On-Site's facility, employing the prototype of the VariClave. Samples of the biological indicators recovered after each of the series of tests were either processed on-site or utilizing all appropriate precautions, transmitted via over night express to an independent laboratory for analysis. We have reviewed the test data and attest that the results presented are a true reflection of those we obtained as part of our on-site analysis and those provided by the independent laboratory.

If you have questions or require further information concerning either the protocols for and the results from these investigations, please feel free to contact us.

Sincerely,

Ira Salkin, Ph.D., F(AAM)
President
Information From Science LLC

Ed Krisiunas, MT(ASCP), CIC, MPH
President
WNWN International, Inc

OnSite "VariClave" Beta Testing and Analysis
November – December 2006

Introduction

Evaluation of the VariClave was organized into four distinct phases; De-bug and Operational Qualification, Round One Beta Testing, Fine Tuning of Variable Parametric Settings, and Round Two Beta Testing. Beta test protocols were designed and administered by Ira Salkin, Ph.D.,F(AAM) and Edward Krisiunas, MT(ASCP), CIC, MPH, while the other two phases of testing were conducted by OnSite Sterilization's staff under their direction.

De-Bug and Operational Qualification

A prototype VariClave was fabricated and assembled along with its control panel and operating software. In cooperation with supporting vendors, OnSite personnel evaluated all components of the system to address operating issues, add desired features and ensure reliable performance.

The initial operating set points for temperature, pressure, residence time and were set based on the minimum regulatory requirements.

Round One Beta Test Phase

Round One Beta Testing commenced on November 14, 2006. The protocols for testing were designed and administered by Dr. Ira Salkin and Mr. Edward Krisiunas. The OnSite staff participated in preparing the test loads and retrieving samples at their direction

In brief, the test protocols included the use of spores strips seeded with 10^4 and 10^5 spores of *Geobacillus stearothermophilus* and strips seeded with 10^4 spores of *Bacillus atrophaeus*. These were inserted at three different points, as indicated in the tables, within red bags containing simulated medical waste. In addition, self-contained biological indicators (24 hr readout) with 10^5 spores of *G. stearothermophilus* were placed in the middle and bottom of the red bags. Spore strips seeded with 10^4 spores of *G. stearothermophilus* were inserted into the barrel of 3 cc syringes in studies of 3.5 gal sharps containers. In addition, 3 cc syringes, loaded with 2 cc suspensions of 10^4 spores of *G. stearothermophilus* were placed into the same 3.5 gal sharps containers which were three-quarters filled with unused syringes. Self-contained biological indicators with 10^4 spores of *G. stearothermophilus* were placed at the indicated locations in 17 gal sharps containers which were three-quarters filled with empty vacuatiner tubes. Strips containing 10^4 spores of both test organisms were also employed in studies of solidified, non-autoclavable suction canisters. One strip of each indicator organism was inserted into the middle of the solidified contents of the suction canisters. The self-contained biological indicators with 10^5 spores of *G. stearothermophilus* were incubated on-site and growth/no growth read after 24

hrs of incubation. All other biological indicators were sent off-site for qualitative analysis.

The test results, analysis and conclusions appear below. The data clearly indicate that the processing parameters, i.e. temperature, pressure, residence time, based upon minimum regulatory requirements were inadequate to sufficiently heat the waste material throughout the load in order to inactivate the biological indicatators positioned deep within the loads. It was further found that the containerization and composition of the waste material, i.e. various size sharps containers and suction canisters, had a dramatic and material effect on the residence time required to achieve inactivation of the bacterial spores.

These test results illustrate the need for comprehensive testing of any autoclave used for the treatment of medical waste in order to establish equipment specific set points that ensure treatment.

ROUND ONE BETA TEST RESULSTS
NOVEMBER 14-16, 2006

November 14, 2006
One-hour residence time, Weight 198 pounds
Minimum 250°F and 15 psig with one pre-vacuum cycle.

Red bags containing simulated waste

Sample Location	Cart 1 – Bag 1	Cart 1 – Bag2	Cart 2 – Bag 1	Cart 2 – Bag2
Top	Ba-1e4-NG Gs-1e4-lost Gs-1e5-G	Ba-1e4-NG Gs-1e4-NG Gs-1e5-NG	Ba-1e4-NG Gs-1e4-G Gs-1e5-G	Ba-1e4-NG Gs-1e4-Lost Gs-1e5-NG
Middle	Ba-1e4-G Gs-1e4-G Gs-1e5-G	Ba-1e4-NG Gs-1e4-G Gs-1e5-G	Ba-1e4-NG Gs-1e4-G Gs-1e5-G	Ba-1e4-NG Gs-1e4-G Gs-1e5-G
Bottom	Ba-1e4-G Gs-1e4-G Gs-1e5-G	Ba-1e4-NG Gs-1e4-G Gs-1e5-G	Ba-1e4-G Gs-1e4-G Gs-1e5-G	Ba-1e4-G Gs-1e4-G Gs-1e5-G

Ba =*Bacillus atrophaeus*
Gs= *Geobacillus stearothermophilus*
1e4 = Concentration of 1 X 10^4
1e5 = Concentration of 1 X 10^5
G = Growth of spore; NG = No growth of spores
All samples were spore strips from either Sterilator or Raven
Top = Placement of strips near the top of the bag of simulated waste
Middle = Placement of strips near the middle of bag of simulated waste
Bottom = Placement of strips at the bottom of bag of simulated waste
All bags located at bottom of carts

OnSite "VariClave" Beta Testing and Analysis
November – December 2006

Additional Data – 1e5 *G. stearothermophilus* SCBI – 24 hr readouts – placed at the middle and bottom of bags = all showed growth

Sharps containers approximately half-filled with needle/syringes

Sample Location	Cart 1 – Sharp 1	Cart 1 – Sharp 2	Cart 1 – Sharp 3	Cart 2 – Sharp 1	Cart 2 – Sharp 2	Cart 2 – Sharp 3
Middle	Gs-1e4-S-G Gs-1e4-St-G	Gs-1e4-S-G Gs-1e4-St-G	Gs-1e4-S-NG Gs-1e4-St-G	Gs-1e4-S-G Gs-1e4-St-G	Gs-1e4-S-G Gs-1e4-St-G	Gs-1e4-S-NG Gs-1e4-St-G
Bottom	Gs-1e4-S-G Gs-1e4-St-G	Gs-1e4-S-G Gs-1e4-St-G	Gs-1e4-S-NG Gs-1e4-St-G	Gs-1e4-S-G Gs-1e4-St-G	Gs-1e4-S-NG Gs-1e4-St-G	Gs-1e4-S-NG Gs-1e4-St-G

Gs= *Geobacillus stearothermophilus*
1e4 = Concentration of 1 X 10^4
S= Spore Suspension
St = Spore strip
G=Growth of spores; NG = No growth of spores
Middle = Placement in the middle of sharps container – 3.5 gal containing syringe/needles
Bottom = Placement in the bottom of sharps container – 3.5 gal containing syringe/needles
Sharps container 1 – in bag at bottom of cart; sharps container 2 in bag at middle of cart and sharps container 3 in bag at top of cart

Additional Data – 1e4 *G. stearthermophilus* SCBI ampoules placed at the bottom, middle and top of 17-gal sharps containers three-quarters filled with empty vacutainer tubes = all showed growth

Conclusions – Red Bags –

1. Spores of *B. atrophaeus* were inactivated when located at the top or middle of bags, but generally were not "killed" when situated at the bottom.
2. Spores of *G. stearothermophilus*, i.e., 1e4 and 1e5 strips, were inactivated when situated at the top of #2 bags in carts 1 or 2.
3. Spores of *G. stearothermophilus* were not inactivated when located in the middle or bottom of all bags or at the top of #1 bags in carts # 1 and 2.
4. Spores of *G, stearothermophilus* in 1e5 SCBI were not killed when situated at the middle and bottom of all bags.
5. The results obtained with *B. atrophaeus* are consistent with their greater sensitivity to moist heat, especially as these indicators were located towards the top of the bags which would afford them greater exposure to treatment conditions.
6. The inactivation of *G. stearothermophilus* spores located at the top of both #2 bags was not expected at the indicated treatment parameters. It is

interesting that with bags #1 and 2 located next to each, at the bottoms of both carts, the spores were killed in one bag but not the other.

Conclusions – Sharps Containers –

1. Spores of *G. stearothermophilus* on strips within the syringe barrels were not inactivated regardless of the location of the syringes within the containers or the location of the containers.
2. Spores of *G. stearothermophilus* in suspensions within the syringe barrels were inactivated when the containers were located at the top position (container 3).
3. Spores of *G. stearothermophilus* in suspensions within the syringe barrels were not inactivated when the containers were located in the middle or bottom of the load (containers 1 and 2).
4. The use of spore strips within the syringe barrels does not appropriately simulate actual working conditions and therefore the results may not be relevant to the treatment capabilities of the Variclave.
5. The data from the use of spore suspensions are probably more reflective of the capabilities of the system to inactivate potential pathogens when syringes are discarded as waste.

Conclusions – Self-contained Biological Indicators –

1. Growth was noted with SCBI containing either 1e4 or1e5 concentrations of *G. stearothermophilus* and support the data found with spores of this bacterium in bags and sharps containers

November 15, 2006
Two-hour residence time, Weight 165 pounds
Minimum 250°F and 15 psig with one pre-vacuum cycle.

Red bags containing simulated waste

Sample Location	Cart 1 – Bag 1	Cart 1 – Bag 2	Cart 2 – Bag 1	Cart 2 – Bag 2
Top	Ba-1e4-NG Gs-1e4-NG Gs-1e5-NG	Ba-1e4-NG Gs-1e4-NG Gs-1e5-NG	Ba-1e4-NG Gs-1e4-NG Gs-1e5-NG	Ba-1e4-NG Gs-1e4-NG Gs-1e5-NG
Middle	Bs-1e4-NG Bs-1e4-NG Gs-1e5-NG	Ba-1e4-NG Gs-1e4-NG Gs-1e5-NG	Ba-1e4-NG Gs-1e4-NG Gs-1e5-NG	Ba-1e4-NG Gs-1e4-NG Gs-1e5-NG
Bottom	Ba-1e4-NG Gs-1e4-NG Gs-1e5-NG	Ba-1e4-NG Gs-1e4-NG Gs-1e5-NG	Ba-1e4-NG Gs-1e4-NG Gs-1e5-NG	Ba-1e4-NG Gs-1e4-NG Gs-1e5-NG

Ba =*Bacillus atrophaeus*
Gs= *Geobacillus stearothermophilus*

1e4 = Concentration of 1 X 10^4
1e5 = Concentration of 1 X 10^5
G= Growth of spores; NG = No growth of spores
All samples were spore strips from either Sterilator or Raven
Top = Placement of strips near the top of the bag of simulated waste
Middle = Placement of strips near the middle of bag of simulated waste
Bottom = Placement of strips at the bottom of bag of simulated waste
All bags located at bottom of carts

Additional Data – 1e5 *G. stearothermophilus* SCBI – 24 hr readouts – placed at the middle and bottom of bags = No growth with one exception

Sharps containers approximately half-filed with needle/syringes

Sample location	Cart 1- Sharp 1	Cart 1 – Sharp 2	Cart 1 – Sharp 3	Cart 2 – Sharp 1	Cart 2 – Sharp 2	Cart 2 – Sharp 3
Middle	Gs-1e4-S-NG Gs-1e4-St-G	Gs-1e4-S-NG Gs-1e4-St-G	Gs-1e4-S-NG Gs-1e4-St-NG	Gs-1e4-S-NG Gs-1e4-St-G	Gs-1e4-S-NG Gs-1e4-St-G	Gs-1e4-S-NG Gs-1e4-St-NG
Bottom	Gs-1e4-S-NG Gs-1e4-St-G	Gs-1e4-S-NG Gs-1e4-St-G	Gs-1e4-S-NG Gs-1e4-St-G	Gs-1e4-S-NG Gs-1e4-St-G	Gs-1e4-S-NG Gs-1e4-St-G	Gs-1e4-S-NG Gs-1e4-St-NG

Gs= *Geobacillus stearothermophilus*
1e4 = Concentration of 1 X 10^4
S= Spore Suspension
St = Spore strip
G=Growth of spores; NG = No growth of spores
Middle = Placement in the middle of sharps container – 3.5 gal containing syringe/needles
Bottom = Placement in the bottom of sharps container – 3.5 gal containing syringe/needles
Sharps container 1 in bag at bottom of cart; sharps container 2 in bag at middle of cart and sharps container 3 in bag at top of cart

Additional Data - 1e4 *G. stearthermophilus* SCBI ampoules placed at the bottom, middle and top of 17-gal sharps containers three-quarters filled with empty vacutainer tubes = no growth was found in the ampoule at the top of the container but growth was found with the indicators at the middle and bottom

Conclusions – Red Bags –

1. Exposure of bags with simulated waste for two hours at temperature caused the inactivation of spores (with one exception) of both *B. atrophaeus* and *G. stearothermopilus.*

2. No growth was found regardless of the concentration of the spores, the use of strips or SCBIs and the placement of the indicators within the bags.

3. Extending the exposure period from one to two hours obviously resulted in total inactivation of the bacterial spores within bags containing simulated waste.

Conclusions – Sharps Containers –

1. *G. stearothermophilus* spores were inactivated in all containers when suspensions were employed as the biological indicators.

2. *G. stearothermophilus* spores on strips within the syringes were also inactivated in the container at the top of the load (container 3).

3. *G. stearothermophilus* spores on strips were not inactivated in containers # 1 and 2 in either of the carts.

4. As noted with the bags of simulated waste, exposure of sharps containers for two hours resulted in the total inactivation of the suspensions of spores and even spores on strips in containers located at the top of loads.

Conclusions – Self-contained Biological Indicators –

1. SCBI containing 1e5 concentration of *G. stearothermophilus* spores placed in the bags of simulated medical waste were all negative, i.e., no growth.

2. SCBI containing 1e4 concentration of G. stearothermophilus spores was negative when situated at the top of 17-gal sharps containers but were positive, i.e., growth, when placed in the middle or bottom of the container.

3. Extending the exposure period from one to two hours enhanced the inactivation of spores at either 1e4 or 1e5 within SCBIs

November 15, 2006

Three-hour residence time, Weight 84 pounds
Minimum 250°F and 15 psig with one pre-vacuum cycle

Sample Location	*B. atrophaeus*	*G. stearothermophilus*
Canister 1 – SafeSorb	Growth	Growth
Canister 2 – SafeSorb	Growth	Growth
Canister 3 – SafeSorb	Growth	Growth
Canister 1 – LTS plus	Growth	No Growth
Canister 2 – LTS plus	No Growth	No Growth
Canister 3 – LTS plus	No Growth	No Growth

B. atrophaeus and *G. stearothermophilus* – 1e4 spore strips inserted in the middle of the solidified canisters
SafeSorb = Solidifying agent without treatment chemical
LTS plus = Solidifying agent containing sodium hypochlorite treatment chemical

Bags containing the canisters (two per bag) were all situated at the bottom of the carts

Additional Data – SCBI ampoules containing 1e4 suspension of *G. stearothermophilus* spores inserted into canisters containing only water– Growth was noted in all indicators within 24 hrs of exposure to treatment conditions.

Conclusions – Suction Canisters

1. Three hour exposure at the indicated temperature was not sufficient to inactivate spores of either test organism on strips in solidified (SafeSorb) or in ampoules in water within canisters.
2. Spores of both test organisms on strips in canisters solidified with LTS-plus were inactivated (one exception).
3. Results from previous experiments with LTS-plus have indicated that inactivation of spores could be achieved without exposure to autoclave conditions. Therefore, it is likely that the data from this experiment reflect the action of the chemical rather than that of the three hour exposure to the Variclave treatment parameters.

Overall Conclusions from all Round One Beta Tests –

1. The Variclave when operated for 2 hr at 250^{o}F and 15 psig is capable of effectively treating medical waste in standard red bags, as well as sharps in 3.5 gal sharps containers.
2. Mixed results were obtained with bags and sharps containers when exposed for 1 hr at these same temperature and pressure settings.
3. Suction canisters containing water or a non-chemical solidifying agent were not effectively treated by exposure for 3 hrs at 250^{o}F and 15 psig.
4. While spores were inactivated in canisters with LTS-plus, the results were probably due to the treatment chemical by itself or possibly to the combination of the chemical and the elevated temperature.
5. It would appear that vacutainer tubes effectively insulated *G. stearothermophilus* spores in the SCBLs in the 17-gal sharps containers.
6. The data from the studies on November 14 and 15 raise questions as to the operating parameters employed by other autoclaves and those contained in the regulations of many states.

Fine Tuning of Variable Parametric Settings

Based on the results obtained in Round One Beta Testing, OnSite personnel conducted studies to establish the appropriate process protocols and algorithms for the VariClave's Autorratic Variable Parametric Settings.

The results of the Round One Beta tests verified the fact that residence times required for treatment varied with the heat transfer properties of the waste to be processed, i.e., mass (weight), containerization, moisture content and degree of efficiency of air removal and steam penetration. They further indicated that fine tuning of the operational protocols and parametric settings would require a new approach rather than using the minimum regulatory requirements as the foundation for residence time settings.

The technique devised to find the appropriate machine settings includes the use of self-contained electronic temperature recorders placed in the most difficult locations in the load to heat. The temperature data from these recorders can be graphed to examine the temperature versus time relationship in heating the waste. Biological challenge would be provided in the form of Raven ProSpore® ampoules in concentrations of 10^4, 10^5 and 10^6 placed with the temperature recorders.

Six distinct test loads were developed for use in fine tuning the settings. First, three levels of challenge were established; Red Bag including sharps containers up to 3.5 gallons, Red Bag plus sharps containers larger than 3.5 gallons and Red Bag plus suction canisters. Then, for each of these, two typical loads would be prepared, one load weighing approximately 90 pounds and another weighing approximately 250 pounds. Testing would then establish the residence time required for effective treatment of each of these six loads. This timing would then be used to set the endpoints of the curve for the 'variable residence time by weight' algorithm for each waste challenge.

Empirical observation determined the number of pre-vacuum cycles required to ensure that the waste containers were compromised to permit steam penetration.

The six test loads were run at extended residence times. The data and graphs from the temperature recorders revealed the residence time required to heat the waste of each challenge to 250°F. This time was then extended by the known D-value of the biological indicators sufficiently to establish a residence time for 6 $\log_{10}$ inactivation of the bacterial spores.

Finally, the challenge loads were re-tested using those residence time and protocol settings to ensure actual 6 $\log_{10}$ reduction. The graphs for these tests are contained in Appendix A, "Fine Turing Operating Matrix and Graphs".

Round Two Beta Test

Round two Beta testing commenced on December 11, 2006. The protocols for testing were designed and administered by Dr. Ira Salkin and Mr. Edward

Krisiunas. The OnSite staff participated in preparing the test loads and retrieving samples at their direction.

The tests were performed with the cycle protocols and Automatic Variable Parametric Setting algorithms set as established during the Fine Tuning process. These are;

Temperature, Minimum 250°F (121°C)
Pressure, Minimum 15psig

Red Bag Cycle
- One, Phase One Pre-vacuum cycle
- Automatic Variable Parametric Settings; For loads less than or equal to 90 lbs the residence time is 60 minutes Above 90 lbs, the residence time is extended by approximately 11.25 seconds per pound.

Red Bag & Sharps Cycle
- Two, Phase One Pre-vacuum cycles
- Automatic Variable Parametric Settings; For loads less than or equal to 90 lbs the residence time is 120 minutes Above 90 lbs, the residence time is extended by approximately 16.5 seconds per pound.

Red Bag & Canister Cycle
- Three, Phase One Pre-vacuum cycles
- Automatic Variable Parametric Settings; For loads less than or equal to 90 lbs the residence time is 342 minutes Above 90 lbs, the residence time is extended by approximately 76.5 seconds per pound.

ROUND TWO BETA TEST RESULSTS

DECEMBER 11-13, 2006

Test procedures – In brief, self-contained ampoules (Raven Prospore biological indicators) holding 10^4 spores and 10^6 spores of *Geobacillus stearothermophilus* were situated within red bags containing simulated medical waste and 17 gal sharps containers holding mixtures of unused sharps and vacutainer tubes. In addition, 3cc syringes, loaded with 2cc suspensions of 10^4 spores and 10^6 spores of *Geobacillus stearothermophilus* (Sterilator Company), were placed into 3.5 gal sharps containers holding unused syringes. Finally, spore strips seeded with 10^4 spores and 10^6 spores of *Geobacillus stearothermophilus* were placed into solidified non-autoclavable and autoclavable suction canisters. The self-contained spore ampoules were incubated on-site and growth/no growth read after 48 hrs incubation. The test syringes and spore strips were sent off site for qualitative analysis.

OnSite "VariClave" Beta Testing and Analysis
November – December 2006

Red Bag Cycle (including 3.5 gallon Sharps Containers)
81 minute residence time, Weight 205 pounds
Minimum 250°F and 15 psig, one pre-vacuum cycle

Red Bags[A]	10^4 Spore Ampoules	10^6 Spore Ampoules
Top	48 hr – No growth	48 hr – No growth
Middle	48 hr – No growth	48 hr – No growth
Bottom	48 hr – No growth	48 hr – No growth

[A] Data presented represent results obtained with multiple bags containing simulated medical waste situated in both VariClave carts. The self-contained spore ampoules were positioned, as indicated, at the top, middle and bottom of the simulated load in each bag

Sharps Containers- 3.5 gal[A]	Syringes - 10^4 suspension	Syringes – 10^6 suspension
Top	7 day – No growth	7 day – No growth
Middle	7 day – No growth	7 day – No growth
Bottom	7 day – No growth	7 day – No growth

[A] Data presented represent results obtained with multiple sharps containers which were sealed and included within red bags containing simulate medical waste. The bags were then placed at the top, middle and bottom of the waste loads, in each of the two VariClave carts. The syringes holding spore suspensions at the indicated concentrations were inserted at the bottom of the containers and then overlaid with empty syringes prior to placing the containers into red bags.

Red Bag + (17 Gallon) Sharps Container Cycle
140 minute residence time, Weight 162 pounds
Minimum 250°F and 15 psig, two pre-vacuum cycles

Sharps Cortainers – 17 gal[A]	10^4 Spore Ampoules	10^6 Spore Ampoules
Middle	48 hr – No growth	48 hr – No growth
Bottom	48 hr – No growth	48 hr – No growth

[A] Data presented represent results obtained with multiple sharps containers situated in both of the VariClave carts. The self-contained spore ampoules were placed in the middle and bottom of containers holding a mixed load of unused sharps and vacutainer tubes. Note that red bags and 3.5 gal sharps containers, as employed in the red bag cycle described above, were exposed during these tests. No growth was again found with the self-container spore ampoules and spore suspensions within syringes.

Suction Canister Cycle
502 minute residence time, Weight 216 pounds
Minimum 250°F and 15 psig, three pre-vacuum cycles

Suction Canister[A]	10^4 Spore Strip	10^6 Spore Strip
NA[B] Canister - SafeSorb	7 day – No growth	7 day – No growth
NA Canister – LTS Plus	7 day – No growth	7 day – No growth
Autoclavable Medela[C]	7 day – No growth	7 day – No growth

[A] Data presented represent results obtained with multiple suction canisters situated in both of the VariClave carts. The strips were placed in the middle of 2.4 liter suction canisters which had been solidified with either SafeSorb or LTS Plus.

[B] NA = Non-autoclavable suction canisters

[C] Medela autoclavable suction canisters – as with the non-autoclavable canisters, the spore strips were inserted into the middle of the solidified contents.

Conclusions

The VariClave, when operated at the set points including the automatic, variable residence time feature as described on page 9, has been proven to provide efficacious treatment of RMW regardless of its composition, containerization, weight or volume.

APPENDICES

A. FINE TUNING
OPERATING MATRIX AND GRAPHS

B. PHOTOGRAPHS

C. THE THREE PHASE PROCESS

APPENDIX A

VARICLAVE FINE TUNING
OPERATING MATRIX AND GRAPHS

OnSite
STERILIZATION, LLC

VariClave® Set Point Matrix

6 $\log_{10}$ Reduction

Cycle Selection		Phase One			Phase Two			Phase Three	Total Cycle Time
Type / Weight	Pre-Heat Time	Steam/Vacuum Time‡ (including repeats)	Repeat	Total Time	Waste Heating Time	Waste at Temperature (Log K) (Time: 12 min)†	Total Residence Time	Post Vacuum and Cool Down Time	
Red Bag									
Chart #1 73 LBS	18 min	21 min	1	37 min	39 min	21 min	60 min	10 min	107 min
Chart #2 243 LBS	20 min	21 min	1	41 min	72 min	18 min	90 min	15 min	146 min
Red Bag & Sharps									
Chart #3 98 LBS	18 min	39 min	2	57 min	102 min	23 min	125 min	15 min	197 min
Chart #4 240 LBS	14 min	40 min	2	54 min	127 min	37 min	164 min	18 min	237 min
Red Bag & Canisters									
Chart #5 91 LBS	28 min	54 min	3	79 min	224 min	30 min	360 min	16 min	455 min
Chart #6 264 LBS	13 min	59 min	3	72 min	429 min	19 min	548 min	16 min	636 min

‡ Steam/Vacuum Time begins when the chamber first reaches 250°F (121°C) and ends after programmed repeats of steam and vacuum.
† Hold Time begins when internal waste temperature has reached 250°F (121°C)

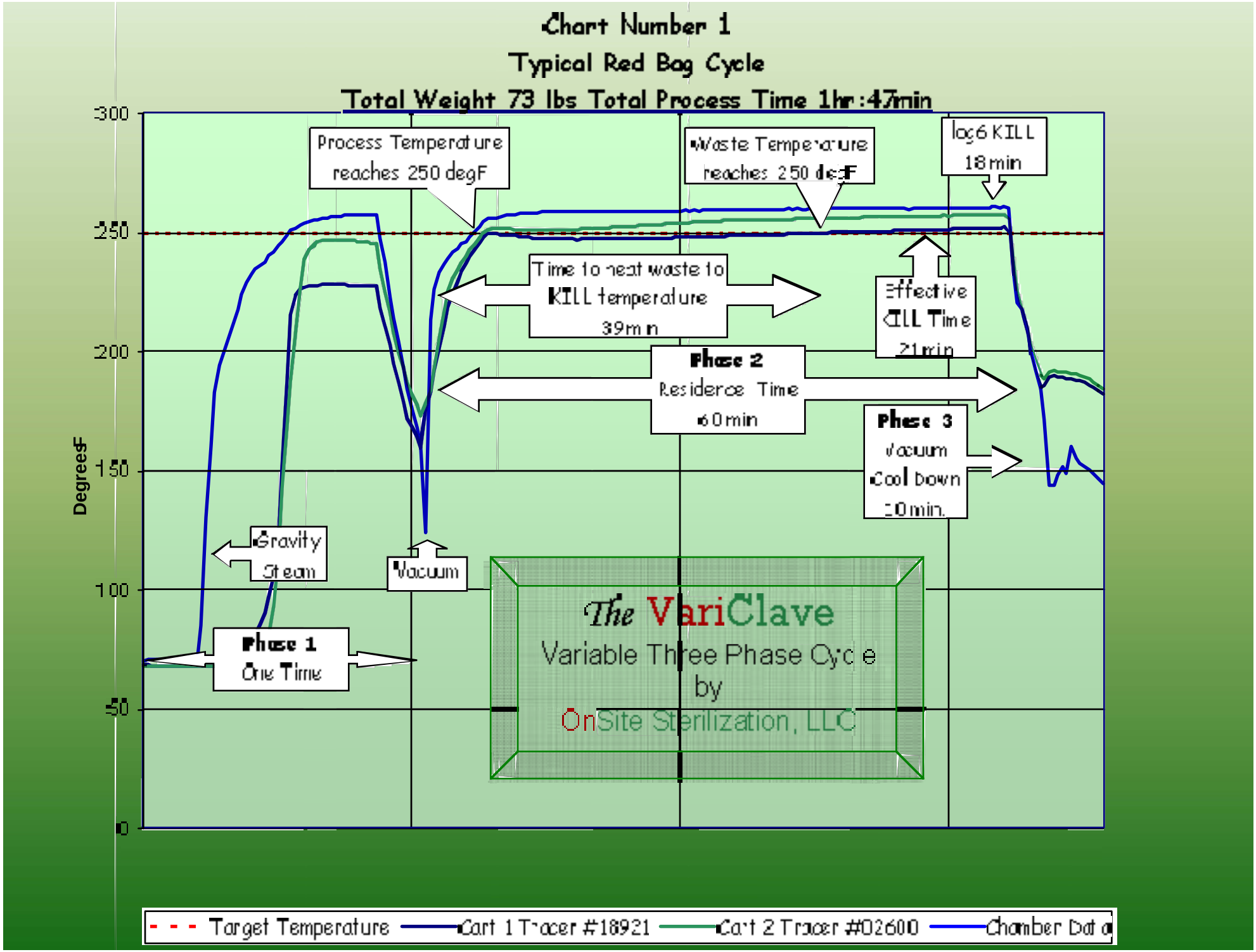
Chart Number 1
Typical Red Bag Cycle
Total Weight 73 lbs Total Process Time 1hr:47min
300
250
200
150
100
50
0
Degrees F
Process Temperature reaches 250 degF
Waste Temperature reaches 250 degF
log6 KILL 18 min
Time to heat waste to KILL temperature 39min
Effective KILL Time 21min
Phase 2 Residence Time 60min
Phase 3 Vacuum Cool down 10min.
Gravity Steam
Vacuum
Phase 1 One Time
The VariClave
Variable Three Phase Cycle
by
OnSite Sterilization, LLC
Target Temperature
Cart 1 Tracer #18921
Cart 2 Tracer #02600
Chamber Data

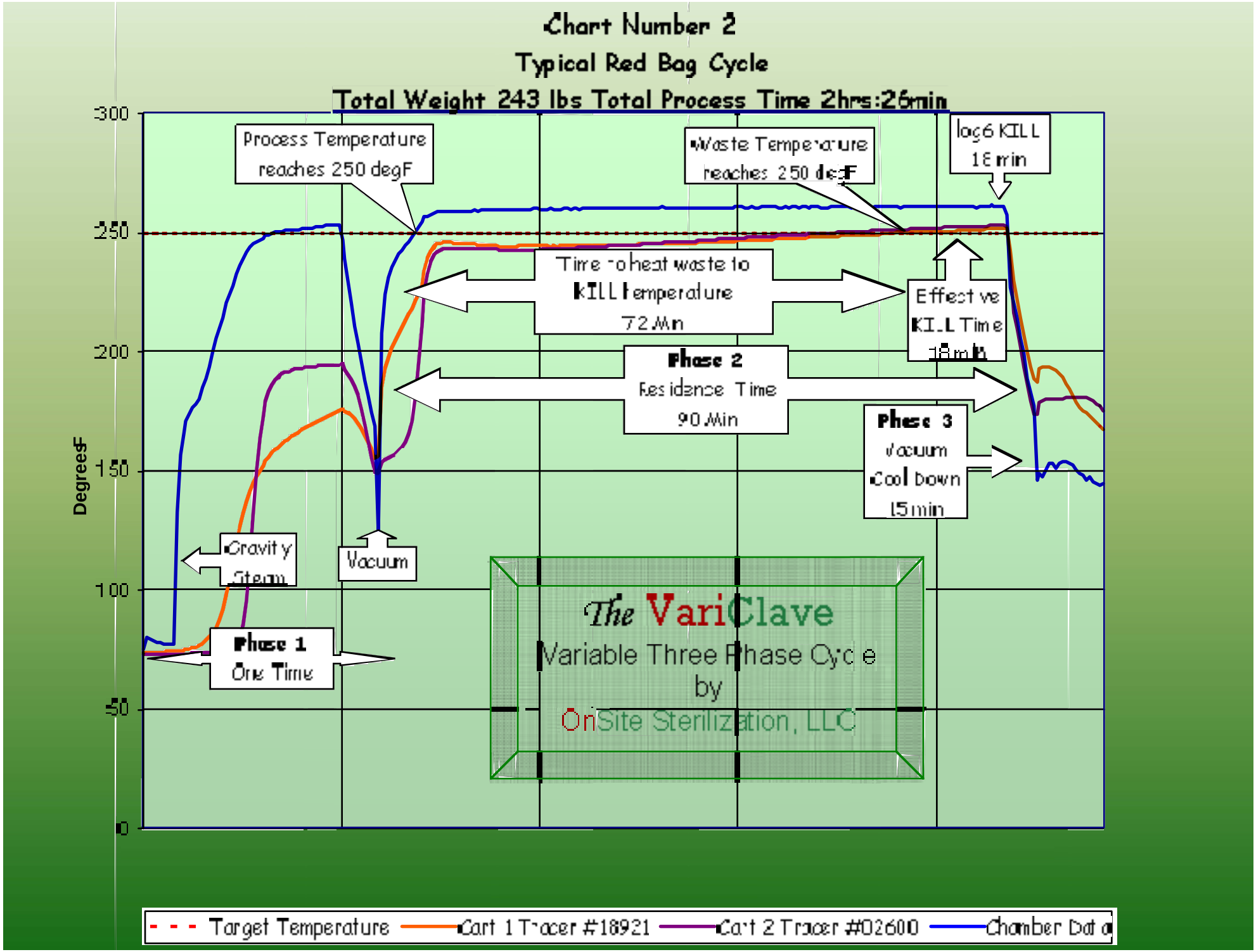
Chart Number 2
Typical Red Bag Cycle
Total Weight 243 lbs Total Process Time 2hrs:26min
300
250
200
150
100
50
0
DegreesF
Process Temperature reaches 250 degF
Waste Temperature reaches 250 degF
log6 KILL 18 min
Time to heat waste to KILL temperature 72 Min
Effective KILL Time 18 min
Phase 2 Residence Time 90 Min
Phase 3 Vacuum Cool down 15 min
Gravity Steam
Vacuum
Phase 1 One Time
The VariClave
Variable Three Phase Cycle
by
OnSite Sterilization, LLC
Target Temperature
Cart 1 Tracer #18921
Cart 2 Tracer #02600
Chamber Data

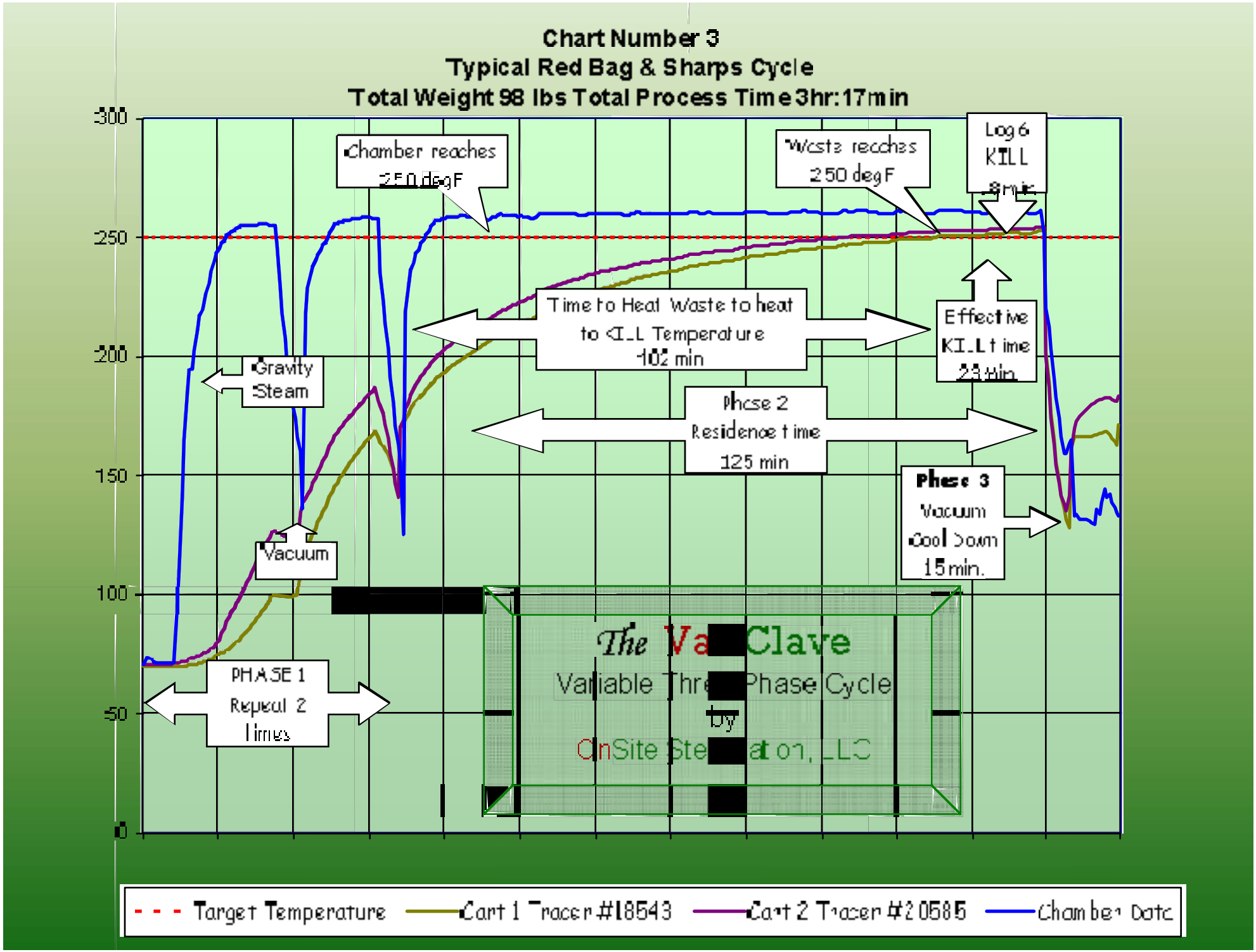
Chart Number 3
Typical Red Bag & Sharps Cycle
Total Weight 98 lbs Total Process Time 3hr:17min
300
250
200
150
100
50
0
Chamber reaches 250 degF
Waste reaches 250 degF
Log 6 KILL 8min.
Time to Heat Waste to heat to KILL Temperature 102 min
Effective KILL time 23 min.
Gravity Steam
Phase 2 Residence time 125 min
Phase 3 Vacuum Cool Down 15 min.
Vacuum
PHASE 1 Repeat 2 Times
The VaClave
Variable Three Phase Cycle
by
OnSite Sterilization, LLC
Target Temperature
Cart 1 Tracer #18543
Cart 2 Tracer #20585
Chamber Data

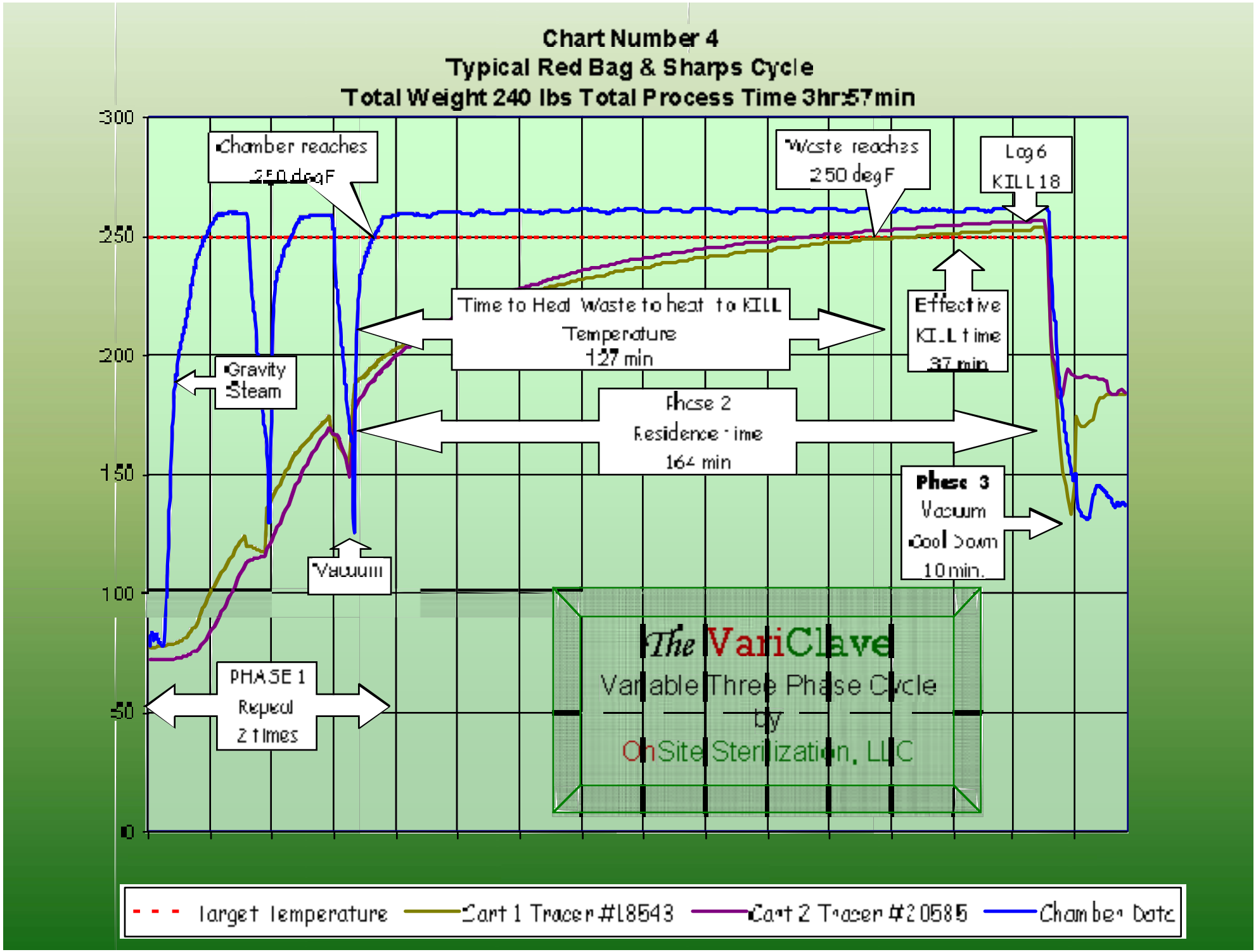
Chart Number 4
Typical Red Bag & Sharps Cycle
Total Weight 240 lbs Total Process Time 3hr:57min
300
250
200
150
100
50
0
Chamber reaches 250 degF
Waste reaches 250 degF
Log 6 KILL 18
Time to Heat Waste to heat to KILL Temperature 127 min
Effective KILL time 37 min
Gravity Steam
Phase 2 Residence Time 164 min
Phase 3 Vacuum Cool Down 10 min.
Vacuum
PHASE 1 Repeat 2 times
The VariClave
Variable Three Phase Cycle
by
OnSite Sterilization, LLC
Target Temperature
Cart 1 Tracer #18543
Cart 2 Tracer #20585
Chamber Data

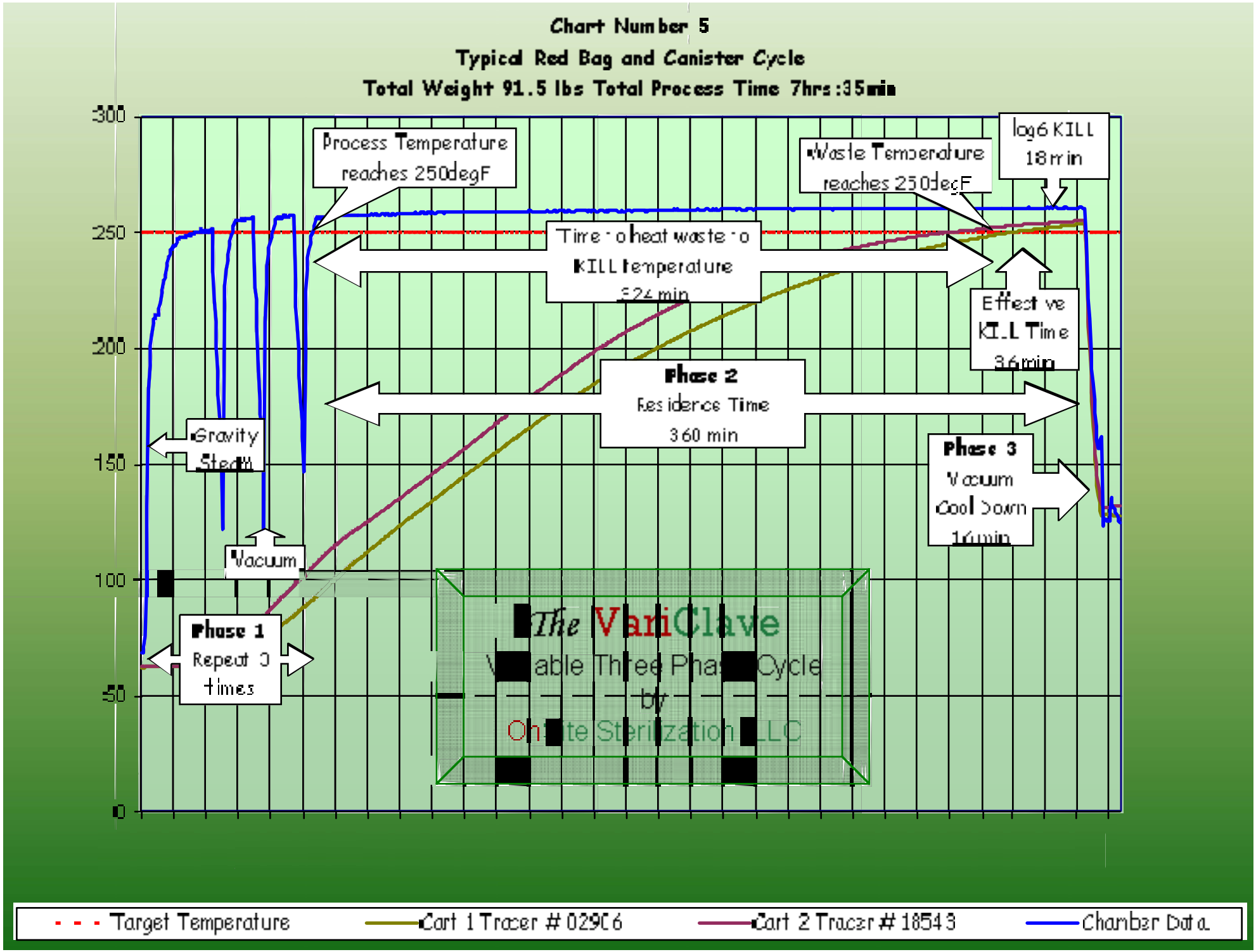
Chart Number 5
Typical Red Bag and Canister Cycle
Total Weight 91.5 lbs Total Process Time 7hrs:35min
300
250
200
150
100
50
0
Process Temperature reaches 250degF
Waste Temperature reaches 250degF
log6 KILL 18min
Time to heat waste to KILL temperature 124 min
Effective KILL Time 36min
Phase 2
Residence Time
360 min
Gravity Steam
Vacuum
Phase 1
Repeat 3 times
Phase 3
Vacuum Cool Down
16min
The VariClave
Variable Three Phase Cycle
by
Onsite Sterilization LLC
Target Temperature
Cart 1 Tracer # 02906
Cart 2 Tracer # 18543
Chamber Data

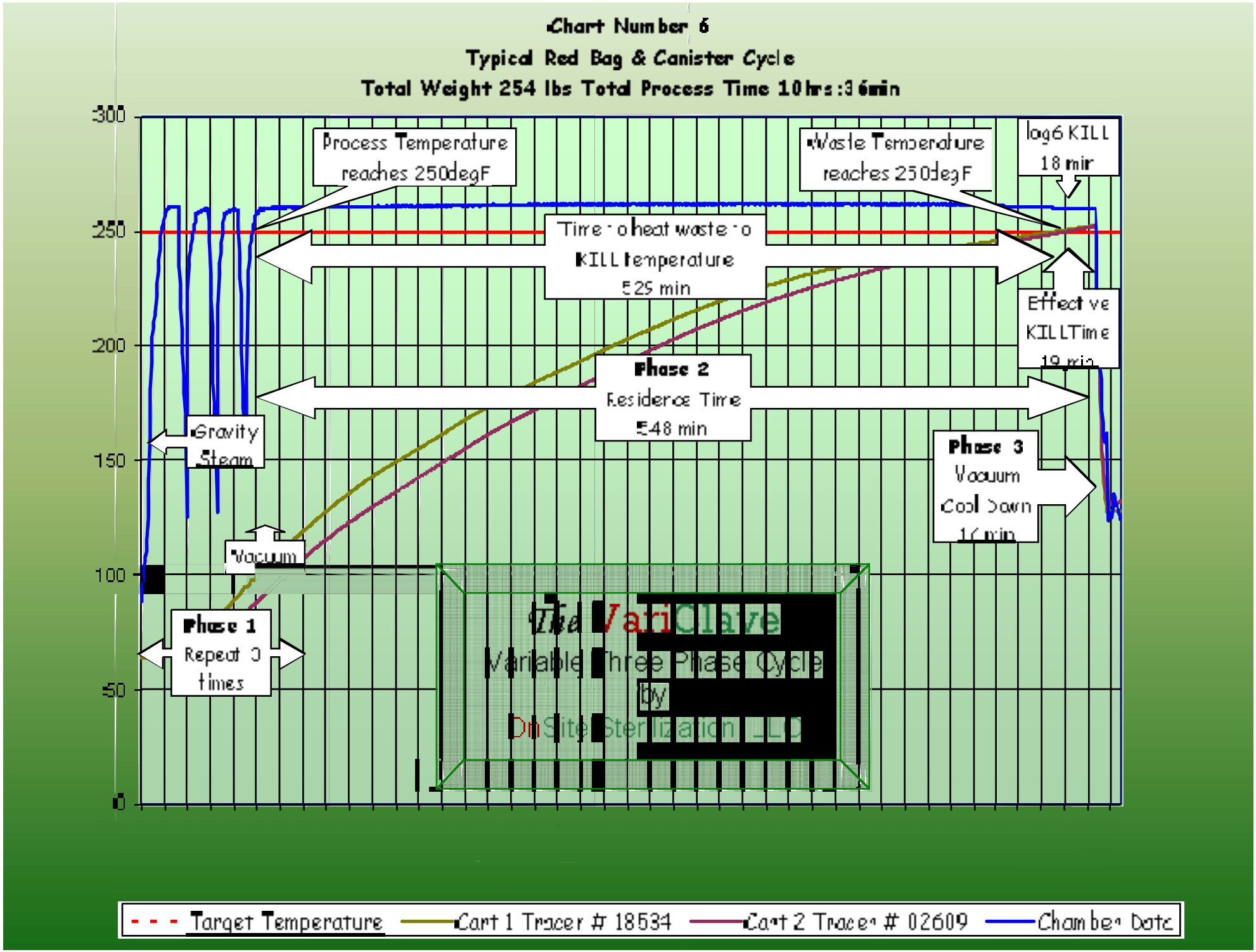
Chart Number 6
Typical Red Bag & Canister Cycle
Total Weight 254 lbs Total Process Time 10 hrs :36min
300
250
200
150
100
50
0
Process Temperature reaches 250degF
Waste Temperature reaches 250degF
log6 KILL 18 min
Time to heat waste to KILL temperature 525 min
Effective KILL Time 19 min
Phase 2 Residence Time 548 min
Gravity Steam
Vacuum
Phase 1 Repeat 3 times
Phase 3 Vacuum Cool Down 17 min
The VariClave
Variable Three Phase Cycle
by
On Site Sterilization LLC
Target Temperature
Cart 1 Tracer # 18534
Cart 2 Tracer # 02609
Chamber Data

APPENDIX B

PHOTOGRAPHS

Figure #1. View of the different types of biological indicators, i.e., spore ampoules, spore strips, syringes load with spore suspensions, employed in the efficacy studies of the Variclave.

Figure 2. An enlarged view of the 10^4 and 10^6 spore ampoules employed in the efficacy studies of the VariClave.

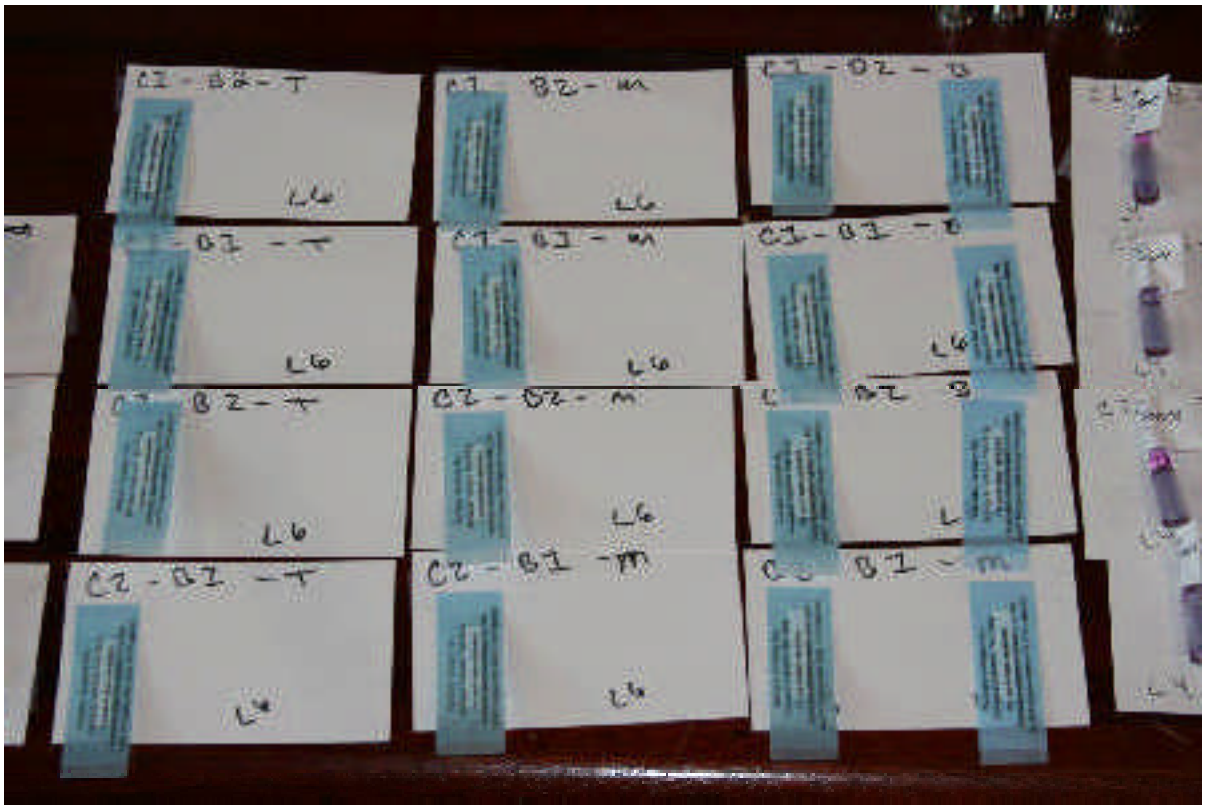

Figure #3. An enlarged view of the 10^4 and 10^6 spore strips used in the efficacy tests of the VariClave.

Figure #4. Small (3.5 gal) sharps container used in the testing.

Figure #5 – Small (3.5 ga) sharps containers loaded with biological indicators and unused sharps – to allow for view, tops were removed.

Figure #6 – Seventeen gallon sharps container loaded with biological indicators and unused vacutainer tubes – to allow for view, top was removed

Figure #7 – Preparation of suction canisters with different solidifying agents

Figure #8 – Data tracers as used to gather temperature data within the autoclave and within the test containers

Figure #9 – VarClave cart partially loaded with red bags, 3.5 gal and 17 gal sharps containers.

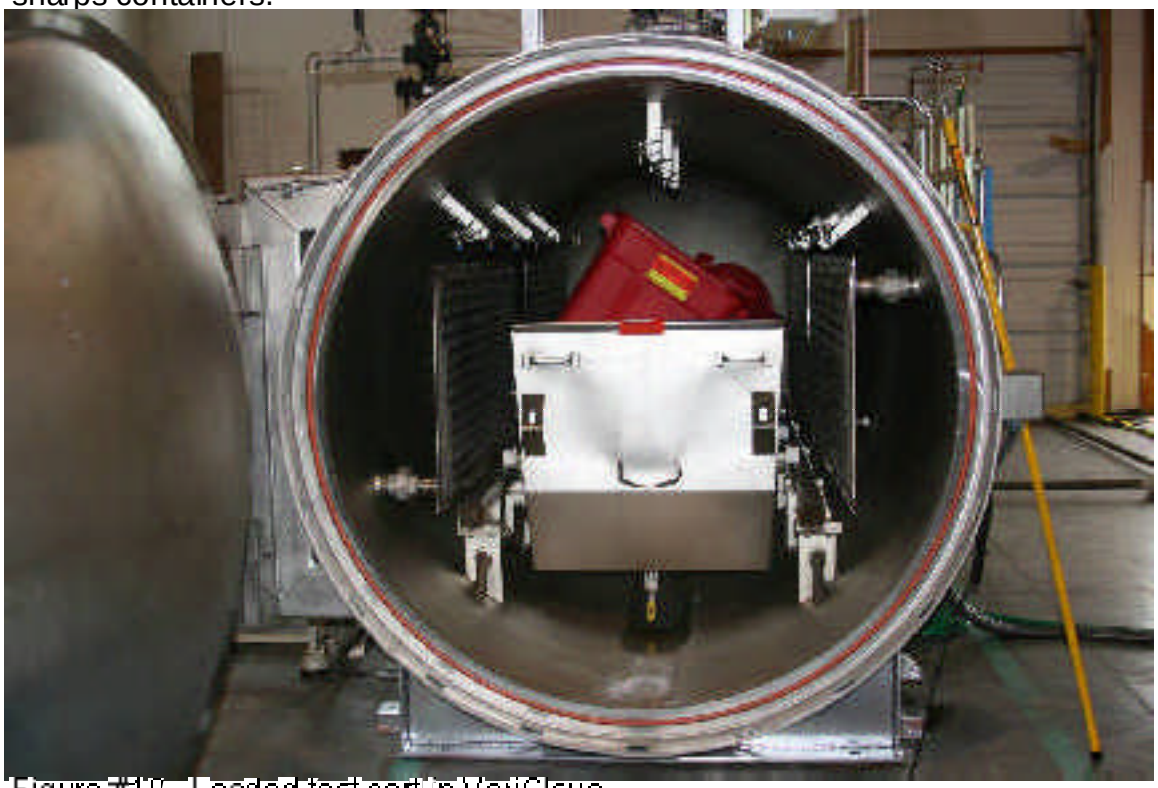

Figure #10 – Loaded test cart in VariClave

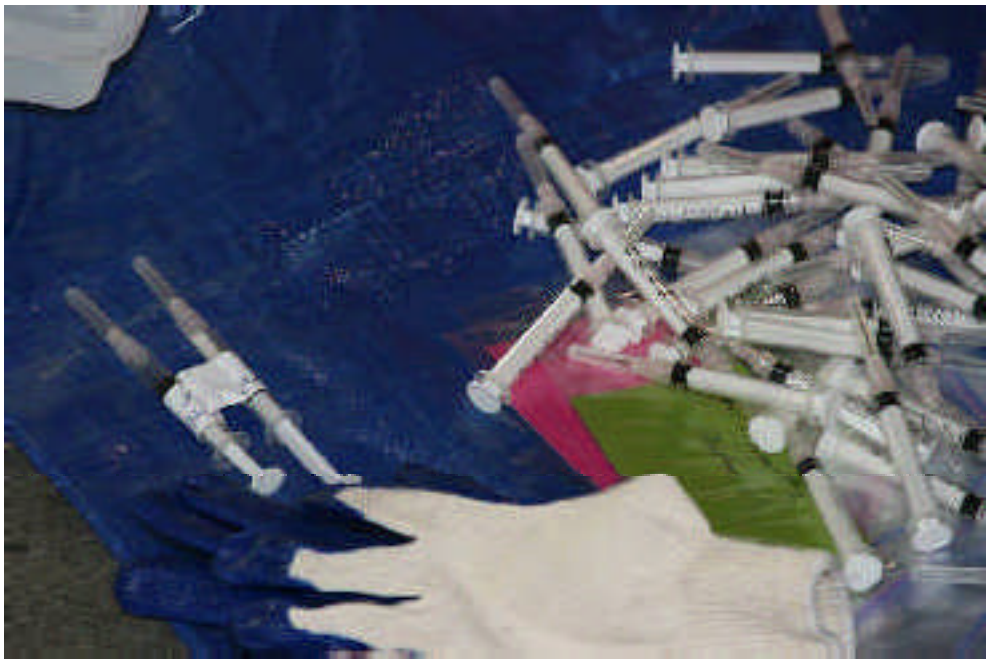

Figure #11 – Retrieval of syringes containing spore suspension after treatment

Figure #12 – Autoclavable suction canister on left and non-autoclavable one on right after treatment in the VariClave

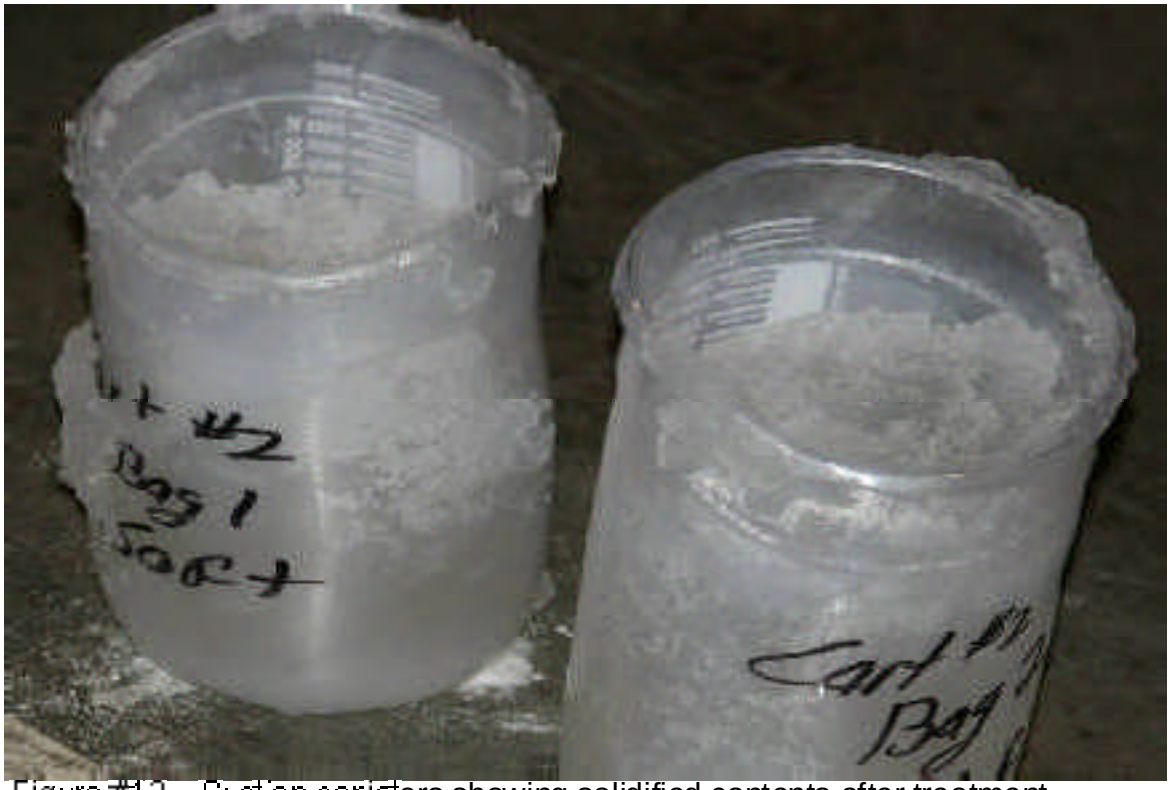

Figure #13 – Suction canisters showing solidified contents after treatment

Figure #14 – VarClave control panel as employed in the tests.

APPENDIX C

THE VARICLAVE THREE PHASE PROCESS

THE VARICLAVE THREE PHASE PROCESS

The VariClave Three Phase process was developed to address issues relative to its efficacy in processing medical waste, as well as environmental and workplace safety concerns. The variable timers and set points for each Phase and each Cycle Type have been established through exhaustive testing.

PHASE 1

The first component of this phase is the gravity steam portion in which steam is introduced into the chamber by gravity, displacing the air and preheating the stainless steel shell. This is followed by a steam pressure segment, during which the steam pressure in the vessel is increased to a minimum of 15psig and 250°F. A variable timer maintains this condition for a period sufficient to kill any airborne pathogens (ABP) that may be present within the chamber. Finally, a vacuum is drawn on the vessel to remove any tramp air and prepare for Phase 2. This heating and vacuum regimen is repeated twice for the 'Red Bag & Sharps' cycle and three times for the 'Red Bag & Suction Canister' cycle. These repetitions are necessary in order to effectively compromise the waste containers for the removal of air and distribution of steam during the second phase.

PHASE 2

This phase in the operation of the VariClave begins with the introduction of steam into processing chamber under vacuum, filling the chamber and interstitial spaces in the waste. Steam pressure and temperature are maintained at a minimum of 15psig and 250°F for a residence time sufficient to heat and hold the waste at 250°F for disinfection. The chamber pressure being vented to the atmosphere signals the end of Phase 2.

PHASE 3

Phase 3 has been included to prepare the bins and materials they contain to be safely removed from the chamber. The phase begins with the operation of the vacuum pump to remove residual moisture from the chamber and the waste. After relieving the vacuum, fresh water is sprayed on the sides of the bins to cool them for safe removal. The cycle ends with a final operation of the vacuum pump to reset the door seal and remove any moisture remaining from the cooling water spray.

The Three Phase Cycle with automatic variable parametric settings results in; (1) treatment of potential ABPs, (2) effective steam impingement by compromising containment systems, (3) efficient treatment by varying residence time based on weight and characterization to the load, (4) a cool to the touch bin for safe removal and (5) no steam plume or odor when the chamber door is opened.

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Destruction of Spores on Building Decontamination Residue in a Commercial Autoclave[▽]

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The U.S. Environmental Protection Agency conducted an experiment to evaluate the effectiveness of a commercial autoclave for treating simulated building decontamination residue (BDR). The BDR was intended to simulate porous materials removed from a building deliberately contaminated with biological agents such as *Bacillus anthracis* (anthrax) in a terrorist attack. The purpose of the tests was to assess whether the standard operating procedure for a commercial autoclave provided sufficiently robust conditions to adequately destroy bacterial spores bound to the BDR. In this study we investigated the effects of several variables related to autoclaving BDR, including time, temperature, pressure, item type, moisture content, packing density, packing orientation, autoclave bag integrity, and autoclave process sequence. The test team created simulated BDR from wallboard, ceiling tiles, carpet, and upholstered furniture, and embedded in the BDR were *Geobacillus stearothermophilus* biological indicator (BI) strips containing 10^6 spores and thermocouples to obtain time and temperature profile data associated with each BI strip. The results indicated that a single standard autoclave cycle did not effectively decontaminate the BDR. Autoclave cycles consisting of 120 min at 31.5 lb/in^2 and 275°F and 75 min at 45 lb/in^2 and 292°F effectively decontaminated the BDR material. Two sequential standard autoclave cycles consisting of 40 min at 31.5 lb/in^2 and 275°F proved to be particularly effective, probably because the second cycle's evacuation step pulled the condensed water out of the pores of the materials, allowing better steam penetration. The results also indicated that the packing density and material type of the BDR in the autoclave could have a significant impact on the effectiveness of the decontamination process.

In the event of a terrorist attack on a building in which biological weapons, such as *Bacillus anthracis* (anthrax), might be used, much of the porous material in the building may be shipped somewhere for disposal after decontamination. Such material is collectively termed "building decontamination residue" (BDR). Although the BDR may be disinfected or decontaminated prior to shipment, it may need additional decontamination to ensure that the contaminating agent has been destroyed, or because of heightened political sensitivities (e.g., a stigma attached to the waste) the BDR may need to be handled as if it were still contaminated. There are no mandated action levels for residual spores in such material, and the emergency response personnel or on-scene coordinators typically work with relevant state regulators to determine what constitutes proper BDR disposal. Much of the BDR might be tightly packed and possibly wet. The U.S. Environmental Protection Agency has initiated a research program to investigate issues related to the proper disposal of BDR (8).

Autoclaves are commonly used to effectively treat regulated medical waste by exposing the waste to steam at elevated pressures and temperatures for extended periods of time (e.g., 31.5 lb/in^2 and 275°F for 40 min) (6). However, it is not known whether the standard operating procedure for a commercial autoclave provides sufficient time, temperature, and pressure to adequately destroy residual bacterial spores bound to BDR.

The primary objective of this study (10) was to establish whether the standard operating conditions for a commercial medical waste autoclave are sufficient to destroy bacterial spores potentially found on BDR, and if not, what modifications to the standard operating procedure could be recommended to ensure complete spore destruction. The secondary objective of this study was to investigate the time and temperature dependence of destruction of *Geobacillus stearothermophilus* spores as a function of autoclave operating conditions and BDR composition. *G. stearothermophilus* was chosen because it is widely available and commonly recommended (11) for validation of moist heat sterilization.

MATERIALS AND METHODS

Autoclave description. The Environmental Protection Agency conducted the tests on 4 to 6 March 2005 at the Healthcare Environmental, Inc., facility located in Chemung, NY, approximately 90 miles from Albany. This facility can treat up to 24 tons of medical waste per day using two identical autoclaves that are 8 ft in diameter and 32 ft long, which accept large metal bins (80 in. by 54 in. by 69 in.) on rollers. Each autoclave (Bertoch model 896) can process six bins with a total mass of approximately 3,000 to 4,000 lb per cycle.

The State and Territorial Association on Alternate Treatment Technologies (STAATT) produced a document (11) that established a framework or guidelines that defined efficacy criteria for medical waste treatment technology and delineated the components required to establish an effective state medical waste treatment technology approval process. This document recommended that all medical waste treatment technologies achieve 6 logs or greater microbial inactivation of mycobacteria and 4 logs or greater reduction of spores. Approximately 37 states use the STAATT criteria for the treatment of regulated medical waste

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FIG. 1. Bulkhead flange for temperature measurement.

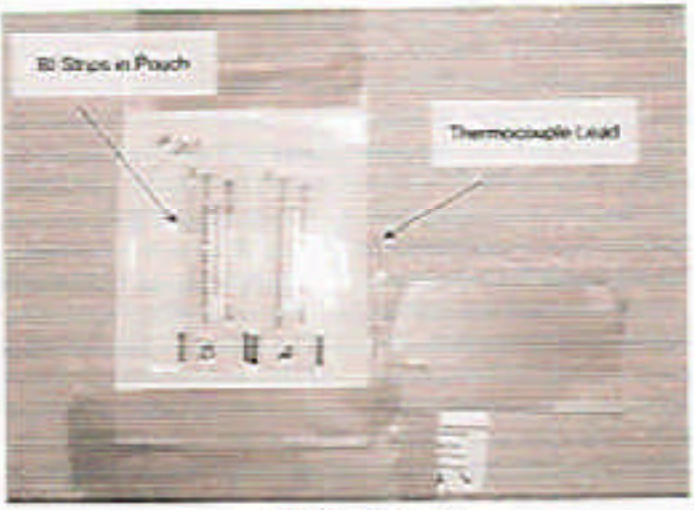

FIG. 2. BI pouch.

of biohazardous waste. Effective in December 2003, the STAATT criteria are also used for autoclave technologies. While BDR may not be classified as regulated medical waste, a commercial autoclave rather than a bench-scale autoclave was investigated because of the quantities of BDR that may be generated in the event of biological contamination.

The nominal autoclave operating cycle time is 40 min plus cool-down time to prepare for subsequent loads. At the start of each cycle, the autoclave is sealed and air is evacuated for 5 min using a vacuum pump to obtain a pressure of approximately −10 lbf/in^2. Steam is then injected until the desired operating pressure and temperature are reached, typically within approximately 5 min, and this operating pressure and temperature are maintained. The nominal operating conditions during the cycles are 31.5 lbf/in^2 and 275°F. Steam is injected through three ports at the top of the autoclave, located at the front, center, and rear. The steam is injected over distributor plates that cause turbulent, fluidized steam flow throughout the autoclave. At the end of each cycle, the steam is evacuated again by pulling vacuum on the autoclave.

Testing approach. Autoclave performance was judged based on two primary test variables: measurements obtained with thermocouples and viability determined with biological indicator (BI) test strips containing 10^6 spores of *G. stearothermophilus* embedded within each load of simulated BDR material tested. The testing comprised a series of test runs with different conditions in one of the facility's autoclaves (unit A1).

For each test run, 24 thermocouples were embedded in the BDR material to record the time/temperature profile at different locations within the load. Additional control thermocouples not embedded in BDR recorded the temperature inside and outside the autoclave (for the sake of data completeness and as an additional diagnostic for operation of the temperature measurement instrument). The thermocouple wires passed into the autoclave through a custom flange plate with a Swagelok bulkhead fitting packed with high-temperature RTV-silicone sealant (Fig. 1). The real-time temperatures was monitored and recorded at each sampling point using a GEC Instruments model 5277C temperature measurement system and type "T" thermocouples. Temperatures were recorded to a hard disk at approximately 10-s intervals.

A BI pouch was paired with a thermocouple at each test location (Fig. 2). Each BI pouch contained two *G. stearothermophilus* (ATCC 7953) lot 3167092I expiration date, January 2007, $D_{121°C}$ value [i.e., the time it takes for a 1-log kill at 121°C], 1.5 min; $D_{132°C}$ value, 0.34 min) indicator strips, labelled A and B, encased in a GS Medical Packaging self-seal pouch (catalog no. 2221006). Each BI strip contained 10^6 spores on Schleicher & Schuell filter paper (catalog no. 470) and was encased in a glassine peel-open envelope. Raven Biological Laboratories, Inc. manufactured the BI strips and assembled the BI pouches. After the test, the A strips were analyzed to determine growth using the United States Pharmacopeia viable spore count procedure (12). The strips were removed from their pouches, transferred into tryptic soy broth with bromocresol purple indicator, and incubated at 55 to 60°C for 7 days. If examination of the A strips showed that there were viable spores, a population assay was performed with the corresponding B strips using United States Pharmacopeia biological indicator (spore strip) population determination (12). The population was determined after 24 h of incubation at 55 to 60°C in tryptic soy agar. Three types of control BI test pouches were also used in the test: BI test pouches fully exposed to the autoclave conditions (but not embedded within BDR), BI test pouches packaged and handled like other BDR test pouches but not autoclaved, and duplicate BI test pouches (i.e., two pouches placed next to each other in the BDR). Figure 3 shows the position of the fully exposed controls.

The following variables were identified as variables that had a potential impact on penetration of hot steam into the BDR and therefore had an impact on the ability to destroy spores: item type (wallboard, ceiling tile, carpeting, upholstered furniture); moisture content of the autoclaved material (wet, dry); autoclave packing density (loose, dense); packing orientation (horizontal, vertical); opening autoclave bags prior to the cycle; autoclave temperature and pressure (31.5 lbf/in^2 and 275°F, 45 lbf/in^2 and 292°F); time in autoclave (up to 2 h); and multiple sequential autoclave cycles.

The test matrix shown in Table 1 was designed to investigate the effects of each of these variables.

Item types. Unpainted wallboard (LaFarge regular grade 0.5-in.-thick drywall) was cut into sections that were approximately 2 ft by 2 ft. Sample BDR bags were

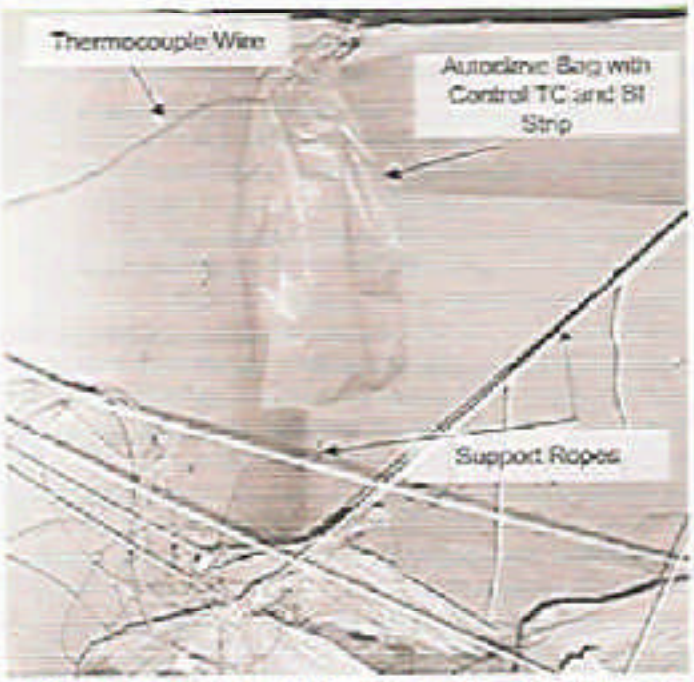

FIG. 3. Control placement (carpet and ceiling tile shown). TC, thermocouple.

TABLE 1. Test matrix

Run	Type of BDR*	Packing arrangement	Pressure (lb/in²)	Temp (°F)	Time (min)
1	Mixed	Loose, horizontal	31.5	275	120
2	Wallboard	Dense, horizontal	45	292	120
3	Carpet	Loose, dense, large roll	31.3	275	120
4	Mixed (including sofa)	Loose, horizontal	45	292	75
5	Mixed	Loose, vertical	31.5	275	40 (two sequential runs)
6	Mixed	Loose, vertical, open bags	31.5	275	40 (two sequential runs)

* Mixed material consisted of wallboard, ceiling tile, and carpet.

prepared by placing five of these sections face to face in autoclave bags. Wallboard was tested both wet and dry. To this context, *dry* refers to as-is condition at ambient humidity with no additional moisture added. Wet samples were submerged in a tank of water for 30 s and placed on a drain rack for 5 min before they were placed in the bag. Dry test bags weighed approximately 34 lb, and wet bags weighed approximately 37 lb. Samples were double bagged in 1.6-mil polypropylene autoclave bags (to represent likely practices that would be found during an emergency response), and the bags were individually goosenecked and taped shut using duct tape. A section of nylon rope was attached to the gooseneck to allow personnel to easily and safely load and unload the bags from the autoclave bins. Three types of wallboard bags were created. Some test bags (called "one-sample" bags) were assembled with one thermocouple and one test strip pouch placed together between the second and third wallboard sections. Other test bags (called "three-sample" bags) were assembled with three thermocouples paired with three test strip pouches placed between the first and second, second and third, and fourth and fifth wallboard sections. Additional bags were prepared without thermocouples and BIs and were used as fillers.

Ceiling tiles (Armstrong Contractor Series model 542 0.625-in.-thick ceiling panels) were cut into sections that were approximately 2 ft by 2 ft. Samples were prepared like the wallboard samples; however, the bags contained nine sections (2 ft by 2 ft) placed face to face. Dry test bags weighed approximately 23 lb, and wet bags weighed approximately 31 lb. "One-sample" bags contained one thermocouple and one test strip pouch placed together between the fourth and fifth ceiling tile sections. "Three-sample" bags contained three thermocouples paired with three test strip pouches placed between the second and third, fourth and fifth, and seventh and eighth ceiling tile sections. Additional bags were prepared without thermocouples and BI test pouches and were used as fillers.

Carpet (Mannington Nepenthe II fine commercial grade carpeting with Nylon 6,6 fibers) was tested in two configurations, small and large rolls. For small rolls, the carpet was cut into strips that were 26 in. wide and 20 ft long, representing how carpet would most likely be removed from a building. Some samples were soaked with a hose-end sprayer. After soaking, samples were rolled and placed on end to allow freeflowing water to drain. Small rolls were bagged like the wallboard and ceiling tiles. Dry test bags weighed approximately 26 lb, and wet bags weighed approximately 40 lb. As a worst-case model, larger sections of carpet that were 6 ft wide and 24 ft long were also tested. Only large rolls that were wet were prepared, and they weighed approximately 200 lb, the maximum size that could be reasonably handled by two workers. The large rolls were wrapped in polypropylene, and all seams were sealed with duct tape. For the small carpet rolls, "one-sample" and "three-sample" bags were prepared. "One-sample" bags contained one thermocouple and one test strip pouch placed together at the approximate midpoint of the radius of the carpet roll. "Three-sample" bags contained three thermocouples paired with three test strip pouches placed two laps from the top, at the midpoint of the radius, and two laps from the center of the carpet roll. Additional bags were prepared without thermocouples and BI test pouches and were used as fillers. The autoclave bins at the two ends of the group of bins used for each run were filled with BDR material without instruments to provide thermal mass and to minimize the impact of any cold spots within the autoclave.

To represent upholstered furniture, a dry, wool, queen size sleeper sofa was autoclaved in run 4. Four thermocouples and four test strip pouches were paired and embedded at the following locations in the sofa: one sample each was inserted into holes that approximately 6 in. deep in a back cushion and a seat cushion (the holes were then covered with duct tape); one sample was placed inside the folded sleeper mattress; and one sample was placed between the seat cushions. Although surface contamination of upholstered furniture is the most likely scenario, the BI strips were embedded in the upholstered furniture to simulate a worst-case scenario. The sofa was wrapped in polypropylene with all seams sealed with duct tape and then placed in the autoclave on a sheet of plywood.

Packing density. Wallboard was tested using two packing densities. In low-density packing, six bags were placed in a bin, forming a single layer at the base of the bin; some surfaces of all bags were exposed to autoclave temperatures. In high-density packing, 25 bags were placed in each bin, forming layers approximately three to four levels deep. In this arrangement, some bags were exposed directly to autoclave conditions, while others were buried in the load in the bin. Ceiling tiles were tested only using a low-density arrangement, as described above for wallboard. Runs of densely packed ceiling tiles were deleted from the test matrix because densely packed BDR material could not be brought up to autoclave temperatures within the 120 min specified in the test plan. Carpet was tested in three configurations. Small rolls, approximately 1 ft in diameter and 26 in. long, were placed in bags. Six bags were placed in a bin for low-density packing, and 25 bags were placed in a bin for high-density packing. In addition, a large, intact roll of carpet 6 ft long and approximately 1.5 ft in diameter was tested in one run. Figure 4 shows the dense and loose packing arrangements.

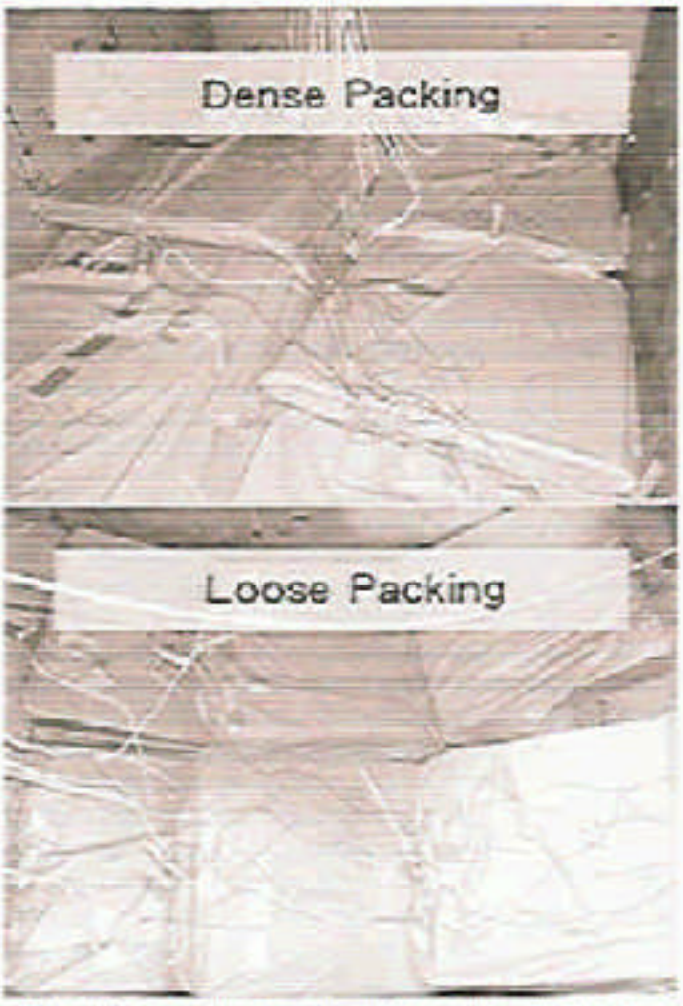

FIG. 4. Dense and loose packing arrangements (wallboard shown).

FIG. 5. Vertical BDR positioning (wallboard and ceiling tile shown).

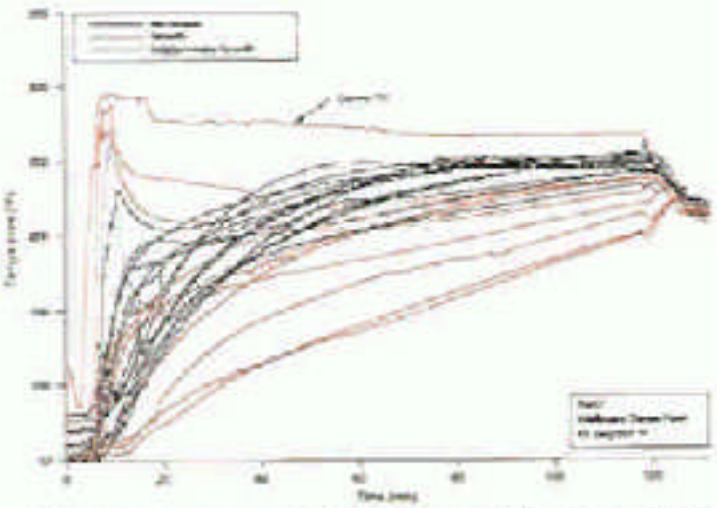

FIG. 7. Temperature and wallboard spore viability for run 2. TC, thermocouple; psig, lb/in^2.

Packing orientation. Material was tested both lying horizontally (Fig. 4) in the autoclave bins and positioned vertically, with all sides exposed. Material was positioned vertically by tying two ropes to the top of a bag and attaching the ends of the ropes to opposite sides of the autoclave bin wall, as shown in Fig. 5. The bags were hung vertically to simulate a rack system and to position bags so that all sides were exposed to steam. The test team theorized that hanging the BDR upright would keep it from compressing from its own weight and allow steam condensate to drain more easily as it formed. If these hypotheses were correct, these conditions would facilitate steam penetration and more effective heating of the material in the autoclave.

Open bags. All BDR was double bagged in 1.8-mil polypropylene autoclave bags. The bags were individually gooseneked and sealed with duct tape. This procedure was adopted based on packaging information from the State Department, Sterling, Va., mail facility anthrax cleanup (1). After autoclaving, some of the bags had closely ruptured due to temperature and pressure changes. However, in many cases, bag wallboard bubbled and became delaminated in the autoclave, but it was not clear if they had fully opened. To test if the bag opening had an effect on decontamination, two bags in run 5 and all of the bags in run 6 were opened prior to autoclaving by slicing open two sides of each bag with a utility knife.

Autoclave conditions. The test plan initially established a minimum run time of 40 min at an elevated temperature (270°F), which is the standard operating conditions for the Healthcare Environmental autoclave. Previously published data indicate that holding material for 15 min at 250°F is required to ensure moist heat sterilization (2–4, 7). Therefore, the test plan called for extending the run time to more than 40 min so that the 250°F temperature target was reached at all, or at least most, embedded thermocouples. Even if the 250°F target had not been reached, the test plan established a maximum run time of 120 min to enable the autoclave to process multiple test runs each day. Runs 1, 2, and 3 were terminated at 120 min, before all the thermocouples reached the target temperature. Run 4 was stopped at 75 min because the sofa temperature was rising above the temperature of the autoclave, indicating that there was a potential exothermic reaction in the sofa. Because the reaction and possible associated hazards were not well understood, the run was terminated. The BI strips in the sofa all showed no growth, indicating that the run 4 conditions were sufficient to decontaminate upholstered furniture. In runs 5 and 6, two 40-min runs were conducted in sequence.

Multiple short cycles. As steam in the autoclave was evacuated at the end of runs 2, 3, and 4, the test team observed that as the vacuum was drawn, most thermocouple readings converged toward a single temperature. It was not known if this resulted from increased turbulence during the postvacuum cycle, from condensed water being drawn out of BDR under vacuum, or from some combination of these and other factors. To further investigate this phenomenon, in runs 5 and 6, two complete normal autoclave operating cycles were run in succession. Each cycle consisted of prevacuum, steam pressurization, and postvacuum phases. The cycles were conducted with no time between them, and the autoclave remained sealed throughout both cycles.

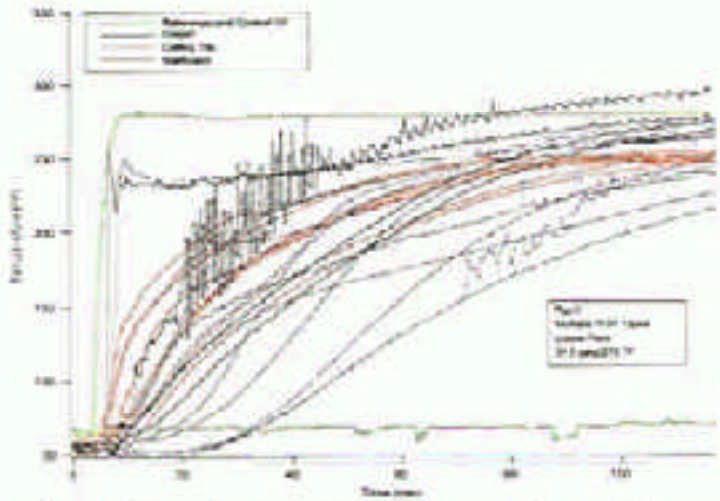

FIG. 6. Time and temperature data (loose packing). TC, thermocouple; psig, lb/in^2.

RESULTS

Figures 6, 7, 8, 9, 10, 11, and 12 show plots of the time and temperature data recorded during each of the six runs. Some of the figures (Fig. 6 and 10) also include readings from the control thermocouple inside the autoclave, the reference thermocouple outside the autoclave, and the autoclave set point pressure and temperature.

Figure 6 shows the time and temperature data from run 1. Note that in run 1 there was a significant amount of noise on several of the thermocouple channels, believed to result from condensation accumulating in the thermocouple connection fittings (Fig. 1). After run 1, the bundle of thermocouple wire outside the flange was positioned so that gravity prevented condensate from collecting, and in subsequent runs there was only a minimal amount of noise. It should be noted that although the temperature of the control thermocouple rapidly approached the autoclave operating temperature, the temperature of many of the thermocouples never reached the target

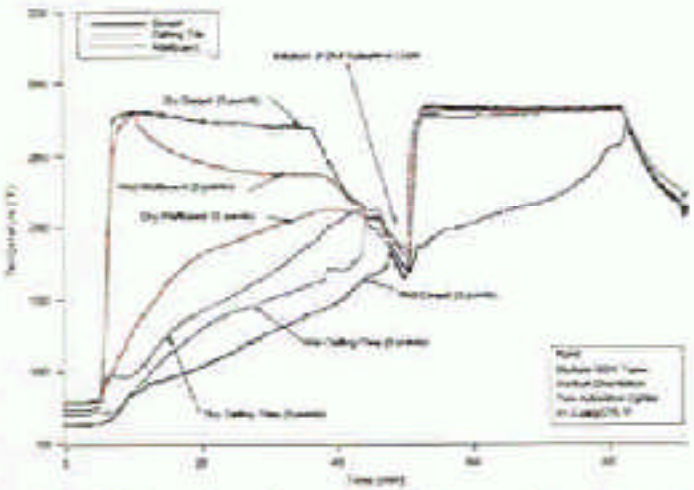

FIG. 8. Effect of second autoclave cycle on reaching the target temperature (average measurements). psig, lb/in^2.

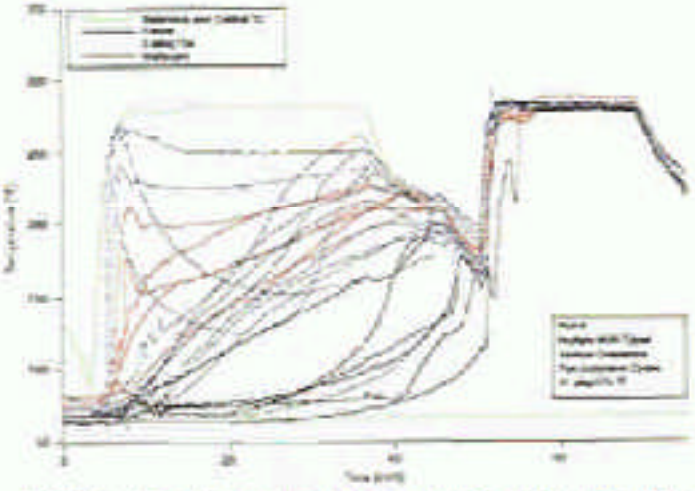

FIG. 10. Effect of second autoclave cycle with cut bags. TC, thermocouple; psig, lb/in^2.

temperature, 250°F. The BI viability measurements obtained in run 1 were consistent with the temperature measurements (i.e., the BI strips that were at locations where 250°F was maintained for 15 min showed no growth).

Run 2 (Fig. 7) consisted of subjecting only densely packed wallboard to the higher autoclave pressure and temperature. Again, even at the higher temperature, the temperature of many of the thermocouples never reached 250°F. Wallboard is composed mostly of $CaSO_4 \cdot 2H_2O$ and loses moisture at temperatures between 212 and 302°F (5). This dehydration step could have contributed to the slow heatup for wallboard, although the bulk density or packing density of the wallboard could also have been a factor. The control temperature dropped in run 2, which was explained later by the fact that the bag containing the control BI strip and thermocouple came loose and fell into the bin, reducing its exposure to the steam. This run was not repeated due to time constraints, but the autoclave facility process monitors exhibited no change at that time, which convinced the investigators that the problem was with the one thermocouple. In addition, the temperature signals converged at the end of the run. This observation led to the hypothesis that a second autoclave cycle might be effective.

The data shown in Fig. 7 are color coded to indicate if the BIs associated with each thermocouple were viable at the conclusion of run 2. A viable spore designation was used if growth was observed in both the growth test and the assay analysis. Decontamination or a no-viable-spore designation was used if no growth was found in the test with an initial population consisting of 10^6 spores. In a limited number of cases, the growth test indicated a positive result; however, the subsequent assay analysis revealed no quantifiable population (<100 CFU). These data series were labeled indeterminate. Note that for the sample locations at which the temperature was maintained at 250°F for 15 min the data consistently showed that there was no growth on the corresponding BI strips, while the data for most of the sample locations that did not meet these time and temperature targets showed that there was growth.

Figure 8 shows the effect of the second autoclave cycle (run 5), based on average temperature data for each bag. During this run, bags containing various materials were placed upright

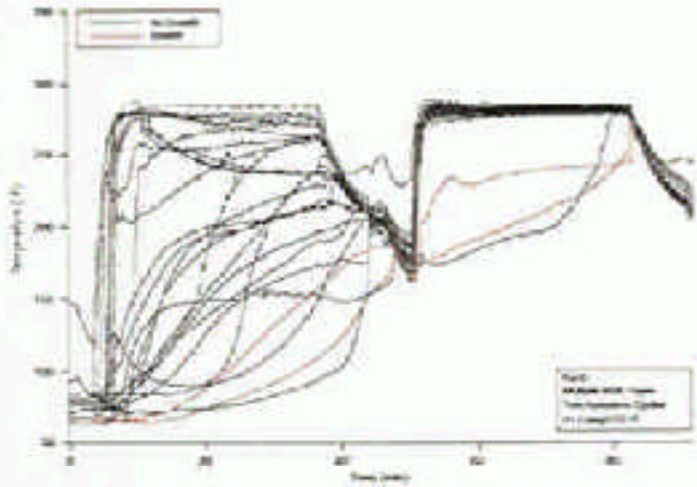

FIG. 9. Effect of second autoclave cycle on spore survivability. psig, lb/in^2.

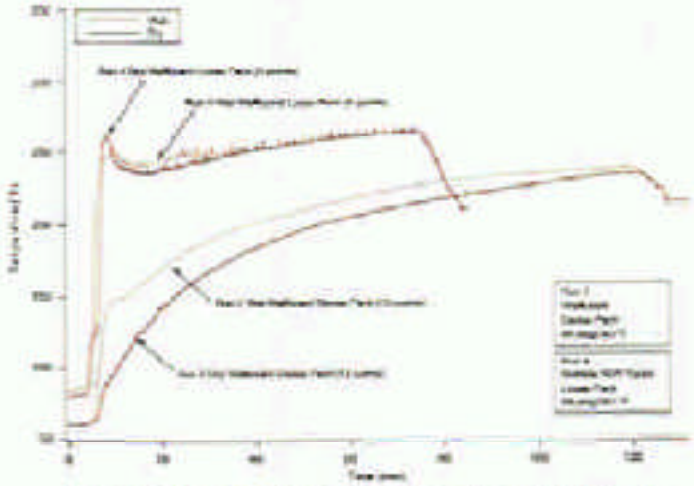

FIG. 11. Effect of packing density for wallboard. psig, lb/in^2.

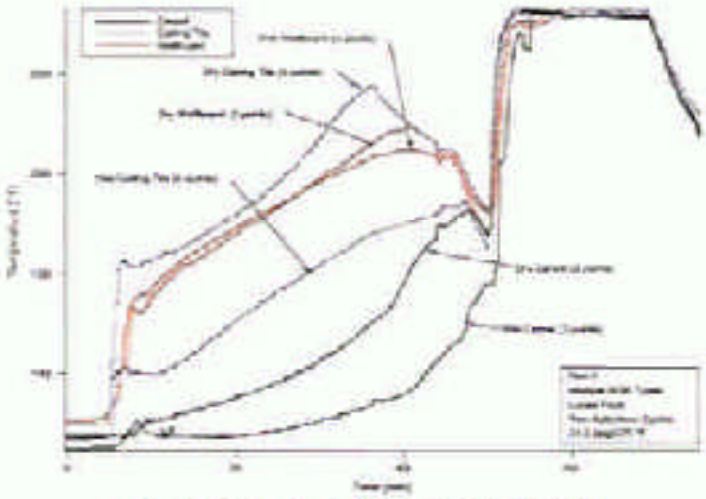

FIG. 12. Effect of moisture content. psig, lb/in^2.

to maximize exposure during the autoclave cycle, and then a second cycle was performed, complete with evacuation and repressurization. At the beginning of the second autoclave cycle, almost all of the temperatures converged to the operating temperature of the autoclave. We believe that when the cold, porous BDR material was exposed to the steam during the first cycle, condensate formed in the pores, limiting steam penetration and subsequent heat transfer. With the pores of the material full of water, heat was transferred to the interior of the material mostly through conduction, which was slow, and the steam could not penetrate very well into the material. At the initiation of the second autoclave cycle, the evacuation step pulled the condensate out of the pores, so that when steam was reapplied, it effectively penetrated the preheated material and the temperature reached the operating temperature of the autoclave. The only thermocouples at which the necessary temperatures were not reached were in the wet carpeting. It was unclear whether the bag with the wet carpet burst open during run 5. This led to run 6 being performed with all bags cut open prior to loading. Figure 9 shows the spore viability data for run 5. As before, the samples that did not achieve the necessary time/temperature for decontamination exhibited residual spore viability.

Figure 10 shows the time and temperature data for run 6, where the bags were cut open prior to autoclave loading and two sequential autoclave cycles were performed. In this case, the temperature of all thermocouples reached the required temperature for the time necessary for spore destruction, and this finding was supported by the fact that none of the run 6 BI strips showed any growth. It was not always obvious whether any given bag ruptured during the cycle, so no definitive conclusions could be made about the effect of changing bag material as a means to promote bag burst during the autoclave cycle. However, these observations did suggest that packing BDR using bags made from a material that melts or opens during autoclaving might ensure good steam penetration.

Figure 11 shows the effect of packing density when wallboard was processed. Clearly, high-density packing reduced the effectiveness of the autoclaving. It appears that an autoclave facility processing BDR should minimize packing density so that steam can readily penetrate into each bag in the load.

Figure 12 shows the effect of initial moisture on heating BDR (except for wet carpet, which was not present in run 1). The wet ceiling tile heated significantly more slowly than the other BDR types probably because the micropores of the ceiling tiles completely filled with water. The other item types showed similar heating profiles. This finding supports the hypothesis that initial condensation of steam in the pores of the ambient-temperature BDR limits heat transfer.

DISCUSSION

In this paper we describe an empirical study to evaluate whether moist heat and steam can successfully reach all surfaces of porous building materials and furnishings with sufficient potency that deeply absorbed bacterial spores may be inactivated and to determine the operational parameters needed to achieve this. While spore strips can present "easily handled" challenges to this process, it is important to acknowledge that weaponized spores, or even live nonweaponized spores, likely behave differently. Given the dangerous nature of biological weapon agents and the severely restricted access and stringent safety protocols necessary to handle live agents, these tests had to be performed with a simulant, such as *G. stearothermophilus*. However, *G. stearothermophilus* is commonly used as a simulant for agents such as *B. anthracis*, particularly for studies of technologies utilizing thermal treatment methods to kill the spores (9). It must be remembered that in all likelihood, any BDR brought to a disposal facility would have been previously decontaminated and would probably contain very small numbers of viable spores, so testing with BIs that contained 1×10^6 spores represented a worst-case scenario.

Based on the results of these tests, heating the BDR to 250°F for 15 min at the sampling locations resulted in no viable spores. The most effective spore destruction was obtained with a loose packing arrangement, dry BDR material, a higher autoclave operating pressure and higher temperature, multiple autoclave cycles performed in sequence, and bags cut open prior to loading.

The optimal practices for processing BDR in a commercial autoclave are as follows: place BDR so that all surfaces are exposed to the autoclave conditions; maintain a loose packing arrangement for the materials; and use plastic film bags that allow steam penetration.

The material that was successfully decontaminated included wet wallboard, dry wallboard, wet ceiling tiles, dry ceiling tiles, dry carpet, and dry upholstered furniture. Wet carpeting was successfully decontaminated only when cut bags and two sequential autoclave cycles were used.

Our conclusions regarding autoclave operating conditions are as follows: treatment for 120 min at 31.5 lb/in^2 and 275°F decontaminated wallboard, ceiling tiles, and dry carpet when the materials were loaded as recommended; treatment for 75 min at 45 lb/in^2 and 292°F was sufficient to decontaminate dry upholstered furniture, although there were not sufficient runs with upholstered furniture to determine whether less rigorous conditions would also result in spore destruction; treatment for 75 min at 45 lb/in^2 and 292°F decontaminated wallboard and ceiling tiles when the materials were loaded as recommended;

and two standard autoclave cycles consisting of 40 min at 31.5 lb/in^2 and 275°F in sequence decontaminated wallboard, ceiling tiles, and dry carpet when the materials were loaded as recommended. It may be possible to shorten the second cycle and still destroy the spores in the BDR; a third cycle may be necessary for wet carpet.

The most important recommendation based on these tests is to use at least two sequential autoclave cycles. In this study the second cycle had a profound effect on the time/temperature profile of the BDR materials processed. The steam evacuation step between cycles appears to be the critical step for ensuring effective decontamination of porous materials in an autoclave.

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This paper was reviewed by the U.S. Environmental Protection Agency and approved for publication. Approval does not indicate that the contents necessarily reflect the views and policies of the agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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ENVIRONMENT AGENCY

WASTE MANAGEMENT LICENSING

TECHNICAL GUIDANCE ON

CLINICAL WASTE

MANAGEMENT FACILITIES

CONSULTATION DRAFT V.1.1 - 24/02/06
APPENDIX 5
Sector Guidance Note IPPC S5.06 - Supplementary
PPC for Clinical Wastes

Limitations of treatment technologies that do not shred or macerate waste before treatment
Page 18 & 19

Note: This document constitutes guidance issued by the Environment Agency to its regulation officers

- These should be analysed in triplicate in thick pour plates
- Plates are incubated in a moist chamber at 60°C for up to 7 days.

For thermal processes the microbial data should be supported by the parallel use of thermal indicator strips or multi-point thermal data loggers to record temperatures through out the waste load wherever possible.

A5-2.10.2.3.2 Validation Criteria

This requires quantitative enumeration of the sub-samples relative to the control run. Qualitative analysis or the use of less than 1×10^6 spores per gram is not appropriate.

For the Control Run(s) the following are required

- The mean number (X_C) of spores recovered from the control samples should be calculated in cfu.
- The $\log_{10}$ of (X_C) should be determined

For the Test Runs the following require determining

- The mean (X_T) number of spores recovered
- The standard deviation (σ) of spores recovered
- The $\log_{10}$ of (X_T)
- The Upper 95% (Lu) confidence interval of (X_T) (this will be $X_T + 1.96\sigma$)
- The $\log_{10}$ of the Upper 95% confidence interval (logLu) of X_T

The following criteria represent the minimum standard that must be achieved:

- ($\log (X_C)-4$) ≥ logLu
- $\log (X_C)$ must be ≥ 5
- For thermal processes all thermal indicator strips should indicate that the required temperature time parameters have been achieved.

These criteria must include the proviso that **ALL** test strips, or spore samples, recovered from the plant must be considered valid. This includes those where contamination has occurred. Significant contamination will therefore require the exercise to be repeated.

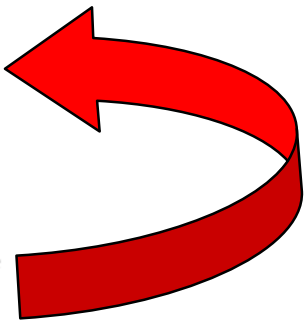
Where these criteria are passed then it is >97.5% probable that any clinical waste will be treated to the minimum standard.

A5-2.10.2.4 For technologies that lack pre-maceration or integral maceration

These technologies may have severe limitations and may lack the technical ability to treat a worst case scenario challenge load of clinical waste.

Where there is no physical action to enable sealed waste containers, and sealed voids in the waste to be punctured, then the treatment is unlikely to penetrate the waste fully.

In general specialist challenge load testing using methodology consistent with that developed by the Health Protection Agency would be required to confirm efficacy. This is beyond the scope of this document.



Static autoclaves, including those with vacuum cycles, are particularly affected by this issue and the waste will require some form of physical pre-treatment (e.g. maceration) to enable effective treatment to take place.

As an indicator of methodology requirements, spore strips should be placed in each of the following:

- Robust rigid 2 litre suction canister/chest drain containers made of thermostable plastic, of variable types containing 1-1.5 litres of fluid and thermally stable gel. The operator should demonstrate that the type(s) chosen represent the worst case challenge load.
- Any other challenging items identified by audit where the penetration of the steam/chemical may be inhibited (for example lengths of tubing, inside syringes bodies in sealed sharps boxes etc).

Testing should cover 36-72 suction canisters and chest drains (in 3-6 test runs), including approximately 6 of each type/brand, each containing two biological indicator strips and two thermal indicator strips.

These containers should then be placed in rigid containers and/or yellow bags of a type to be taken by the plant and mixed with the a typical waste load.

The validation criteria from section A5-2.10.2.2.4 should be applied.

A5-2.10.3 Routine Efficacy Monitoring

All clinical waste treatment devices should be monitored routinely throughout their operational life to ensure that microbial inactivation has occurred and that performance is maintained.

The following is considered to be the minimum requirements for such monitoring.

A5-2.10.3.1 For those plants for which the use of spore strips is appropriate

The minimum frequency of monitoring is specified in Table A5-2.10.1.

Table A5-2.10.1 Routine Monitoring of Microbial Inactivation

Continuous Hourly throughput or batch cycle load.(kg)	Test frequency (first 6 months of operation)	Test frequency (operational, after the first 6 months)	Minimum Number spore strips or sub-samples	Number of Control strips
0-50kg	Monthly	quarterly	5	1
51-500 kg	Fortnightly	Bi-monthly	5	1
501-1000kg	Weekly	monthly	5	1

For thermal processes thermal indicator strips or multipoint data loggers should always be used in parallel where possible.

Either qualitative or quantitative enumeration of spore strips with a certified population may be used.