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Microbiological Evaluation of the STI Series 2000 Medical Waste Treatment Process

January 25, 2005

EXECUTIVE SUMMARY

Microbiological Evaluation

STI Series 2000 Medical Waste Treatment Process

The treatment and destruction of medical waste by the STI Series 2000 Medical Waste Treatment Process (STI) as evaluated utilizing Bacillus atrophaeus spore strips. The STI is designed to shred and treat medical waste to render it unrecognizable. The capabilities of the STI process to treat conventional medical waste have been documented for and approved by regulatory agencies. This testing evaluates the STI as an alternative medical waste treatment technology with the ability to treat pathologic waste.

The STI Series 2000 Medical Waste Treatment Process (STI) is designed to mechanically shred medical waste and treat it through a thermal process. When operated in accordance with all manufacturers' instructions, the system will render the waste unrecognizable and reduce its bioload for safe disposal in the solid waste stream.

To evaluate the efficacy of the STI Series 2000, known concentrations of a biological indicator, i.e., *Bacillus atrophaeus spore strips* were added to test waste loads under routine operating conditions. Samples were processed through the STI Series 2000 and then returned to an independent microbiology laboratory where they were cultured and monitored for the growth of the biological indicator. The optional use of sodium hypochlorite (NaOCl) was not be used during the efficacy testing. For purposes of efficacy testing and evaluation, the optional use of NaOCl solution was replaced with water to demonstrate that the STI system can achieve efficacy solely based on steam treatment. The water injection rate was the same volume as the recommended deodorizing injection rate, specified by STI.

Following treatment, spore strips were sent to an independent lab for qualitative testing. After homogenation of samples, serial dilutions were made of the sample. Controls were evaluated utilizing the same techniques as employed with test samples.

Identical testing was conducting on STI units are two different locations (Muhlenberg Medical Center and LabCorp, both in the state of New Jersey).

No growth of B. atrophaeus was detected in any of the 18 spores test samples indicating a ≥ 6 $\log_{10}$ reduction.

These test results demonstrate the ability of the STI Series 2000 Medical Waste Treatment Process to effectively treat medical waste.

Note on organizations involved in this project

Several organizations were involved in this microbiological evaluation. They include the following:

WNWN International – Burlington, Connecticut

US based company with over 15 years experience in all facets medical waste management at the domestic and international level.

This organization developed the protocols and organized the testing among all parties.

Information From Science – West Sand Lake, New York

US based company provided on-site management of samples and data collection at the test sites.

Sterilator Company – Cuba, New York

US based company producing commercially available spore strips

This organization provided Bacillus atrophaeus spore strips.

BIoCI System – Raleigh, North Carolina

US based company providing bacterial spore strips and products as well as microbiological analytical services related to biological indicator testing.

This organization was responsible for conducting the B. atrophaeus spore strip analysis.

Efficacy Protocol

STI Medical Waste Treatment Process
***Bacillus atrophaeus* spore strip Microbiological Efficacy Protocol**

I. Purpose

The STI Series 2000 Medical Waste Treatment Process (STI) is designed to mechanically shred medical waste and treat it through a thermal process. When operated in accordance with all manufacturers' instructions, the system will render the waste unrecognizable and reduce its bioload for safe disposal in the solid waste stream.

To evaluate the efficacy of the STI Series 2000, under routine operating conditions involving the use of heat generated by injection of steam for waste treatment, with test waste loads spiked with known concentrations of a biological indicator, i.e., *Bacillus atrophaeus*. Samples will be processed through the STI Series 2000 and then returned to an independent microbiology laboratory where they will be cultured and monitored for the growth of the biological indicator. The optional use of sodium hypochlorite (NaOCl) will not be used during the efficacy testing. For purposes of efficacy testing and evaluation, the optional use of NaOCl solution will be replaced with water to demonstrate that the STI system can achieve efficacy solely based on steam treatment. The water injection rate shall be the same volume as the recommended deodorizing injection rate, specified by STI.

II. Process Description

The STI system consists of a lift, an intake hopper, shredding chamber, and jacketed steam treatment auger. Once the waste in introduced into the intake hopper and its cover secured, the packaged waste is scalded with steam prior to shredding. Next, the shredder is engaged to reduce the volume of waste and to expose its maximum surface area to achieve appropriate treatment. The shredded material is then further subjected to additional direct steam impingement in the steam auger section via the delivery of steam to the inside of the auger. The waste is moved through the inclined auger which is surrounded by a steam jacketed chamber. Waste materials are subjected to temperatures at a minimum of 205 degrees F^o (travel temperature range 205 to boiling) for an exposure period of 60 minutes or more.

III. Microbiological Testing

A. Preparation of Biological Indicator and Inoculation of Test Load Samples

1. The biological indicator, *Bacillus atrophaeus* (ATTC 9372), will be prepared by Sterilator Company, Inc. of Cuba, NY using standard laboratory practices i.e. US Pharmacopoeia for the cultivation of microorganisms. Spore strips will contain a population of no less 1e6. Analysis will be performed by BIoCI systems, Inc. of Raleigh, North Carolina.

2.. A total of six (6) control and nine (9) test samples will be prepared, packaged separately into glassine envelopes and then immediately transported, via insulated container maintained at approximately 4^{o}C, to the test site for efficacy testing.

B. Processing of samples through the STI medical waste treatment process – Three (3) test cycles will be conducted on a single day and involve the following types of samples:

1. Samples Types

a. Control samples:

i. Field Controls (**FC** - 3 samples) – Spore strips transported to and from the test site without processing in the STI Series 2000; and

ii. Untreated Controls (**UC** - 3 samples) – Spore strips transported to the test site and processed, without thermal treatment. Also, the addition of sodium hypochlorite for odor control will be replaced by only water during the shredding phase through the STI Series 2000 with a sufficient amount of surrogate of waste to assure transit through the auger.

b. Test Samples

i. Treated Samples (**TS** - 9 samples) - Spores samples transported to the test site and processed through the STI Series 2000 with thermal treatment.

2. Sample processing through the STI

a. Untreated Controls (UC) – One (1) cycle, involving

i. Each of the three (3) samples will initially be placed into an "egg" carrier (perforated plastic container provided by the STI) and the egg introduced into the STI through a sampling port below the shredding chamber. The egg will then become entrained in a minimum volume of waste (similar in composition to the waste generated at the test site) as it is conveyed along the incline screw through the treatment chamber (**without** the use of steam to heat the walls or be injected into jacketed area) until it exits this section at the end of the process..

ii. Upon exiting, each of the samples will be removed from the egg, placed into a quart plastic bag, the bag marked with UC and immediately refrigerated to maintain sample integrity before shipment to the testing laboratory within an insulated container, with cold packs, to maintain the temperature at approximately 4^{o}C. Temperature monitoring strips will be used in all shipments to confirm adequate temperatures and specimen integrity. Temperatures will be periodically recorded.

b. Field Controls (FC)

i. Upon receipt at the test site, each sample will be placed into a quart plastic back, the bag marked with FC and immediately refrigerated to maintain sample integrity before shipment to the laboratory within an insulated container, with cold packs, to maintain the temperature at approximately 4^{o}C.

c. Test Samples (TS) – Three (3) separate tests cycles at full unit capacity, each involving the following procedures -

i. As with the UC, except that each of the three (3) eggs will exposed to thermal treatment through the heated walls and direct injection of steam into the jacked section. As mentioned above, same quantity of water will be injected through the chemical injection for the purposes of testing and evaluation of efficacy.

ii. Upon exiting, each of the samples will be removed from the egg, placed into a quart plastic bag, the bag marked with TS and immediately refrigerated to maintain sample integrity before shipment to the laboratory within an insulated container, with cold packs, to maintain the temperature at approximately 4^{o}C.

During the test procedure, the following data will be recorded:

-Date
-Name of responsible test manager
-Insertion time of each sample into the unit
-Removal time of each sample from the unit
-Additional remarks, observations, or comments
- Treatment parameters i.e., temperatures
-.Temperatures in auger section recorded at 15 minute intervals
-. Residence time will be calculated and recorded for each individual sample
- Waste composition shall be noted for all tests loads

IV. Recovery of Spiked Organisms*

A. Processing of samples - All control and test samples shall be packaged, handled, transported and analyzed in the same manner.

1.For each sample, aseptically transfer strip/disk into a sterile tube containing glass beads and 10mL sterile DI water (yielding 1e–1 dilution).
2. Vortex tube until sample is homogenized.
3. Mix well then serially dilute by transferring 1mL of previously prepared dilution to the next 9mL dilution tube.
4. Plate cooled heat-shocked dilutions, 1mL per plate, overlay with TSA.
5. Invert and incubate plates at 30-35°C for 24-48 hrs.

Dilutions are made so there are between 30-300 colonies.

All results will be reported.

*Reference: BIoCI SOP # 070-L002 - *Bacillus atrophaeus* Spore Strip Population Assay

V. Calculations: Demonstration of Disinfection Efficacy

A. Data from the following tests is necessary to determine the disinfection efficacy of the STI Unit. **All concentrations shall be converted using a logarithmic table before calculations are performed, i.e. $2x10^5$ cfu/ml = 5.301.**

$\overline{X_o}$ = Mean initial concentration ($\log_{10}$) of organisms per BI

$\overline{X_u}$ = Mean concentration ($\log_{10}$) of recoverable organisms per BI after being processed through the unit without the benefit of treatment

X_t = Concentration ($\log_{10}$) of organisms per BI after being processed through the STI Medical Waste Treatment Process with the benefit of treatment.

Disinfection Efficacy Equation: $\mathbf{X_u - \overline{X_t} = X_f}$

X_f = $\log_{10}$ reduction in concentration of organisms due to treatment process

Acceptance Criteria: A 6 log reduction of biological indicator (B. atrophaeus)

Note: All efficacy tests will be conducted, analyzed, and certified by a microbiologist.

11/24/04 EK

Revised 12/5/04 EK

On-site Operational Data

INFORMATION FROM SCIENCE
P. O. BOX 408
WEST SAND LAKE, NEW YORK 12196
VOICE/FAX – 518-674-1713
E-MAIL – IRASALKIN@AOL.COM

EFFICACY TESTING OF STI MEDICAL WASTE TREATMENT SYSTEM

MUHLENBURG MEDICAL CENTER

DECEMBER 8, 2004

The following are the descriptions of each of the three test runs, each involving one (1) egg per run containing the (3) bacterial spore samples:

Test Run # 1

- Responsible test manager – Ira F. Salkin, Ph.D., F(AAM)
- Insertion time – 3:37 pm – Eggs I
- Retrieval times – Egg I = 5:29 pm
- Residence times – Egg I = 1 hr/52 min
- Temperature recorded
  - Time 0 – 208^{o}F – lower clave; 211^{o}F – upper clave
  - Time 15 min – 208^{o}F – lower clave; 212^{o}F – upper clave
  - Time 30 min – 208^{o}F – lower clave; 211^{o}F – upper clave
  - Time 45 min – 209-210^{o}F–lower clave; 211-212^{o}F – upper clave
  - Time 60 min – 209-210^{o}F – lower clave; 214-215^{o}F – upper clave
  - Time 75 min – 211-212^{o}F – lower clave; 214-215^{o}F – upper clave
  - Time 90 min – 214-215^{o}F – lower clave; 214-215^{o}F – upper clave
  - Time 105 min – 214-215^{o}F – lower clave; 215-216^{o}F – upper clave

Test Run # 2

- Responsible test manager – Ira F. Salkin, Ph.D., F(AAM)
- Insertion time – 3:48 pm – Eggs II
- Retrieval times – Egg II= 5:50 pm
- Residence times – Egg II= 2 hr/02 min
- Temperature recorded
  - Time 0 – 208^{o}F – lower clave; 212^{o}F – upper clave
  - Time 15 min – 209-210^{o}F – lower clave; 211-212^{o}F – upper clave
  - Time 30 min – 209-210^{o}F – lower clave; 211-212^{o}F – upper clave
  - Time 45 min – 211-212^{o}F–lower clave; 214-215^{o}F – upper clave
  - Time 60 min – 211-212^{o}F – lower clave; 214-215^{o}F – upper clave

- Time 75 min – 214-215°F – lower clave; 214-215°F – upper clave
- Time 90 min – 214-215°F – lower clave; 215-216°F – upper clave
- Time 105 min – 214-215°F – lower clave; 215-216°F – upper clave
- Time 120 min – 214-215°F – lower clave; 215-216°F – upper clave

Test Run # 3

- Responsible test manager – Ira F. Salkin, Ph.D., F(AAM)
- Insertion time – 4:03 pm – Eggs III
- Retrieval times – Egg III = 6:02 pm
- Residence times – Egg III = 1 hr/59 min

- Temperature recorded
  - Time 0 – 209-210°F – lower clave; 211-212°F – upper clave
  - Time 15 min – 211-212°F – lower clave; 214-215°F – upper clave
  - Time 30 min – 211-212°F – lower clave; 214-215°F – upper clave
  - Time 45 min – 211-212°F–lower clave; 214-215°F – upper clave
  - Time 60 min – 214-215°F – lower clave; 214-215°F – upper clave
  - Time 75 min – 214-215°F – lower clave; 214-215°F – upper clave
  - Time 90 min – 214-215°F – lower clave; 215-216°F – upper clave
  - Time 105 min – 214-215°F – lower clave; 215-216°F – upper clave
  - Time 120 min – 214-215°F – lower clave; 215-216°F – upper clave

Average duration of exposure – all test runs = 1 hr/57 min
Average temperature of exposure – all test runs = 211.8°F – lower clave; 213.6°F – upper clave

4. Return of Samples

At the conclusion of all testing, samples were removed from refrigeration, packaged in an insulated container with frozen cold packs and Fed Exed, overnight to BIoCI Laboratory. Samples were received at the laboratory by 10:30 am the following day.

Submitted by:

Ira F. Salkin, Ph.D., F(AAM)

INFORMATION FROM SCIENCE LLC
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EFFICACY TESTING OF STI MEDICAL WASTE TREATMENT SYSTEM

LABCORP LABORATORIES

DECEMBER 9, 2004

The following are the descriptions of each of the three test runs, each involving one (1) egg per run containing the (3) bacterial spore samples:

Test Run # 1

- Responsible test manager – Ira F. Salkin, Ph.D., F(AAM)
- Insertion time – 1:54 pm – Eggs I
- Retrieval times – Egg I = 3:32 pm
- Residence times – Egg I = 1 hr/38 min
- Temperature recorded
  - Time 0 – 213-214^{o}F – lower clave; 216-217^{o}F – upper clave
  - Time 15 min – 214-215^{o}F – lower clave; 216F-217^{o}F – upper clave
  - All subsequently temperature readings remained constant at 214-215^{o}F – lower clave; 216F-217^{o}F – upper clave

Test Run # 2

- Responsible test manager – Ira F. Salkin, Ph.D., F(AAM)
- Insertion time – 2:01 pm – Eggs II
- Retrieval times – Egg II
- Residence times – Egg II
- Temperature recorded
  - Time 0 – 214-215^{o}F – lower clave; 216F-217^{o}F – upper clave
  - All subsequently temperature readings remained constant at 214-215^{o}F – lower clave; 216F-217^{o}F – upper clave

Test Run # 3

- Responsible test manager – Ira F. Salkin, Ph.D., F(AAM)
- Insertion time – 2:09 pm – Eggs III
- Retrieval times – Egg III = 3:57 pm
- Residence times – Egg III = 1 hr/48 min

- Temperature recorded

    - Time 0 – 214-215^{o}F – lower clave; 216F-217^{o}F – upper clave
    - All subsequently temperature readings remained constant at 214-215^{o}F – lower clave; 216F-217^{o}F – upper clave

Average duration of exposure – all test runs = 1 hr/05 min
Average temperature of exposure – all test runs = 214^{o}F – lower clave; 216^{o}F – upper clave

4. Return of Samples

At the conclusion of all testing, samples were removed from refrigeration, packaged in an insulated container with frozen cold packs and Fed Exed, overnight to BIoCI Laboratory. Samples were received at the laboratory by 10:30 am the following day.

Submitted by:

Ira F. Salkin, Ph.D., F(AAM)

Test Results

BIoCI Systems, Inc.

LABORATORY TEST REPORT

Customer ID:	WNWN (Report # 293-2)
Date issued:	2004-12-15

Test performed:	**BI Population Test - Bacillus Atrophaeus (subtilis) – STI Medical Waste Treatment System – 1000pph**
Test performed by: J. Falkowski	Date: 2004-12-10
Product ID: **SterilatorCompany Inc. biological indicator (in glassine envelope) without waste. Tested at STI System at LabCorp, NJ** ((Lot RA17 Exp 2006-04-26, Label population: 2.3e7 per BI)	

Sample ID	Average CFU			Recovered Population	
	Not Heat-shocked	Heat-shocked	Dilution Factor	Not Heat-shocked	Heat-shocked
Untreated Control #1	39	26	e5	0.39e7 (3.9e6)	0.26e7 (2.6e6)
Untreated Control #2	104	84	e5	1.0e7	0.84e7 (8.4e6)
Untreated Control #3	40	30	e5	0.4e7 (4.0e6)	0.3e7 (3.0e6)

Sample ID	Average CFU			Recovered Population	
	Not Heat-shocked	Heat-shocked	Dilution Factor	Not Heat-shocked	Heat-shocked
Field Control #1	266	203	e5	2.7e7	2.0e7
Field Control #2	425	339	e5	4.3e7	3.4e7
Field Control #3	349	290	e5	3.5e7	2.9e7

Sample ID	Average CFU		Recovered Population
	Not Heat-shocked	Dilution factor	Not Heat-shocked
Exposed S1	0	1.1	Less than 1.1
Exposed S2	0	1.1	Less than 1.1
Exposed S3	0	1.1	Less than 1.1
Exposed S4	0	1.1	Less than 1.1
Exposed S5	0	1.1	Less than 1.1
Exposed S6	0	1.1	Less than 1.1
Exposed S7	0	1.1	Less than 1.1
Exposed S8	0	1.1	Less than 1.1
Exposed S9	0	1.1	Less than 1.1