APPENDIX C

EXAMPLE:
MICROBIAL INACTIVATION TESTING PROTOCOL FOR A GRINDER/CHEMICAL MEDICAL WASTE INACTIVATION PROCESS

PREFACE

The following protocol is provided as an example of the steps and procedures required to determine the level of microbial inactivation of a system that cannot ensure or provide integrity of the biological indicator carrier (i.e., test strip, ampule) through the treatment process to recovery. This protocol is not intended to be all inclusive or meet all the variables or constraints associated with the multiplicity of medical waste treatment technologies. However, the protocol includes the components and the processes that require consideration to ensure the data recovered and numeric calculations made accurately represent the true microbial inactivation level of the treatment process.

This example provides a protocol for a chemical inactivation/ grinding medical waste treatment process that does not allow the retrieval of the biological indicator carrier. For each step in the protocol, an explanation or note is offered (in brackets) to provide rationale or background for the step or process described. For the protocol provided, adherence to good microbial and laboratory practices is essential for researcher and equipment operator safety and for the generation of accurate data.

EXAMPLE:
MICROBIAL INACTIVATION TESTING PROTOCOL FOR A GRINDER/CHEMICAL MEDICAL WASTE TREATMENT PROCESS

I. Materials

A. Bacillus stearothermophilus spores as a suspension of 2×10^{10} initial inoculum. NOTE: B. stearothermophilus spores were chosen as the spore of choice due to the thermophilic nature of B. stearothermophilus and its ability to optimally grow at elevated temperatures. Culturing collected waste samples at 60°C using B. stearothermophilus spores as a biological indicator reduces the number of potential cross contaminants that might arise on a culture plate. A spore suspension of 2×10^{10} initial inoculum was chosen to provide an adequate number of recoverable spores for determining a 4 Log_{10} reduction. Determination of this concentration may require trial runs to ascertain the recovery concentrations.

B. Surrogate waste load constructed to contain by weight: 5% organic material and 95% plastics, cellulose, and glass. Total weight of sample to be between 15 and 20 pounds. NOTE: The surrogate waste load used in this example was constructed to represent the typical medical waste composition that would be treated by this system at the user site location. Surrogate waste loads may also be constructed to replicate medical waste loads which challenge the efficacy of the system. The sample weight of the load was selected as being representative of the feed rate and typical loading conditions of the unit. Weight loads should be constructed to mimic conditions of actual use.

II. Protocols

A. Control Run

1. Add 2×10^{10} B. stearothermophilus spore suspension to surrogate waste load. The spore suspension should be added as to not expose the researcher or equipment operator to the biological indicator. To minimize potential exposures and to adequately disperse the spore suspension throughout the load, the spore suspension could be transferred into four or more separate plastic screw-capped tubes. These tubes could subsequently be equally dispersed throughout the surrogate waste load.

2. Load inoculated surrogate waste into the previously cleaned (decontaminated) treatment unit and run unit without chemical inactivation agent. [The unit should be previously decontaminated to minimize cross contamination from spores originating from previous efficacy testing.]

3. Collect ten one (1) gram samples during the duration of the run (i.e., collect samples at the beginning of waste discharge through final discharge). NOTE: The amount, number and collection frequency of the sample collection will be determined previously by trial runs. The important consideration for this determination is to ensure that during the span of the run, the test data collected provide an accurate reflection of the level of microbial inactivation for the entire load.

4. Place the 1-gram samples immediately upon collection into pre-weighed (combination weight of both liquid and tube) plastic screw cap tubes containing an appropriate neutralizing solution and vortex vigorously for 5 minutes. NOTE: This step is required to neutralize chemical agent activate at the time the waste exits the unit and is necessary to determine actual microbial inactivation the treatment process and minimize the inclusion of residual chemical activity that might be present. The amount, concentration, and exposure time of the selected neutralizing agent must be pre-determined so as to neutralize the specific chemical agent without inhibiting growth of the biological indicator. Collection tubes are pre-weighed, including neutralizing agent, to determine the weight of the actual waste sample collected.

5. Construct an approximate 10-gram composite sample from the 10 representative samples collected in Step 3. [This step provides for the evaluation of the level of microbial inactivation of the entire load without assaying each individual sample taken above.]

6. Decant, sieve, and filter as required to separate solid waste material from the neutralizing liquid. Save liquid effluent. [This step is required to wash bacterial spores from the collected waste sample. Protocols involved in this rinsing step will be determined by trial runs to ascertain the best mechanisms to adequately rinse and separate the solid waste components from the liquid rinse.]

7. Wash and vortex solid materials a second time with neutralizing buffer. Decant, sieve, and filter as required to separate solid waste material from liquid. Combine liquid effluent with that obtained in Step 6. [This step provides an extra wash to collect from the waste as many of the spores as possible.]

8. Filter liquid through Millipore™ filtration unit or equivalent to concentrate retrieved spores on membrane filter. Wash filter with 10 mls of citrate or other appropriate buffer. [This step concentrates retrieved spores to equal the number of spores from 10 grams waste/10 mls buffer or by factoring, the number of spores from 1 gram waste per 1 ml buffer. For example,

plating one ml of the liquid would result in the number of cfu on the plate to be equal to the number spores per one gram of waste.]

a) Triplicate plate 0.1 ml from the 10 ml concentrate in Step 8 above; this dilution represents Plate A. [This step equates to a total dilution of 1:10.]

b) Add 1.0 ml of the 10 ml concentrate in Step 8 above to 9.0 mls of buffer solution (this represents a 1:10 serial dilution and is represented as Dilution Tube B). Triplicate plate 0.1 ml of Dilution Tube B; this dilution represents Plate B. [This step equates to a total dilution of 1:100.]

c) Add 1.0 ml of Dilution Tube B above to 9.0 mls of buffer solution (This represents an additional 1:10 serial dilution and is represented as Dilution Tube C). Triplicate plate 0.1 ml of Dilution Tube C; this dilution represents Plate C. [This step equates to a total dilution of 1:1000).

d) Add 1.0 ml of Dilution Tube C above to 9.0 mls of buffer solution (This represents an additional 1:10 serial dilution and is represented as Dilution Tube D). Triplicate plate 0.1 ml of Dilution Tube D; this dilution represents Plate D. [This step equates to a total dilution of 1:10,000).

B. Test Run

1. Follow protocols in II A. except run the treatment unit with specified chemical inactivation agent concentrations.

2. Upon washing the membrane filter in Step II.8 with 10 mls of buffer:

a) Triplicate plate 1 ml of buffer in Step 2 above via the pour plate method (i.e., 1 ml of spore concentrate into 10-12 mls of liquid agar. Vortex and pour into plate; this represents Plate A^1. [This step equates to no dilution factor, i.e., this number represents the number of spores per gram of waste.]

b) Triplicate plate 0.1 ml of buffer in Step 2 above via the pour plate method (i.e., 0.1 ml of spore concentrate into 10-12 mls of liquid agar. Vortex and pour into plate; this represents Plate B^1. [This step equates to a 1:10 dilution factor.]

c) Add 1.0 ml of the buffer in Step 2 above to 9.0 mls of buffer solution [this represents a 1:10 serial dilution and is represented as Dilution Tube C^1]. Triplicate plate 0.1 ml of Dilution Tube C^1; this dilution represents Plate C^1. [This step equates to a total dilution of 1:100.]

III. Calculations

Using the equations found in Section C3 of "State Guideline for Approval of Alternate Medical Waste Technologies", the following calculations are performed:

A. Calculate initial inoculum in spores per gram waste.

1. 2×10^{10} spores/15 lbs. waste =
2×10^{10} spores/6.8×10^3 grams waste =
3×10^6 spores/gram waste = inoculum = IC

IC = 3×10^6

B. Calculate number of spores recovered.

1. Step One "Control" Data:

	a	b	c
Plate A -	TMTC*	TMTC	TMTC
Plate B -	TMTC	TMTC	TMTC
Plate C -	TMTC	TMTC	TMTC
Plate D -	200 cfu**	210 cfu	190 cfu

*Too Many To Count
**Colony Forming Units

Accounting for the dilution factor of 10,000 for Plate D, the average recovery of viable "Control" spores per gram equals 200 x 10,000 or 2,000,000 spores/gram or 2×10^6 spores/gram.

RC = 2×10^6

2. Step Two "Test" Results:

	a	b	c
Plate A^1 -	50 cfu	48 cfu	52 cfu
Plate B^1 -	5 cfu	4 cfu	6 cfu
Plate C^1 -	1 cfu	0 cfu	0 cfu

The average recovery of viable "Test" spores per gram equals 50 spores per gram (no dilution factor).

$RT = 5 \times 10^1$

C. Calculate Log_{10} Reduction.

1. Step One "Control" Results:

$Log_{10}RC = Log_{10}IC - Log_{10}NR$; where

$Log_{10}RC = Log_{10}(2 \times 10^6 \text{ spores/gram}) = 6.301$
$Log_{10}IC = Log_{10}(3 \times 10^6 \text{ spores/gram}) = 6.477$
$Log_{10}NR = Log_{10}IC - Log_{10}RC$
$Log_{10}NR = 6.477 - 6.301 = 0.176$

$Log_{10}NR = 0.176$

2. Step Two "Test" Results and $Log_{10}Kill$ Calculation:

a) $Log_{10}Kill = Log_{10}IT - Log_{10}NR - Log_{10}RT$, where:

$Log_{10}IT = Log_{10}IC = 6.477$
$Log_{10}NR = 0.176$
$Log_{10}RT = Log_{10}(5 \times 10^1) = 1.699$

b) Log_{10} Reduction ($Log_{10}Kill$), where:

$Log_{10}Kill = 6.477 - 0.176 - 1.699 = 4.602$

$Log_{10}Kill = 4.602$