MICROBIAL SURVIVABILITY TEST FOR MEDICAL WASTE INCINERATOR ASH

Conditional Test Methods (CTM-025)

1. Applicability and Principle

1.1 Applicability. Two test methods will be performed to determine the survivability of microorganisms in the ash during the normal operation of the medical waste incinerator. The quantification of surviving indicator organisms is utilized as an indication of the effectiveness of incineration as a medical waste treatment technology. This test procedure is intended to recover, identify and quantify the indicator organisms used to determine the efficiency of the incinerator. Bacillus stearothermophilus, a spore forming bacterium, is used because this type of organism is typically the most resistant to thermal inactivation, thereby ensuring that more fragile organisms will be destroyed. The following procedures were developed to recover only the indicator organism.

1.2 Principle. With the incinerator operating under recommended conditions, the waste stream is charged with known quantities of Bacillus stearothermophilus spores in items normally found in the medical waste stream and in the insulated pipes. The samples are added to the incinerator with typical medical wastes and are recovered at the end of the burn cycle when ashes are batch-removed. The destruction efficiency of the incinerator is determined by establishing the survivability of the indicator microorganism.

2. Apparatus

Note: Mention of trade names or specific product in this method does not constitute endorsement by the U.S. Environmental Protection Agency.

2.1 Sterilization. Autoclave capable of steam sterilization conditions of 121°C for 15 minutes at 15 psi. Specific apparatus

and reagents that contact the recovered sample shall be sterilized under these conditions by autoclaving or other equivalent method.

2.2Sampling. The direct ash samples are collected from the ash after cooling. A thief sampler is used for collection. The sample is collected into sterile containers and stored at 4°C prior to transportation to the laboratory. The pipe sample is shown in Figure 1 and is composed of the following components:

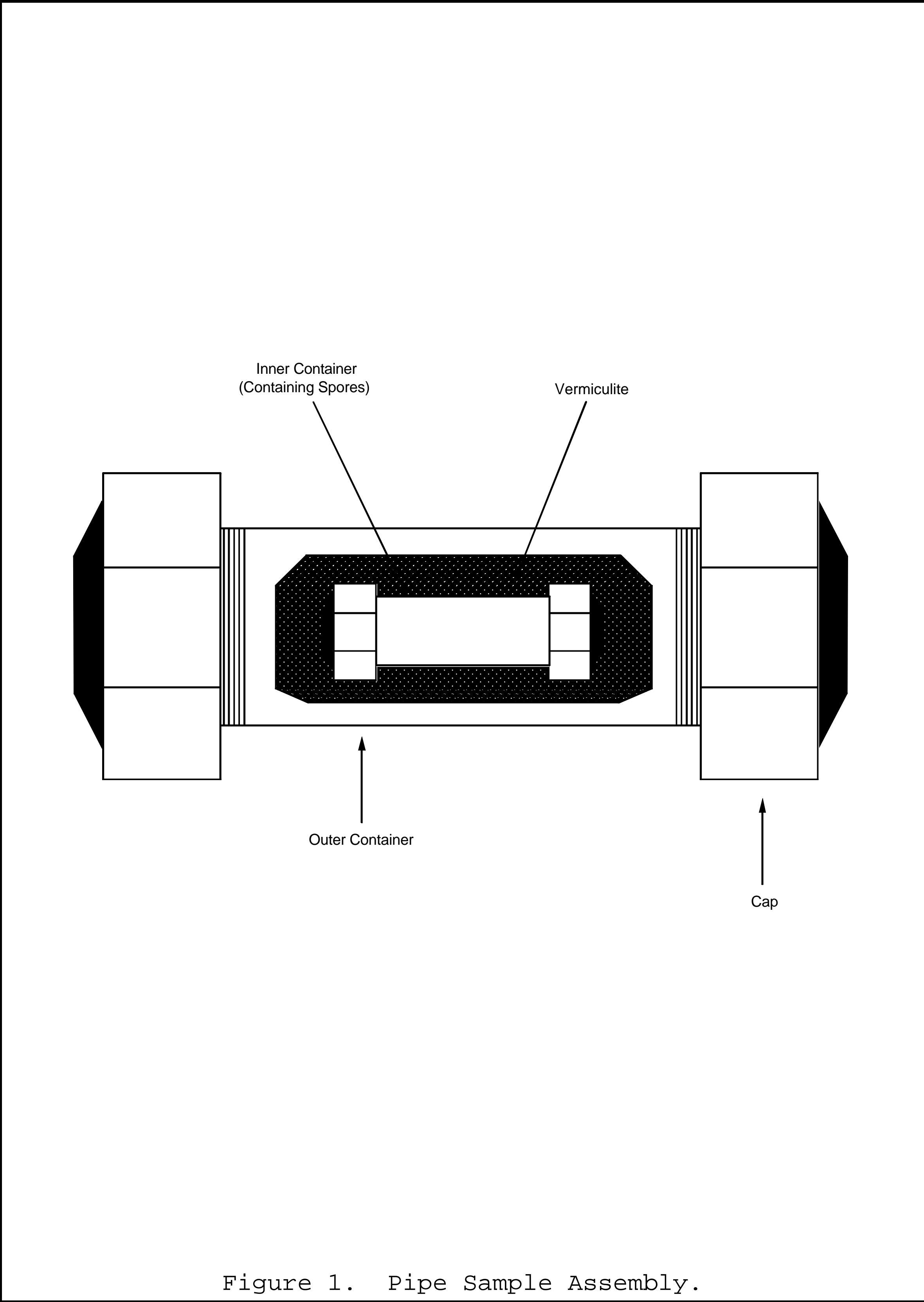

Figure 1. Pipe Sample Assembly.

2.2.1Outer Container. Steel pipe having a two-inch diameter and 6 in. long with end caps to contain the sample. (Note: Since components are commonly available in English units, only English units are specified). The ends of the pipe shall be threaded to attach the metal caps. Etch or stamp each tube for identification.

2.2.2Inner Container. 2-in. length of 3/8-stainless-steel tubing with stainless-steel Swagelok caps. Other leak-free stainless-steel fittings may be used as alternatives.

2.2.3Insulation. Vermiculite, or other thermal insulating material. The insulation shall be such that the inner container is maintained in the center portion of the outer container to protect it from thermal shock.

2.3Sample Recovery.

2.3.1Gloves. Of sufficient quality to protect the tester from microorganisms that may be present in the ash.

2.3.2Sample Retrieval Instruments. Shovel, tongs, thiefs or other instruments for removing an incinerator ash sample and the pipe sample from the ash dump.

2.3.3Sample Storage Containers. Pipe Samples are placed inside plastic bags. Direct ash samples are placed in sterile containers then in plastic bags. All samples are stored at 4°C until transport to the laboratory.

2.4Analysis.

2.4.1Incubator. Air convection type, capable of aerobic incubation at 55 to 65°C.

2.4.2Incubation Tubes (Sterile). Heat-proof tubes or bottles.

2.4.3Water Bath. Capable of maintaining a temperature of 80°C.

2.4.4Petri Dishes (Sterile).

2.4.5Filter Units (Sterile). Nalgene[R] 0.2-μm cellulose nitrate membrane analytical filter units or equivalent.

3.Reagents

3.1Sampling and Sample Recovery.

3.1.1Crushed Ice. For sample storage in the field.

3.1.2Indicator Microorganisms. Bacillus stearothermophilus spores. Each pipe should contain approximately 1 x 10^6 dry spores and approximately 1 x 10^{12} spores per day are being added to the actual waste stream. Dry spores of this quantity may be obtained from ATCC, 12301 Parklawn Drive, Rockville, MD 20857. (Telephone No. 800-638-6597).

B. stearothermophilus is also added to the waste stream at approximately 1 x 10^{12} spores per day. Direct ash samples will be taken each day to determine actual spore survivability or the incinerator destruction efficiency.

3.2Analysis. The analytical reagents will vary according to the culture and identification procedure used. The reagents described are required for the example tests listed in this method. Other reagents suitable for appropriate alternative tests are acceptable.

3.2.1Water. All water used shall be sterile deionized.

3.2.2Spore Reagent (Sterile). Buffered phosphate solution (2.0 M) prepared by dissolving 174.2 g dibasic potassium phosphate (K_2HPO_4) and 136.1 g monobasic potassium phosphate (KH_2PO_4) in water and diluting to 1 liter.

3.2.3Trypkticase Soy Agar Growth Medium. Prepare by mixing 15.0 g pancreatic digest of casein, 5.0 g papaic digest of soy meal, 5.0 g NaCl, and 15.0 g agar with 1 liter of water. Boil to dissolve the agar. Sterilize at 121°C for 15 minutes. This recipe will prepare 10 petri dishes.

4.Procedure

4.1 Sampling.

4.1.1 Sample Preparation.

4.1.1.1 Clean the inner pipe and caps before use. Remove the cap from the spore vial and breakup the spore cake using a clean instrument. Secure one end cap to the inner container. Carefully transfer the crushed cake of 1×10^6 spores into the inner sample container, and seal the other end cap. All sampling instruments (i.e. sampling thief) used to take the actual sample are sterilized. Sterile sample containers for the ash samples are obtained.

4.1.1.2 Secure one end cap to the outer container, and add enough vermiculite to allow the inner container to be positioned in the approximate middle and center of the outer tube. Add additional vermiculite, gently tapping the outer container to effect settling, until full. Secure the other end cap. The spore inoculation techniques are discussed in Appendix A.3 will be used for inoculation of waste items for direct ash sampling. A total of 1×10^{12} spores are added to the waste stream per sampling day.

4.1.2 Incinerator Spike. The incinerator spike will vary according to loading practice. For semi-continuous loading operations, add a set of three samples to the incinerator at the beginning, middle, and end of a normal day's loading period (9 total samples per daily burn). Disperse each sample per set in different sections of the wastes to be charged. For single-charge batch operations, place all 9 samples on the incinerator bed before any wastes are charged. Divide the bed into a grid of 9 equal areas. Place a sample in the approximate center of each grid. Load the wastes charge in a manner that will not disturb the sample arrangement.

4.2 Sample Recovery. The sample recovery will vary according to incinerator ash-removal practice. Recover the samples during ash removal following the cool-down period for batch removal

operations or as they emerge in the ash bin for continuous cleanout operations. <u>NOTE</u>: For units having water-quenched ash bins, care should taken to remove the samples quickly from the bin to prevent contamination. Remove excess debris from the recovered samples, place in a plastic bag, and pack in ice to maintain at or below 4°C. Ensure that the samples are protected from contamination from melted ice.

4.3Analysis.

4.3.1Sample Preparation of Analysis. The samples should be delivered to the laboratory within 96 hours. A pH is performed on the direct ash samples at the time of recovery. Upon delivery to the laboratory the pipe sample is opened, the outer container and vermiculite are discarded after the inner container is recovered. The inner container should be cleaned sufficiently to prevent cross-contamination when opening the sample. The contents of the inner pipe container is then transferred aseptically into sterile test tubes. The inside of the container is rinsed with sterile water and this is placed in the test tube. Ash samples must be thoroughly mixed to ensure homogeneity. Approximately one gram of ash is then added to 3-100 ml aliquots of sterile water. A six log serial dilution of each aliquot will then be prepared.

4.3.2Culture Development. Pour molten trypticase soy agar into 10 petri dishes and allow the agar to harden. Using a separate cellulose nitrate filter, for each serial dilution, filter each concentration from each ash suspension. Using sterile tweezers, remove the filters from each unit and place face up on separate agar plates. Aerobically incubate the plates in an air convection incubator at 65°C for 18 to 24 hours.

4.3.4Identification of Indicator Microorganisms. A variety of tests may be used to identify <u>B. stearothermophilus</u>. As a minimum, techniques to establish that the microorganisms found are gram-positive, rod-shaped, spore-producers should be used.

This may be performed using stain/morphological/biochemical tests or by strip/card testing units for determining biochemical profile.

4.4Quality Control Procedures.

4.4.1Indicator Organisms. Spores from a vial not subjected to the incinerator test shall be dissolved in spore reagent, appropriately aliquoted to yield a final plate count of between 20 and 200 colonies, developed, and enumerated simultaneously with the samples as a control to aid in establishing colony identity.

5.Calculation

5.1Microbial Survivability of Indicator Spores.

$$MS = [1 - (S_r/S_s)]\ 100$$

Eq. 1

where:

MS=Microbial Survivability, percent (to 6 sig. figures.)

S_r=Number of spore colonies counted in the analysis.

S_s=Number of spores in original spike vial.

100 =Conversion to percent.

6.Bibliography

1. American Society for Microbiology. Manual of Methods for General Bacteriology. Washington, D.C. 1981. 524 p.

2.Buchannan, R.E. and N.E. Gibbons. Bergey's Manual of Determinative Bacteriology. Baltimore, MD. The Williams and Wilkins Company. 1974. 1268 p.

EPA MEDICAL WASTE INCINERATION TESTS
PROPOSED ASH SAMPLING PROTOCOL
Revision 2 - 8/7/90

I.PRE-SAMPLING PREPARATION

A.Sifter (see Figure 1A)

1.Scrub the sifter with soapy water and clean with a disinfectant such as Virex 128. Allow the sifter to air dry in a clean, dry area.

2.Disinfect with a 3 percent hydrogen peroxide solution, allow to air dry in a clean, dry area.

3.Cover the can with clean plastic and seal the lid until use.

B.Other

1.Where possible, use disposable items that have been disinfected for sampling. Examples of disposable items are disposable gloves, garments, etc. Use new bottles to store samples.

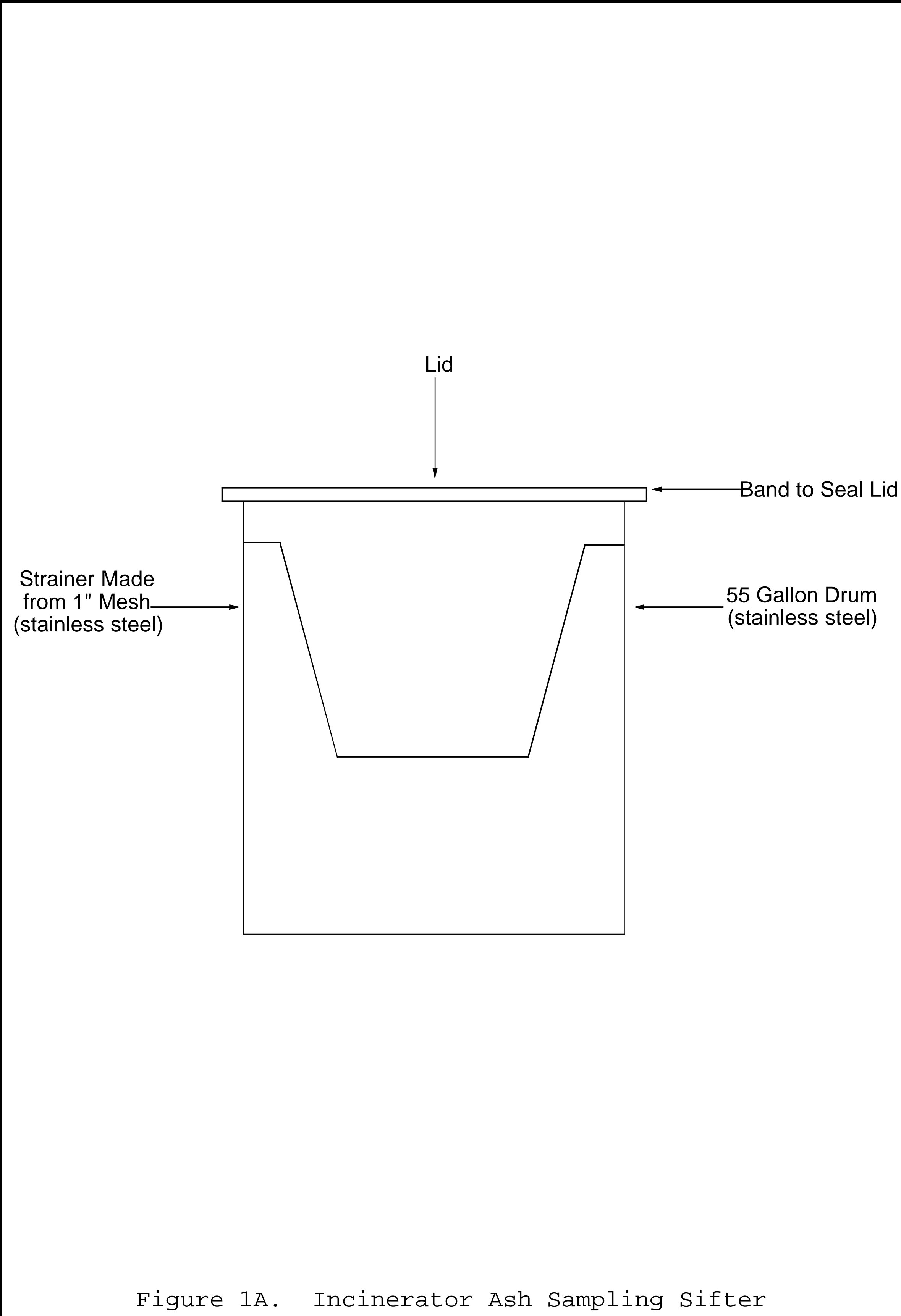

Figure 1A. Incinerator Ash Sampling Sifter

II.ASH SAMPLING PROCEDURE

A.Open the incinerator door.

B.Remove shovel from the storage container and allow to air dry in a clean, dry area.

C.Remove ash from the chamber with a shovel and deposit it into the top of the sifter.

D.Gently sift the ash until fines fall into the bottom of the sifter.

E.Remove the sifter basket and deposit course material into a tared trash can.

F.Repeat Steps C through E until the desired amount of ash is removed. The amount of the ash that is removed will be determined by the hospital's normal ash removal procedures.

G.When the ash has been extracted, weigh and record the amount of course material removed.

H.Place the lid tightly on the sifter.

I. Roll the sifter for a minimum of one minute to uniformly mix ash fines.

J. Remove the lid after the ash has settled.

K. Remove sampling thief (grain sampler, three feet long with 1/2 inch slots) from storage container and allow to air dry in a clean, dry place.

L. Take samples using sampler. Collect four samples into 950 ml bottles by repeatedly inserting sampler into random locations in the ash bed within the sifter drum. Seal the bottles, wipe each with disinfectant and label the bottles immediately. Store labeled bottles in a refrigerator (microbial samples only).

M. The four samples will be sent for analysis as follows:

1. 950 ml for dioxin analysis.

2. 950 ml for metal/carbon/loss on ignition (LOI) analysis.

3. 950 ml for spore enumeration analysis.

4. 950 ml for archive purposes.

III. POST SAMPLING PREPARATION

A. Clean and prepare the utensils for next use. Store them in containers filled with 3 percent hydrogen peroxide solution.

B. Clean and disinfect the sifter for the next use.