MICROBIAL SURVIVABILITY TEST FOR MEDICAL WASTE INCINERATOR EMISSIONS

Conditional Test Method (CTM-026)

1. Applicability and Principle

1.1 Applicability. This test method measures the survivability of a specific indicator organism through the normal medical waste incinerator operation cycle. Microbial spores are the most resistant type of organism to thermal inactivation and are therefore utilized as the surrogate organism to ensure that all microorganisms will be rendered non-viable. This procedure is designed to recover, identify, and quantify the indicator organism and is not intended to recover other microorganisms collected by the sampling train.

1.2 Principle. With the incinerator operating under recommended conditions, it is charged with wastes spiked with known amounts of Bacillus stearothermophilus spores. Emission samples are collected isokinetically in a buffered solution in impingers. The recovered samples are cultured. Colonies of B. stearothermophilus are identified and quantified.

2. Apparatus

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

2.1 Sterilization. Autoclave capable of steam sterilization at 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.

2.2 Sampling. The sampling train is shown in Figure 1 and is composed of the following component parts.

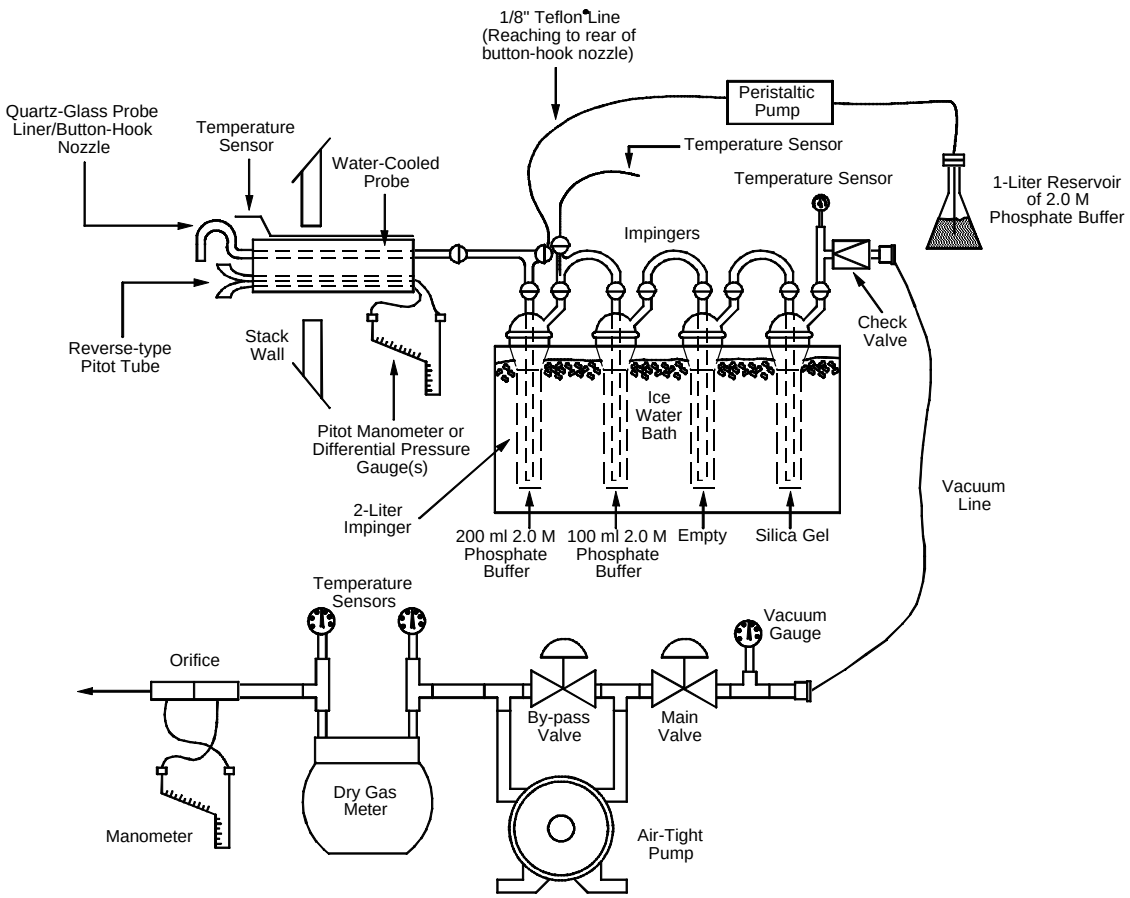

Figure 1. Sampling Train.

2.2.1 Probe Nozzle/Liner. One piece, quartz glass with button-hook design nozzle having a sharp, tapered leading edge. The angle of taper shall be ≤30° and the taper shall be on the outside to preserve a constant internal diameter. The nozzle shall be sized based on the range of velocity heads in the duct (EPA Method 2) so that nozzle size need not be changed to maintain isokinetic sampling rates (see Section 4.1.3.1). The nozzle shall be calibrated according to the procedures outlined in Section 5. Select a suitable liner length (and water-cooled probe) such that all traverse points can be sampled.

2.2.2 Water-cooled Probe. Metal sheath incorporating a circulating water system capable of delivering at least 5.7 liters/min (1.5 gal/min) of tap or ice water to cool the length of the quartz probe liner. It should also have provisions for supporting the S-type pitot tube and thermocouple probe.

2.2.3 Pitot Tube. Same as in Method 5, Section 2.1.3, except refer to Figure 1 in this method.

2.2.4 Differential Pressure Gauges. Same as in Method 5, Section 2.1.4.

2.2.5 Impingers. Four impingers of the Greenburg-Smith design connected in series with leak-free ground glass fittings or any similar leak-free non-contaminating fittings. The first impinger shall have sufficient capacity to contain the total volume of buffer originally charged to this impinger, the moisture catch, and the buffer introduced into the probe. The stem tips of the first, third, and fourth impingers shall be modified by replacing the standard tips with 1.3-cm (0.5-in.) ID glass tubing extended to about 1.3 cm from the bottom of the flask. A temperature sensor shall be inserted into the first impinger and a thermometer or temperature sensor shall be placed at the outlet of the fourth impinger for monitoring the gas stream temperatures at these locations; both shall be capable of

measuring temperature to within 1°C (2°F). Alternatively, functionally equivalent systems are acceptable.

2.2.6 Probe Gas Buffering System. Sterile tubing, pumping system, and sterile buffer reservoir to introduce sterile 2.0 M phosphate buffer, pH -7, into the quartz-glass probe liner in a spray pattern adjacent to the rear of the button-hook nozzle during sampling. Teflon tubing 32-mm (1/8-in.) OD crimped at the end and punched with needle-sized holes has been shown to be suitable. The pumping system should be capable of delivering buffer to the probe at a rate of approximately 15 to 20 ml/min. An acceptable configuration is illustrated in Figure 1.

2.2.7 Metering System. Same as in Method 5, Section 2.1.8.

2.2.8 Barometer. Same as in Method 5, Section 2.1.9.

2.2.9 Gas Density Determination Equipment. Same as in Section 2.1.10 of Method 5.

2.3 Sample Recovery.

2.3.1 Probe Brush. Nylon bristle brush with stainless steel, plastic, or Teflon handle. The probe brush shall have extensions (at least as long as the probe) of similar material. The brush shall be properly sized and shaped to brush out the probe liner and nozzle with the probe gas buffering system installed.

2.3.2 Wash Bottles (sterile). Two glass, polyethylene, or Teflon wash bottles are recommended.

2.3.3 Sample Storage Containers (sterile). Glass containers with leak-free caps, 2-liter.

2.4 Analysis.

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

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

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

2.4.4 Petri Dishes (sterile).

2.4.5 Filter Units (sterile). Nalgene^{R} 0.2-µm cellulose nitrate membrane analytical filter units or equivalent.

3. Reagents

3.1 Sampling.

3.1.1 Water. All water used shall be sterile deionized water.

3.1.2 Collection and Probe Gas Buffering Reagent (sterile). Buffered phosphate solution (2.0 M), approximate Ph 7. Prepare 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. Approximately 2 liters are required for each test run.

3.1.3 Crushed Ice.

3.1.4 Silica Gel. Same as in Method 5, Section 3.1.2.

3.1.5 Stopcock Grease. Heat-stable silicone grease. This is not necessary if screw-on connectors with Teflon sleeves or similar, inert, fittings are used.

3.1.6 Indicator Microorganisms. Bacillus stearothermophilus spores. The theoretical spore charge to the incinerator when mixed with wastes should be at least 10^{12} spores total per sampling run. With advance notice, liquid cultures at this level can be produced by the Fermentation Facility, Department of Biochemistry, University of Alabama at Birmingham, UAB Station, Birmingham, Alabama, 35294 (Telephone No. 205-934-5561). For spiking purposes, cultures will be inoculated into or onto materials normally found in the medical waste stream.

3.2 Analysis. The analytical reagents recommended have been demonstrated to be suitable for culturing and enumerating the indicator spores. Other reagents may also be used if demonstrated to be suitable.

3.2.1 Water. All water used shall be sterile deionized water.

3.2.2 Trypticase 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 100 petri dishes.

3.2.3 1 N Sodium Hydroxide (NaOH). Add 40 g of NaOH to a 1-liter volumetric flask containing water and dilute to volume with water while mixing.

3.2.4 Balance. To measure within 0.5 g.

4. Procedure

4.1 Sampling.

4.1.1 Pretest Preparation. It is suggested that sampling equipment be calibrated and maintained according to the procedures described in Section 3.4 of EPA's Quality Assurance Handbook, Volume III (Citation 4). Clean and hydrogen peroxide/alcohol-disinfect the nozzle/probe liner, first three impingers, all connections, and the nozzle/probe liner brush as follows: Soak in 1.0 N HNO_3 for at least one hour, wash with a laboratory detergent, rinse three times with tap water and three times with deionized water, and finally, rinse with 90 percent ethanol. During storage between cleaning and assembly, plug all component inlets and outlets with similarly-prepared cork or rubber stoppers, or cover with Parafilm^R. Store the probe brush in a clean plastic bag.

4.1.2 Preparation of Collection Train. Assemble the train as in Figure 1, using applicable sections of Citation 4 as a guide. Aseptically add 200 ml and 100 ml of collecting reagent to the first and second impingers, respectively. Transfer approximately 200 to 300 g of silica gel to the fourth impinger. If the stack gas moisture is to be determined using this train, weigh all four impingers to the nearest 0.5 g. Mark the probe

with heat-resistant tape or by other means to denote the proper distance into the stack or duct for each sampling point. Place crushed ice around the impingers.

4.1.3 Preliminary Determinations. Same as in Method 5, Section 4.1.2, with the addition that the total sampling time shall be such that the pH of the first impinger will remain above five.

4.1.4 Leakcheck Procedures.

4.1.4.1 Pretest Leakcheck. This pretest leakcheck is recommended, but not required. Plug the inlet to the first impinger using a sterile plug, and pull a 380-mm Hg (15-in. Hg) vacuum. (A lower vacuum may be used, provided that it is not exceeded during the test.) Then connect the probe to the train and leak check at about 25-mm Hg (1-in. Hg) vacuum. Alternatively, the probe may be leak checked with the rest of the sampling train, in one step, at 380mm Hg (15-in. Hg) vacuum. Leakage rates in excess of 4 percent of the average sampling rate or 0.00057 m^3/min (0.02 cfm), whichever is less, are unacceptable. The leakcheck procedures described in Citation 4 may also be helpful.

4.1.4.2 Leakchecks During Sample Run. If during the sampling run, a component (e.g., impinger) change becomes necessary, a leakcheck as described shall be conducted immediately before the change is made. Leakchecks after component changes are optional.

4.1.4.3 Post-test Leakcheck. A leakcheck is mandatory at the conclusion of each sampling run. The leakcheck shall be done in accordance with the procedures outlined in Section 4.1.4.1, except that it shall be conducted at a vacuum equal to or greater than the maximum value reached during the sampling run. If the leakage rate is acceptable, no correction need be applied to the total volume of dry gas metered. If, however, an unacceptable leakage rate is obtained, the tester shall either record the

leakage rate and correct the sample volume as shown in Section 6.3 of Method 5, or void the sampling run.

4.1.5 Sampling Train Operation.

4.1.5.1 During the sampling run, maintain an isokinetic sampling rate (within 10 percent of true isokinetic unless other wise specified by the Administrator). Nomographs and programmable calculators are available, which aid in the rapid adjustment of the isokinetic sampling rate without excessive computations. For each run, record the data on a data sheet such as the one shown in Figure 5-2 of Method 5. If this train is to be used to determine the moisture content of the sample gas, determine and record the starting volume of the buffer in the probe gas buffering system reservoir. If used, level and zero the manometer. Because the manometer level and zero may drift due to vibrations and temperature changes, make periodic checks during the traverse. Clean the ports prior to the test run to minimize the chance of sampling deposited material. When the stack is under significant negative pressure, take care to close the coarse adjust valve before inserting the probe into the stack to prevent collection solution from backing into the probe. If necessary, the pump may be turned on with the coarse adjust valve closed.

4.1.5.2 Ensure that the temperatures of the primary (and secondary, if applicable) combustion chamber, as well as the stack have reached the requirements specified in the applicable regulations. Begin sampling just before the spiked wastes are added to the incinerator.

4.1.5.3 Before inserting the probe into the duct and beginning sampling, remove the nozzle cap, verify that the pitot tube and probe are properly positioned (the probe at a slight angle to allow buffer and condensed moisture to drain into the first impinger), and start the circulating water and probe gas buffering systems. Insert the probe into the duct and position

the nozzle at the first traverse point with the tip pointing directly into the gas stream. Block off the openings around the probe and port to prevent unrepresentative dilution of the gas stream. Begin sampling by immediately starting the pump and adjusting the flow to isokinetic conditions. Traverse the duct cross-section, as required by Method 1, being careful not to contact the duct walls with the probe nozzle when sampling near the walls or when removing or inserting the probe through the port; this minimizes the chance of extracting deposited material. During the test run, add more ice around the impingers and, if necessary, salt to maintain temperatures of less than 50°C (120°F) in the first impinger and 20°C (68°F) at the silica gel impinger outlet. Also, if a manometer is used, periodically check its level and zero.

4.1.5.4 At the end of the sample run, turn off the coarse adjust valve, remove the probe and nozzle from the stack, and turn off the pump and probe gas buffering system. If this train is to be used to determine the moisture content of the sample gas, determine and record the final volume of the buffer in the probe gas buffering system reservoir. Record the final dry gas meter reading and conduct a post-test leakcheck as outlined in Section 4.1.4.3. Also, leak check the pitot lines as described in Method 2, Section 3.1; the lines must pass this leakcheck in order to validate the velocity head data.

4.1.6 Calculation of Percent Isokinetic. Same as in Section 4.1.6 of Method 5.

4.2 Sample Recovery. Sample recovery procedures are summarized in Figure 2. Allow the probe to cool, ensuring that no foreign contamination enters the nozzle during the process. When the probe has cooled, turn off the water circulating system and wipe off any external matter near the tip of the probe nozzle. Disconnect the probe from the impinger assembly leaving the buffer delivery system inside the probe liner. Using a wash

bottle containing sterile buffer solution and the probe brush, wash the interior of the probe liner, and collect the washings in the sample bottle. Remove the umbilical cord from the last impinger. If using this train to determine the moisture content of the sample gas, weigh each of the four impingers to the nearest 0.5 g. Aseptically transfer the contents of the first three impingers to the sample recovery bottle with sterile buffer rinsings. Check the pH of the sample solution using pH paper, and if necessary, adjust to pH 6.0 to 7.5 with 1 N NaOH. Mark the level of liquid in the sample recovery bottle to check for leakage later. For shipment to the laboratory, pack the bottle in ice and maintain at or below 4°C. Ensure that the sample bottle is sufficiently sealed to prevent contamination from melted ice.

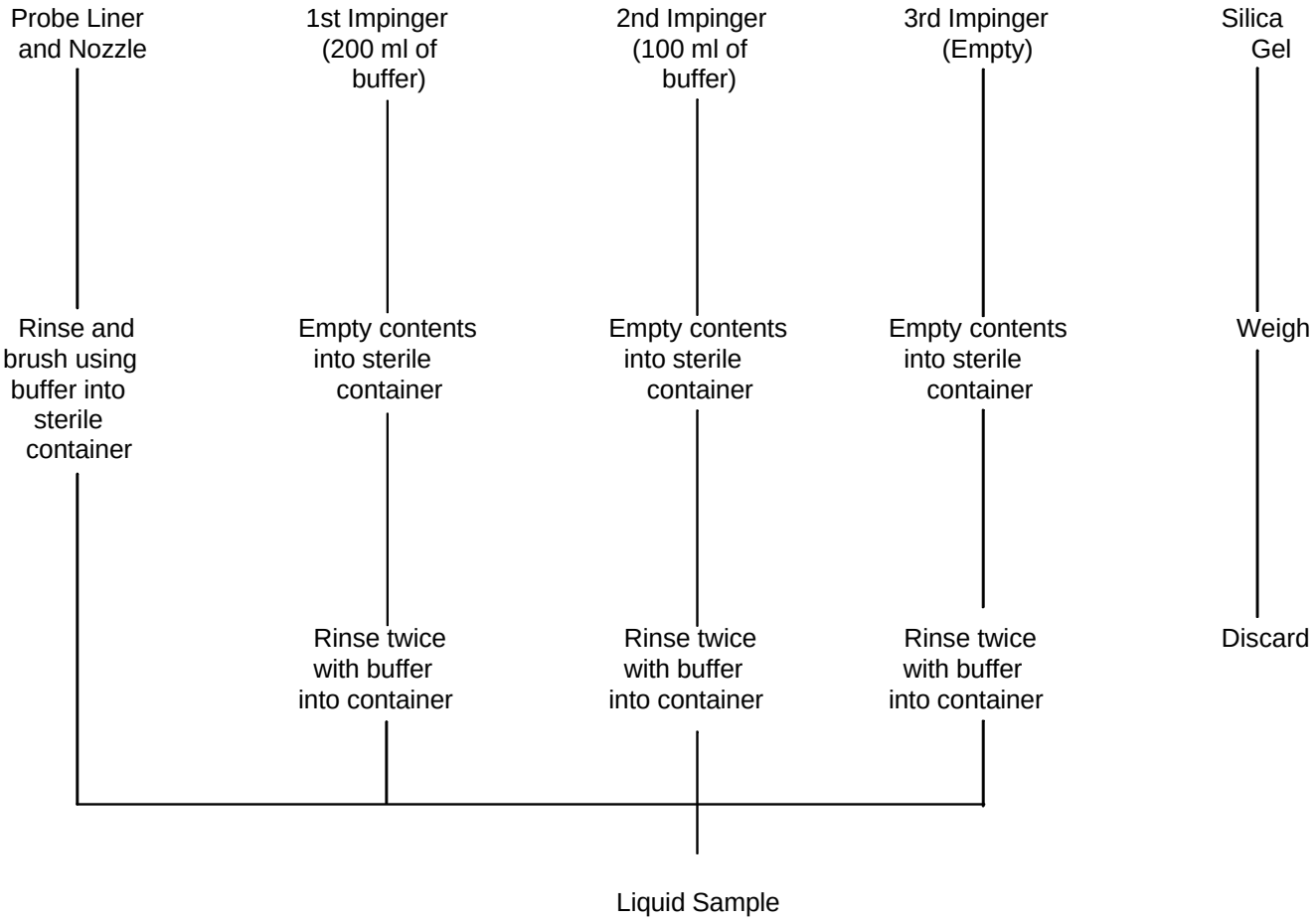

Figure 2. Sample Recovery Scheme for Microbial Testing.

4.3 Sample Preparation and Analysis. The procedures for sample preparation and analysis are summarized in Figure 3.

4.3.1 Sample Solution Aliquots. Note the level of liquid in the sample container and confirm on the analysis sheet whether leakage occurred during transport. Mix the sample solution well and divide into the following aliquots using sterile pipets and/or glassware: three 10-ml aliquots, three 100-ml aliquots, and three equal volume aliquots of the remaining solution. Using sterile water, rinse pipets or other glassware used to transfer the sample solutions into the containers.

4.3.2 Culture Incubation. Pour molten trypticase soy agar into petri dishes. Allow the agar to harden. Using separate filter units, filter each sample aliquot. Rinse each incubation tube with sterile water and pass this rinse through the filter as well. 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.

4.3.3 Quantification. Remove plates from incubator, identify and enumerate the bacterial colonies.

4.3.4 Identification of Indicator Microorganismis. Confirm that the colonies are B. stearothermophilus by testing representative colonies from the plates. A variety of tests, including gram staining and biochemical tests will be used as necessary to correctly identify the colonies.

4.4 Quality Control Procedures.

4.4.1 Meter Orifice Check. The procedures in Section 4.4.1 and 4.4.2 of Method 5 are recommended, but are optional.

4.4.2 Indicator Microorganisms. Aliquots of the spore suspension used to spike the waste shall be filtered, cultured, and quantified simultaneously with the samples to establish the actual spike quantity.

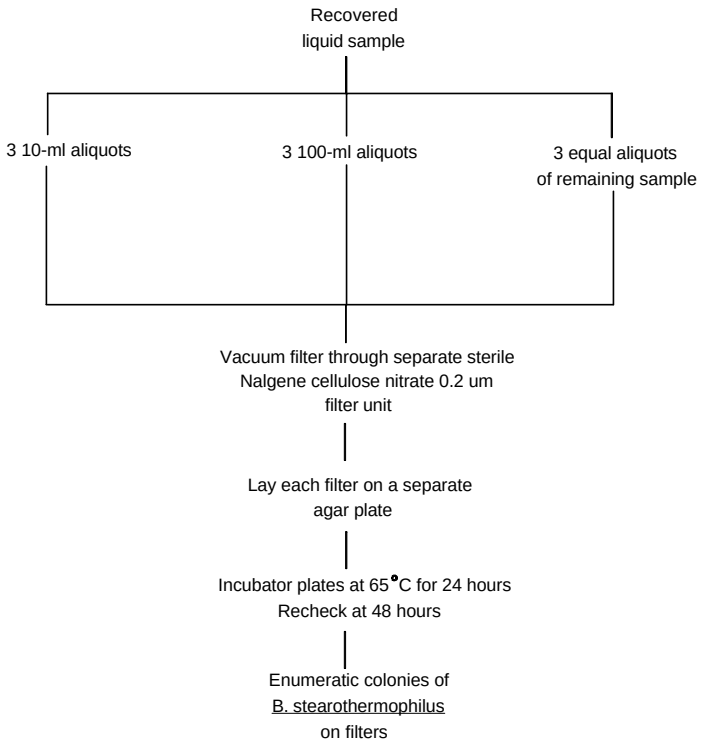

Figure 3. Sample Preparation and Analysis Procedures.

5. Calibrations

Maintain a laboratory log of all calibrations. Calibrate the probe nozzle, pitot tube, metering system, and barometer as described in Method 5, Sections 5.1, 5.2, 5.3, and 5.7.

6. Calculations

Carry out calculations, retaining at least one extra decimal figure beyond that of the acquired data. Round off figures after the final calculation. Other forms of the equations may be used as long as they give equivalent results.

6.1 Nomenclature.

A	=	Cross-sectional area of stack, m^2 (ft^2).
A_n	=	Cross-sectional area of nozzle, m^2 (ft^2).
B_{ws}	=	Water vapor in the gas stream, proportion by volume.
C	=	Concentration of B. stearothermophilus in stack gas, dry basis, corrected to standard conditions, viable spores/dsm^3 (viable spores/dscf).
DE	=	Destruction efficiency, percent.
I	=	Percent of isokinetic sampling.
M_w	=	Molecular weight of water, 18.0 g/g-mole (18.0 lb/lb-mole).
N	=	Number of viable indicator microorganisms in sample.
P_{bar}	=	Barometric pressure at the sampling site, mm Hg (in. Hg).
P_s	=	Absolute stack gas pressure, mm Hg (in. Hg).
P_{std}	=	Standard absolute pressure, 760 mm Hg (29.92 in. Hg).
Q_{sd}	=	Dry volumetric flue gas flow rate at standard conditions, dscm/min (dscf/min).

R	=	Ideal gas constant, 0.06236 (mm Hg)(m^3)/°K)(g-mole) [21.85 (in. Hg)(ft^3)/(°R)(lb-mole)].
S_a	=	Average number of spore colonies counted for three replicate aliquots.
S_e	=	Number of viable spiked spores exiting stack.
S_s	=	Number of viable spores spiked in the waste feed.
T_m	=	Absolute average dry gas meter temperature (see Figure 5-2 of Method 5), °K (°R).
T_s	=	Absolute average stack gas temperature (see Figure 5-2 of Method 5), °K (°R).
T_{std}	=	Standard absolute temperature, 293°K (528°R).
V_a	=	Volume of sample aliquot filtered.
V_g	=	Volume of gas exiting stack during test period, dsm^3 (dscf).
V_{lc}	=	Total volume of liquid collected in impingers and silica gel (difference between total weight of impingers before and after sampling divided by 1 g/ml), ml.
V_{lf}	=	Final volume of liquid sample, ml.
V_m	=	Volume of gas sample as measured by dry gas meter, dm^3 (dcf).
$V_{m(std)}$	=	Volume of gas sample measured by the dry gas meter, corrected to standard conditions, dsm^3 (dscf).
V_{pb}	=	Volume of buffer introduced into the probe, ml.
$V_{w(std)}$	=	Volume of water vapor in the gas sample, corrected to standard conditions, sm^3 (scf).
V_s	=	Stack gas velocity, calculated by Equation 2-9 of Method 2, using data obtained from this method, m/sec (ft/sec).

Y = Dry gas meter calibration factor.

)H = Average pressure differential across the orifice meter (see Figure 5-2 of Method 5), mm H_2O (in H_2O).

P_w = Density of water, 0.9982 g/ml (0.002201 lb/ml).

1 = Total sampling time, min.

13.6 = Specific gravity of mercury.

60 = Sec/min.

100 = Conversion to percent.

460 = Correction to absolute temperature.

6.2 = Average Dry Gas Meter Temperature and Average Orifice Pressure Drop. See data sheet (Figure 5-2 of Method 5).

6.3 Dry Gas Volume. Correct the sample volume measured by the dry gas meter to standard conditions (20°C, 760 mm Hg or 68°F, 29.92 in. Hg) by using Equation 1.

$$V_{m(std)} = V_m\ Y\ (T_{std}/T_m)\ [(P_{bar} + (\Delta H/13.6)]/P_{std}$$
$$= K_1\ V_m\ Y\ [P_{bar} + (\Delta H/13.6)]/T_m \qquad \text{Eq. 1}$$

where:

K_1 = O.3858 °K/mm Hg for metric units,

= 17.64 °R/in. Hg for English units.

NOTE: Equation 1 can be used as written unless leakage is observed during any of the mandatory leakchecks.

6.4 Volume of Water Vapor.

$$V_{w(std)} = V_{lc}\ (\rho_w/M_w)\ RT_{std}/P_{std} = K_2 V_{lc} \qquad \text{Eq. 2}$$

where:

K_2 = 0.001333 m^3/ml for metric units,

= 0.04707 ft^3/ml for English units.

NOTE: V_{lc} is equal to the final weight of the impingers minus the initial weight of the charged impingers and the buffer introduced into the probe (V_{pb}).

6.5 Moisture Content. Same as in Method 5, Section 6.5.

6.6 Number of Indicator Microorganisms Collected. If possible, use the set of replicate sample values that yields between 20 and 200 counts per plate for conducting these calculations.

$$N = S_a \, V_{lf}/V_a \qquad \text{Eq. 3}$$

6.7 Concentration of Indicator Organisms Collected.

$$C = N/V_{m(std)} \qquad \text{Eq. 4}$$

6.8 Flue Gas Flow Rate at Standard Conditions.

$$Q_{sd} = 60 \, (1-B_{ws}) \, v_s \, A \, (T_{std}/T_s) \, (P_s/P_{std}) \qquad \text{Eq. 5}$$

6.9 Volume of Gas Exiting Stack During Test Period.

$$V_g = Q_{sd} \, \theta \qquad \text{Eq. 6}$$

6.10 Number of Viable Spores Exiting Stack During Test Period.

$$S_e = C \, V_g \qquad \text{Eq. 7}$$

6.11 Destruction Efficiency of Incinerator for Indicator Spores.

$$DE = [1 - (S_e/S_g)] \, 100 \qquad \text{Eq. 8}$$

6.12 Isokinetic Variation. Same as in Method 5, Section 6.11.

6.12.13 Acceptable Results. Same as in Method 5, Section 6.12.

7. Bibliography

1. Same as in Bibliography of Method 5.

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

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

4. Quality Assurance Handbook for Air Pollution Measurement Systems, Volume III, Stationary Source Specific Methods. U.S. Environmental Protection Agency, Office of Research and Development. Research Triangle Park, NC, 27711. EPA-600/4-77-027b. August 1977.