Designation: E 884 – 82 (Reapproved 1993)

Standard Practice for Sampling Airborne Microorganisms at Municipal Solid-Waste Processing Facilities[1]

This standard is issued under the fixed designation E 884; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice covers sampling of airborne microorganisms at municipal solid-waste processing facilities, hereafter referred to as facilities. Investigators should consult Practice D 1357 for the general principles of conducting an air-sampling program.

1.2 This practice applies only to sampling airborne bacteria and fungi, not viruses. Since sampling airborne viruses is significantly more difficult than sampling bacteria and fungi, reliable methods of sampling viruses are not yet available.

2. Referenced Documents

2.1 *ASTM Standards:*

D 1356 Terminology Relating to Atmospheric Sampling and Analysis[2]

D 1357 Practice for Planning the Sampling of the Ambient Atmosphere[2]

2.2 *Other Standards:*

Microbiological Methods for Monitoring the Environment, Water and Wastes[3]

Air Sampling Instruments for the Evaluation of Atmospheric Contaminants[4]

3. Definitions

3.1 *microbiological aerosol*—an airborne particle partially or exclusively composed of microorganisms including bacteria and fungi.

3.2 For definitions of other terms used in this practice, refer to Terminology D 1356.

4. Summary of Practice

4.1 Concentrations of selected airborne bacteria and fungi are determined using both liquid impinger and multi-stage impactor samplers.

4.2 Procedures are included for selecting sampling locations; determining numbers of samples, types of microorganisms to be sampled, intervals between sample collection and analysis; choosing sampling equipment; preserving samples; and reporting results.

5. Significance and Use

5.1 Bacteria and fungi present in municipal solid wastes (as well as in other forms of waste) may become airborne as dusts during waste processing. Several investigations to determine the health significance of these microbiological aerosols have been hindered by the lack of standardized procedures for sampling airborne bacteria and fungi in an industrial environment and by the absence of standards for assessing their health significance. Because it is difficult to correlate airborne levels of bacteria and fungi with epidemiological data, this standard is designed to permit the formation of a data base to aid in the assessment of the health significance of airborne microorganisms. It is intended that the use of this practice will improve sampling precision and thereby facilitate comparisons between sampling results.

6. Apparatus

6.1 Two types of samplers are used in each sampling program for microbiological aerosols at waste processing facilities **(5)**.[5]

6.1.1 *Multi-Stage Impactor*, for collection of airborne microbes on agar plates. It is recommended that an impactor be used for sampling all of the types of bacteria and fungi listed in 10.6.1.[6]

6.1.2 *All-Glass Impinger*, for collection of airborne microbes in a liquid medium. It is recommended that an impinger be used for sampling fecal coliforms and for determination of total plate count.[7]

6.2 *Air Sampling Pumps*, providing approximately 40 L per min (1.4 CFM) free-flow capacity.

6.3 Additional equipment such as carts, stands, and tool

[1] This practice is under the jurisdiction of ASTM Committee D-34 on Waste Management and is the direct responsibility of Subcommittee D34.01 on Sampling and Monitoring.

Current edition approved July 30, 1982. Published May 1983.

[2] *Annual Book of ASTM Standards*, Vol 11.03.

[3] Available from the National Technical Information Service, 5285 Port Royal Road, Springfield, Va. 22161. Request EPA-600/8-78-017.

[4] Available from American Conference of Governmental and Industrial Hygienists, 6500 Glenway Avenue, Building D-5, Cincinnati, Ohio 45211.

[5] The boldface numbers in the parentheses refer to the list of references at the end of the method.

[6] The six-stage and two-stage microbiological samplers manufactured by Anderson Samplers, Inc. have been found to be satisfactory.

[7] Air sampling impinger No. 7540 manufactured by Ace Glass, Inc. (AGI 30) has been found to be satisfactory.

boxes are routinely used during dust-sampling programs.

7. Reagents and Materials

7.1 *Agars for Use with the Multi-Stage Impactor*:

7.1.1 *Littman Oxgall*, for total number of fungi present and for identification of the following species of fungi: (*a*) *Aspergillus flavus* and (*b*) *A. fumigatus.*

7.1.2 *Vogel and Johnson*, selective for *Staphylococcus aureus.*

NOTE 1—A fungicide such as nystatin should be used with these agars.

7.1.3 *Levine eosin methylene blue*, specific for enterics including *Klebsiella* spp. (Note 1)

7.1.4 *Trypticase soy*, for total bacteria count. (Note 1)

7.2 *Liquid Media for Use in Impingers*:

7.2.1 *Lactose Broth with Antifoam A*, for analysis of fecal coliform and total plate count.

7.2.2 The exact amount of Antifoam A to be added should be determined prior to field sampling. Sufficient antifoam should be added to prevent loss of fluid from the impinger, but excess should be avoided.

7.3 *Media Preparation*:

7.3.1 Conduct the following according to *Microbiological Methods for Monitoring the Environment, Water and Wastes* **(14)**: (*a*) laboratory quality assurance, (*b*) selection and use of laboratory apparatus, (*c*) washing and sterilization, and (*d*) preparation of culture media.

7.3.2 Preincubate all sampling media to determine if contamination has occurred and to dry the agar surface. Excessive evaporation from the media or excessive contamination of the exterior surfaces of the petri dishes must be guarded against during this preliminary incubation.

7.3.3 Media level in the sampling container is critical to collection efficiency.

7.3.3.1 *Impactor*—The petri dishes must be of such a size that the agar surface is at the manufacturer's specified distance below each stage. The manufacturer of the Andersen impactor specifies 27 mL of agar per standard Andersen petri dish. The agar surface must be smooth and free of bubbles to ensure an even air flow.

7.3.3.2 *Impinger*—For the all glass impinger, 20 mL of broth is recommended **(17).** Autoclave impingers, and then aseptically add 20 mL of sterile broth. Mark its level on the impinger, and record any significant loss during sampling. After sampling, the volume must be reconstituted to the original or the actual volume carefully calculated because a known volume must be used for quantitative work.

8. Precautions

8.1 Due to the nature of municipal refuse, common sense dictates that some precautions should be observed when sampling dusts at municipal solid-waste processing facilities. Recommended safety practices include wearing hard hats, safety shoes, safety glasses, gloves, and respirators as well as washing hands before eating or smoking.

9. Sampling

9.1 *Location and Number of Sampling Sites*:

9.1.1 All sampling shall be carried out during normal plant operations.

9.1.2 Use not less than two sampling locations inside the facility at work sites or zones where employees are most likely to be exposed to airborne dust concentrations **(7).** (Note 2) Among these locations, those where sampling equipment can be located without interfering with facility operations shall be preferred.

NOTE 2—Examples of potential sampling locations are (*a*) on a tipping floor near or on a front end loader; (*b*) at a hand-picking station along a conveyor belt; and (*c*) along catwalks or platforms in frequent use by employees.

9.1.3 Outside the facility, locate at least one sampling site 300 m (1000 ft) upwind from the facility and at least one sampling site 100 m (330 ft) downwind from the facility. Measure the distances upwind and downwind from the same point, the point at which the emissions leave the facility or, in the case of multiple discharge points, from a central point equidistant from the discharge points.

9.1.4 Carefully measure and record the actual distances of the sampling sites from the points of emission and wind direction and velocity.

9.2 *Position of Sampling Inlet*—Locate the sampling inlet(s) 1.5 m (5 ft) above the floor level to approximate the breathing zone of a worker or other person exposed to the dusts. Locate the vacuum pumps where they will not disturb the air flow patterns around the sampling inlet(s).

9.3 *Number of Samples*:

9.3.1 Inside the facility, collect not less than 5 replicate samples at each sampling site.

9.3.2 Outside the facility, collect not less than 3 replicate samples at the upwind site(s) and not less than 5 replicate samples at the downwind site(s).

9.3.3 Wide variations in reported microbiological aerosol levels within facilities make it unlikely that the collection of five samples will yield a tight distribution of results; therefore, where economically feasible, it is recommended that the sample size be increased to more than five.

9.4 *Air Temperature*:

9.4.1 Collect samples when the air temperature at the sampling site is above 5°C (40°F).

9.4.2 At temperatures below 5°C (40°F), the sampling medium may crystallize, thus affecting recovery of microorganisms.

10. Procedure

10.1 Record air temperature and relative humidity for each location sampled.

10.2 Label all impingers to denote sampling run and location. Label all petri dishes to denote sampling run, location, and stage of impactor.

10.3 *Air-Flow Rates*:

10.3.1 Determine the air-flow rate by an in-line flow meter. Where this is not possible, calibrate air-flow rate with a gas-flow meter according to the procedure described in Ref **(16).** The recommended flow rate for the Andersen impactor is 28.3 L/min. The optimum flow rate for the all-glass impinger is 12.5 L/min.

10.3.2 Maintain a constant air-flow rate through the sampler during the sampling time. Before sampling, allow the vacuum pump to warm up for not less than 1 min. Use clamps, T-shaped

connectors, and in-line membrane filters with 1-mm pore size to pull filtered air through the pump during the warmup without pulling air through the sampler. Select clamps and T-shaped connectors that will not alter the flow rate through the samplers.

10.3.3 Secure all connections to keep the air loss less than 4 % of the average sampling rate or less than 0.00057 m^3/min (0.02 ft^3/min), whichever is smaller. Measure the leakage-flow rate with a suitable dry-gas meter connected to the discharge side of the vacuum pump while the inlet to the sampling apparatus is plugged and a 380-mm (15-in. Hg) vacuum is drawn. A lower vacuum may be used provided it is not exceeded during sampling.

NOTE 3—Many of the vane-type air sampling pumps (including the one furnished for use with the Andersen sampler) use a needle valve to control the air flow through the sampler by bleeding in air that bypasses the sampler. The air flow through the pump is therefore constant, and a meaningful measure of the flow through the sampler can only be made at this location in the sample stream.

10.4 *Sampling time*—The length of time needed to collect each sample is dependent upon the type of sampler used and the concentration of microbiological aerosols present in the air. Trial sampling runs may be necessary to determine if a satisfactory plate loading can be obtained within the limitations of the equipment used.

10.4.1 For the all-glass impinger operating at a flow rate of 12.5 L/min, the normal sampling time is 20 min.

10.4.2 When using a multistage impactor, choose the sampling time to avoid overloading the impaction plates, that is, the loading on any of the plates should not exceed 300 colonies per plate. The sampling time for the multistage impactors will vary depending on the medium used for sampling collection and the concentration of airborne dust. Suggested initial sampling times for the various media are in Table 1.

10.5 *Care During Sampling and Transport*—Collect, pack, transport, and manipulate the sample prior to analysis in a manner that safeguards against any change in the microbial activity in the sample, such as, extreme heat and cold and radiation, including sunlight. Use the proper media to ensure preservation of the sample until its identification. If samples must be shipped prior to analysis, positive controls should be included with each shipment. Federal regulations must be followed when they apply to these shipments.

10.5.1 *Care During Sampling with the Impactor*:

10.5.1.1 Carry out impactor loading and unloading in an atmosphere of minimal microbial activity, preferably in a portable polyethylene glove bag or a similar container. Invert the petri dishes immediately when the sampler is unloaded. Sanitize the impactor with a 70 % alcohol solution and dry thoroughly between samplings. Do not sanitize in the glove bag. To provide a control check for contamination, load and unload the impactor without sampling using a set of trypticase soy agar petri dishes, and then subject these petri dishes to the same processing steps and analytical procedures applied to the samples.

TABLE 1 1 Suggested Initial Sampling Times

Type of Media	Suggested Initial Sampling Time, min.[A]
Littman-Oxgall	1.5
Vogel and Johnson	8
Levine eosin methylene blue	10
Trypticase soy	0.5

[A] The initial sampling times suggested above are based on reported concentration levels in an enclosed facility. These times are subject to adjustment based upon the initial test results.

10.5.1.2 Minimize uneven distribution of colonies on the plates by centering the plates on the three pegs in each stage of the impactor and, once loaded, handling the impactor carefully to maintain this position.

10.5.2 *Care During Sampling with the Impinger*:

10.5.2.1 Include a negative (sterile) control with the impingers to determine whether the samples become contaminated while in transit or at the test site.

10.5.3 Preserve all samples by placing each one in a closed container at 4 ± 2°C immediately after taking them. Protect the plates from direct contact with the ice to prevent contamination.

NOTE 4—Sealed ice packets have been found to be satisfactory and convenient for this purpose.

10.5.4 Return the samples to the laboratory as soon as possible and not later than 6 h after sampling. Process the samples and place in a incubator as soon as possible.

10.5.5 For impinger samples, rinse the neck of the impinger and add this material to the sample. The volume of the rinse solution must be measured so that the final sample volume is known.

10.6 *Identification of Colonies*:

10.6.1 Analyze for the types of bacteria and fungi listed in 10.6.1.1-10.6.1.4. This is a minimum list of bacteria and fungi recommended for identification and quantification. Individual investigators may wish to sample for additional organisms. Among the other microorganisms that have been sampled at facilities are *Aspergillus niger*, *Mycobacterium* species and other members of the *Actinomycetales* order.

10.6.1.1 Total plate count (impactor) (Note 5),

10.6.1.2 Total plate count (impinger) (Note 5),

10.6.1.3 Bacteria, (*a*) Fecal coliforms, (*b*) *Klebsiella* species, (*c*) *Staphylococcus aureus*, and

10.6.1.4 *Fungi*, (*a*) *Aspergillus fumigatus* and (*b*) *Aspergillus flavus.*

NOTE 5—The sum of total bacteria counts and total fungi counts should be reported separately.

10.6.2 Agar plates and impinger solutions are to be processed by standard microbiological procedures such as those described in Refs. **1-3, 9, 13,** and **14**.

10.6.3 Identify representatives of both typical and atypical colony morphology. The microorganisms are being recovered from a stressed environment, consequently the colony morphology on the various media may not be typical.

11. Enumeration of the Microorganisms

11.1 *Impactor Samples*:

11.1.1 Count impactor plates as specified by the manufacturer of the impactor. Correct impactor plate counts using the positive hold conversion table supplied by the manufacturer.

11.1.2 When multistage impactors are used, report results for each stage.

11.1.3 Because the loading varies among the impactor plates, report which stages have less than 30 and more than 300 colonies.

11.1.4 If none of the plates had more than 30 colonies, report the computed counts as an estimated total count.

11.1.5 If at least one of the plates had more than 300 colonies, report the computed counts as greater than (>) the calculated concentration of total count.

11.1.6 Plates containing more than 399 colonies are reported as too numerous to count (TNTC).

11.1.7 Plates containing spreading colonies must be so reported on the data sheet. If "spreaders" exceed one-half of the total plate area, the plate should not be used and the results for the plate should be reported as "No results, spreaders."

11.1.8 Colonies can be counted on representative portions of plates if spreading colonies constitute less than one-half of the total plate area following the procedure in *Microbiological Methods for Monitoring the Environment, Water and Wastes* **(14).**

11.2 *Impinger Samples*:

11.2.1 Serial dilution procedures should be used to adjust the sample concentration prior to further processing. Choose a dilution that will provide 30 to 300 colonies per plate.

11.2.2 Standard plate counts should be determined and recorded as specified in Paragraph 5.6, Part III, of Ref **(14)**. Report all results as the number of colony-forming units per cubic metre of air.

12. Report

12.1 Report all concentrations as the number of colony-forming units (that is, particles that contain at least one living organism) per cubic metre of air.

12.2 For each site and each type of microorganism analyzed, report the mean and standard deviation of the logtransform of the concentrations according to the procedure described in Part IV (Quality Assurance) of *Microbiological Methods for Monitoring the Environment, Water and Wastes* **(14)**.

NOTE 6—It is recognized that the standard deviation may be quite large, particularly, if minimum sample sizes are used, but the reporting of standard deviations will at least indicate the spread of the data and will be useful in putting perspective on the sampling results.

12.3 The report of results should include the following:

12.3.1 Concentrations at each sampling site,

12.3.2 Descriptions of the number and location of sampling sites,

12.3.3 Activities at sampling sites,

12.3.4 The temperature and relative humidity of the collected air at the sampling site,

12.3.5 The flow rates,

12.3.6 The sampling times,

12.3.7 The holding time and temperature between the time of sampling and the time of assay,

12.3.8 Descriptions of the microbiological procedures used in the identification of organisms,

12.3.9 The suppliers and lot numbers of the various medias used, and

12.3.10 The colony counts, if any, recovered on "control" petri dishes described in 10.5.1.1.

12.4 Additional meteorological parameters such as solar radiation, precipitation, wind speed, and wind direction may also influence recoveries and should be reported where possible.

13. Precision and Accuracy

13.1 The information in this section is derived from the data collected at a facility and analyzed by L. Lembke and R.N. Kniseley at Ames Laboratory, U.S. Department of Energy **(12)**.

13.2 *Repeatability* (single-laboratory)—The coefficient of variation of total plate counts obtained with gelatin milk broth **(16)** in the all-glass impinger was estimated to be 0.38 for an observed aerosol concentration of 10^4 colony-forming units per cubic metre of air in an operating municipal solid-waste processing facility. The coefficient of variation of counts obtained with 5 % sheep blood agar plates in the six-stage Andersen impactor was estimated to be 0.23 for an observed aerosol concentration of 10^4 colony-forming units per cubic metre of air in an operating municipal solid-waste processing facility.

13.3 *Accuracy*—No procedure presently exists to unequivocably determine the accuracy of this method. At Ames Laboratory, simultaneously operated all-glass impingers yielded colony counts that indicated a strong linear relationship between samplers over an observed aerosol concentration of 1.1×10^3 to 2.8×10^7 colony-forming units per cubicmetre of air in an operating municipal solid-waste processing facility. Simultaneously operated six-stage impactors also yielded colony counts that indicated a strong linear relationship between samplers over an observed aerosol concentration of 3.9×10^3 to 1.9×10^5colony-forming units per cubic metre of air in an operating municipal solid-waste processing facility.

REFERENCES

(**1**) *Compendium of Methods for the Microbiological Examination of Foods*, American Public Health Assn. (APHA), Section 31.93, Washington, D.C., 1976.

(**2**) *Standard Methods for the Examination of Water and Wastewater*, American Public Health Assn. (APHA), Fourteenth Edition, Washington, D.C., 1976.

(**3**) *Standard Methods for the Examination of Dairy Products*, American Public Health Assn. (APHA), Eleventh Edition, Washington, D.C., 1960.

(**4**) Andersen, A. A., *New Sampler for the Collection, Sizing and Enumeration of Viable Airborne Particles*. J. Bacteriol., Vol 76, 1958, pp. 471–484.

(**5**) Brachman, P. S.; Erlich, R.; Eichenwald, H. F.; Cabelli, V. J.; Kethley, C. W.; Madin, S. H.; Maltman, J. R.; Middlebrook, G.; Morton, J. D.; Silver, I. H.; and Wolfe, E. K., "Standard Sampler for Assay of Airborne Microorganisms," *Science*, Vol 144, 1964, (3642): p. 1295.

(**6**) Chatigny, Mark A., "Sampling Airborne Microorganisms", *Air Sampling Instruments for Evaluation of Atmospheric Contaminants*, Fifth Edition, American Conference of Government of Industrial Hygienists, Cincinnati, Ohio, 1978.

(**7**) Corn, M. and Esman, N. A., "Workplace Exposure Zones for Classification of Employee Exposures to Physical and Chemical Agents," *Amer. Industrial Hygiene Assoc. Journal*, Vol 40, 1979, pp. 47–56.

(**8**) Dimmick, R. L. and Akers, A. B., *An Introduction to Experimental Aerobiology*, Wiley-Interscience, New York, N.Y., 1969.

(**9**) Emmons, C. W., Binford, C. H., and Utz, J. P., *Medical Mycology*, Lea and Feibiger, Philadelphia, Pa., 1963.

(**10**) Gregory, P. H., *The Microbiology of the Atmosphere*, John Wiley & Sons, New York, N.Y., 1973.

(**11**) Larson, E. W., Demchak, P., and Dominik, J. W., "Comparisons Between the Single-Stage Impaction Device and Multistage Liquid Impinger Systems for Sampling and Sizing Biological Aerosols," *Airborne Transmission and Airborne Infection*, ed. by Hers, J. F., Ph. and Winkeler, K. C., John Wiley & Sons, New York, N.Y., 1973.

(**12**) Lembke, L. L., Kniseley, R. N., Van Nostrand, R. C., and Hale, M. D. "Precision of the All-Glass Impinger and the Andersen Microbial Impactor for Air Sampling in Solid-Waste Handling Facilities," *Applied and Environmental Microbiology*, Vol 42, 1981, pp. 222–225.

(**13**) Raper, K. B. and Fennell, D. I., *The Genus Aspergillus*, Robert E. Krieger, Huntington, New York, 1977.

(**14**) *Microbiological Methods for Monitoring the Environment, Water and Wastes*, EPA-600/8-78-017, U. S. Environmental Protection Agency, December 1978. Available from National Technical Information Service.[3]

(**15**) Wells, W. F., *Airborne Contagion and Air Hygiene*, Harvard University Press, Cambridge, Mass., 1955.

(**16**) White, L. A., Hadley, D. J., Davids, D. E., and Naylor, R., "Improved Large-Volume Sampler for the Collection of Bacterial Cells from Aerosol," *Applied Microbiology*, Vol 29, 1975, pp. 335–339.

(**17**) Wolf, H. W., Skaliy, P., Hall, L. B., Harris, M. M., Decker, H. M., Buchanan, L. M., and Dahlgren, C. M., "Sampling Microbiological Aerosols," *Public Health Monograph*, No. 60, U. S. Department of Health, Education, and Welfare, Public Health Service Publication No. 686, Washington, D.C., 1959.