7.3 Mechanical/Chemical References

1. Jetté L.P. and S. Lapierre. 1992. Evaluation of a mechanical/chemical infectious waste disposal system. *Infection Control and Hospital Epidemiology*, (13)7:387-393.

2. Denys, G.A. 1989. Microbiological evaluation of the medical Safetech mechanical/ chemical infectious waste disposal system. Presented at the 89th annual meeting of the American Society of Microbiology, New Orleans, LA, May 16.

8.0 ASSESSMENT OF INDICATOR MICROORGANISMS

Table 1 summarizes the microorganisms used in the cited medical waste treatment and/or bioemissions testing studies. The list is comprised of organisms including vegetative bacteria, vegetative fungi (molds and yeasts), viruses, mycobacteria, and bacterial endospores. From many years of germicide research, as detailed in the published literature, a scale of resistance relative to the major groups of microorganisms has emerged. This general scale of resistance, from least to most resistant, is as follows: vegetative bacteria, vegetative fungi and fungal spores, viruses, mycobacteria, and bacterial spores. Recent research data (Cole, unpublished results) confirmed a correlation between the inactivation of bacterial spore challenges in waste treatment processes with the inactivation or kill of significant challenges of the less resistant groups of microorganisms. Thus, the inactivation of a significant challenge of bacterial endospores is a reliable indicator of the effectiveness of a treatment system.

Bacillus stearothermophilus and *Bacillus subtilis* var. *niger* are two indicator organisms whose spores are commonly used to evaluate the effectiveness of medical waste treatment processes, because they are intrinsically resistant to chemical and physical inactivation, are non-pathogenic, and are easy to isolate on a variety of laboratory media. *B. stearothermophilus* is not commonly found in medical waste, and can be easily recovered from medical waste because it is thermophilic and requires high incubation temperatures (55°C) at which most other microorganisms will not grow.

These same bacterial spores can also be used to evaluate emissions from waste treatment processes. A study was recently conducted for bioemissions from a medical waste compaction process. [1] In the study, a prototype mechanical infectious waste compaction unit was assembled and tested for airborne bacteria released by compressing wastes spiked with known quantities of *Bacillus subtilis* var. *niger*. A series of AGI-30 impingers was used to monitor airborne bacteria releases during operation, and contact agar plates were used to monitor surfaces on and around the unit after

operation. Results from this study showed that airborne indicator spores were emitted from the unit during the compaction process.

8.1 Indicator Microorganism Reference

1. Emery, et al. 1992. Release of bacterial aerosols during infectious waste compaction: an initial hazard evaluation for health care workers. *American Industrial Hygiene Association Journal*, 53(5):339-345.

Table 1[a]. Medical Waste Treatment Technologies Test/Indicator Organisms [*]

Treatment Technology	Test/Indicator Organism
Incineration	*Bacillus stearothermophilus* spores
	Bacillus spp
	Staphylococci
	Staphylococcus aureus
	Pseudomonas fluorescens
	Bacillus subtilis
	Bacillus subtilis var. *niger* spores
	Salmonella
	Total bacteria
	Total coliforms
	Fecal coliforms
	Serratia marcescens
Steam Autoclave	*Bacillus pumilus* (NAS Associates)
	Vaccinia virus strain WR
	Bacillus stearothermophilus spores
	Bacillus stearothermophilus spores NCA1518
	Bacillus subtilis var. *niger* spores
	Serratia marcescens
	T1 coliphage

[*] **Cited in the technical literature**

Table 1[b]. Medical Waste Treatment Technologies Test/Indicator Organisms [*]

Treatment Technology	Test/Indicator Organism
Microwave	*Bacillus subtilis* ATCC 6633
	Enterobacter cloacae ATCC 23355
	Klebsiella pneumoniae ATCC 23357
	Serratia marcescens ATCC 8100
	E. coli ATCC 25922
	Pseudomonas aeruginosa ATCC 27853
	Staphylococcus aureus ATCC 25923
	S. epidermidis ATCC 12228
	Proteus mirabilis (clinical isolate)
	Enterococcus (clinical isolate)
	Alpha streptococcus, Group D.
	Streptococcus faecalis
	Saccharomyces cerevisiae
	Escherichia coli
	Bacillus subtilis var. *niger*
Mechanical/Chemical	*Bacillus subtilis* var. *niger* (ATCC 6633)
	Candida albicans (ATCC 10231)
	Enterococcus faecalis (ATCC 29212)
	Mycobacterium fortuitum (ATCC 6841)
	Serratia marcescens LSPQ 3028 (pigmented)
	Bacteriophages ΦX174, f2
	Bacillus subtilis (clinical isolate)
	Enterococcus faecalis (ATCC 29212)
	Mycobacterium fortuitum (ATCC 3571)
	Serratia marcescens (pig.clin.iso.)
	Acinetobacter anitratus (ATCC 33498)
	Aspergillus sp. (clinical isolate)
	Candida albicans (clinical isolate)
	Pseudomonas aeruginosa (ATCC 27853)
	Salmonella sp. (clinical isolate)
	Staphylococcus aureus (ATCC 29213)

[*] **Cited in the technical literature**

9.0 SUMMARY OF BIOAEROSOL MONITORING METHODS

Table 2 shows the sampling methods used for microbiological monitoring in the cited studies. Standard stack gas sampling techniques using impingers and volumetric sampling through filters were used in the incineration studies. One exception was the direct use of a Casella slit sampler for stack gas. The steam autoclave studies used AGI-30 impingers and sieve samplers; and the mechanical/chemical studies used AGI-30 impingers, Mattson-Garvin slit-to-agar samplers, Andersen cascade impactor (sieve) samplers, and gravity settle plates. Settle plates, however, have severe limitations as they do not sample air volumetrically and their reliability for most bioaerosol sampling is questionable due to long settling times required for small particles < 7μm, such as bacteria and fungal spores. Such particles may remain entrained in even minimal airflow. Therefore, settle plates are not recommended for bioaerosol research studies. AGI-30s were used to successfully monitor infectious waste compaction. In a mechanical/chemical study, as well as compactor evaluation, contact agar plates were used to assess air to surface contamination. The plates are 16 cm^2 plates with raised agar surfaces which are pressed onto the surface being monitored. The plates are then capped and incubated at proper temperatures. Surfaces can also be monitored with sterile swabs or rinsed with sterile saline.

The origin of the liquid impinger sampler for airborne bacteria is obscured by cooperative efforts of numerous laboratories between 1939 and 1946. Tyler and Shipe reported on the development and evaluation of AGIs from studies performed from 1948 to 1952. [1] Later, the AGI-30 (having a jet-to-base distance of 30 mm) was recommended as a standard sampler by a committee of aerobiologists. [2] Recently, a National Institute of Occupational Safety and Health (NIOSH) bioaerosol chamber study evaluated the collection efficiency of eight bioaerosol samplers relative to the AGI-30 as a reference sampler. [3]

As shown in Table 3, the slit and sieve impactors, and all-glass impingers are commonly used bioaerosol samplers. The American Society for Testing Materials (ASTM) *Standard Practice for Sampling Airborne Microorganisms at Municipal Solid-Waste Processing Facilities* requires both the multi-stage impactor and All-Glass Impinger (ASTM E884-82). [4] Additionally, the National Sanitation Foundation's Standard Number 49 (Class II Biohazard Cabinetry) requires the use of AGI-30s and slit-to-agar samplers for spore challenge tests for personnel, product, and cross-contamination protection. [5] Also, report of a recent investigation to characterize occupational fungal exposure successfully utilized 2-stage cascade impactors and AGI-30 impingers. [6]

RTI, in cooperation with the U.S. EPA, recently developed and evaluated an indoor air quality test kit (IAQTK).[7] The IAQTK is comprised of a number of air quality monitoring instruments which includes the Mattson/Garvin slit-to-agar bioaerosol sampler, with different plate rotation motors (5, 15, 20, and 60 mins). The IAQTK instruments were evaluated in a large field study of sixteen indoor environments in both commercial and residential buildings.[8] The Mattson/Garvin was found to accurately detect varying levels of airborne bacteria and fungi when results were compared to those from a high-volume sampler and Andersen cascade impactor.

9.1 Bioaerosol Monitoring Methods References

1. Tyler, M.E. and E.L. Shipe. 1959. Development and evaluation of the all-glass impinger. Bacterial aerosol samplers. *Applied Microbiol*; 7:337-349.

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

3. Jensen, P.A., et al. 1992. Evaluation of eight bioaerosol samplers challenged with aerosols of free bacteria. *Am Ind Hyg Assoc J*; 53 (10):660-667.

4. American Society for Testing and Materials. 1987. *Standard Practice for Sampling Airborne Microorganisms at Municipal Solid-Waste Processing Facilities* (E884-82). ASTM, Philadelphia, PA.

5. National Sanitation Foundation. 1987. *Standard Number 49, Class II (Laminar Flow) Biohazard Cabinetry.* (NSF), Ann Arbor, MI.

6. Lenhart, S.W. and E.C. Cole. 1993. Respiratory illness in workers of an indoor shiitake mushroom farm. *Appl Occup Environ Hyg*; 8:112-119.

7. Green, D.A, K.E. Leese, and E.C. Cole. 1992. Development and Evaluation of an Indoor Air Quality Test Kit. RTI technical report No. 94U-4479-005/03F, EPA Contract No. CR-815509-01-0.

8. Cole, E.C., K.K. Foarde, K.E. Leese, D.A. Green, D.L. Franke, D.F. Naugle and M.A. Berry. 1992. Indoor Air Quality Monitoring in Carpeted Environments. RTI technical report No. 94U-4479-005/04F. EPA Contract No. CR-815509-01-0.

Table 2. Summary of Bioemissions Monitoring Methods for Medical Waste Treatment and Compaction *

Treatment Technology	Source	Microbiological Monitoring Method
Incineration	Stack gas	Cooled glass probe with impingers Aluminum tubing with Shipe impingers Hurricane samplers with filters Modified exhaust blowers with filters Staplex sampler with filters Casella slit sampler with steel probe
	Ash, waste	Grab samples
Steam Autoclave	Exhaust/ Ambient air	AGI-30s (All glass impingers) Sieve impactor samplers
Microwave	None cited	None cited
Mechanical/Chemical	Ambient air	AGI-30s Andersen cascade impactors Gravity settle plates
	Surfaces	Contact agar plates
Compaction	Machine/ Ambient air	AGI-30s
	Surfaces	Contact agar plates

* **Cited in the technical literature**

Table 3. Commonly Used Bioaerosol Samplers*

Sampler Type	Principle of Operation	Sampling Rate (lpm)	Recommended Sample Time
1. Slit impactor	Impaction on rotating plate	30-700	5-60 min
2. Sieve impactor			
a. single-stage portable	impaction on agar; "rodac" plate	90 or 185	0.5 or 0.3 min
b. single-stage impactor	impaction on agar; 100 mm plates	28.3	1 min
c. two-stage impactor	impaction on agar; 2-100 mm plates	28.3	1-5 min
3. Membrane filters	Filtration	1-2	15-60 min or 8 hr
4. High-volume	Filtration	140-1400	5 min-24 hr
5. High-volume	Electrostatic collection into liquid	up to 1000	variable
6. All-glass impinger (AGI-30)	Impingement into liquid	12.5	5-20 min
7. Centrifugal impactor	Impaction on agar; plastic strips	~40	0.5 min

* Modified from *Guidelines for the Assessment of Bioaerosols in the Indoor Environment*, American Conference of Governmental Industrial Hygienists, 1989, Cincinnati, OH.

10.0 RECOMMENDATIONS

10.1 Indicator Microorganisms

Spores of *Bacillus stearothermophilus* and *Bacillus subtilis* var. *niger* are commonly used to evaluate the effectiveness of medical waste treatment processes because they are intrinsically resistant to inactivation, they are non-pathogenic, and are easy to isolate and identify in the laboratory. *B. stearothermophilus* is not commonly found in medical waste and is easily recovered because it is thermophilic and requires incubation temperatures (55°C) at which most other microorganisms cannot grow. *B. subtilis* var. *niger* has a characteristic pigmentation. In consideration of these characteristics, and a review of the cited studies and published practices, it is recommended that both *B. stearothermophilus* and *B. subtilis* var. *niger* spores be used as microbiological challenges in the evaluation of biological emissions from alternative medical waste treatment processes.

10.2 Monitoring Methods

As a review of the cited literature shows, the All-Glass impinger (AGI-30) is recognized as a bioaerosol sampling standard and is recommended as the primary method for the evaluation of microbiological emissions from medical waste treatment processes.

Bioemissions monitoring of a medical waste treatment process may require extended sampling intervals. Also, there are events such as the grinding period in a mechanical/chemical process, or the door opening in a steam autoclave process, which may have greater potential for bioaerosol emissions. The Mattson-Garvin (M/G) slit-to-agar sampler can operate for up to one hour intervals at a time. The inlet of the sampler can accommodate an extension probe for sampling, which expands its capacity to monitoring areas of limited access. Also, the rotation of the agar plate during sample collection facilitates the detection of sudden changes in bioaerosol concentrations which may be event related. The NIOSH study compared the M/G sampler to the AGI-30 with bioaerosols in a chamber and found that the collection efficiency of the M/G was only marginally lower than the AGI-30. The ASTM standard practice for monitoring solid waste processing facilities requires AGI-30s and multiple-stage impactors, and the NSF Standard 49 requires AGI-30s and slit-to-agar samplers for monitoring biohazard cabinetry. In addition, RTI uses both the M/G and 2-stage Andersen samplers routinely for indoor air biocontamination investigations and considers them to be reliable bioaerosol monitoring methods. Thus, the Mattson-Garvin slit-to-agar sampler and the Andersen 2-stage impactors are recommended in conjunction with AGI-30s for the evaluation of bioemissions from medical waste treatment processes.