1.0 INTRODUCTION

This document identifies techniques to assess the microbiological efficacy of various medical waste treatment technologies and provides general guidance on the proper operating procedures. Each treatment technology vendor should provide guidance for the operation and maintenance of the specific devices. This document is not intended to provide step-by-step instructions for operation of any equipment, but is intended as a technical guide for use by Federal, State, and local agency personnel, and medical waste management personnel.

This document summarizes the technical information related to the operation and maintenance of a number of currently available medical waste treatment technologies. Chapter 2 presents information specific to testing medical waste incinerators. Chapter 3 presents information specific to steam autoclave treatment. Chapter 4 discusses various options for chemical treatment of medical waste. Chapter 5 provides guidance for assessing non-ionizing radiation (microwave and shortwave radiofrequency) treatment. Chapter 6 discusses occupational health and safety issues associated with medical waste treatment, both onsite and offsite. General facility maintenance guidelines are presented in Chapter 7.

1.1 WASTE CHARACTERISTICS

The Medical Waste Tracking Act (MWTA) of 1988 defined medical waste as any solid waste which is generated in the diagnosis, treatment, or immunization of human beings or animals, in research pertaining thereto, or in the production or testing of biologicals. Descriptions of specific classes of medical wastes are listed in Title 40 of the Code of Federal Regulations (CFR) Part 259 and include:

- Class 1 - CULTURES AND STOCKS

  Cultures and stocks of infectious agents and associated biologicals, including cultures from medical and pathological laboratories, cultures and stocks of infectious agents from research and industrial laboratories, wastes from the production of biologicals, discarded live and attenuated vaccines, and culture dishes and devices used to transfer, inoculate, and mix cultures (hereinafter referred to as microbiological waste).

- Class 2 - PATHOLOGICAL WASTES

  Human pathological wastes, including tissues, organs, and body parts, and body fluids that are removed during surgery and autopsy or other medical procedures, and specimens of body fluids and their containers.

- Class 3 - HUMAN BLOOD AND BLOOD PRODUCTS

  Waste human blood and products of blood, items saturated and/or dripping with human blood; or items that were saturated and/or dripping with human blood that are now caked with dried human blood; including serum, plasma, and other blood components, and their containers, which were used or intended for use in either patient care, testing and laboratory analysis, or the development of pharmaceuticals. Intravenous bags are also included in this category.

- Class 4 - USED SHARPS

  Sharps that have been used in animal or human patient care or in medical, research, or industrial laboratories, including hypodermic needles, syringes, pasteur pipettes, scalpel blades, blood vials, test tubes, needles with attached tubing, and culture dishes (regardless of presence of infectious agents). Also included are other types of broken or unbroken glassware that were in contact with infectious agents, such as used slides and cover slips.

- Class 5 - ANIMAL WASTE

  Contaminated animal carcasses, body parts, and bedding of animals that were known to have been exposed to infectious agents during research (including research in veterinary hospitals), production of biologicals, or testing of pharmaceuticals.

- Class 6 - ISOLATION WASTES

  Biological waste and discarded materials contaminated with blood, excretion, exudates, or secretions from humans who are isolated to protect others from highly communicable diseases or isolated animals known to be infected with highly communicable diseases.

- Class 7 - UNUSED SHARPS

  Unused sharps include the following unused, discarded sharps as a class of regulated medical waste; hypodermic needles, suture needles, syringes, and scalpel blades.

Table 1.1 presents the medical waste types appropriate for treatment by each of the major medical waste treatment technologies. All types of wastes may be treated by incineration, however, a special permit is required to incinerate low level radioactive waste or hazardous or cytotoxic waste. However, incineration does not change the radioactive characteristics of medical waste, thus the ash from incineration of radioactive medical waste will remain radioactive. No other treatment technology may be used for radioactive,

Table 1.1 Medical Waste Types Appropriate For Treatment By Technology

TECHNOLOGY	CLASS 1	CLASS 2	CLASS 3	CLASS 4	CLASS 5	CLASS 6	CLASS 7	RADIO-ACTIVE	HAZ AND CYTOTOXIC
INCINERATION	X	X	X	X	X	X	X	X[1]	X[1]
STEAM AUTOCLAVE	X	X[2]	X	X	X[2]	X	X		
CHEMICAL TREATMENT	X	X[2]	X	X	X[2]	X	X		
MICROWAVE	X	X[2]	X	X	X[2]	X	X		
RADIOFREQUENCY	X	X[2]	X	X	X[2]	X	X		
GAMMA IRRADIATION	X	X[2]	X	X	X[2]	X	X		

1 The treatment of radioactive antineoplastic and hazardous waste which are mixed with medical wastes can be treated with incineration, however, special permits are usually required for this type of treatment. Additionally, incineration does not inactivate radioactive waste. Thus the ash from these processes may be radioactive and/or contain hazardous constituents.

2 Technology not recommended for treatment of body parts because the density of the waste may prevent adequate treatment. Grinding the waste may increase treatment efficacy however, the grinding process may present aesthetically unacceptable results

hazardous or cytotoxic wastes. Steam autoclaving is appropriate for most other wastes with the exception of body parts or animal carcasses, which are too dense to allow for steam penetration. Animal carcasses and body parts are also excluded from treatment by mechanical/chemical, microwaves, radiofrequency, and gamma irradiation for aesthetic reasons.

1.2. TREATMENT DEFINITIONS

"Treatment" as defined by Title 40 CFR Part 259 is "any method, technique, or process designed to change the biological character or composition of any medical waste so as to reduce or eliminate its potential for causing disease." There are, however, several levels of microbial inactivation as discussed in the following subsections.

1.2.1 Microbial Inactivation

Microbial inactivation as used in this document refers to the effects of physical or chemical processes that render microorganisms incapable of multiplication. Such processes may either kill the organisms or injure them to the extent that effective repair and subsequent growth is not possible. The evaluation of effectiveness of medical waste treatment technologies tested during the EPA investigation is presented in Table 1.2.

1.2.1.1 Level I Microbial Inactivation

Level I microbial inactivation destroys most disease causing microorganisms. It indicates kill of at least 10^5 vegetative bacteria and fungi, fungal spores and viruses. It implies the inability to inactivate mycobacteria and bacterial spores. It may be accomplished by a variety of physical or chemical processes. It is similar to disinfection which is defined by the Office of Prevention, Pesticides, and Toxic Substances (OPPTS) in terms of performance requirements based upon standard test methods for each major group of microorganisms other than bacterial spores. For example, products labeled as "disinfectants" for use on hard surfaces require kill "...on 59 out of each set of 60 carriers" using the Association of Official Analytical Chemists (AOAC) Use Dilution Method (DIS/TSS-1, 1/22/82) with specific vegetative bacteria.

1.2.1.2 Level II Microbial Inactivation

Level II microbial inactivation is defined as significant inactivation of all microorganisms with the exception of bacterial spores. This indicates the inactivation of at least 10^5 mycobacteria in addition to Level I inactivation. It implies a measure of "tuberculocidal" activity.

Table 1.2 Evaluation of Level of Microbial Inactivation Achieved by Medical Waste Treatment Technologies

WASTE TREATMENT TECHNOLOGIES	MICROBIAL INACTIVATION			
	Level I[a]	Level II[b]	Level III[c]	LevelIV[d]
STEAM AUTOCLAVE				
Lab Test Results[1]	yes	yes	yes	no
Field Test Results[2]	yes	yes	yes	yes
MICROWAVE				
Field Test Results[3]	NT	NT	yes	no
RADIO FREQUENCY				
Field Test Results[4]	NT	NT	yes	no
CHEMICAL				
Lab Test Results[5]	yes	yes	yes*	yes*
Field Test Results[6]	yes	yes	no**	no**

a Inactivation of 10^5 vegetative bacteria, and fungi
b Inactivation of 10^5 mycobacteria
c Inactivation of at least 10^4 B. subtilis (heat); or at least 10^4 B. stearothermophilus (chemical)
d Inactivation of at least 10^6 B. stearothermophilus 10^6 or greater
1 Benchtop and gravity displacement autoclaves, 121^0 C, 15 psi
2 Prevacuum system, 138^0 C, 30 psi; Double door gravity system, 163^0 C, 80 psi
3 Microwave treatment system (6 units at 2450 MHz each)
4 Short wave RF system, 11 - 13 MHz
5 Chemical only, sodium hypochlorite 1000 ppm and 3000 ppm FAC, prolonged exposure ($\geq$ 3 hrs)
6 Chemical/mechanical systems, sodium hypochlorite 1000, 2000, 3000 ppm FAC
NT Not tested
* Dependent on Prolonged exposure (>3 hrs)
** Not achieved under normal operating conditions (< 3 hrs exposure)

1.2.1.3 Level III and IV Microbial Inactivation

Level III indicates the kill of microbial life forms as evidenced by the inactivation of at least 10^4 indicator spores which have death curves similar to human pathogenic spores. Thus, *B. subtilis* spores may be used to indicate Level III microbial inactivation for moist heat treatment, since they also exhibit thermal death data similar to species of the pathogenic spore-forming *Clostridium*.

Level IV indicates the kill of microbial life forms as evidenced by the inactivation of 10^6 bacterial indicator spores recognized as most resistant to the treatment process. For example, the inactivation of at least 10^6 spores of the bacterium *B. stearothermophilus*, recognized as most resistant to moist heat, is an indication of Level IV inactivation by steam autoclaving.

1.3 OPERATION EVALUATION

The treatment of medical waste is intended to render the waste noninfectious or less infectious prior to disposal. The effectiveness of the treatment may be measured by the kill or inactivation of suitable viable indicator microorganisms in regulated or surrogate medical waste. Surrogate medical waste (unused biomedical products) should be comprised of a variety of materials expected to occur in regulated medical waste, such as plastic, glass, rubber, metal, fabric, paper, etc.

1.3.1 Test Organism Selection

With the exception of gamma irradiation, it is generally accepted that if the treatment process inactivates a specific level of resistant bacterial spores, other types of microorganisms (bacteria, fungi, viruses, mycobacteria) will also be inactivated. Thus when choosing an indicator organism to use for routine effectiveness testing, the organism should be chosen based on the desired level of treatment the process is expected to achieve.

For treatment technologies relying on thermal inactivation of microorganisms (incineration, steam autoclaving, nonionizing irradiation) spores of *B. subtilis* (ATCC 9372, ATOCC 6633) can be used to verify Level III microbial inactivation and *B. stearothermophilus* (ATCC 12980, ATCC 10149) may be used to verify Level IV microbial inactivation.

For treatment technologies that rely on chemical inactivation of microorganisms, spores of *B. stearothermophilus* (ATCC 12980, ATCC 10149) may be used to determine the level of treatment achieved for Level III, and for Level IV. *B. stearothermophilus* provides a measure of resistance to chemical inactivation very similar to that of *B. subtilis*, and may be readily recovered and isolated from medical waste treatment systems due to its thermophilic growth property.

Indicator organisms for gamma irradiation treatment of medical waste have not been tested or verified. Some viruses appear to be the most resistant organisms for this technology. However, animal viruses are difficult to use as indicator organisms because of their extensive maintenance requirements in the laboratory.

1.3.2 Test Organism Procurement

Indicator spore strips and spore suspensions should be purchased from reputable suppliers. When the organisms are received the packaging and containers should be visually inspected and the date of receipt, lot number and expiration date recorded in a log book. The log book should be maintained for inspection by the individual responsible for quality assurance for the facility. After being logged into the system, all spores should be refrigerated (2 to 8 °C) or stored under other recommended conditions until used.

1.3.3 Test organism quantitation

Commercially prepared spore strips and spore suspensions are supplied with a quality assurance statement that gives the mean number of spores per strip or per mL of suspension. As long as the spores have been shipped and stored properly this quantitative data remains valid until the date of expiration.

1.3.4 Test load preparation

The test load containing the indicator organisms should be prepared in a manner to allow it to be placed into the treatment system with a normal waste load and be recovered easily. The preparation and recovery of appropriate test organism loads becomes more challenging when the treatment cycle includes a waste destruction step and/or significant dilution. In that situation, the treated solid waste must be processed for surviving organisms..

For thermal inactivation, a minimum challenge of 10^4 *B. subtilis* spores should be used to verify Level III microbial inactivation. To verify Level IV microbial inactivation, a minimum 10^6 *B. stearothermophilus* spores should be used.

For mechanical/chemical treatment monitoring, enough *B. stearothermophilus* spores should be included in the test organism challenge to demonstrate a minimum kill of 10^3 per gram of treated waste solids. Additionally, testing should include representative organic matter (e.g., agar plates, whole blood, serum, plasma, etc.) that comprises at least 5 percent of the total waste challenge by weight.

1.3.5 Test load exposure

The waste treatment system should be tested under normal operating conditions. The test load should be placed in the system with a normal waste load and recovered after a standard treatment cycle. The recommended efficacy testing frequency for standard operating

procedure is presented in Table 1.3. All technologies should be evaluated bi-weekly with the exception of incineration. Incinerators should be evaluated quarterly because of the expense and time required for testing. If normal operating procedures are changed, the treatment process should be revalidated.

1.3.6 Organism recovery

Recovery of indicator organisms should be accomplished easily. The methods used should recover the maximum number of viable (injured and noninjured) indicator organisms. This includes selecting the appropriate nutrient medium, and time and temperature of subsequent incubation. *B. subtilis* and *B. stearothermophilus* both may be recovered on soybean-casein digest agar or broth (or equivalent). *B. subtilis* should be incubated at 32 °C for at least 72 hours. *B. stearothermophilus* should be incubated at 55 °C, also for at least 72 hours.

Table 1.3 Recommended Frequency of Efficacy Testing By Technology

Technology	Recommended Frequency
Incineration	Quarterly, unless procedures change or repairs are made to the equipment
Steam Autoclaving	Bi-weekly, unless procedures change or repairs are made to the equipment
Chemical Disinfection	Bi-weekly, unless procedures change or repairs are made to the equipment
Microwave	Bi-weekly, unless procedures change or repairs are made to the equipment
Radiofrequency	Bi-weekly, unless procedures change or repairs are made to the equipment

1.3.7 Quality Control Procedures

Quality assurance and quality control procedures are essential for insuring medical waste treatment efficacy testing is performed properly. This section presents some guidance for documenting these procedures. Published quality assurance practices such as those found in the American Public Health Association's (APHA, 1989) *Standard Methods for the Examination of Water and Wastewater* may be used.

1.3.7.1 Organisms

Microorganisms should be purchased from reputable suppliers, and upon receipt, inspected, logged, and stored at 2 to 8 °C or as otherwise directed until ready to use. The log should include the date, lot number, expiration date. Spore strips or spore suspensions should not be used beyond their indicated expiration date.

1.3.7.2 Media

Microbiological media may be bought commercially or prepared in the laboratory. Media may be used as liquid broth in tubes or bottles, or agar prepared in petri plates or tubes as agar slants. Commercially prepared media is quality controlled prior to shipment, and upon receipt at the facility it should be logged, inspected, and stored at the required temperature. Likewise, dehydrated media for laboratory preparation should be logged, inspected, dated, and stored appropriately.

Laboratory prepared media should be made according to the manufacturer's directions. All media containers should be dated when first opened. Ingredients should be weighed on calibrated laboratory balances, and suspended in distilled, deionized water. Media should be sterilized in a steam autoclave operated at 121 °C and 15 psi pressure. Items to be autoclaved may also be tagged with temperature sensitive autoclave sterility indicator tape. Each autoclave cycle should be logged with date, time of cycle, type of exhaust, and temperature. All laboratory prepared media should be incubated following preparation as a sterility check. All media should be inspected before use, and if found contaminated, discarded.

1.3.7.3 Reagents

As with microbiological media, reagents may be purchased or prepared in the laboratory. Purchased reagents should be inspected upon receipt, logged, dated, and stored according to manufacturer's direction. Laboratory prepared reagents also should be logged, placed in appropriate clean containers, and labeled with date of preparation, name of reagent, expiration date if applicable, and the initials of the preparer. Phosphate buffer dilution water (PBDW) may be prepared according to the method described in the *Official Methods of Analysis of The Association of Official Analytical Chemists* (1990), or an equivalent used.

1.3.7.4 Equipment

Incubators and refrigerators should be equipped with thermometers calibrated against a reference thermometer traceable to standardization against a National Institute of Standards and Technology (NIST) standardized thermometer. Correction factors should be assigned to each thermometer, acceptable temperature ranges assigned to each piece of equipment, and

temperatures, deviations, and corrective actions recorded on a daily basis as equipment is used. The pH meter should be standardized prior to each use with standard pH 4.0 and pH 7.0 buffer solutions. Balances should be calibrated with standard balance weights.