- Level II criteria provides a sufficient degree of microbial inactivation;

- Level III criteria may conflict with lesser inactivation criteria already defined by the state; and

- Level III or IV criteria can be applied, if necessary, to those medical waste streams requiring an additional margin of safety.

Arguments for recommending Level III criteria were as follows:

- Level III criteria serve as a margin of safety from the variables inherent in the treatment of medical waste (i.e., waste packaging, waste composition, waste density, and factors influencing the homogeneity of the treatment process);

- Segregation of some medical waste categories (i.e. laboratory cultures) requiring Level III treatment would be impractical if Level II criteria were in effect;

- Medical waste treatment equipment industry already achieves Level III criteria; and

- Level II or Level IV criteria may still be allowed dependent on the technology application or waste type processed.

It was the consensus (not unanimous) of the committee that Level III be required of all emerging medical waste technologies. The committee took the position that Level III criteria were to be established as a benchmark and as such, were applicable to all medical waste treatment devices. The committee realized that there might be circumstances under which a state may allow relaxation of the Level III requirement.

The committee rejected the allowance for exception to Level II standards for those technologies that could be termed "counter-top" devices designed for a specific medical waste category. Relaxation from Level III to Level II criteria was not considered warranted on the basis of the equipment's:

- Inability to inactivate spores;

- Designation as a small quantity treatment device;

- Designation for treating minimally contaminated medical waste categories; or

- Exhibiting difficulty to demonstrate microbial inactivation through designated protocols (i.e., a needle thermal-destruction device).

The committee realized that there might be circumstances under which a state may allow relaxation of the Level III requirement. These exceptions would by necessity need to be made on a case-by-case basis, would require the equipment manufacturer to provide a rationale for relaxation, and would require adequate supporting documentation to substantiate that rationale.

The committee also debated if laboratory wastes (i.e. discarded cultures and stocks of pathogenic agents) should require sterilization (i.e. meet Level IV criteria) on the basis that these wastes may contain high concentrations of known pathogens. The committee took the position that Level III criteria remained the standard for all medical waste categories. The committee emphasized, however, that laboratories should be aware that cultures and stocks of disease-causing agents may require sterilization before disposal. In addition to guidelines set by the Centers for Disease Control in Biosafety in Microbiological and Biomedical Laboratories, (1993) and standards of the College of American Pathologists (CAP), some states require laboratory cultures to be incinerated or autoclaved (i.e., steam sterilized) before leaving the laboratory or before being disposed of. Although no specific recommendations for medical waste disposal are made under the Clinical Laboratory Improvement Amendments (CLIA), medical waste disposal practices are receiving increased scrutiny during routine inspections.

2.3 Representative Biological Indicators

In the absence of an ultimate pathogen surrogate to represent all defined microbial groups, the selection of pathogen surrogates representing vegetative bacteria, fungi, parasites, viruses, mycobacteria, and bacterial spores was considered necessary to define and facilitate any state approval process. Criteria defining surrogate selection should include that any surrogate recommended:

- Not affect healthy individuals;
- Be easily obtainable;
- Be an ATCC registered strain, as available;
- Be easily cultured and maintained; and
- Meet quality control requirements.

Microorganism strains obtained from the American Type Culture Collection (ATCC) and methods prescribed by the Association of Official Analytical Chemists (AOAC) assist in fulfilling these recommendations by (1) providing traceable and pure cultures of known characteristics and concentration and (2) providing recognized culturing protocols and detailed sampling and testing protocols.

Provided in Table II are the biological indicators recommended by the committee for testing microbial inactivation efficacy in medical waste treatment processes. The selection of these representatives was based on each microorganism:

- Meeting, where possible, the criteria established above;
- Representing, where possible, those organisms associated with medical waste; and
- Providing a biological challenge equivalent to or greater than that associated with microorganisms found in medical waste.

Biological indicators selected to provide documentation of relative resistance to an inactivating agent should be chosen after evaluation of the treatment process as it relates to the conditions used during comparative resistance research studies described in the literature. Literature studies support the assertion that the degree of relative resistance of a microorganism to an inactivating agent can be dependent on various factors (i.e., pH, temperature). Conditions used in literature studies that demonstrate a relatively high degree of resistance of a particular microorganism may be significantly different to the conditions found within the treatment process. A comparison of the conditions used in the literature to those used in the treatment process should be made to determine if relative microbial resistance can be altered (i.e., lowered) as a result of treatment process conditions.

The committee emphasized that although the microorganisms selected represent pathogen surrogates, these selected surrogates may have the potential to be pathogenic under certain conditions. As such, the committee recommended that all testing be conducted using recognized microbial techniques. For those pathogen surrogates that still retain some higher degree of pathogenicity (e.g., Cryptosporidium, Giardia, and Mycobacteria), efficacy testing should be conducted only by qualified laboratory personnel.

TABLE II - RECOMMENDED BIOLOGICAL INDICATORS

Vegetative Bacteria	-	Staphylococcus aureus (ATCC 6538) Pseudomonas aeruginosa (ATCC 15442)
Fungi	-	Candida albicans (ATCC 18804) Penicillium chrysogenum (ATCC 24791) Aspergillus niger
Viruses	-	Polio 2, Polio 3 MS-2 Bacteriophage (ATCC 15597-B1)
Parasites	-	Cryptosporidium spp. oocysts Giardia spp. cysts
Mycobacteria	-	Mycobacterium terrae Mycobacterium phlei Mycobacterium bovis (BCG) (ATCC 35743)

Bacterial Spores	-	B. stearothermophilus (ATCC 7953) B. subtilis (ATCC 19659)

The committee recommended that one or more of the representative microorganisms from each microbial group be used in efficacy evaluation. Specific criteria for the selection of these microorganisms are provided below in Table III:

TABLE III - BIOLOGICAL INDICATOR SELECTION CRITERIA

Vegetative Bacteria	-	Staphylococcus aureus and Pseudomonas aeruginosa were selected to represent both gram-positive and gram-negative bacteria, respectively. Both are currently required by the Association of Official Analytical Chemists (AOAC) use-dilution method and both have been shown to be resistant to chemical inactivation.
Fungi	-	The selection of Candida albicans and Penicillium chrysogenum was based on reported data indicating these organisms representing yeast and molds, respectively, are the most resistant to germicides. Although Trichophyton mentagrophytes is the AOAC test organism for molds, Penicillium chrysogenum is reported to be more resistant to germicides. The inclusion of Aspergillus niger as an indicator organism was based on its familiarity as a common mold.
Viruses	-	Lipophilic (enveloped) viruses are less resistant to both thermal and chemical inactivation than the hydrophilic (nonenveloped) viruses. As such, enveloped viruses such as HIV, Herpes simplex virus and Hepatitis B virus are less resistant than enveloped viruses such as Poliovirus, Adenovirus, and Coxsackievirus. Polio 2 (attenuated vaccine strain) and Polio 3 virus were selected based on their relative higher chemical and thermal resistance. Additionally, the use of an enterovirus (e.g., Polio 2 or Polio 3) can provide a stringent measure of efficacy for irradiation treatment processes. MS-2 bacteriophage was selected as a Hepatitis virus surrogate in that this bacteriophage offers a comparable degree of chemical and thermal resistance, is safe to handle and easy to culture.

Parasites	-	Both Cryptosporidium spp. oocysts and Giardia spp. cysts are used as test organisms to demonstrate germicidal effectiveness. Cryptosporidium has been demonstrated to have a higher chemical resistance and Cryptosporidium spp. oocysts are more readily available than Giardia spp. cysts. Both are significantly pathogenic (both have an infectious dose of 10 cysts) and care is advised when using these microorganisms as parasitic biological indicators.
Mycobacteria	-	Mycobacterium phlei has a demonstrated measure of disinfectant resistance, is a rapid grower and is pigmented for easy identification. M. bovis (BCG) is used in the AOAC Tuberculocidal Method and is analogous to M. tuberculosis in that it is in the same group or complex. Individuals exposed to M. bovis (BCG, ATCC strain) may skin test convert although no actual infectivity or disease occurs. Risk of exposure would come from those mechanisms that grind the waste. Mycobacterium terrae is equivalent to M. tuberculosis in resistance to chemical inactivation. In Europe it is recommended for disinfectant testing. M. terrae does not grow as rapidly as M. bovis or M. tuberculosis.
Bacterial Spores	-	Both B. stearothermophilus and B. subtilis spores are commonly used as biological indicators for both thermal and chemical resistance. B. stearothermophilus spores exhibit more thermal and chemical resistance than spores from B. subtilis.

After discussion on the rationale for selection of the representative biological indicators presented above, consensus by the committee was attained on recommending the use of these biological indicator strains for treatment technology efficacy testing.

2.4 Quantification of Microbial Inactivation

Establishing the mechanisms to quantify the level of microbial inactivation is essential in developing the format and requirements of the guidance protocols. As presented and discussed, microbial inactivation ("kill") is equated to "Log_{10}Kill" which is defined as the difference between the logarithms of number of viable test microorganisms before and after treatment. This definition is translated into the following formula:

$Log_{10}Kill = Log_{10}(cfu/g\ Introduced) - Log_{10}(cfu/g\ Recovered)$

where:

$Log_{10}Kill$ is equivalent to the term Log_{10} reduction;

"Introduced" is the number of viable test microorganisms introduced into the treatment unit;

"Recovered" is the number of viable test microorganisms recovered after treatment; and

"cfu/g" are colony forming units per gram of waste solids.

A $Log_{10}Kill$ of 6 or greater is equivalent or less than a one millionth [0.000001] survival probability in a microbial population or a 99.9999% reduction or greater of that population.

Using the Level III definition recommended by the committee as shown in Table I, a $Log_{10}Kill$ of 6 (e.g., 6 Log_{10} reduction) is required of vegetative bacteria, fungi, lipophilic/hydrophilic viruses, parasites, and mycobacteria and a $Log_{10}Kill$ of 4 (e.g., 4 Log_{10} reduction) is required of B. stearothermophilus or B. subtilis spores. Employing the above equation to quantify microbial inactivation will require the consideration of the methods of biological indicator introduction and recovery. For those treatment processes that can maintain the integrity of the carrier (i.e., ampules, plastic strips) of the desired microbiological test strain, commercially available biological indicators of the required strain and concentration can be easily placed, recovered, and cultured to demonstrate efficacy. Quantification is evaluated by growth or no growth of the cultured biological indicator. For example, if an ampule that contained 1×10^4 B. stearothermophilus spores were treated, retrieved, and cultured, no growth would demonstrate a 4 Log_{10} reduction.

For those treatment mechanisms that cannot ensure or provide integrity of the biological indicator carrier, quantitative measurement of efficacy requires a two-step approach. The purpose of the first step is to account for the reduction of microorganisms due to equipment design (i.e., dilution of indicator organisms or physical entrapment).

This first step, the "Control", is typically performed using microbial cultures (i.e., liquid suspensions) of predetermined concentrations necessary to ensure a sufficient microbial recovery at the end of this step. The microbial suspension is added to a standardized surrogate medical waste load that is processed under normal operating conditions without the addition of the microbial inactivation agent (i.e., heat, chemicals). Standard loads may vary depending on the various treatment challenges (i.e., high moisture content, high organic load, high density) required of the equipment. After processing, waste samples are collected and washed to recover the biological indicator organisms in the sample. Recovered microorganism suspensions are plated to quantify microbial recovery. The number of viable microorganisms recovered serves as a baseline quantity for comparison to the number of recovered microorganisms from wastes processed with the microbial inactivation agent. The required number of recovered viable indicator microorganisms from the "Control" must be equal to or greater than the number of

microorganisms required to demonstrate the prescribed Log reduction as defined in Level III (i.e., a 6 Log_{10} reduction for vegetative microorganisms and a 4 Log_{10} reduction for spores). See Appendix A (Section C3) and Appendix C for a detailed process description.

This step can be defined by the following equation:

$$Log_{10}RC = Log_{10}IC - Log_{10}NR$$

where:

$Log_{10}RC$ > 6 for vegetative microorganisms and > 4 for bacterial spores;

$Log_{10}RC$ is the number of viable "Control" microorganisms (in colony forming units per gram of waste solids) recovered in the non-treated processed waste residue;

$Log_{10}IC$ is the number of viable "Control" microorganisms (in colony forming units per gram of waste solids) introduced into the treatment unit; and

$Log_{10}NR$ is the number of "Control" microorganisms (in colony forming units per gram of waste solids) not recovered in the non-treated processed waste residue.

Rearranging the equation above enables the calculation of microbial loss due to dilution, physical manipulation, or residue adhesion during the treatment process. $Log_{10}NR$ represents an accountability factor for microbial loss and is defined by the following equation:

$$Log_{10}NR = Log_{10}IC - Log_{10}RC.$$

The second step ("Test") is to operate the treatment unit as in the "Control" run with the selected biological indicators, but with the addition of the microbial inactivation agent. After processing, waste samples are collected and washed as in the "Control" to recover any viable biological indicator organisms in the sample. From data collected from the "Test" and "Control", the level of microbial inactivation (i.e., "$Log_{10}Kill$") can be calculated by employing the following equation:

$$Log_{10}Kill = Log_{10}IT - Log_{10}NR - Log_{10}RT$$

where:

$Log_{10}Kill$ is equivalent to the term Log_{10} reduction;

$Log_{10}IT$ is the number of viable "Test" microorganisms (in colony forming units per gram of waste solids) introduced into the treatment unit. $Log_{10}IT = Log_{10}IC$;

$Log_{10}NR$ is the number of "Control" microorganisms (in colony forming units per gram of waste solids) not recovered in the non-treated processed waste residue; and

$Log_{10}RT$ is the number of viable "Test" microorganisms (in colony forming units per gram of waste solids) recovered in treated processed waste residue.

Appendix C (Section III) serves to illustrate the application of the equations presented above.

Formulas used in the discussion above for the quantification of microbial inactivation were modified from those used by Illinois EPA in their final regulations (June 1993) entitled "Potentially Infectious Medical Wastes" (see Selected Bibliography).

After discussion on the use and application of the formulas and calculations presented above, consensus by the committee was unanimous on recommending the use of the formulas and methods of calculation in the enumeration of medical waste treatment technology efficacy.

3.0 PROCESS FOR APPROVING MEDICAL WASTE TREATMENT TECHNOLOGIES

State approval of an emerging medical waste treatment technology is necessary to ensure that the technology can effectively and safely treat medical waste. From discussions, the completed approval process can be viewed as fulfilling, where applicable, three components:

- Approval of the technology by the state to ensure the technology is effective in safely inactivating microorganisms to specified criteria;
- Granting site approval to verify the sited equipment meets approved specifications and efficacy requirements under actual operating conditions; and
- USEPA FIFRA pesticide registration requirements, as applicable, for those medical waste treatment technologies that use chemicals as the microbial inactivator.

Each of these components requires information be supplied to states demonstrating that the treatment technology is effectively treating medical waste by established criteria and that the process is environmentally sound and occupationally safe. Information necessary for proper review of medical waste treatment technologies is provided for each component described below.

3.1 Biological Inactivation Efficacy: Establishing Protocols

Methodology employed to determine efficacy of the technology will, by necessity, need to be developed by the equipment manufacturer to assure the protocols are congruent with the treatment method. Protocols developed for efficacy testing should incorporate recognized standard procedures such as those found in Test Methods for Evaluating Solid Waste, Physical/ Chemical Methods and Standard Methods for the Examination of Water and Waste Water (see Selected Bibliography).

In establishing testing criteria to evaluate efficacy, the composition of the waste load(s) tested is critically important. Depending on the treatment mechanism, efficacy may vary with waste load composition (i.e., organic content, density, moisture or liquid content). Although the committee recognized that waste composition may affect efficacy results considerably, establishing specific requirements for challenge loads for all existing, pending, and future treatment technologies is not practical or necessarily all inclusive. The committee recommended that the equipment manufacturer prescribe those types of medical wastes that present the greatest challenge to efficacy of the equipment and present protocols that adequately evaluate efficacy under normal operating conditions. On submittal for evaluation by the state, the manufacturer's prescribed waste types and testing protocols could be accepted or modified at the discretion of the reviewing agency.

Dependent on the treatment process and efficacy protocols used, other factors may also influence the evaluation results. As such, the committee could not define specific protocols, but recommended that protocols evaluating medical waste treatment systems specifically delineate or incorporate:

- Waste compositions that typify actual waste to be processed;
- Waste types that provide a challenge to the treatment process;
- Comparable conditions to actual use (i.e., process time, temperature, chemical concentration, pH, humidity, load density, load volume);
- Assurances that biological indicators (i.e., ampules, strips) are not artificially affected by the treatment process;
- Assurances of inoculum traceability, purity, viability and concentration;
- Dilution and neutralization methods that do not affect microorganism viability;
- Microorganism recovery methodologies that are statistically correct (i.e., sample collection, number of samples/test, number of colony forming units/plate); and
- Appropriate microbial culturing methods (i.e., avoidance of microbial competition, the selection of proper growth media and incubation times).

Based on the results obtained from challenge load testing, the medical waste treatment technology may be limited in its application to not treating all categories or types of medical wastes. Physical or aesthetic characteristics may also predicate the limitations applied or the conditions of the equipment's use. If certain medical waste categories are excluded from the treatment process, the state should specify for the manufacturer (vendor) and the user of the equipment the waste segregation parameters that will be employed to prohibit the waste from treatment and the mechanisms of treatment/disposal to be utilized for these excluded wastes.

Consideration should also be given to the equipment's use in a particular setting when applying challenge load testing. The composition of the challenge load would be conceivably different and more challenging if a particular application treats a medical waste stream containing a higher proportion of a waste type or composition that is difficult to treat by that process. Conversely, challenge loads for technologies whose primary application is hospital medical waste, might be relaxed if that technology was applied only to waste generated by physician offices. Efficacy testing protocols may also require modification dependent on the size or throughput of the equipment. Multiple testing points might be required due to the waste volume processed or the treatment process.