Biohazardous Waste Basics

A Guide for Handling & Disposal of Biological Wastes Generated in the UT Research & Diagnostic Service Environment

Background & Regulatory Summary

Biohazardous waste includes any waste item that is contaminated with a biological material that is an infectious disease transmission risk or an environmental release risk (i.e., recombinant DNA).

In the state of Tennessee, some forms of biohazardous waste are defined as "medical wastes" and are regulated for disposal purposes by the Tennessee Department of Environment and Conservation (TDEC). These regulated "medical wastes" include the following categories which may be applicable to UT researchers:

1. Wastes generated by hospitalized patients who are isolated to protect others from communicable diseases;

2. Cultures and stocks of infectious agents, including specimen cultures from medical and pathological labs, cultures and stocks of infectious agents from research and industrial labs, wastes from the production of biological, discarded live and attenuated vaccines, and culture dishes and devices used to transfer, inoculate and mix cultures;

3. Waste human blood and blood products such as serum, plasma and other blood components;

4. Pathological wastes (i.e., tissues, organs, body parts, and body fluids) that are removed during surgery and autopsy;

5. All discarded sharps (i.e., hypodermic needles, syringes, Pasteur pipettes, broken glass, scalpel blades) used in patient care or which have come into contact with infectious agents during use in medical, research or industrial labs;

6. Contaminated carcasses, body parts, and bedding of animals that were intentionally exposed to pathogens in research in the production of biologicals, or in the *in vivo* testing of pharmaceuticals.

Unlike hazardous chemical or radioactive waste, there is no one federal agency that clearly defines and regulates biohazardous waste. Several agencies associated with research funding have unique waste disposal requirements that may go above and beyond what TDEC regards as regulated "medical waste". Therefore, it is the researcher's responsibility to have a general knowledge of biosafety regulations & guidelines and how they apply to their work and the waste that is generated through the research and diagnostic service process.

Please review the regulatory/agency information in the following table. If your work will involve generating any of the wastes previously described, or any of the wastes in the table, then you will most likely need to segregate and manage some portion of your research waste as biohazardous waste.

Questions about biohazardous waste treatment and disposal?
Please contact the Biosafety Office at 974-1938 or 974-9836.

Other Agencies with Biohazardous Waste Requirements

Regulation	Activities covered by this standard	Biohazardous wastes
OSHA's Bloodborne Pathogens Standard	Work with human-derived materials including clinical and unfixed anatomical specimens, human cells and cell lines.	Those wastes that are contaminated to the extent where fluids can drip off or flake off of waste; liquid wastes; fresh (unfixed) tissues; sharps.
NIH Guidelines for Research Involving Recombinant DNA Molecules	Work with molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or molecules that result from the replication of those previously described; regardless of whether work meets "exempt" criteria or not, all recombinant DNA work is to be carried out in accordance with biosafety level 1 containment practices at a minimum.	All contaminated solid and liquid wastes including sharps.
CDC/NIH "Biosafety in Microbiological and Biomedical Laboratories" (BMBL)	Lab and animal studies involving work with microorganisms that can cause disease in humans; under certain circumstances, lab and animal studies involving microorganisms that are infectious to animals; diagnostic laboratory operations involving human or animal clinical specimens.	All cultures, stocks and items contaminated with these materials; in some cases, animal bedding and carcasses; biohazardous sharps.
USDA APHIS Permits	Work with any animal or plant-derived materials or pathogens that require an APHIS permit to receive or retain the material.	Permits will outline specific waste treatment requirements for the material in question. However, this usually involves segregation and biological inactivation of the material prior to disposal.

Managing Lab Waste as "Biohazardous" as a Prudent Practice

The unique research enterprise at The University of Tennessee covers a wide range of activities including agricultural, environmental, and a wide array of medically-oriented research. Some of these activities may fall outside the regulatory guidelines established by the Federal and State Government.

Applying a universal precautions approach (i.e., managing all research biological materials as if they are an infectious disease or environmental release risk) in conducting these varied research activities is a prudent standard. The University of Tennessee Institutional Biosafety Committee and Office of Research recommend that otherwise unregulated research lab biological wastes be managed as biohazardous waste. This action will assure that *all* biological research materials are inactivated or managed in a manner that isolates the exposure risk for the general public and the environment.

Attached Resources

Attachment A- Managing Serological Pipettes for Disposal

Attachment B- Transporting Biohazardous Waste

Attachment C- Autoclave Validation & Biohazardous Waste Treatment Procedure

Biohazardous Waste Categories

There are 4 general categories of biohazardous wastes based on the physical form of the waste. Each form must be segregated, identified, decontaminated and disposed of in an appropriate manner for the form in order to minimize occupational exposure and environmental release risks.

Biohazardous waste in any form should not be left untreated and unsecured in areas that are accessible to the public (i.e., left in hallways). Only lab personnel should remove treated biohazardous waste from the lab area and transport it to waste holding areas for final disposal.

1. SOLID BIOHAZARDOUS WASTE (non-sharps)

In the research lab or field environment, this includes any non-sharp item that is contaminated with human or animal diagnostic specimen material (i.e., body fluids, tissue debris), any microbiological culture material (including recombinant DNA).

Examples include but are not limited to:

- Gloves and other disposable PPE contaminated with specimen or culture material.
- Plasticware such as pipettes or pipette tips, culture plates, specimen vials, etc. that are contaminated with biological specimens, bacterial and cell culture material, or nucleic acids.
- Towels and bench paper that are biologically contaminated (Note: Bench paper that is used in areas where samples or cultures are opened and manipulated must be regarded as biologically-contaminated and therefore removed and managed as solid biohazardous waste)
- All culture or sample containers that are contaminated with biological materials
- Tubes of blood (note: glass blood vials that could break easily upon disposal should be segregated as sharps waste; see below)

Storage

This type of waste must be collected for final treatment and disposal in a leak-proof container lined with a bag of moderate thickness to prevent punctures. The collection container must have a lid or other means of closure and the container must be labeled with the biohazard symbol regardless of the lab's operating biosafety level.

Bench top containers should be used for collection of small quantities of contaminated dry goods (i.e., pipette tips, centrifuge tubes, etc.). Small plastic containers or wire bag racks lined with a biohazard bag are suitable for bench top collection. These containers do not need to have a lid (unless waste is contaminated with a pathogen) but daily disposal of the bag into a larger collection container such as the one shown to the right is strongly recommended.

Wastes Requiring Special Considerations

"Breakable" biohazardous wastes

Tubes of blood and other "breakable" biohazardous waste can be troublesome to manage properly and safely for treatment and disposal. For small amounts of "breakable" biohazardous waste, these items may be placed in sharps containers for disposal. However, if your lab generates a large amount of "breakable" biohazardous waste, please contact the UTK/UTIA Biosafety Officer for assistance with finding solutions for safer waste management.

Serological pipettes
All plastic pipettes, regardless of contamination status should be segregated from other lab wastes because they readily puncture waste and trash bags which increases spill potential. Please see Attachment A at the end of this document for procedures for effective segregation, treatment and disposal of serological pipettes.

Treatment and disposal

The purpose of solid biohazardous waste treatment is biological inactivation in a manner that reduces hazardous exposure risk for lab personnel and the environment. This is generally achieved by autoclave treatment of waste or treatment and disposal through a medical waste disposal contractor (i.e., licensed medical waste hauler) who will autoclave or incinerate the waste. Under the TDEC regulations, wastes are to be "rendered non-infectious by sterilization techniques prior to disposal". This means that all items contaminated with a potentially infectious material must be autoclaved or managed through a medical waste disposal contractor for disposal.

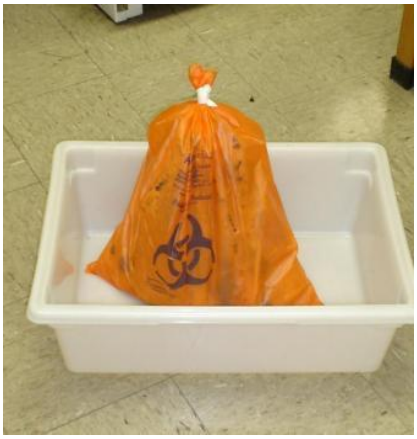
Disinfection of items such as serological pipettes contaminated with human cells does not preclude the need to manage these items as biohazardous waste for final treatment and disposal.

On-site Autoclave Treatment

Autoclavable waste bags must be used in biohazardous waste collection containers. Bags must be placed in a secondary container (i.e., tray with raised sides), which is placed on a cart for movement to the autoclave facilities.

Practice notes on biohazard bags:

- Biohazard bags are a one-way means of disposal. Do not "dump" the contents from one biohazard bag into another as this action spreads contamination and increases your exposure to this waste.
- Biohazard bags need to be contained at all times during the collection, treatment, and disposal process. Some lab items may puncture bags and this can lead to leaks and spills. Bags awaiting autoclave treatment should be stored in trays, tubs, buckets, etc. (The only exception to this practice is when small quantities of biohazardous wastes that do not contain liquids are collected temporarily in benchtop containers.)
- Biohazard bags must not be used for collection of other hazardous wastes (i.e., ethidium bromide gels).



Biohazardous waste bags awaiting treatment should always be stored in pans or secondary containment to prevent spills!

Ethidium bromide waste is to be collected in a leak-proof container with a lid lined with a sturdy, non-descript bag. The UT Hazardous Waste label should be placed on the storage container as well. No biohazard labels!

If your lab works with human-derived materials or other materials that are an infectious disease risk for humans (i.e., BSL-2), you must use bags that bear the biohazard symbol. The bags should have a built-in heat indicator that allows for verification of autoclave treatment. Otherwise, autoclave indicator tape should be placed on the bag before autoclave treatment.

If your lab does not work with human-derived materials or other materials that are an infectious disease risk for humans (i.e., BSL-1), you may use bags that do not have the biohazard symbol. However, all biohazardous waste must be clearly identified as such from the point of generation through the point of treatment or biological inactivation. Therefore, if you chose to use autoclave bags without a biohazard symbol, you must employ some other means to identify the material as a biohazard. An example of how this can be achieved is placing the waste in a designated, biohazard-labeled secondary container for storage and transport. The waste bag can then be transferred from the biohazard-labeled secondary container to a regular autoclave pan for final placement in the autoclave.

Autoclave treatment of this waste must be performed in accordance with the biohazardous waste treatment parameters established for the autoclave. Note: Only personnel who have received training regarding the operation of the autoclave should use this device.

If the treated wastes meet the TDEC definition of "medical wastes", or if bags are red/orange in color and/or have a biohazard symbol on them, they must be placed in designated "Autoclave-treated Waste" bins. These bins are large white waste receptacles, are clearly labeled, and are typically in or near autoclave rooms. This action is required because these treated wastes must be segregated from other solid wastes. The treated wastes are subsequently relocated by Facilities Services to a designated dumpster to be hauled directly to an approved landfill per TDEC requirements.

Medical Waste Disposal Contractor

Some departments have a contract for pickup and disposal of waste through a medical waste disposal contractor. In this case, the same procedures apply as above. However, bags do not have to be autoclavable, nor should they be placed in a non-see-through black bag.

Field Generation

Waste should be collected and stored as previously outlined. Contact the UTK/UTIA Biosafety Officer or UTIA Safety Officer for assistance with identifying disposal options. If you plan to transport waste for treatment and disposal, please refer to guidelines at the end of this document for this activity.

2. LIQUID BIOHAZARDOUS WASTE

This includes bulk quantities of blood, blood products, body fluids from human and animal research origin and culture media. Note: Disposable primary containers or sample containers containing small quantities of liquids (less than 10 mls) should be managed as solid biohazardous waste.

Storage

These liquids must be stored in closed, leakproof containers while awaiting treatment and disposal. Collection vessels should be secured so that they can not be tipped over. Secondary containment is strongly recommended and can be achieved by placing the vessel in a bucket or deep tray.

Storage vessels or the secondary container must be labeled with the biohazard label if the liquids will not be treated and disposed of within the shift. If disinfectant is added to the vessel, provide labeling so that the chemical hazard is identified as well. For instance, if your collection flask contains waste cell media and bleach, you should place biohazard label on the flask (or secondary container) as well as the words "bleach-treated cell culture materials" to properly identify both the chemical and biological hazard.

Treatment and disposal

Liquid wastes may be treated and disposed of by either one or the other of the following methods:

- **Chemical treatment of liquids with disinfectant; disposal via lab sink**
  Disinfectants may be used for "treatment" of liquid biological waste to prohibit growth of microorganisms. Here is an example for the use of household bleach.

  Add household bleach to the collection vessel so that the bleach makes 10% to 15% of the final volume. Allow a contact time of at least 30 minutes. Carefully discharge the mixture to the sanitary sewer by way of the lab sink, then thoroughly rinse down the sink with water. Remember to wear splash goggles, gloves, and a lab coat for handling of bleach and bleach-treated liquids.

  NOTE: ***Diluted bleach solutions may go down the drain in most cases. However, many chemicals used for disinfection cannot be discarded down the train. Contact EH&S at 974-5084 to determine if sink disposal of disinfectants other than diluted bleach solutions is acceptable.***

- **Autoclave treatment of liquids; disposal via lab sink**
  Place the closed collection vessel in a secondary container and transport by cart to the autoclave facilities. Treat by autoclave using the liquids cycle. (Remember to loosen or remove the closure on the vessel before placing in autoclave.) Discharge cooled, treated liquids to the sanitary sewer by way of the lab sink. Note: Only personnel who have received training regarding the operation of the autoclave should use this device.

Safety Note: PLEASE do not autoclave liquids containing chemical disinfectants!

3. BIOHAZARDOUS SHARPS

A biohazardous sharp is any device that is sharp enough to puncture the skin and that is contaminated with a biological material that is an infectious disease transmission risk, or an environmental release risk (i.e., recombinant DNA). Examples include but are not limited to:

- Needles, disposable syringes, capillary tubes & scalpels contaminated with human or animal blood
- Microscope slides contaminated with unfixed human or animal specimen materials
- Pasteur pipettes contaminated with cell culture waste media
- Small glass/broken tubes of blood or microbiological cultures.

SAFETY NOTE ON NEEDLES & MEDICAL DEVICES WITH A NEEDLE ATTACHED…

State waste regulations do not specifically address the disposal of hypodermic needles and medical devices with a needle attached (i.e., tuberculin syringes) unless these items are contaminated with blood or potentially infectious materials. However, it is strongly recommended that ALL needles and medical devices with a needle attached be disposed of in biohazardous sharps containers to protect those who may come in contact with these items during the disposal process. If such devices are contaminated with radioactive materials- follow Radiation Safety procedures for disposal of such items. If such devices are contaminated with hazardous chemicals, contact the Environmental Health and Safety (EH&S) Office at 974-5084, or the UTIA Safety Officer at 974-1153 for guidance with disposal.

Storage

Biohazardous sharps containers are those containers which are specifically designed for the collection and disposal of biohazardous or medical sharps. (Recycled food or reagent containers are NOT acceptable for collection and disposal of biohazardous sharps!)

A biohazardous sharps container is:

- constructed of puncture-resistant material,
- leak-proof on the sides and bottom,
- marked with the biohazard symbol, and
- has a restricted opening to prevent items from coming back out of the container, and to prevent someone from sticking their hand inside.

To protect yourself and others in your work area, place biohazardous sharps in a properly assembled (i.e., lid installed) biohazard sharps container immediately after use. This can be achieved by placing sharps containers within arms reach of where biohazardous sharps are used.

SAFETY NOTE: Do not recap needles. Do not bend or break sharp devices. Do not overfill sharps containers or use force to get an item into a sharps container.

Treatment and disposal

All sharps containers must be permanently closed and disposed of when ¾ full or whenever items do not freely fall into the container.

Disposal of biohazardous sharps containers will be accomplished through a medical waste disposal contractor coordinated through EH&S. Please do not dispose of biohazardous sharps containers in the trash, regardless of treatment status.

Collection will be on the same schedule as chemical waste. (Knoxville campus on Wednesdays at Walters waste room from 1-2 pm and SERF waste room from 2-3 pm. Ag Campus on quarterly waste pickup days and by appointment by calling the UTK/UTIA Biosafety Officer at 974-1938.)

Sharps containers must be permanently closed and wiped down with a disinfectant prior to removal from the lab and for disposal through EH&S. If there are any liquids present in the biohazardous sharps container, it must be placed in a leak-proof secondary container with a secure lid (and a biohazard label) for transport to the waste collection site. Upon arrival at the waste room, the container must be placed in the large, cardboard waste container by the generator.

Medical Waste Disposal Contractor

The same procedures apply as above.

Field Generation

Waste should be collected and stored as previously outlined. Contact the UTK/UTIA Biosafety Officer or UTIA Safety Officer for assistance with identifying disposal options. If you plan to transport waste for treatment and disposal, please refer to guidelines at the end of this document for this activity.

Do you have long Pasteur pipettes that are contaminated with potentially infectious material or recombinant DNA?

Tall sharps containers with an opening large enough to safely deposit these items are strongly recommended.

SAFETY NOTE ON BROKEN GLASS…

If broken glass is biologically-contaminated, it must be managed as a biohazardous sharp for disposal. However, some instances occur when the broken glass does not fit in a sharps container. In these events, please call the UTK/UTIA Biosafety Officer for assistance.

Broken glass that is not contaminated with a hazardous material should be placed in a suitable puncture-resistant container for disposal. (Storage of broken glass in trash bags is NOT acceptable!) Disposable and reusable broken glass containers are available through most lab supply companies.

Only lab personnel should remove broken lab glass from the lab area and transport it to waste holding areas for disposal.

4. PATHOLOGICAL WASTE

This includes all unfixed human organs, tissues and body parts except for teeth. It also includes unfixed animal tissues and carcasses that have been:

- exposed to human-derived materials (i.e., cells),
- experimentally challenged with agents infectious to humans or recombinant organisms,
- and other circumstances as deemed appropriate through the biological risk assessment process.

Storage, treatment and disposal

This type of waste must be double-bagged in biohazard bags that bear a biohazard symbol. Bags must be stored in a manner that will minimize the potential for release of fluids during the storage and handling process. Storage of bags in a tray with sides, or secondary storage of bags in a sturdy plastic zipper bag is strongly recommended. Remember that these items must be labeled with the biohazard symbol. These items must be incinerated (not autoclaved) for disposal unless other provisions apply. A medical waste disposal contractor should be used for pickup and disposal of these materials. Contact the UTK/UTIA Biosafety Officer (974-1938) for further assistance with disposal of items in this category. For assistance with disposal of formalin-fixed or chemically preserved tissues, please contact EH&S or the UTIA Safety Officer.

Final Note on "Mixed Wastes"

Some lab analyses may involve treatment or exposure of biological materials to chemical compounds or radioactive materials. Examples may include radioisotope labeling of genetic material in culture or cells, and exposure of cells or research animals to carcinogens or diagnostic processes involving radiation hazards. In these situations, mixed wastes are likely to be produced that will require special consideration for collection, handling and disposal. Biohazardous waste treatment and disposal techniques alone are not likely be suitable for "mixed" wastes. When planning studies that will generate "mixed wastes", please contact the appropriate safety office for assistance in determining your waste handling procedures.

Environmental Health and Safety Office	974-5084
UTIA Safety Office	974-1153
Radiation Safety Office	974-5580
UTK/UTIA Biosafety Office	974-1938

Attachment A
Recommended Practices: Disposal of Plastic Serological Pipettes

All plastic pipettes, regardless of contamination status should be segregated from other lab wastes because they readily puncture waste and trash bags which increases spill potential.

Disposal recommendations: Pipettes that ARE NOT biologically-contaminated

The following recommendation applies to waste pipettes that ARE NOT contaminated with body fluids, cell debris, or other materials that may contain infectious agents or recombinant DNA molecules.

Pipettes should be placed in a dedicated container that is lined with a sturdy trash bag, and configured in such a way that pipettes are oriented in one direction. The container should be clearly marked "waste pipettes only-NO biohazardous waste" or comparable wording to ensure that all personnel are notified of the intent of the container. To dispose of the pipettes, the trash bag should be tied closed and transferred to a cardboard box (if it's not stored in one already). The box should be taped closed and "trash" should be written on the box. It is highly recommended that lab personnel remove the box directly to a nearby dumpster. In some buildings, custodial personnel will remove the box from the lab.

Disposal recommendations: Pipettes that ARE biologically contaminated

Waste pipettes that ARE contaminated with body fluids, cell debris, or other materials that may contain infectious agents or recombinant DNA molecules must be segregated, stored, treated and disposed of as biohazardous waste. There are various ways that this can be achieved as long as the basic principles of biohazardous waste handling and disposal are followed. Here are examples of acceptable practices:

- Cardboard boxes are convenient collection containers but they are not leak proof and cannot be disinfected. Therefore, if you use a cardboard box as your collection container, you must use it as a one-time collection container only. Additionally, you should restrict the use of cardboard boxes to circumstances where they will be filled and replaced frequently.
- Pipette washers or 5-gallon buckets may be lined with a biohazard bag and used for pipette segregation. In this scenario, please ensure that the outside of the container has a biohazard label. When the bag is full, pipettes can be treated by autoclave or disposal through the medical waste hauler.
    - If treating by autoclave: After autoclave treatment, bags of pipettes should then be placed in a designated "Autoclave-treated Waste" containers.
    - If disposing through the medical waste contractor: Transfer the bag of pipettes to a contractor-approved container and close container per provided instructions.
- Waste pipettes may also be collected in a receptacle containing disinfectant (i.e., pipette washer) at the time of use. A biohazard label and identification of the disinfectant should be on the receptacle. The pipettes should be placed in the receptacle so that the contaminated tips are submerged in the disinfectant. At the conclusion of procedures, the pipettes can be drained and transferred from the receptacle to a biohazard bag for treatment by autoclave, or disposal through the medical waste hauler.
    - If treating by autoclave: After autoclave treatment, bags of pipettes should then be placed in a cardboard box. The box must then be securely taped closed and clearly marked as "trash".
    - If disposing through the medical waste hauler: Transfer the bag of pipettes to a cardboard box. The entire box must be closed and placed in a biohazard bag for treatment and disposal through the medical waste hauler.

Please contact the Biosafety Officer at 974-1938 if you are using a different system to manage your pipettes to determine if it meets the biohazardous waste handling requirements.

Attachment B
Procedure for Transporting Biohazardous Wastes

Whenever possible, biohazardous wastes should be treated and disposed of on-site. However, generation of biohazardous wastes in the field is often unavoidable and handling and transport will be necessary. Please follow the steps below in order to manage and dispose of these materials safely.

1. Wastes that are generated in the field must be segregated and collected using the same principles as outlined for the lab environment.
2. Bags of waste and sharps containers should be closed before removal from the site. (Bulk quantities of liquid waste should not be transported if at all possible. Contact the UTIA/UTK Biosafety Officer for assistance if the need arises to transport such material.)
3. Bags of waste and sharps containers must be placed in a leak-proof secondary container with a secure lid (i.e., latchable, secured with tape, etc.) for transport to treatment facilities. The secondary container must be labeled with a biohazard symbol and an emergency contact name and phone number.
4. Use a University-owned vehicle whenever possible for transport. Store and secure the transport container in a location in the vehicle whereby if an accident were to occur, the container or its contents will not be an exposure risk to the driver or the environment. For example, if transporting materials by car or van, store the container in the back seat or cargo bay. Secure the container with bungee cords or belts to keep the container upright and stable.
5. When you arrive at your destination, transport the waste into the facility using the shortest available route, and move the materials with the aid of a cart. Do not use public elevators if at all possible and avoid traveling with the waste through common public areas. Do not touch door handles, elevator buttons or other common contact surfaces with gloved hands. (Use the one-gloved hand technique, or get assistance from other staff for opening doors, etc.)

Attachment C
Autoclave Validation for Biohazardous Waste Treatment

In accordance with local and state regulations, all biohazardous waste must be biologically-inactivated before it is disposed of as regular trash. This can only be achieved if the waste is exposed to the right temperature for the right amount of time. Optimally, the waste should be exposed to: 121 degrees C, at a pressure of 15 PSIG for at least 20 minutes.

Autoclave gauges are not always accurate, and autoclave tape only indicates the presence of hot steam. Therefore, the autoclave must be validated on a regular basis with a biological indicator ampoule or chemical integrator strip that clearly demonstrates that the appropriate conditions were achieved for sterilization.

Responsibility for validating autoclave performance lies with those who use the autoclave for treating biohazardous waste. It is strongly recommended that a designated individual be identified among the lab staff who will be responsible for the validation of the autoclave and the training of personnel who use this equipment.

UT Biosafety Recommended Practice for Autoclave Validation and Use

Each quarter all autoclaves used to treat biohazardous waste will be validated using 3M Comply Thermalog™ Steam Chemical Integrator strips, which will be provided by the Biosafety Office upon request (974-1938 or 974-9836). Chemical integrator strips test the time, temperature, and quality of steam exposure and are calibrated to mimic the results obtained using the traditional *Bacillus stearothermophilus* biological indicator tests (i.e., spore tests). However, chemical integrator strips give immediate results rather than requiring the 24-48 hour post-autoclave incubation period necessary for spore germination and growth. Based on prior parallel validation testing experiments, there is no difference between the chemical integrators and biological indicators. However, the Biosafety Office will perform spore tests along with the chemical integrators if validation problems/inconsistencies arise.

One misconception that often arises is that biological waste is inactivated as long as the autoclave chamber achieves the proper conditions. However, remember that autoclave performance is affected by load size (volume) and load contents. For example, 121 degrees C for 15 minutes may be adequate to sterilize 2 L of media but not 20 L of media. Thus, achieving proper chamber conditions does not always guarantee sterilization for the entire load. This is especially true for bagged biohazardous waste. Complete inactivation of bagged biowaste can be a challenge and often requires a few additional procedural precautions. The table below illustrates two common problems likely to negatively impact the complete inactivation of bagged biowaste and tips to remedy these problems.

Problems	Solutions
Inadequate steam penetration and exposure	Biohazard bags are not usually steam permissive, so: 1. Be sure that bags are **open**. Do not close the bags by binding or tying prior to autoclaving. 2. Add a small amount of warm water to the bag to generate steam within the bag. 3. Increase the sterilization time.
Overstuffing the chamber	Bagged biowaste is often bulky or high volume, so: 1. Do not overfill biohazard bags. 2. Be sure that the autoclave fits the task. Do not "cram" large bags into autoclaves that are too small to accommodate the load. 3. Do not autoclave multiple biohazard bags.

To summarize the details specified above, the Biosafety Office has developed a standard protocol for the validation of autoclaves used to treat biohazardous waste. The autoclave validation procedure is outlined below:

Autoclave Validation Instructions

1. **Place a full, medium-sized biohazard bag (e.g. 25" x 35") into an autoclavable secondary container.**
2. **Add 1 cup (~250ml) warm water to the bag.** This is recommended for all bagged waste, but it is required if the contents are not likely to generate steam within the bag (e.g. bag contains mostly dry plastic disposables).
3. **Place a 3M Comply Thermalog™ Steam Chemical Integrator strip inside the bag near the center of the bagged contents.** Make sure that there is a mechanism for retrieving the strip after the validation test. For example, affix a piece of twine to the strip and tape one end of the twine to the outside of the bag.
4. **Place bag into the autoclave, leaving the top of the bag open** to facilitate adequate steam penetration into the bag. Make sure that the bag is opened widely at the top and that it is not bound, tied, twisted. Also, take care not to obstruct the opening of the bag by "cramming" the bag against the walls or ceiling of the autoclave chamber.
5. **Autoclave the biohazard bag for a minimum of 30 minutes at 250F/121C.** 30 minutes is the recommended minimum, but sterilizations of >1 hour are not abnormal depending on the autoclave and load volume/contents.
6. **After cycle completion, note the status of the chemical integrator.** A successful test is achieved only if the blue indicator line reaches any portion of the "Safe" window.
7. **Document the validation test.** Documentation should include the following:
    - Date and time of test
    - Load contents
    - Parameter setting for autoclave
    - Type of test and results of test
    - Name of person performing test
    - Failed attempts and remedial actions as necessary
8. **If the validation was successful the biohazard bag may be tied up and discarded into a non-see-through trash bag for final disposal.** If the validation was not successful, repeat the test and add 10 minutes to the sterilization cycle. Integrator strips are single-use only. Use a new strip for each retest.

The autoclave settings that are validated as necessary to achieve effective sterilization of wastes should be communicated to the Biosafety Office so that a database of autoclave validation results can be maintained. Additionally, the Biosafety Office will generate a sign indicating the validated cycle and time settings to be printed and posted by the autoclave tester. Autoclaves that are not validated for biohazardous waste will also be posted accordingly. Examples of autoclave postings are provided on the following page:

This autoclave is currently approved for BIOHAZARDOUS WASTE TREATMENT using the following parameters:

Cycle Setting

Liquids 6

Cycle Time

55 minutes*

* This is the validated time for ONE bag of waste. You must add 10 minutes cycle time for each additional bag.

REMEMBER:

- **Bags must be in autoclavable secondary container.**
- **Bags must be left OPEN for treatment.**
- **One cup of WARM WATER must be added to each bag.**

This validation expires: 6/10/2007
Validation performed by: Cathy Scott
Questions? Call 974-1938 or 974-9836

THIS AUTOCLAVE IS NOT CURRENTLY VALIDATED FOR BAGGED BIOHAZARDOUS WASTE!

Please contact the Biosafety Office at 974-1938 or 974-9836 for more information on this autoclave or for autoclave validation instructions.

Because autoclaves present a number of hazards, only those personnel who have received on-site training by the lab's designated trainer should operate an autoclave. A general procedure for autoclave treatment of solid biohazardous waste (non-sharps) is as follows:

Biohazardous Waste Treatment by Autoclave

1. **Use secondary containment (i.e. cart) for transporting waste bags to the autoclave for treatment** to reduce the possibility of a spill during transport.
2. **Add one cup of water to each bag** to facilitate air displacement and enhance steam generation.
3. **Leave bags open** to facilitate steam penetration.
4. **Place bags in autoclavable secondary containment pan for autoclave treatment** to reduce the possibility of a spill during treatment.
5. **Follow waste cycle parameters established for the autoclave** to assure effective decontamination of waste.
6. **Unload waste after cycle is complete** and chamber pressure has returned to 0 PSIG. **Do NOT override safety features to open the autoclave.**
7. **Use autoclave gloves and appropriate eye protection** to avoid injury from contact with hot surfaces or liquids when removing waste from autoclave.
8. **Tie or band the treated bags closed** to reduce the possibility of a spill.
9. **Package waste in non-see-through bag or container** for final disposal as regular trash.

Do NOT autoclave wastes that are contaminated with hazardous chemicals!