

# Biohazardous Waste Disposal

**Presented by:**

**Biosafety (HSEWB) &  
The Faculty of Science and Engineering**



**Training Material Available on the OHS website:**  
<http://www.yorku.ca/dohs/prog-biosafety.html>

# Objectives

- To ensure consistency of proper biohazardous waste disposal for each lab
- Topics:
  - Definitions & Background
  - Regulated Biomedical Waste Disposal
  - Biohazardous Waste Treatment and Disposal:  
Microbiological Waste

# Biohazardous Waste Disposal

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## Definitions & Background



# Waste Control Laws

## Ministry of Environment, Ontario Government:

- Environmental Protection Act
  - O. Regulation 347, Waste Management
  - **Guideline C-4: Biomedical Waste Management**
  - Guideline C-17: Non-Incineration Technologies

## City of Toronto:

- Sewer By-law
- Littering and Dumping of Refuse

**Fines and Jail Time If Not Compliant**

# What is Biohazardous Waste?

- Waste that may contain or have been contaminated with infectious agents
- The government refers to Biohazardous Waste as “**Biomedical Waste**” and specifically defines what it is
- Ministry of Environment enforces regulation (O. Reg 347, C4 Guidelines)



# What is Biomedical Waste?

## From the C4 Guidelines:

- **Microbiological Waste**  *only stream we can 'treat' at York*
  - Human anatomical waste
  - Human blood waste
  - Animal anatomical waste
  - Animal blood waste
  - Sharps Waste
  - Cytotoxic Waste
  - Waste in contact with human blood waste that is infected or suspected of being infected with any infectious substance (human), or
  - Waste containing or derived from one or more wastes described above
- 
- "Regulated waste" that must be shipped out*

# Biohazardous Waste Disposal

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Regulated Biohazardous Waste: Biomedical





## Will My Biohazardous Waste Be Shipped Out?



No

Yes

**Solid waste:** e.g. used lab disposables such as petri plates, contaminated glassware, gloves, paper towels, pipette tips, microcentrifuge tubes, etc.

**Liquid Waste:** e.g. inoculated broth/culture, cell culture waste, etc.

### Can Treat Using Lab Protocols

- Disinfection
- Autoclaving



**Regulated Biomedical Waste:** e.g. Needle tips/ razors, blood vials, animal carcasses, human or animal anatomical waste and blood, human or animal blood contaminated disposables, animals in formalin, etc.

### Prepare Waste For Shipping

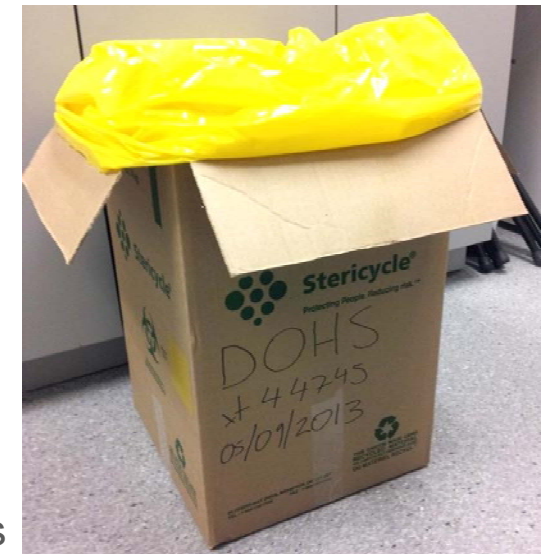
- Biomedical Waste properly recorded, packaged, & stored





# Disposal: Regulated Biomedical Waste

- ONLY use approved waste boxes
  - All supplies available at Farquharson Science Stores
  - Double line the box with a yellow biohazard bag
  - Tape box bottom with strapping tape
  - **Label box with Lab PI, Date, and Contact # with permanent marker**
  - Place appropriate sticker on box
  - Once the box is full:
    - Use twist-ties to seal the yellow biohazard bag
    - Use strapping tape to seal box at least 2" down sides
    - Place box in the science store fridge or Vivaria Freezer
    - LSB Researchers: Contact Brad Sheeller for pick-up: 647.999.9806
  - Do not overfill box (14kg max)



# Disposal: Regulated Biomedical Waste

Place Anatomical Waste sticker on box if needed:



# Disposal: Regulated Biomedical Waste

## Sharps

- razor blades, needles, scalpels...and **any** sharp objects contaminated with biohazardous waste
- Discard syringes immediately into a sharps container
  - **No need** to detach needle from plastic tube
  - **Do not** bend, shear, recap the needle



# Disposal: Regulated Biomedical Waste

## Sharps

- Label the container with the **lab PI, a lab phone number, and date**. Use a permanent marker.
- When a sharps container filled to the line, close the container and safely transport it to Farquharson Science Stores. **Do not** over fill past the line.
- Place inside lined waste box (labelling rules apply)
- LSB Researchers: Contact Brad Sheeller to pick-up when full



# Biohazardous Waste Disposal

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**Biohazardous Waste Treatment & Disposal:  
Microbiological Waste**



# What is Microbiological Waste?

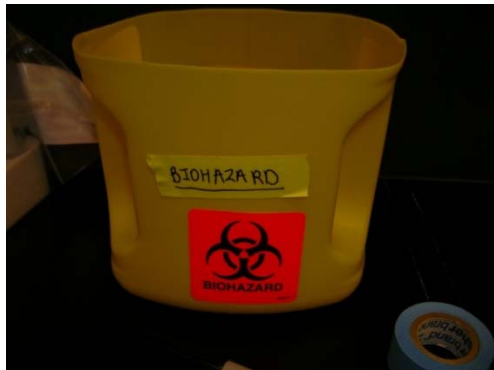
## From the C4 Guidelines:

- Human or animal cultures
  - Stocks or specimens of microorganisms
  - Human diagnostic specimens (excluding urine, feces)
  - Disposable laboratory material that has come into contact with one or more of the items listed above
- Only waste stream that York U can treat by:
    1. **Chemical Disinfection**
    2. **Physical Disinfection (Autoclaving)**



# Storage: Microbiological Waste

- ALWAYS LABEL WASTE CONTAINERS
  - Custodians may pick up waste if not labeled
  - Emergency Response Personnel need to know
  - Clear waste bags should NOT be outside of a labeled container if unautoclaved



# Disinfection: Definitions 101

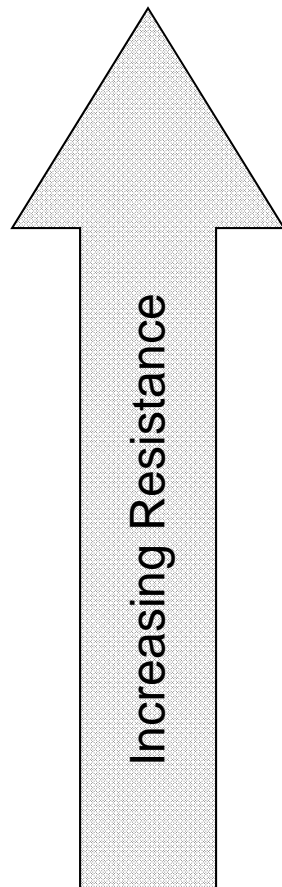
- **Sterilization:** *Destroy/eliminate **all** microbes with the intent to protect against recontamination*
- **Disinfect:** *Destroy/eliminate **all** non-spore forming microbes*
- **Decontamination:** *Disinfection/sterilization of contaminated articles*
- **Antiseptic:** *Prevent/stop the growth & action of microbes*
- **Sanitize:** *Reduce the number of microbes to a safe level*
- **-Cide:** *destroys/eliminates (bactericide, fungicide, etc)*
- **-Static:** *prohibit growth but may not kill (Bacteriostatic, etc)*



# Disinfection: Why Disinfect & Sterilize?

- Minimize risk of contamination
  - Prepares media and reagents for experiments
  - Prevents unwanted microbial growth (e.g. cell cultures, agar plates)
- Minimize risk of exposure in the lab
  - Routine surface decontamination
  - Treatment of biohazardous waste
  - Immediate spill cleanup

# Disinfection: Disinfectant Resistance



- Prions
- Bacterial spores
- Coccidia (*Cryptosporidium mycobacterium*)
- Nonlipid viruses (hepatitis A, polio)
- Fungi
- Rickettsiae, Chlamydiae
- Vegetative bacteria
- Lipid-containing viruses (e.g. HIV)

# Disinfection: Disinfectant Considerations

- **Surface topography:** *rough surfaces harder to clean*
- **Temperature:** *high temps may inactivate disinfectant*
- **Organic load:** *higher load requires higher disinfectant concentration*
- **Concentration:** *low concentrations may not disinfect , too high concentration may be hazardous*
- **Contact time:** *short contact times may not disinfect*

# Disinfection: Types of Disinfection

- Chemical Disinfection
  - 6 classes
- Physical Disinfection
  - Thermal (autoclave, incineration)
  - Filtration (water treatment)
  - Irradiation (UV, gamma radiation)

# Disinfection: Chemical Disinfectants Classes

- **Halogens (e.g. Chlorine, Iodine)**
- **Alcohol**
- Phenolics
- Quaternary ammonium compounds (e.g. lysol)
- Aldehydes (e.g. glutaraldehyde, formaldehyde)
- Hydrogen peroxide

# Disinfection: Common Lab Disinfectants

- 5-10% household bleach solution
  - 5% for 1 hour / 10% for 30 mins
  - advantages: very effective, affordable
  - disadvantage: easily inactivated by organics, corrosive
  - **Must change solution often and make fresh batches**
- 70% ethanol (EtOH) solution
  - advantages: very effective, affordable
  - disadvantage: flammable, does not kill spores

# Disinfection: Common Usage

Agent	Disinfectant (Examples)
Vegetative bacteria	5% bleach, 70% EtOH, quaternary ammonia, 6% hydrogen peroxide
Mycobacteria and fungi	10% bleach, 70% EtOH, phenolic compounds, 6% hydrogen peroxide
Spore-forming bacteria	10% bleach, glutaraldehyde, formaldehyde, 6% hydrogen peroxide
Enveloped viruses	2% bleach, 70% EtOH, quaternary ammonia, 6% hydrogen peroxide
Non-enveloped viruses	10% bleach, 6% hydrogen peroxide, glutaraldehyde, formaldehyde

# Disinfection: Physical Disinfection

- Thermal
  - Steam Sterilization (e.g. autoclave)
    - advantages: nontoxic
    - disadvantages: burn hazard, expensive
  - Incineration
    - disadvantage: not available on-site
- UV radiation
  - advantages: no chemical hazard
  - disadvantages: skin/eye exposure hazard, limited use (effectiveness is questionable)



# Autoclave Use

## The Hands-on Training Guide



# Disinfection: Autoclave

- Pressurized device that uses heat, steam and pressure to achieve sterilization or decontamination



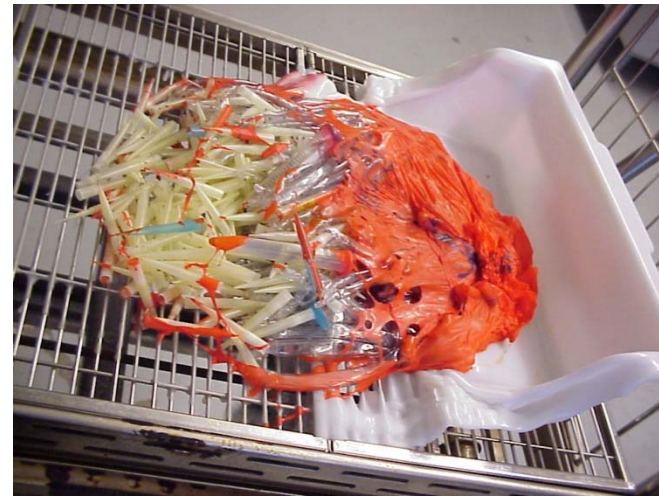
# Autoclave Overview

- Typically operated at 121°C, 15psi, for 15-45 minutes.
- Allows the heating of liquids above boiling point.
- Uses moist heat (steam) to increase efficiency of sterilization.
- Heat is used to coagulate proteins, which destroys microorganisms and any potential biohazard.

# Autoclave Hazards

- Tremendous pressure from steam in chamber provides explosive potential.
- High temperatures creates potential for burns and scalding.
- Potential exposure to hazardous vapours
- Inadequate decontamination allows for the potential of biological hazards to contaminate personnel and the environment.

# Autoclave Hazards



# What you CAN autoclave?

## From the C4 Guidelines:

- Human or animal cultures
- Stocks or specimens of microorganisms
- Human diagnostic specimens (excluding urine, feces)
- Disposable laboratory material that has come into contact with one or more of the items listed above
- Autoclaving is also used for:
- Items for sterilizations such as; glassware media, aqueous solutions



# What you CANNOT Autoclave

- DO NOT Autoclave:
  - BIOMEDICAL WASTE (except Microbiological)
    - Including human anatomical or blood waste, animal anatomical or blood waste, cytotoxic waste, or any waste in contact with these waste products (including sharps waste).
  - RADIOACTIVE WASTE
  - HAZARDOUS CHEMICAL WASTE
    - This includes anything contaminated with a toxic, volatile, corrosive, or mutagenic chemical
      - (e.g.) bleach, formalin, glutaraldehyde, ethidium bromide
    - Check MSDS beforehand



# Autoclaves at York

- Lumbers Room 120A
- Farquharson 227A
- 3<sup>rd</sup> Floor Life Sciences Building





# Steps to Autoclaving

- Preparing your items for autoclaving
- Loading the autoclave
- Choosing the cycle settings
- Unloading the autoclave
- Aborting Autoclave

# Autoclaving: Preparing your items

- Sign into log book
  - Keeps track of autoclave use for maintenance records
  
- Use personal protective equipment!
  - Eye protection
  - Heat resistant gloves
  - Lab coat



# Autoclaving: Preparing your items

- Use a primary container
  - Container comes into direct contact with the contaminated or non-sterilized material or fluid
  - Do not fill more than 2/3 of holding capacity
  - Must NOT be a tightly sealed container (might explode)
    - **MUST allow steam penetration**



# Autoclaving: Preparing your items

- Use a primary container - **MUST** allow steam penetration
  - Bottles:
    - Loosen screw caps or use self venting caps
    - Cap open containers with aluminum foil or muslin

Loosen screw caps



# Autoclaving: Preparing your items

- Use a primary container - **MUST allow steam penetration**
  - For waste bags:
    - Do not pack or compress contents, do not knot or seal the bag
    - Label with Date, Lab PI, Lab Phone extension
    - Use a chemical integrator
    - Do not knot or seal bag: can fold excess over but keep open





# Autoclaving: Preparing your items

## Chemical Integrators

- The steam sterilization process is the function of three basic parameters: time, temperature and steam penetration
- Chemical Integrators are a good way of testing these parameters
  - Black band **must** be within the 'Accept' area
  - If the black band **does not** reach the 'Accept' area, re-autoclave the load:
    - Increase the sterilization temperature, time, or steam penetration

Non-autoclaved



Accept  
(throw in bin)



Reject  
(re-autoclave)



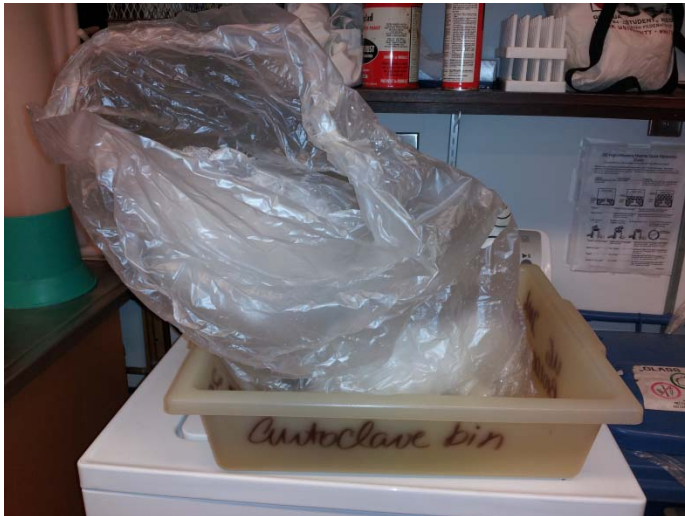
# Autoclaving: Preparing your items

- Place the Chemical Integrator (CI) **centrally within** the waste bag
- Use the extenders to place the CI:
  - It avoids direct contact with waste
  - Attach the CI to one end of the extender. The extender can be autoclaved
- **Note: Not every bag of waste per load must receive a CI.**
  - Place CI in the bag which occupies the most challenged position in the load.



# Autoclaving: Preparing your items

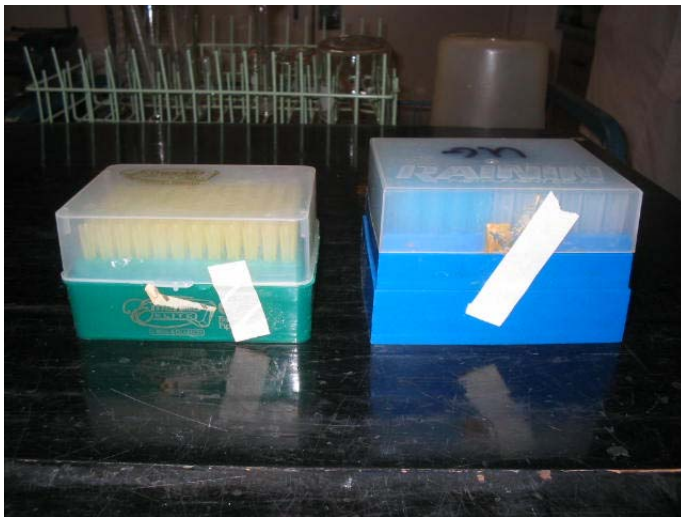
- Use a secondary container
  - Used to contain any spills
  - The sides of the secondary container must be sufficiently high to contain any spill that may occur
  - Tray MUST be autoclave safe





# Autoclaving: Preparing your items

- Use temperature sensitive tape
  - Will indicate that high temperature has been achieved
  - Will not prove that decontamination or sterilization was successful
  - Will assist in keeping track of autoclave and non autoclaved items



Before



After

# Autoclaving: Loading the autoclave

- Be cautious if autoclave was recently used
  - Loading rack may be hot
  - Use heat protective gloves
- Make sure loading rack is locked on cart
  - Rack may slide out unexpectedly if not locked



# Autoclaving: Loading the autoclave

- Farquharson:

- Tighten door so that prongs are fully extended
- If noise and/or steam escapes, abort the cycle and tighten the door more



- Lumbers

- Keep the door button pushed until the ready prompt is on the screen



# Autoclaving: Which cycle to use

- Autoclaves run 3 types of cycle programs
- The type of cycle depends on what is being autoclaved:

Liquid/Slow exhaust	* For autoclaving liquids * Prevents liquids from boiling over
Solid/Gravity	* Best for wet waste
Solid/Vacuum	* Best for dry waste

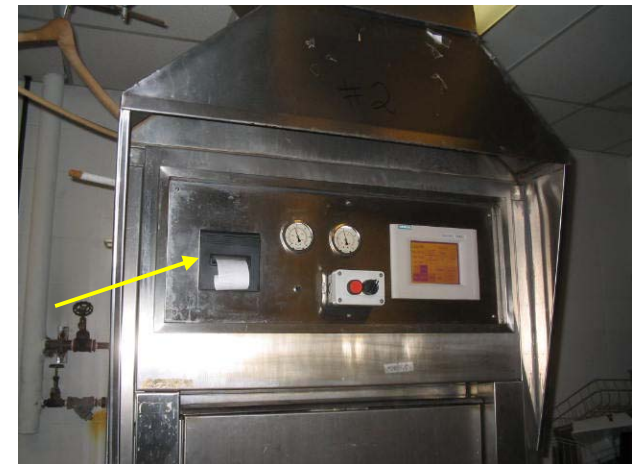
- Notes
  - When in doubt, use Solid/Gravity for waste
  - **Do not** autoclave liquids with a solid load

# Autoclaving: Choosing a Cycle

- For Liquids:
  - 20 mins / litre of liquid, 5 mins per additional litre
- For Solids:
  - Glassware (empty): 20 mins
  - Instruments (utensils): ~30 mins
  - Biohazardous Garbage: at least 60 mins
    - Use biological test strips to optimize duration
    - Can decrease time if biological testing proves effective
    - Using a pre-vac cycle can assist in steam penetration (LSB autoclaves use these cycles)

# Autoclaving: Unloading autoclave

- Use PPE
- Wait for **autoclave** to state END CYCLE before opening door
- When opening, stand away from door opening
  - Make sure no one is standing by door opening
- Farquharson: turn door knob slowly and open door slightly to allow steam to escape
- Lumbers: make sure your hands are not above the top vents





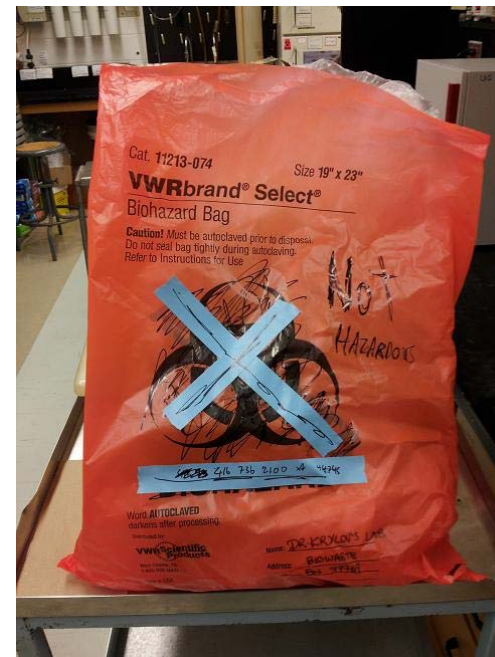
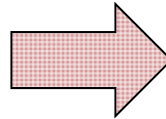
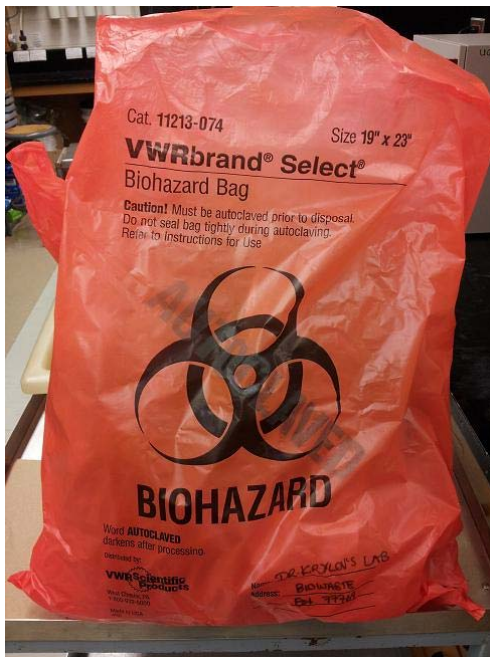
# How to Autoclave – Unloading autoclave

- Make sure rack is locked on to cart
- Analyze chemical integrator (if failed, redo sterilization) and note results in log book
- Please put back heat resistant gloves for other users
- Keep autoclave doors shut when not in use



# Waste Disposal: Treated Waste

- Unmark any biohazard signs and words that may be seen on waste bags!
- City waste collectors have complained and refused to pick up our waste in past



Note: It is best to use clear autoclave bags



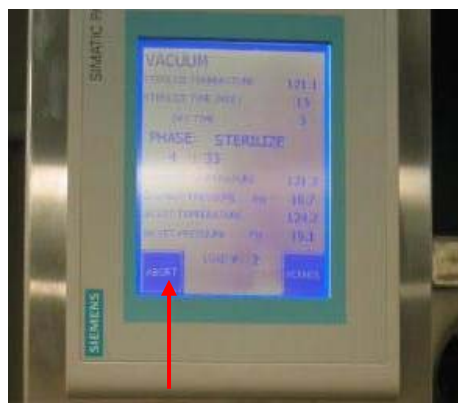
# Waste Disposal: Treated Waste

- **Autoclaved** bags to be put into the red bins outside the autoclave room
- Unmark all biohazardous symbols
- Always label waste bags (**Date, Lab PI and contact #**)
- Do not over fill red bins



# How to Autoclave – Aborting

- Only qualified personnel should attempt to troubleshoot an autoclave
- Farquharson
  - Large autoclave: push abort button
  - Small autoclave: (note do not use for waste!) need to manually advance through the autoclave cycle
- Lumbers
  - Push abort button
- Life Science Building
  - Push off button



# Autoclave: Performance Indicators

- How to know if autoclave is functioning correctly:

Physical	<ul style="list-style-type: none"><li>- Annual testing by certified technician</li><li>- Pressure, Temperature, Cycle times, recorded on paper</li></ul>
Chemical	<ul style="list-style-type: none"><li>- Heat sensitive autoclave tape</li><li>- Not an indicator of successful sterilization, useful to keep track of autoclaved and unautoclaved items</li></ul>
Biological	<ul style="list-style-type: none"><li>- Tests ability of autoclave to sterilize effectively</li><li>- <i>Bacillus stearothermophilus</i> spore strips often used because they are resistant to steam sterilization.<ul style="list-style-type: none"><li>- EZ Test (SGM Biotech) (Fisher Sci #29801 074)</li><li>- 3M Attest Rapid Readout Biological Indicators</li><li>- Steris Verify Integrator Laminated and EO Integrators</li></ul></li></ul>

# Autoclave Issues/Concerns?

## Contact:

- Your Supervisor

## Farquharson and Lumbers:

- Brad Sheeller, 647.999.9806, FSE
- Maria Mazzurco, Bioogy, Lumbers 106, ext. 22657
- Debbie Freele, Biology, Lumbers 115, ext. 22655

## LSB

- Lab/Investigator in charge of autoclave at the time
- Brad Sheeller, 647.999.9806, FSE

## General Inquiries: York Biosafety Officer:

- Jay Majithia, HSEWB, ext. 44745

# Disposal: Mixed Waste

Chemical



Radiological



Biological



- *Try to keep waste streams separate as much as possible*
- *Limit experiments that create mixed waste streams, where possible.*
- *Contact DOHS if unsure how to handle any waste stream.*



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Treat according to radiation waste protocol, then as biohazard.



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Chemically disinfect biological waste first. If biological material cannot survive or is not infectious, treat as chemical waste.



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Treat in order (radiation, biological, chemical). Contact DOHS for assistance

# End

Thank you, Please remember to keep record of your training in the lab group's safety binder



**Training Content maintained by Health, Safety and Employee Well-Being**

<http://www.yorku.ca/dohs/prog-biosafety.html>

**Inquiries –**

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