

University of Houston  Clear Lake

## **Biotechnology Waste Autoclaving & Management**

Training created from the UT Health Science Center Biosafety  
Officer Training course materials,

and (bio) medical waste regulations found in:

30 TAC 330 Subchapter Y - TCEQ

25 TAC 1 Subchapter K - TDSHS

49 CFR 173.134(5) - DOT

### **Types of Medical Waste** from biomedical research and educational institution research laboratories

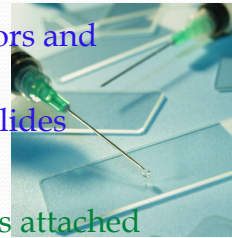
- **Animal Waste** – carcasses, parts, blood & blood products, and bedding of animals intentionally exposed to pathogens
- **Microbiological Waste** – discarded
  - Cultures and stocks of infectious agents and associated biologicals
  - Cultures of specimens from research labs
  - Live and attenuated vaccines, excluding empty containers
  - Used disposable culture dishes and used disposable devices used to transfer, inoculate or mix cultures
- **Pathological Waste** – human materials
- **Human blood, blood products, and body fluids**
- **Sharps** – see next slide

# Sharps

Any object contaminated with a pathogen or that may become contaminated with a pathogen and also capable of cutting or penetrating skin or a packaging material.

Sharps include, but are not limited to the following items when contaminated:

- Razor blades, Scalpel blades, Disposable razors and scissors
- Glass pipettes, specimen tubes, microscope slides
- Broken glass



Hypodermic needles and syringes with needles attached are considered sharps with or without contamination.

# Treatment Methods (onsite)

All Regulated waste must be rendered non-infectious prior to disposal. They should be placed in properly labeled containers and then treated by:

1. Chemical disinfection-on inanimate surfaces, usually done with liquid waste and reusable equipment
2. Moist heat disinfection - microwave radiation of internally shredded waste with moist heat; or sealed containers with radiowaves & then shredding
3. Thermal Inactivation - dry heat, which requires a much higher temperature than Steam disinfection.
4. Steam Disinfection/Sterilization- Subjecting waste to steam under pressure, which is the typical autoclave use.

## Chemical Disinfection

Chemical disinfection is generally used for **liquid** wastes

- EPA registered disinfectant
  - 1:10 bleach solution (fresh container)
  - 70-100% Isopropanol
  - Phenols / Lysol – can be added to wash
- Immerse Completely for 10-15 minutes
- Disinfectant must be thoroughly drained prior to disposal if used on solids.
- Human and animal parts need to be macerated after disinfection.



## Steam Disinfection/Sterilization via Autoclave

- Autoclaving provides an economical process of killing pathogenic microorganisms through saturation with moist heat (steam) under pressure, and is also called Steam Sterilization.
- This process makes the microorganisms nonviable by destroying (denaturing) essential proteins and structures
- Effective method of treatment if **All Three** factors are met:
  1. Time
  2. Temperature
  3. Pressure

## Steam Disinfection/Sterilization

Autoclave Parameters for effective treatment:

Autoclave Parameters	Temp.	Press.	Time	QC Indicator
General	121°C (250°F)	15 psi	15 min, or longer depending on load size	Geobacillus stearothermophilus, SteamPlus Sterilization Integrator
Prions (highest resilience)	132°C (270°F)	30 psi	4.5 hrs	same
<b>Dry Heat Tabletop Autoclave</b> (when steam is not working or items cannot be penetrated by steam) May have to be approved for Pathological & Sharps	170°C (338°F)		1 hr	Bacillus Atrophaeus
	140°C (284°F)		3 hrs	Same
	121°C		16+ hr	Same

Parameters depend on the microorganism being treated

## Waste Collection Containers

**Rigid Container** that is leak resistant

- Impervious to moisture (the bio bag) and strong enough to prevent tearing and bursting
- Orange heat resistant bags for Autoclave
- Absorbent to capture any free liquids\*
- Closed collection container for aerosols



**Proper labeling**

- Biohazard Symbol Labels on Containers
- If you can't treat it immediately and need to leave the waste, place a tag on the bag labeling it with the following:
  - Contact Name
  - Contents - agent and materials
  - Date generated (bag filled)



## Autoclave Waste Handling

Biohazardous waste materials to be autoclaved must be placed in a properly labeled, heat resistant, autoclavable bag.

- These are usually orange. Not all biohazard bags are made heat resistant for autoclaving (i.e., red ones meant for off-site incineration and disposal).
- *Do not put bleach in the bags*, nor bleach waste first as this damages the autoclave metal and gasket seals.
- If waste does not contain moisture, **add ½ cup of water** for required\* steam
- Keep the bags open or loosely taped **while autoclaving\*\*** so that steam can get in



## Autoclave Waste Handling



- Color change tape only detects that the temperature was met, not if the pressure or length of time was met.
- Bags should be left to cool for several minutes before removing from autoclave
- Once cool, securely close biohazard bag and place into opaque black bag which has (large white trash bin pictured) secondary containment



# Efficacy Monitoring

## Methods for Quality Control, Parameter Monitoring:

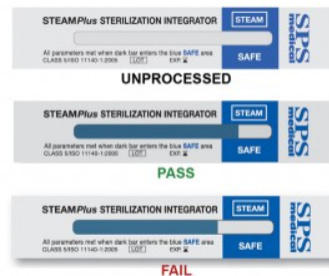
- Autoclave temperature tape - tells desired temperature reached, not time or pressure
- Parameter monitoring - pressure, time and temperature
- **Record every load on autoclave use log**
- Perform Efficacy monitoring (Biological activity check)
  - **50-99 lbs total generated on campus/month - monthly**
  - 100 - 200 lbs/month - biweekly
  - >200 lbs/month - weekly
- Routine parameter monitoring may be substituted for biological monitoring for autoclaves which have a continuous readout and record of operating parameters. If this is done, cut and tape readout to autoclave log with other required information (date, initials, weight...)

# Efficacy Monitoring


- Parameter monitoring is required to ensure autoclave is working properly.
- This is done using SteamPlus Integrator strips.
- **Time, Temperature, and Steam Pressure** are monitored
- **Indicator should be placed in the bag with the waste to verify waste treatment, not near the door.**
- Autoclaves with pinwheel plots require biological monitoring with *Verify* biological monitoring vials containing live *GeoBacillus Stearothermophilus*



Efficacy Testing Strips we use to ensure time, temperature, and saturated steam are met



# Efficacy Test Log

University of Houston  Clear Lake AUTOCLAVE EFFICACY TESTING

Testing Schedule  
 lbs/mo Frequency  
 < 50 Semester  
 50-99 monthly  
 100 2 weeks  
 200 weekly  
 & after maintenance

All 3 Required for Effective Treatment  
 Parameter monitoring substituted for  
 biological monitoring. (Min Values)

QC Monitoring Results from  
 Indicator placed inside Bag

Autoclave Number	Treatment Date	Name Print	Initials	Time (15-30 min)	Temperature (121°C/250°F)	Pressure (15psi)	Month	Monthly Total Treated (lbs)	Write Results (Live agent & type) or attach Indicator Strip to sheet

For compliance with 30 TAC Chapter 330 - Special Waste from Health Care Related Facilities. For questions, call EH&S at x.2106; Equipment issues or supplies call x.3751

# Autoclave Parameter Log Example

University of Houston  Clear Lake AUTOCLAVE TESTING LOG



SteamPlus Sterilization Integrator Strips

Testing Schedule  
 lbs/mo Frequency  
 < 50 Semester  
 50-99 monthly  
 100 2 weeks  
 200 weekly  
 and after repairs



Minimum Values Required for Effective Treatment

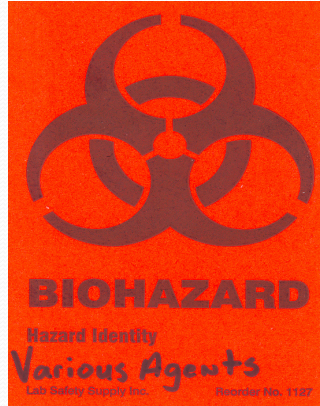
QC Monitoring Results

Autoclave Number	Treatment Date	Name Print	Initials	Time (15-30 min)	Temperature (121°C/250°F)	Pressure (15psi)	Month	Monthly Total Treated (lbs)	Place Strip in Bag with waste. Attach Indicator Strip in this
2	9/18/18	Lisa Coen	LC	30min	121°C	15psi	Sept		
3	9/18/18	Lisa Coen	LC	30min	121°C	15psi	Sept		
1	9/18/18	Lisa Coen	LC	NA	NA	NA	Sept	NA	Autoclave not working
							September	64 lbs	



## Autoclave Rooms are BSL-2

- It is important to remember that all autoclave rooms are BSL-2 (biosafety level 2) rooms per the CDC's BMBL.
- Indicates a biohazard is present such as Hepatitis B virus, HIV, the salmonellae, and Toxoplasma spp. Also includes human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown [include applicable verbage]
- Indicates that *NO FOOD/DRINKS or cosmetics* are allowed in the room



## Autoclave Limitations

### Can't autoclave:

- Toxins
- Chemicals or disinfectants
- Non-heat stable materials / some plastics will melt
- Radioisotopes
- No animal carcasses or feces
- Must have water in bag to work or add water and loosely tie to allow steam to penetrate bag.
- Must do periodic indicator testing with Geobacillus stearothermophilus or tape or vial chemical indicator

## Personal Protective Equipment

Personal Protective Equipment (PPE) is required by OSHA, Lab Prudent Practices, the State Office of Risk Management, and the Bloodborne Pathogens Standard (BBP) if exposure to chemicals, hazardous materials, or blood/OPIM is anticipated and where occupational exposure remains after institution of engineering and work practice controls.

- PPE should be replaced immediately if torn
- PPE should not be worn outside the lab area
- Long pants or long skirts and closed toe shoes only,
- NO shorts, short skirts, sandals, or peep toe shoes.

**Lab coats** must be worn to protect clothing/exposed skin from contact with blood/OPIM.



## Personal Protective Equipment

**Gloves** - must be worn when hand contact with blood or OPIM is anticipated or when handling contaminated items

- Verify gloves are compatible with your specific application, process and materials before using
- Always wear heat resistant gloves and keep your face away from the door when loading and unloading the autoclave



**Safety glasses** (with side shields) to protect the eyes.

**Face shields** to protect mucous membranes, such as the nose, mouth and eyes from contact with any blood or OPIM during sample manipulation

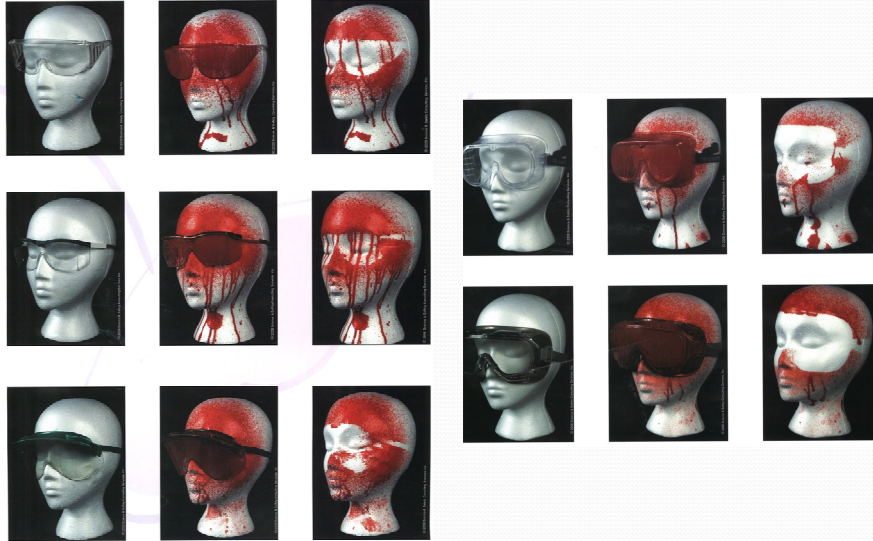


**Surgical mask** to protect the nose and mouth.

- The last 2 PPE should be used in addition during procedures that are likely to generate splashes or sprays of blood or OPIM

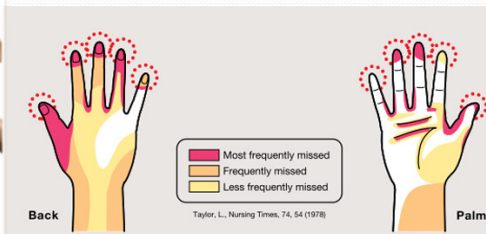


## PPE: Eyewear Limitations



## Hand Hygiene

- Frequent and effective hand washing are essential in reducing transmission of bloodborne pathogens as well as other bacteria such as Methicillin resistant Staphylococcus aureus (MRSA) and Vancomycin Resistant Enterococcus (VRE). Always wash before leaving work area.
- Sing the Happy Birthday song while rubbing hands together for 20 seconds.
- Demo
- Sanitizer Limitations



## Employee Safety Practices

- Avoid compressing bags which may create aerosols
- Waste should be stored *inside* the autoclave room and not in the hallways, in rigid, leak proof containers such as a bag inside a box, bin, or secondary containment tray/tub
- Sharps (needles, glass pipettes or other pipettes that can puncture bags, broken glass or plastic\*, microscope slides, etc.) placed in an autoclavable red sharps container
- Biological Waste should not be left for “someone else” to autoclave
- Biological waste containing hazardous chemicals (flammables, disinfectants) **should not be autoclaved**. Call EH&S at 281-283-2106 for collection of these items

## Employee Safety Practices

- Be a conscientious worker:
  - Keep work areas clean and free of hazards
- Use Standard Precautions:
  - Treat all human blood and body fluids as if known to be infectious
- No eating, drinking, or applying cosmetics in the work area
- Frequent hand washing
- Decontaminate work surfaces daily, and have disinfectant available in the room(s)

## Common Biosafety Deficiencies

Common deficiencies include:

- Food or drink consumed or stored in autoclave area
- Autoclave log incomplete (or not present)
- Sterility monitoring not performed
- Training records incomplete
- Minors in the work place
- Absence of disinfectant indicates surfaces are not disinfected

## Autoclave Use Instructions:

1. Use heat indicating autoclave bags or place indicator tape on the autoclave bag. Make sure bags are designed for autoclave use or they will melt and you will have to clean up the mess.
2. **Do not store waste for someone else to autoclave.** If you must leave, place a tag on the bag labeling it with all required information:
  - Biohazard logo with specimen/agent name (contents)
  - Name or PI name and room # came from, and the dateThis is necessary because the bag may need opened some to allow both pressure and moisture to penetrate (steam sterilization). Do you want to handle or open someone else's unknown biohazards?
3. Place waste inside autoclave. Use secondary containment (pan) under the bag. *If waste does not contain any moisture, put ½ cup of water inside bag for steam generation.*

## Autoclave Use Instructions:

4. Autoclave according to manufacturer's instructions for a minimum 30 minutes, at a minimum operating temperature and pressure of 250 deg. F and 15 psi. (refer to chart)
5. Log usage activity in the autoclave usage log
6. When cycle is finished, inspect sterilizing tape, and visually check bags to ensure autoclave was working correctly. Check that the bag is open, moisture is present, and for color change of bag text.
7. Wait a few minutes for waste to cool, then remove with **heat resistant gloves**.
8. Place waste in black opaque bags **held steady inside a bucket**. Seal autoclave bag, then black bag with tape or tie in a knot.
9. Place waste in **trash cans labeled "Autoclaved Waste Only"**
10. Perform routine efficacy monitoring and record results in logbook

## Blood Borne Pathogens (BBP)

### Slides applicable for Biotechnology Labs Created from:

UH Bloodborne Pathogens Training

UT Health Science Center Houston Bloodborne Pathogen  
Awareness Training

OSHA's Revised Bloodborne Pathogens Standard Training, 2001

## Governing Laws & Regulations

### OSHA

- Bloodborne Pathogen Standard found in 29 CFR 1910.1030, originally adopted in 1991
- Needlestick Reduction Act modified it in 2001
  - Education of and selection of sharps injury reduction devices (like self-sheathing needles)
  - Keeping a (contaminated) sharps injury log

### TDSHS Bloodborne Pathogen Control 25 TAC 96

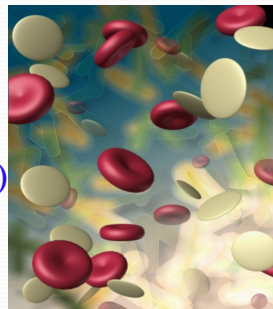
- Exposure Control Plan required to minimize government agency employee's exposure to BBP.

## What is a Bloodborne Pathogen?

**Any pathogenic microorganism that is present in human blood, or other potentially infectious materials (OPIM) that can cause disease in humans.**

**Some examples include:**

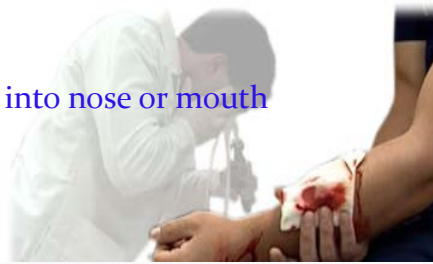
- Hepatitis B Virus (HBV)
- Hepatitis C Virus (HCV)
- HIV / Aids
- *Treponema pallidum* (syphilis)
- Herpes Virus
- HTLV-I
- *Mycobacterium tuberculosis*



## BBP – Routes of Transmission

Bloodborne Pathogens may be transmitted by:

- Puncture wounds caused by sharp objects
  - Broken glass, misuse of or accidental puncture by needle
- Contact with open wound or broken skin
  - Risk increases with prolonged contact or larger areas of broken/damaged skin
- Splash into the eyes
- Sprayed or aerosolized and into nose or mouth



## BBP – Example Injury Statistics

- This is a summary of the UT Health Statistics:
  - Percentage of reported potential BBP exposure injury events (out of all reported injuries)
    - Residents 92%
    - Students 87%
    - Employees 26%
  - Of those BBP injuries, these were the types
    - Needlestick 50% of all BBP injuries
    - Cut (Other) 30%
    - Known BBP Exposure 20%



## Minimizing or Eliminating Hazards

- Engineering controls
- Safe Work Practices & Universal Precautions
- Signs and labels
- Personal Protective Equipment
- Proper hygiene
- Proper waste disposal procedures
- Spill cleanup
- Exposure Control Plan which identifies the above information



## Engineering Controls

Engineering Controls are devices that isolate or remove the BBP from the workplace. They can include:

- Handwashing Facilities
- Leak-proof containers for storage and transportation
- Sharps Injury Reduction Devices such as retractable syringes, self-sheathing needles and needless systems used
- Biosafety cabinet (BSC) with HEPA filter & Directional air flow



## Sharps Injury Prevention

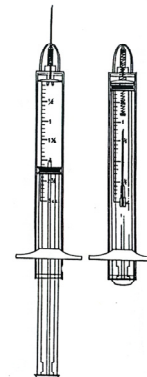
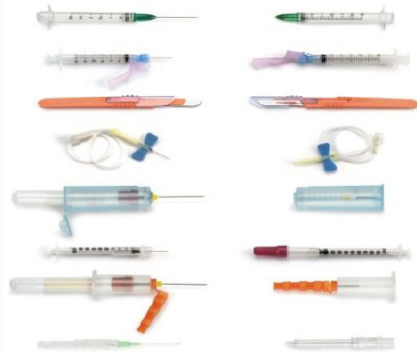


- **Eliminate** the use of sharps when possible
- If elimination is not possible, **use engineered sharps devices** that reduce injury (slides following)
- And use **Safe Sharps Practices** at all times:
  - **NEVER recap** a used needle!
  - **NEVER bend** or manipulate sharps
  - **NEVER pass sharps** by hand between people
  - **Do NOT attempt to catch** falling sharps if dropped
  - **Use a puncture resistant tray** to transport sharps
  - Don't fill container more than  $\frac{3}{4}$  full



## Sharps Injury Reduction Devices

Hypodermic syringes with “Self-Sheathing” or “Retractable Technology”



## Safe Work Practices

- **Universal Precautions**-treat all human blood and body fluids as if they are infectious.
- **No smoking, eating, drinking, applying cosmetics or contact lenses in the work area**
- **Decontaminate work surfaces** – frequently, after spills, with 10% bleach or 70% Isopropanol
- **Hand washing** – frequent, adequate
- **NEVER recap needles!!**



## Signs, Labels and Color Coding

Observe warning signs in biohazard work areas or on laboratory doors that alert of hazards present. Door signage also shows the appropriate PPE needed in the laboratory.

Inside the lab, warning labels must be placed on all containers of waste, refrigerators, and freezers that contain blood or other potentially infectious material. These labels must have the word "BIOHAZARD" on them.

AUTHORIZED PERSONNEL ONLY			
Room: <b>Room # and Building Name</b>	EMERGENCY CONTACT NUMBER(S)		
Principal Investigator: <b>Dr. WWW XXX</b>			
Safety Designee: _____			
 BIOHAZARD	 BIOSAFETY LEVEL 2	 REPRODUCTIVE TOXIN	 CANCER HAZARD
 SAFETY SHOWER/ EYEWASH	 PPE Required	 GLOVES	 Eye Protection Required
		 NO FOOD	 NO SANDALS
OTHER EMERGENCY CONTACT NUMBERS			
POLICE EMERGENCY: 911	POLICE NON-EMERGENCY: 713-743-3333		
FIRE SAFETY: 713-743-1635	ENVIRONMENTAL HEALTH AND RISK MANAGEMENT: 713-743-5858		
			 UNIVERSITY OF TEXAS HEALTH SYSTEM PLANT OPERATIONS PPE 10-10-10

## First-Aid Response

- Remember Universal Precautions
- Encourage self-care
- Use PPE
- Avoid applying pressure without barrier
- Apply routine first aid
- Clean site of injury with soap and flush with warm water for at least 15 minutes
- Flush mucous membranes with water or saline for at least 15 minutes



## Spill Clean-Up

### **If you spill blood or OPIM:**

- Place paper towel(s) or other absorbent material on top
- Wet that with disinfectant and let sit for 10 minutes
- Pick up broken glass pieces with tongs or dustpan or piece of plastic
- Then wipe up spill from outward to inward

### **Never pick up broken glass with your hands, even while wearing gloves.**

- Contaminated broken glass should go in a sharps container.
- Disinfected broken glass can go in broken glass container.

## Post Exposure Follow-up

- **Seek medical treatment**
- **Employees must report exposure to supervisor and EHS within 24 hrs via [Incident Report form](#)**
- Risk Management ext. 2106
- Some incidents are reportable to the CDC or the TDSHS within 24hrs, some within a week
- **Blood tests**
- **Post exposure prophylaxis**
- **Counseling**

## Resources

### **UH Biosafety Manual**

<http://www.uh.edu/ehs/about/manuals/Biosafety-Manual-06-2016.pdf>

### **Occupational Health and Safety Administration**

<http://www.osha.gov/SLTC/bloodbornepathogens/index.html>

### **Centers for Disease Control and Prevention**

<http://www.cdc.gov/niosh/topics/bbp/>

# Questions?

Environmental Health & Safety Department  
ext. 2106

[https://www.uhcl.edu/about/administrative-offices/  
environmental-health-safety/](https://www.uhcl.edu/about/administrative-offices/environmental-health-safety/)

- Lisa Coen -  
(281)283-2107  
coen@uhcl.edu