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## Biological Safety Training




Biosafety Office: 413-545-2682

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## Environmental Health and Safety

Ensuring a safe and healthful environment for living, learning and working

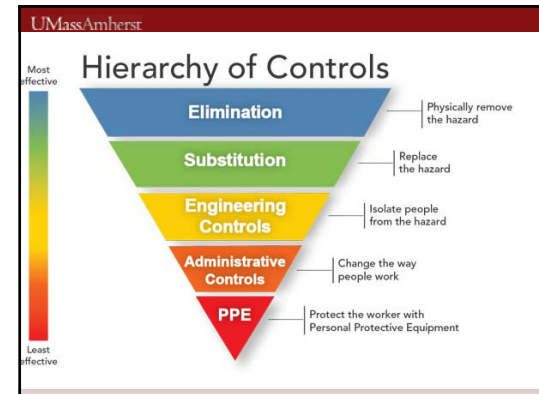


EHS staff works 24/7 to ensure safety of this campus

Call 413-545-2682 with any questions or visit <http://www.ehs.umass.edu/>

Biological Safety staff works to:

- Ensure that biological research is conducted in a safe fashion
- Ensure that biological research meets regulatory requirements



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## Biological Agents & Routes of Exposure

Biological agents:

- Bacteria, viruses, fungi, rickettsia
- Other microorganisms and/or their associated toxins

Can be transmitted to humans via:

- Ingestion, inhalation, mucous membrane exposure, inoculation (e.g. needlestick)



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## Biological Agents & Routes of Exposure

Working with biological agents may create risks to:

- workers
- environment
- community

Biosafety regulations were developed to mitigate all of these risks



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## Risk Groups

Risk Group Classification	NIH Guidelines
Risk Group 1	Agents not associated with disease in healthy adult humans. (ex. <i>Escherichia coli</i> K-12)
Risk Group 2	Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available. (ex. <i>E. coli</i> O157:H7)
Risk Group 3	Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk). (ex. HIV)
Risk Group 4	Agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk). (ex. Ebola virus)

## Biosafety Levels

Each biosafety level has its own specific containment controls that are required :

- Laboratory Practices
- Laboratory Facilities
- Safety Equipment



## Biosafety Level Chart

Biosafety Level	BSL-1	BSL-2	BSL-3	BSL-4
Sample Organisms	<ul style="list-style-type: none"> <li>• <i>E. Coli</i> K-12 Strain</li> <li>• <i>Lactobacillus acidophilus</i></li> <li>• <i>Bacillus subtilis</i></li> </ul>	<ul style="list-style-type: none"> <li>• <i>E. Coli</i> O157:H7</li> <li>• <i>Staphylococcus aureus</i></li> <li>• HIV</li> <li>• Hepatitis B virus</li> </ul>	<ul style="list-style-type: none"> <li>• Yellow Fever Virus</li> <li>• West Nile Virus</li> <li>• <i>Mycobacterium tuberculosis</i></li> </ul>	<ul style="list-style-type: none"> <li>• Ebola Virus</li> <li>• Marburg Viruses</li> </ul>
Facility Requirements	<ul style="list-style-type: none"> <li>• Doors for access control</li> <li>• Sink for hand washing</li> <li>• Sanitizable surfaces and frequent disinfection</li> <li>• Screens on windows</li> <li>• Sharps</li> </ul>	<ul style="list-style-type: none"> <li>• Biosafety cabinet for aerosol procedures</li> <li>• Centrifuge safety caps</li> <li>• Sharps containers</li> <li>• Biosecurity</li> </ul>	<ul style="list-style-type: none"> <li>• Pass-through autoclave with Bioseal required</li> <li>• Negative air flow</li> <li>• HEPA filter exhaust</li> </ul>	<ul style="list-style-type: none"> <li>• Pass-through autoclave with Bioseal required</li> <li>• Highest security</li> </ul>

Biosafety Level	BSL-1	BSL-2	BSL-3	BSL-4
Safety glasses	✓	✓	✓	✓
Gloves	✓	✓	✓	✓
Proper shoes	✓	✓	✓	✓
Buttoned lab coats	✓	✓		
Tyvek suit			✓	✓
Respiratory protection			✓	✓

## NO

- Cell Phones
- Ear Buds
- Mouth Pipetting
- Loose Hair
- Handling Contact Lenses
- Applying Cosmetics
- Shorts/Skirts
- Eating/Drinking
- Animals (pets)
- Plants (house plants)

## What You Work With Can Make You Sick

Follow safe lab practices—and don't bring germs home with you.

**Always wash your hands** with soap and water...  
 • Right after working in the lab  
 • Just before you leave the lab

**Avoid contamination** while in the lab.  
 Don't eat, drink, or use things in your mouth (such as pens)  
 Don't touch your mouth or eyes  
 Don't put on cosmetics (like lip balm) or handle your contact lenses

**Don't carry dangerous germs** from the laboratory home with you.  
 Leave personal items outside of the lab so they don't contaminate them: cell phones, car keys, labels or laptops, MP3 player

**Keep work items off** of bench areas where you do experiments: backpacks, sandwiches, pencils, pens

**Leave lab supplies inside** the lab.  
 If you need take supplies out of the lab, keep them in a separate bag so you don't contaminate anything else

**Leave your experiment inside the lab so you can stay healthy outside the lab.**

EDC

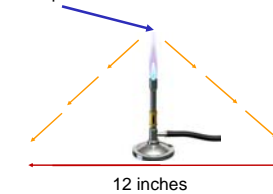
## Engineering Controls

- Biosafety cabinet used for procedures likely to generate splashes or aerosols
- Centrifuge must have sealed rotor head or centrifuge safety cups
- Autoclaves are large pressure cookers used to sterilize media and biological waste.



## Engineering Controls

Acceptable

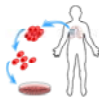


Better



## Working with Human, Mammalian Cells and Tissues

- Always follow Universal/Standard Precautions when working with human blood, body fluids, tissues, primary cell lines etc.
- ATCC (American Type Culture Collection) does not screen for every possible organism (mycoplasma, viruses) in the cell lines that it sells
- Handle all human cell lines, (including all cancer cell lines) at biosafety level 2
- Self inoculation, needle stick, is a potential risk with human cancer cell lines. There has been one reported case of a tumor from a needle-stick



## NIH Guidelines for Research Involving Recombinant DNA or Synthetic Nucleic Acid Molecules



- Register the research with Institutional Biosafety Committee
- The principal investigator must make sure that his/her laboratory complies with the NIH Guidelines for such research
- Non-compliance can result in UMass losing all NIH funding\*
- Injuries/ incidents to rDNA or synthetic nucleic acids must be reported to the Biosafety Officer ASAP
- Proper containment of these materials must be maintained. They are not allowed to enter the food chain, water supply, air stream, etc.
- Proper disposal requires destruction of the research material by autoclaving or incineration before the final product is discarded
- Example: Fish are maintained in sealed tanks and destroyed by autoclaving or incineration, they are not allowed to go "down the drain"

## HOW TO REMOVE YOUR GLOVES PROPERLY



Pull one glove near your wrist towards your finger tips until the glove folds over



Carefully grab the fold and pull towards your finger tips, as you pull, turn the inside of the glove outwards



Slide your finger from your glove free hand under the remaining glove. Turn you finger 180 degrees and pull the glove outwards and towards your finger tips



While you do this, the first glove will be encased in the second glove. Dispose of the gloves properly and...

**WASH YOUR HANDS**

## Personal Protective Equipment (PPE)

### Gloves

- No latex or powder gloves
- Change frequently
- Do not reuse; Discard used gloves immediately (if contaminated put in biohazard waste)
- Do not touch door handles with gloves; Never wear outside lab
- Report any allergies immediately to supervisor and EH&S
- Always wash hands after removing the gloves



## Secondary Containment

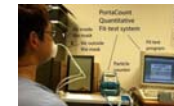
Always transport biological specimens in secondary containment



## Personal Protective Equipment (PPE)

### Respirator

- UMass has a Respiratory Protection Program
- All respirator users on campus (N-95, half-face, full-face, powered respirators) will be enrolled in this program
- Use of respirators on UMass campus is not recommended without a fit test
- Visit website <https://ehs.umass.edu/respiratory-protection-program> or call 413-545-2682



## Biological Safety Cabinet



## What's Wrong With This Picture?



## OSHA Bloodborne Pathogen Standard

29 CFR 1910.1030

- Human Blood
- Human Blood Components
- Human Tissues
- Human Cell Lines



Image by SucceedManagementSolutions, LLC



Bloodborne Pathogens

## OSHA 1910.1030 Bloodborne Pathogens Standard

- Federal standard to protect workers that may be exposed to human materials (blood, serum, tissues) that may have **HIV, Hepatitis B and Hepatitis C**
- Free vaccinations to Hepatitis B for employees that are at risk of exposure
- Free medical evaluations for incidents of exposure
- Implementation of engineering and work practice controls
- Proper labelling of risks



## Lab Acquired Infections (LAIs)

- The causative incident for most LAI's is unknown.....**ONLY 20% are from a recognized incident**

- Aerosols most likely route of exposure



- Always be careful when manipulating biological materials

### Use of

- ✓ Centrifuge
- ✓ Vortex
- ✓ Blender
- ✓ Homogenizer
- ✓ Sonicator

### Common lab procedures

- ✓ Vigorous shaking
- ✓ Pouring, Spraying
- ✓ Opening lyophilized cultures
- ✓ Flaming loops/needles
- ✓ Changing animal bedding

## Accidental Exposure Procedure

Needle sticks, Cuts, Animal bite/scratch or Splash to eye/mouth

**CALL 911 if a serious accident (UMPD: 413-545-3111)**

1: Stop work immediately and wash injured or exposed area ASAP with soap and water for at least **15 minutes**

2: Do not ignore any exposure; Always seek medical treatment at University Health Services (UHS) or an Emergency room for post exposure evaluation/treatment within **2 hours of incident**



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## Accidental Exposure Procedure

Needle sticks, Cuts, Animal bite/scratch or Splash to eye/mouth

3: All incidents/accidents must be reported to Environmental Health and Safety (EH&S) (413-545-2682) and lab supervisor; NIH may need to be notified by EH&S

Complete and submit the Laboratory Incident Report Form: <http://www.ehs.umass.edu/lab-incidents-and-lab-incident-report-form>

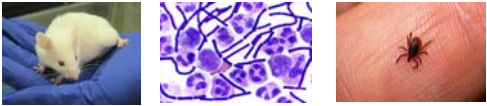
4: Complete and submit the Notice of Injury (NOI) form to the UMass Amherst Human Resources (HR) within 48 hours of the incident: <http://www.umass.edu/humres/notice-injury-form>



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## Medical Surveillance

- If immunosuppressed (taking steroids, pregnant, no spleen, etc.), you are more at risk
- If ill, seek medical attention and inform Doctor what you work with (animals, microbial agents etc.)



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## Principles of Sterilization and Disinfection

A **Sterilization** procedure is one that kills all microorganisms, including high numbers of bacterial spores. Sterilization can be accomplished by autoclaving.

Sterilizing	Best	This is to decontaminate a space: No pathogens left alive & zero contamination.
Disinfecting	Better	This is cleaning for Health: Removing some germs, creating a healthier space.
Cleaning	Good	This is for appearance: Dirt, grime, and debris removal.

**Disinfection** is generally a less lethal process than sterilization. It eliminates nearly all recognized pathogenic microorganisms but not necessarily all microbial forms (e.g., bacterial spores) on inanimate objects.

Reference: Principles in Microbiological and Biomedical Laboratories

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## Selection and Use of Disinfectants

Disinfection is the process that eliminates disease causing micro-organisms on inanimate objects, with the exception of bacterial spores

Bacterial spores ( <i>Bacillus anthracis</i> , <i>Clostridium tetani</i> etc.)
Mycobacteria ( <i>Mycobacterium tuberculosis</i> , <i>Mycobacterium avium</i> etc.)
Non-enveloped viruses (Polio, Rhinoviruses etc.)
Fungi ( <i>Candida</i> , <i>Aspergillus</i> , <i>Cryptococcus</i> etc.)
Vegetative Bacteria ( <i>Staphylococcus</i> , <i>Salmonella</i> , <i>E.coli</i> etc.)
Enveloped Viruses (Herpes simplex virus, measles virus, mumps virus, influenza virus, rubella virus etc.)

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## Comparison Chart For Commonly Used Disinfectants in Labs

<b>Alcohols</b> <b>70% Ethanol (v/v %)</b> 	<ul style="list-style-type: none"> <li>Contact time of 10 min</li> <li>Challenging due to evaporation</li> <li>Not effective for blood spills</li> </ul>
<b>Chlorine</b> <b>10% Bleach (v/v %)</b> 	<ul style="list-style-type: none"> <li>Effective for blood spills</li> <li>Contact time of 10 min for surfaces</li> <li>Contact time of 30 min for liquid waste</li> <li>Irritant to skin and mucous membranes</li> <li><b>Must make 10% working solution fresh daily</b></li> </ul>

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## Small Scale Biological Spill Clean up Procedure

Call EH&S for assistance with larger spills 413-545-2682


- Allow aerosols/dust to settle
- Use appropriate PPE
- Never pick up broken glass or sharps with bare hands; use tongs, forceps etc.
- Do not pour disinfectant directly onto the area of spill
- Cover the spill with paper towels or absorbent material
- Pick up as much of the spill as possible and place in biohazard waste
- Add appropriate disinfectant and follow manufacturer's recommendations for contact time
- Reapply disinfectant if necessary; wipe down vertical surfaces also
- Place all clean-up materials in regular waste (second disinfection)



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### Bleach Facts

- Buy 5.25% concentration or higher; if concentration is not listed on the container, **don't use it**
- Check code on bottle for expiration date
- Code cipher: **E216145**  
Read the last three digits on the right (Julian calendar date)  
**145= May 25<sup>th</sup>**
- Read the next digit (to left) = year manufactured; **16 = 2016**
- Ignore other digits/letters
- Concentrated bleach is only stable for one year from date of manufacture (E216145 = 5/25/2016 which is the date it was made)
- Read labels thoroughly



Patented formula splashes less so bleach goes where you want it.  
Powerfully whitens whites better than detergent alone.  
Great for deep cleaning your laundry and your home, with greater control as

Not for sanitization or disinfection. To sanitize and disinfect, use Clorox® Regular-Bleach.

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### Biological Waste

**Solid**


- Recombinant DNA or synthetic nucleic acid materials (soil, plants, bacteria, animals, etc.)
- Materials containing or contaminated with blood or body fluids;
- Pipettes and pipette tips;
- Animal waste; specimens
- Disposable labware (flasks, tubes, agar plates, bottle, vials)
- Contaminated gloves, other PPE, wipes etc.

**Liquid**

- Cell culture waste
- Other liquid biological waste

**Mixed Biological/Chemical**

- Call EH&S for information



Biohazard Box Waste

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### Decontamination of Biological Liquids

**Biological Liquid Waste can be:**

- Cell culture waste
- Other liquid biological waste

100 mL Concentrated Bleach + 900 mL Liquid Waste


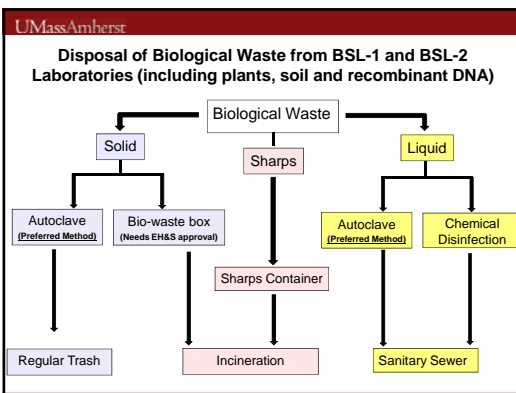


Contact Time: 30 minutes

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### Biological Waste

- All Biological waste must be kept in a leak proof and covered container labeled with a biohazard symbol
- When the container is not in use, keep the lid closed
- Transport full bags in a secondary container; never place directly on floor as it can lead to a spill/leak
- All biological waste must be deactivated before final disposal (autoclaved, chemically disinfected or sent off-site for incineration)

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### Autoclaving Biological Waste


**End Point:** achieve sterilization; kill all living organisms

**Mode of Action:** Steam

**Parameters:**

- Time (minimum) 60 min
- Temperature 121°C (250°F)
- Pressure 15 psi

- Do not autoclave chemicals or radioactive materials
- Do not use as a reaction vessel for experiments



### Autoclaving Biological Waste

- Only use clear autoclavable bags
- Only use autoclavable secondary containers (Nalgene or Stainless Steel)
- Add 50 -100 ml of water into the bag to promote steam production
- Do not overfill the bags; remove the bag from the waste receptacle when it's approximately 2/3<sup>rd</sup> full



### Autoclaving Biological Waste

- Tie bags loosely; Do not tape the bag shut (leave room for a gloved finger to fit into neck)
- Loosen caps for liquid containers or use vented caps
- Do not overload the autoclave
- Place completed label (below) on cooled bag

NON-INFECTIOUS AUTOCLAVED BIOLOGICAL WASTE FROM UMASS AMHERST TREATED IN ACCORDANCE WITH MA-DEP AND MA-DPH REGULATIONS  
 DATE TREATED: \_\_\_\_\_  
 BUILDING: \_\_\_\_\_  
 PRINCIPAL INVESTIGATOR: \_\_\_\_\_



### What Happened?



### Autoclaving Biological Waste

Select appropriate cycle for your biological waste (use preprogrammed cycles if available)

**Remember: Do not use fast exhaust or gravity cycle for autoclaving liquids; liquids will boil over and cause a spill**

- **Required cycle time for biological waste is 60 min**

**Remember: cycle time will increase depending upon number of bags and quantity of biological waste**



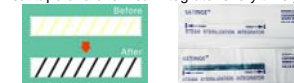
### Autoclaving Biological Waste: Cycle Complete

- Wear PPE (heat resistant gloves, buttoned lab coat, covered shoes, safety goggles/ face shield)
- Stand behind the autoclave door before opening
- Allow liquids to cool down for at least 10 min before removing them
- After autoclaving, place "Autoclave Label" on all autoclaved clear bags of biological waste
- Before final disposal, place all autoclaved clear bags (with the autoclave stickers on them) inside a black trash bag



### Autoclaving Biological Waste: Verification

- Chemical Verification: Verifies if autoclave achieved the temperature of 121°C (250°F) during the cycle
- Use chemical tape or a chemical integrator every time you run a cycle



- Biological Verification: Verifies if autoclave achieved sterilization
- Use biological spore vials every month



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### Complete the Autoclave Log Sheet – Required by Law

UMass Amherst Biological Waste Treatment Log - Do not leave any column blank

Date	Quantity	Container Type	Antiseptic	Cycle #	Principal Investigator	Print Year Name		Quality Control Results	
						Year	Signature	Spore Positive	Spore Negative
12.7.16	1	<input checked="" type="checkbox"/> Sharp <input type="checkbox"/> Fluids <input type="checkbox"/> Other	<input checked="" type="checkbox"/> Biological Waste <input type="checkbox"/> Sterile Supplies	# 4	C. Rogers		Judy LaDuc	0 Yes	0 Yes**
		<input type="checkbox"/> Sharp <input type="checkbox"/> Fluids <input type="checkbox"/> Other	<input type="checkbox"/> Biological Waste <input type="checkbox"/> Sterile Supplies				Judy LaDuc	0 Yes	0 Yes**
		<input type="checkbox"/> Sharp <input type="checkbox"/> Fluids <input type="checkbox"/> Other	<input type="checkbox"/> Biological Waste <input type="checkbox"/> Sterile Supplies					0 Yes	0 Yes**

\*Rerun the autoclave cycle with a new indicator. If the indicator does not change, run spores to validate correct operation. If spores fail, post an "Out of Order" sign on the autoclave; Notify responsible person and/or call autoclave repair service.


Contact EH&S for alternative waste disposal methods if another autoclave is not available.

\*\* If checking "Yes" and performing spore test, document results on this page as well as on the spore testing log at the back of the booklet. 

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### Documentation: Good Laboratory Practice

- Principles help to define and standardize the planning, performance, **recording**, reporting, monitoring and archiving processes within research institutions
- Entries must be made in ink (**Blue** or **Black**)
- Cross out mistakes using a single line

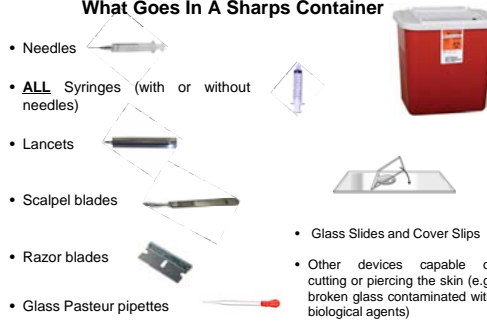


Ref: World Health Organization; GLP HANDBOOK, Chapter 2 • Good Laboratory Practice Training

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### What Goes In A Sharps Container

- Needles
- ALL** Syringes (with or without needles)
- Lancets
- Scalpel blades
- Razor blades
- Glass Pasteur pipettes
- Glass Slides and Cover Slips
- Other devices capable of cutting or piercing the skin (e.g. broken glass contaminated with biological agents)




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### Safety with Sharps

**WARNING**  
Needle stick injury can expose you to infectious diseases such as Hepatitis and HIV. TO AVOID INJURY...

- Do not force sharps into container
- Do not put fingers inside container
- Do not remove needle
- Do not bend or break needle
- Do not recap needle

Keep your hands behind the sharp tip.



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### Safety with Sharps Containers

Stop using sharps container when 2/3 full or filled to FULL line.

Do Not Shake Container to Make More Room!

Close sharps container as instructed on label. Different containers have different closures.

MAKE SURE container is not overfilled or damaged.

CHECK that container is large enough to fit your sharp.



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### Sharps Containers Disposal

- Visit EH&S website <http://www.ehs.umass.edu/>
- Click on "Request It" (see yellow arrow)
- Click on "Hazardous Waste Pickup" link on the dropdown list





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## Sharps Containers Disposal

• Complete the required fields and submit the form

Hazardous Waste Pickup Request

Name:

Phone Number:

Principal Investigator (request on behalf of):

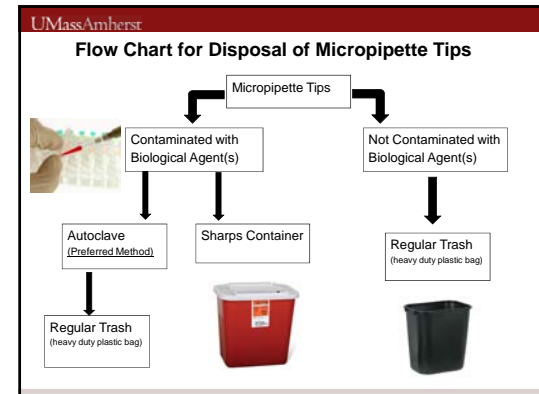
Department:

Pick-up Location:

Item # | chemical substance (identify all constituents and concentrations) | # containers / amount / unit OR specify other

1 | Sharps Container | 1 | 1 | other

submit



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**'Glass Only Box' only if clean, dry, and NOT CONTAMINATED with biological or chemical hazards**

- Glass slides
- Cover slips
- Test tubes
- Glass beakers
- Glass flasks
- Glass vials
- Other Glass items
- Serological pipettes
- Broken or Discarded

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## Shipping Biological Materials

Certain biological materials are regulated under the Department of Transportation (DOT) and the International Air Transit Authority (IATA) as hazardous materials and require specific packaging, labeling, and documentation

Use eShipGlobal program at: <http://www.ehs.umass.edu/shipping-hazardous-materials-instructions>

Contact EHS at 413-545-2682 with any questions

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## Field Research

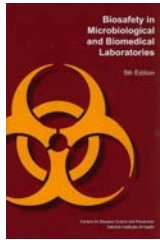
**Have Contingency Plan for:**

- **Physical hazards** extreme heat, extreme cold, noise, weather
- **Biological hazards** vector-borne diseases, venomous wildlife and insects, and poisonous plants
- **Chemical hazards** exposure to pesticides

**Field Research Considerations**

- Vaccination against potential pathogens (e.g. Tetanus, rabies)
- Training on when and how to use PPE

## Resources



- CDC/NIH Biosafety Manual free PDF download at:  
[http://www.cdc.gov/od/OHS/biosfty/bmb15/BMBL\\_5th\\_Edition.pdf](http://www.cdc.gov/od/OHS/biosfty/bmb15/BMBL_5th_Edition.pdf)
- UMass Environmental Health and Safety Biological Safety Website:  
<http://www.ehs.umass.edu/biological-safety>
- UMass Environmental Health and Safety Fact Sheets  
<http://www.ehs.umass.edu/fact-sheets>

## American Biological Safety Association



- ABSA was founded in 1984 to promote biosafety as a scientific discipline and serve the growing needs of biosafety professionals throughout the world.
- <https://absa.org/>
- Risk Group Database
- Laboratory Acquired Infection (LAI) Database