

Biosafety Practices and Procedures

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<http://www.qu.edu.qa/offices/research/brc/biosafety/index.php>

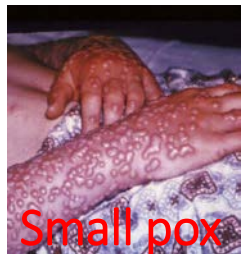
Definitions

BiOhazard

An agent of biological origin that has the capacity to produce negative effects on **humans, plants or animals.**

BiOsafety

The application of: lab practices and procedures, specific lab facilities and safety equipment to protect against exposure to potentially infectious material



What is a Biohazardous Material?

Bacteria

Viruses

Parasites

Fungi

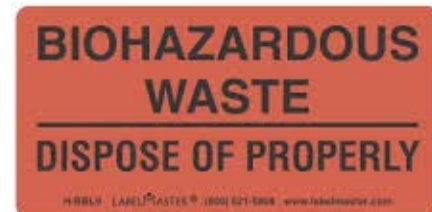
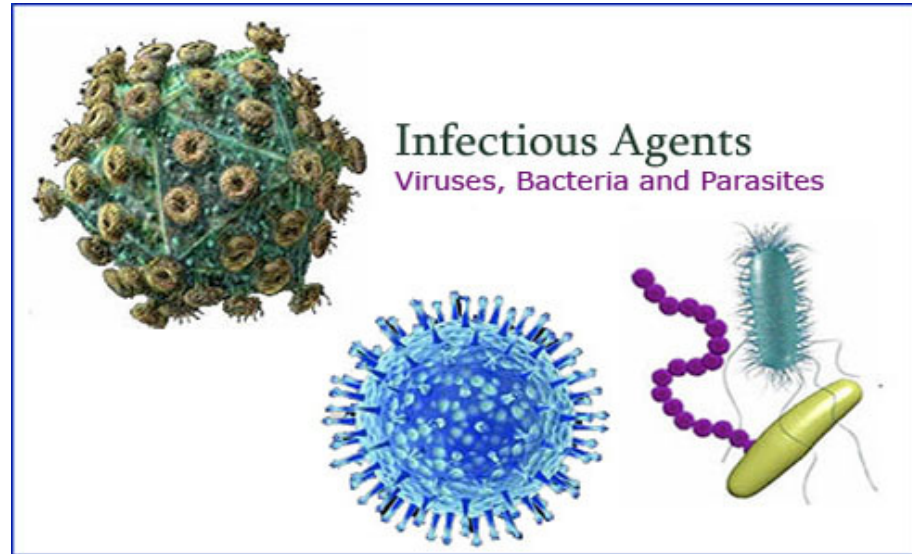
Prions

Human and Non-Human Primate Material

Recombinant DNA

Animals

Biological Toxins



How Can You Be Exposed?

Inhalation: 80% of lab acquired infections (LAI)

Perform experiments in biological safety cabinets

Use respiratory protection

Remaining 20% LAI are a combination of:

Percutaneous

Avoid sharps when possible, use plastic pipettes and disposable glassware

Mucous Membrane (eyes, nose and mouth)

Wash hands often, avoid touching face

Ingestion

Wash hands often, No eating, drinking, smoking or applying cosmetics



**All exposures must be reported to the PI,
Biosafety Officer, Office of Environmental Safety**

Hierarchy of Control Measures

- Administrative Level
- Engineering Level
- Work Practices
- Personal Protective Clothing or Equipment (PPE)

Administrative Level

- Authorization
- Written biosafety procedures required for the experimental procedures and equipment including inventory of biological agents or materials (Biosafety guidelines, MSDS, others)
- Laboratory personnel biosafety training
- Medical Surveillance (BSL 2 and above)
 - Immunization

Engineering Level

- Biosafety cabinets
- Animal containment caging systems
- Safety equipment (filtered or sealed equipment)
- Ventilation system
- Containment facilities
- **Eye washers**
- **Showers**
- Disinfectants






Personal Protective Equipment (PPE)

- Provides barrier against skin, mucous membrane or respiratory exposure to infectious material during procedures
- Prevent spread of contamination
- **Does not eliminate the hazard**
- Integrity wanes with use (i.e., change gloves frequently)



**Differs whether you
are working in
BSL1, 2, 4 or 4!**

Biosafety Levels

- BSL-1:**  agents not known to cause disease (in healthy adults)
- BSL-2:**  agents associated with mild disease (seasonal influenza, Adenoviruses, etc.)
- BSL-3:**  indigenous/exotic microorganism associated with human serious disease and with potential for aerosol transmission (H5N1 Influenza, MERS)
- BSL-4:** dangerous/exotic agents of life threatening nature with no available vaccine or treatment (EBOLA)

BSL 1: Work Practices and Procedures

- Applications
- **Non-infectious** agent and tissue culture, media preparation: E.coli K-12, Transgenic Plants, Plasmids, Fungi, Mold, Yeast.
- Prevent Cross Contamination
 - Keep cultures covered
 - **Flame instruments and containers**
 - Use sterile media and equipment
 - Keep hands or face away from cultures

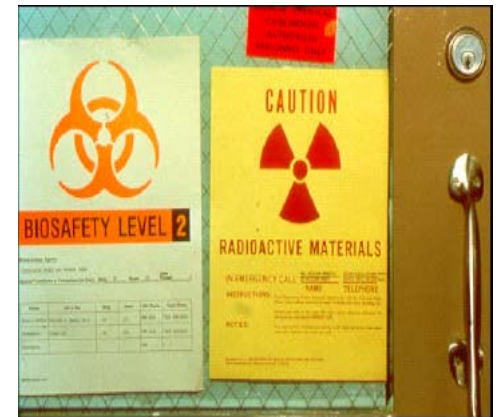
BSL 1: Work Practices and Procedures

- **Biosafety Procedures**

- May be conducted on open bench, usually with flame one.
- Wash hands often
- No mouth pipetting
- No eating or drinking in lab
- Minimize aerosol generation
- Decontaminate work surfaces
- Wear applicable PPE

BSL 2: Work Practices and Procedures

- Increasing emphasis on safety procedures and practices
- Increasing need for staff training
- Increasing need for competent supervision
- Biohazard signs and labels posted at entry door and regulated waste containers, respectively.
- Use of PPE as a barrier to exposure: lab coat, gloves, eye and face protection
- Some work on open bench allowed



BSL 2: Work Practices and Procedures

- **Reduce aerosol** generation by performing work in a **biosafety cabinet**:
 - Homogenizing
 - Vortexing
 - Vigorous mixing
 - Pipetting infectious liquids
 - Sonication
 - Pouring
- If breach occurs:
 - Evacuate lab, post spill sign
 - With appropriate PPE and disinfectant, decontaminate centrifuge, buckets, other items or areas



Correct Use of Biosafety Cabinets

- **Start Up**

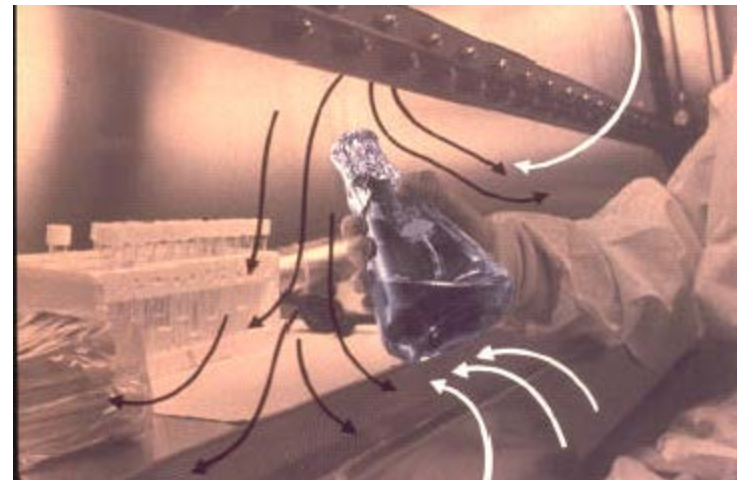
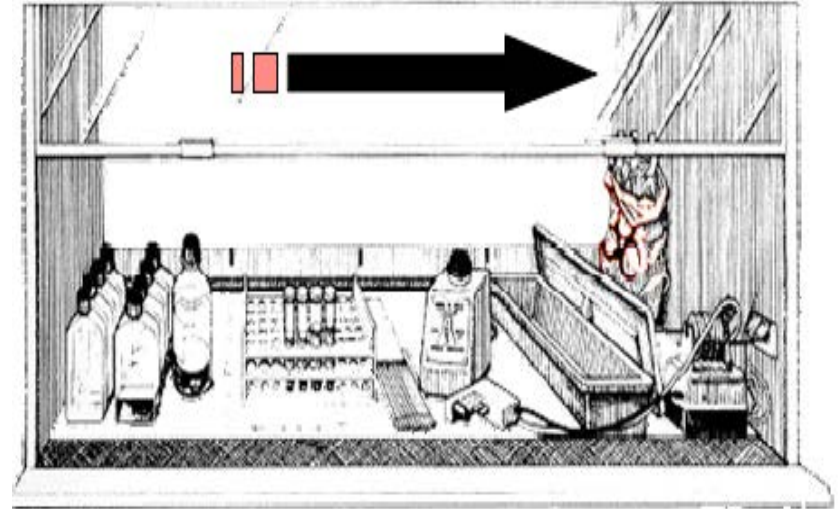
- Turn off ultraviolet light (if so equipped) as soon as you enter the room.
- Turn on all blowers and BSC illumination lights.
- Allow five minutes of operation to purge system; check flow alarm system audio and visual alarm function (if so equipped).
- Decontaminate readily accessible interior surfaces and items with a disinfectant (appropriate for the agents or suspected agents present) before loading and wait at least 10 minutes prior to start of work.



Correct Use of Biosafety Cabinets

- **During Use**

- Load supplies prior to work.
- Disinfect supplies before putting them in the cabinet.
- Do not overload cabinet.
- Separate clean and dirty side (better to use different hoods).
- Work in center of work area.
- Do not block front or rear grills.
- Minimize disruption of airflow (turbulence).
- Clean up spill promptly.
- Discard waste within the cabinet.



Correct Use of Biosafety Cabinets

- **Shut Down**

- Decontaminate and remove all items from interior work area.
- Decontaminate readily accessible interior surfaces with a disinfectant appropriate for the agents or suspected agents present.
- Turn on ultraviolet light (if so equipped).
- Allow five minutes of operation to purge system. Then wait at least 10 minutes.
- Turn off BSC blower.



Correct Use of Biosafety Cabinets

All BSCs that are used for handling biohazardous materials must be **recertified annually**

Use of Biosafety Cabinets

- **DO NOT** use Bunsen burners or open flames:
 - Fire hazard
 - Can damage HEPA filter
 - Interferes with proper air flow
 - Open flames react with disinfectants (flammables)

Safe Use of Centrifuge

- Use **sealed** tubes, rotors, and safety cups/buckets that are sealed with O-rings
- Inspect tubes, O-rings and rotors for wear, and buckets for cracks, chips, erosion, etc.
- Do not use aluminum foil to cap centrifuge tubes
- Clean and maintain gaskets and O-rings
- Change O-rings if compromised
- Load/unload centrifuge tubes, rotors and accessories in BSC
- Small, low speed centrifuges may be placed in a BSC; however, high speed centrifuges pose additional hazards
- Do not overfill tubes
- Balance buckets, tubes and rotors properly before centrifugation
- Wait 5 minutes (or 30 mins. for high speed centrifuge) after each run before opening
- Do not decant or pour off supernatant. Use a vacuum system with appropriate in-line reservoirs and filters



Reminder: Always Minimizing Aerosols

- Use careful pipetting practices
- Avoid drops onto hard surfaces
- Wipe up spills promptly with appropriate disinfectant
- For ejection of liquid from micropipette
 - No blowout
 - No pressure ejection
 - Use wall contact
- Use capped tubes when mixing, blending, or vortexing
- Pour liquids carefully
- Avoid bubbles

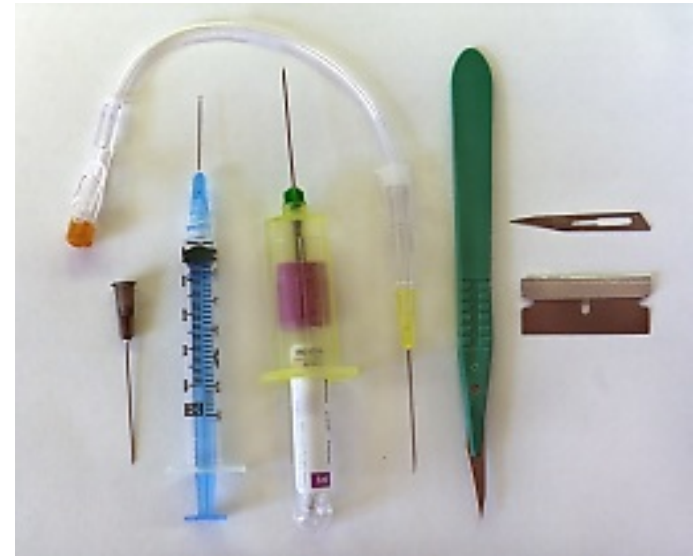
Careful Pipetting Techniques

- Never blow out last drop in pipette
- Use pipetting aids with filters
- Never mix by suction and expulsion (mix by vortex)
- Discharge liquid down side of container, using tip-to-wall contact
- Work over plastic-backed absorbent matting (ensure it doesn't slide forward or backward blocking air grill)



Use Extreme Care with Sharps

- **Avoid use sharps unless absolutely required as part of a process**
- Percutaneous exposure risk
 - Employ safe work practices
 - **Utilize safe sharp devices**
- Aerosol exposure risk
 - **Use biosafety cabinet for removal of air from needle**
- Use mechanical methods for needle removal
- **Never bend, recap or manipulate sharps by hand**
- **Keep hands away from needle**
- **Dispose all sharps in red sharps container.**



Vacuum Line HEPA Filter Protection



- Placed in between the vacuum system and the aspiration flasks

Reminder: Biohazardous Waste Includes...

Blood and Blood Products Especially of human and NHP

Cultures/Stocks of Infectious Agents and Associated
Biologicals

Animal Carcasses and Bedding

Sharps

Glass / Volumetric Pipettes

Primary and Established Human and Animal Cell Lines

BL1 and BL2 Waste Disposal Procedures

Liquid:

chemically treated prior to drain disposal

10% (total volume) sodium hypochlorite solution (Bleach, wescodyne, etc) for 20-30 minutes

Solid:

BL1 and BL2 non recombinant waste

- Dispose of directly into red/orange plastic waste containers or cardboard boxes lined with red biohazard bags

Recombinant DNA

- Any viable organism containing recombinant DNA must be rendered noninfectious prior to disposal
- Steam sterilization, chemical inactivation or UV.

BSL2 Waste Disposal

Liquid

Inactivate all liquid waste with then dispose in appropriate containers or autoclave

Solid

Autoclave all solid waste

Avoid using Sharps and Glass if possible

Sharps Disposal

All sharps should be disposed of directly into an approved sharps container immediately after use

When the sharps container is 2/3 full:

- Place in biohazard waste container

- Arrange for pick up from Building Services

CONTAINERS FOR WASTE DISPOSAL



CHEMICAL

NOTE: A Chemical Waste Disposal Form must accompany all waste containers

Liquid:

- Aqueous solutions containing toxic metals
- Concentrated acidic solutions (place in thick glass or plastic containers)
- Concentrated alkaline solutions (place in plastic containers)
- Mercury
- Ag salts (recycled)
- Used vacuum pump oil (recycled)

Gross solid:

- Silica and alumina gels

Solid:

- Contaminated PPE
- Kimwipes
- Chemicals no longer needed or wanted may remain in their original containers

Recycle:

- Organic solvents
- Halogenated organic solvents

Request a hazardous materials pick-up on-line at the following web page:

<http://capsnet.usc.edu/EHS/HazWastePickUpForm.cfm>



CLEAN GLASS

- Intact or broken glass not contaminated with chemical or biological agents
- Rinse 3 times and deface label before disposal
- Use heavy, puncture-resistant cardboard lined with plastic bag

CONTAMINATED GLASS

- Glass contaminated with chemicals only
- Use heavy, puncture-resistant cardboard lined with plastic bag
- Label box "Contaminated Glass"
- No microscope slides

BIOMEDICAL

Solid Material:

- Contaminated with human/animal fluids/blood or other biohazards e.g. gauze, paper towels, plastic-backed absorbents or bench coat, etc.
- Petri dishes
- Plastic pipettes
- Plastic pipette tips
- Plastic Vacutainer tubes
- Culture vials
- Live or attenuated vaccines
- Gloves and other Personal Protective Equipment worn while working with biohazardous material or animals

Tabletop container:

- All items may be placed in small tabletop container, EXCEPT serological pipets.
- Place smaller waste bags into larger biohazardous waste can
- Do not overfill! NO SHARPS!

Liquids:

Decontaminate by an approved method (e.g. 10% bleach, 20 minute contact time); dispose down sink



PATHOLOGICAL

- Organs, tissues, and body parts removed by trauma, surgery, autopsy, or other medical procedure
- Animal carcasses contaminated with infectious materials
- Place materials in leak-proof bag



SHARPS

- Needles
- Razor blades, scalpels
- Microscope slides
- Glass pipettes
- Dental wires
- Glass Pasteur pipettes
- Blood vials (glass Vacutainer tubes)
- Any contaminated material which can puncture or penetrate the skin or a Red Bag



PHARMACEUTICAL

Outdated and/or empty vials, broken ampoules, etc.



CHEMOTHERAPY

Outdated and/or empty vials, broken ampoules, etc.



OSHA Bloodborne Pathogens Training

Human blood, unfixed tissue, primary human cell culture, other potentially infectious materials



Bloodborne Pathogens

What are the three bloodborne pathogens you need to be concerned about and what is the risk of transmission each?

BLOODBORNE PATHOGENS

The average risk for transmission after a percutaneous exposure is approximately

HEPATITIS B (HBV)	30%
HEPATITIS C (HCV)	3 %
HIV	0.3%

Bloodborne Pathogens

Hepatitis B

**the most highly transmissible
but greatly reduced by vaccine use!!**

Hepatitis C

**No vaccine, but early identification and treatment offers
some hope**

HIV

Early treatment greatly reduces the risk

Exposure Prevention strategies

Vaccination

Work Practices/Standard Precautions

PPE/Engineering Controls

Exposure response

Education/policies

Hepatitis B vaccine

Typically, must be offered to all employees with exposure risk

A 3 shot series- must complete

Antibody status checked after series completed

No need for routine “boosters”

Work Practice Controls

- Standard Precautions
 - Treat all blood and body fluids as infectious!
 - Gloves for contact with blood, body fluids, moist mucous membranes, non-intact skin, secretions and contaminated items
 - Other Personal Protective Equipment (PPE) as needed
 - Remove PPE when task completed and moving to a clean area
 - Disinfect hands after contact with blood/body fluids/tissue, even if gloves are used
 - Disinfect equipment/surfaces per policy

Post Exposure Response

How can you be exposed?

- Percutaneous
- Mucous membrane
- Broken Skin
- Animal Bites

Immediate Response

- First Aid- wash area

Reporting- Environment and Biosafety office -All animal bites must be reported.

Follow-up required

First Aid and Spill kits

- Each lab is provided with a first aid, a biological and chemical spill kits.
- Make sure to locate them in your lab, read the instructions on how to use these kit, and regularly check the components to see they are full and intact.
- Guide on how to use spill kit is provided on the BRC biosafety page.





**SAFETY
FIRST**