Biological Laboratory Safety
University of Colorado at Boulder

Environmental Health and Safety
www.colorado.edu/ehs
(303) 492-6025
ehsbio@colorado.edu
Introduction

The purpose of this training module is to familiarize the Principal Investigator and lab personnel with good microbiological practices which include recognizing risk groups for biological materials, appropriate containment levels, and personal protective clothing and equipment.
What is Biosafety?

- The measures employed to avoid infecting oneself, others or the environment when handling biohazard materials
- Practicing good microbiological techniques is the single most effective means of preventing exposures
Why Biosafety Practices?

- Protection for:
  - workers
  - "products"
  - co-workers
  - lab support personnel
  - lab or field animals
  - environment
Definition

Biohazard

An agent or material of biological origin that has the capacity to produce deleterious effects on humans, i.e. microorganisms, toxins and allergens derived from those organisms; and allergens and toxins derived from higher plants and animals.

Examples:

- Viruses, bacteria, fungi, and parasites
- Blood and body fluids, as well as tissues from humans and animals
- Transformed cell lines or human cells
Definition

Pathogen

- A disease causing agent.
- Most are infectious microbes, such as bacteria or viruses, which are capable of causing disease. Other parasites, such as fungi and protozoans, are also considered pathogens. Because not all microbes are harmful, pathogens refer specifically to those that can cause disease or other harm.

Examples:
- Viruses, bacteria, fungi, and parasites
Definition

**Infectious Agent**

*An agent of biological origin that is transmissible through contact, ingestion, inhalation, or animal and insect bites that may cause disease. If they are pathogenic then they will cause an infectious disease.*
Definition

Zoonotic Disease

- An infectious disease that can be transmitted from animals to humans.
- A number of infectious diseases, including viruses, bacteria, and parasites, can be transmitted from animals to people through a variety of infection routes, including animal bites, vectors (i.e., insects), and animal-to-human contact (i.e., inhalation of respiratory droplets or skin-to-skin contact). Some examples of common zoonotic diseases include lyme disease, rabies, ringworm, and plague.
Classification of Agents

- The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid (rsNA) Molecules established a classification and assigned human etiological agents into four risk groups on the basis of hazard and requirements for each biosafety level of containment. http://oba.od.nih.gov/rdna/nih_guidelines_oba.html

- The CDC guideline, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) uses the World Health Organization (WHO) recommendations for an agent risk group classification for laboratory use that describes four general risk groups requirements for each biosafety level of containment. http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
Which Guidelines Should I Follow?

For all rsNA activities:

For all non-rsNA activities:
- The CDC guideline, Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition: http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
Risk Group Classifications

A list of Risk Group Agents can be found in the NIH Guidelines:
A list of Agent Summary Statements can be found in the BMBL:

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Risk Group 1</td>
<td>Agents that are not associated with disease in healthy adult humans.</td>
<td>(No or low individual and community risk) A microorganism that is unlikely to cause human or animal disease.</td>
</tr>
<tr>
<td>Risk Group 2</td>
<td>Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are <em>often</em> available.</td>
<td>(Moderate individual risk; low Community risk) A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but Effective treatment and preventive measures are available and the risk of spread of infection is limited.</td>
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## Risk Group Classifications

Currently, UCB prohibits the possession or use of Risk Group 3 or 4 Agents

<table>
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<tbody>
<tr>
<td>Risk Group 3</td>
<td>Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions <em>may be available</em> (high individual risk but low community risk).</td>
<td>(High individual risk; low community risk) A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective Treatment and preventive measures are available.</td>
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<tr>
<td>Risk Group 4</td>
<td>Agents that are likely to cause serious or Lethal human disease for which preventive or therapeutic interventions are <em>not usually available</em> (high individual risk and high Community risk).</td>
<td>(High individual and community risk) A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.</td>
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Risk Group Agents

- **Examples of Risk Group 1 Agents:**
  - *Bacillus subtilis*
  - *Escherichia coli* K-12
  - *Pichia pastoris*

- **Examples of Risk Group 2 Agents:**
  - *Acinetobacter baumannii*
  - *Salmonella typhimurium*
  - *Staphylococcus aureus*
  - *Cryptosporidium* including *C. parvum*
  - amphotropic and xenotropic strains of murine leukemia virus
  - Hepatitis A, B, C, D, and E viruses
  - Epstein Barr virus
  - Human cells, blood, blood products, bodily fluids, tissue
Risk Group Agents

Examples of Risk Group 3 Agents:
- *Burkholderia (Pseudomonas) mallei, B. pseudomallei*
- *Yersinia pestis*
- *Histoplasma capsulatum, H. capsulatum var.. duboisii*
- Flaviviruses (Togaviruses) - Group B Arboviruses
- Prions
- Retroviruses

Human immunodeficiency virus (HIV) types 1 and 2
Human T cell lymphotrophic virus (HTLV) types 1 and 2
Simian immunodeficiency virus (SIV)

Examples of Risk Group 4 Agents:
- *Ebola virus*
- *Smallpox*
- Herpesvirus simiae (Herpes B or Monkey B virus)
- Hemorrhagic fever agents and viruses as yet undefined
 Routes of Transmission

- Most common routes of transmission in laboratory which can lead to laboratory acquired infections:
  - direct skin, eye or mucosal membrane exposure to an agent;
  - parenteral inoculation by a syringe needle or other contaminated sharp, or by bites from infected animals and arthropod vectors;
  - ingestion of liquid suspension of an infectious agent, or by contaminated hand to mouth exposure;
  - inhalation of infectious aerosols; and
  - vectors, such as mosquitoes, ticks, etc.
Risk Assessment

Once the investigator has decided on the agent or recombinant molecule, then he or she must conduct an assessment of risk.

1) Identify agent hazards and perform initial assessment of risk which includes:
   - agent in use/use of recombinant DNA
   - virulence/pathogenicity/infectious dose
   - environmental stability e.g. temperature, sterility, UV sensitivity
   - mode of transmission e.g. contact, ingestion, inhalation, animal bite
   - quantity/concentration/volume used (additional requirements >10L, notify Biosafety Officer)
   - vaccine/treatment availability
   - allergenicity
2) Identify laboratory procedure hazards which may include:
   - use and disposal of sharps
   - aerosol generating procedures e.g. sonication, vortexing, homogenization, flow cytometry, pipetting
   - volume of culture used (additional requirements >10L, notify Biosafety Officer)
   - transport of agents
   - use of chemicals and radionuclides
   - use of animals
   - work on open bench top
   - disposal of infectious waste
3) Make a determination of appropriate biosafety level of containment and select additional precautions indicated by risk assessment.

4) Evaluate proficiencies of staff regarding safe practices and integrity of safe equipment.

5) Review risk assessment with Biosafety Officer, subject matter expert, and IBC.
Containment

- The objective of a good lab biosafety program is the containment of potentially harmful biological agents and materials.
- Describes safe methods, facilities and equipment for managing infectious materials in the lab where they are being handled or maintained.
- Reduces or eliminates exposure of lab workers, other persons, laboratory animals, and the outside environment to potentially biohazardous agents and materials.
Biosafety Levels of Containment

There are four Biosafety Levels:

- **BSL1** - agents not known to cause disease
- **BSL2** - agents associated with human disease which are easily treatable or prevented by vaccination
- **BSL3** - indigenous/exotic agents associated with human disease and with potential for aerosol transmission
- **BSL4** - dangerous/exotic agents of life threatening nature
Biosafety Levels of Containment

As the level Moves UP

- the risk of the organism to humans, animals, plant and/or the environment increases
- the procedural and facility requirements increases
- the level of containment required increases
- the degree of protection for personnel, the environment and the community increases
### BMBL Summary of Biosafety Levels

#### Biological Laboratory Safety Training

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**Table 2. Summary of Recommended Biosafety Levels for Infectious Agents**

<table>
<thead>
<tr>
<th>BSL</th>
<th>Agents</th>
<th>Practices</th>
<th>Primary Barriers and Safety Equipment</th>
<th>Facilities (Secondary Barriers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not known to consistently cause diseases in healthy adults</td>
<td>Standard microbiological practices</td>
<td>No primary barriers required.</td>
<td>Laboratory bench and sink required</td>
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<td>PPE: laboratory coats and gloves; eye, face protection, as needed</td>
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<tr>
<td>2</td>
<td>• Agents associated with human disease</td>
<td>BSL-1 practice plus:</td>
<td>Primary barriers:</td>
<td>BSL-1 plus:</td>
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<tr>
<td></td>
<td>• Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure</td>
<td>• Limited access</td>
<td>• BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials</td>
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<tr>
<td></td>
<td></td>
<td>• Biohazard warning signs</td>
<td>PPE: Laboratory coats, gloves, face and eye protection, as needed</td>
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<tr>
<td></td>
<td></td>
<td>• &quot;Sharps&quot; precautions</td>
<td></td>
<td>Autoclave available</td>
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<td></td>
<td></td>
<td>• Biosafety manual defining any needed waste decontamination or medical surveillance policies</td>
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<td>3</td>
<td>• Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure</td>
<td>BSL-2 practice plus:</td>
<td>Primary barriers:</td>
<td>BSL-2 plus:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Controlled access</td>
<td>• BSCs or other physical containment devices used for all open manipulations of agents</td>
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<td></td>
<td></td>
<td>• Decontamination of all waste</td>
<td>PPE: Protective laboratory clothing, gloves, face, eye and respiratory protection, as needed</td>
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<tr>
<td></td>
<td></td>
<td>• Decontamination of laboratory clothing before laundering</td>
<td></td>
<td>Physical separation from access corridors</td>
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<tr>
<td>4</td>
<td>• Dangerous/exotic agents which post high individual risk of aerosol-transmitted laboratory infections that are frequently fatal, for which there are no vaccines or treatments</td>
<td>BSL-3 practices plus:</td>
<td>Primary barriers:</td>
<td>BSL-3 plus:</td>
</tr>
<tr>
<td></td>
<td>• Agents with a close or identical antigenic relationship to an agent requiring BSL-4 until data are available to redesignate the level</td>
<td>• Clothing change before entering</td>
<td>• All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure suit</td>
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<tr>
<td></td>
<td>• Related agents with unknown risk of transmission</td>
<td>• Shower on exit</td>
<td></td>
<td>Separate building or isolated zone</td>
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<td></td>
<td></td>
<td>• All material decontaminated on exit from facility</td>
<td></td>
<td>Dedicated supply and exhaust, vacuum, and decontamination systems</td>
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<td>Other requirements outlined in the text</td>
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General Lab Requirements

- An Approved Institutional Biosafety Committee (IBC) Application is Required for All Biological Research

- Knowledgeable Principal Investigator and Lab Personnel
  - aware of potential hazards
  - proficient in practices & techniques

- Administrative Controls
  - appropriate signage (emergency and biohazard notifications signs, biohazard, biohazardous waste, non-infectious stickers, etc.)
  - training
General Lab Requirements

- **Biosafety Levels of Containment (BSLs)**
- **Laboratory Practice & Technique**
  - standard practices
  - special practices
- **Safety Equipment (Primary Barriers)**
- **Facility or Laboratory Design and Construction (Secondary Barriers)**
General Lab Requirements

- Biosafety Cabinets (BSCs) – Required for BSL-2 whenever aerosol generating procedures are performed
- Personal Protective Clothing
  - gloves
  - lab coats
  - eye and face protection
- Pipetting Devices
- Safety Centrifuge Cups and Rotors
Primary Barriers

Safety Equipment (e.g. Biological Safety Cabinet) and Personal Protective Equipment (PPE) are used to protect lab personnel working directly with infectious materials (e.g. gloves, safety glasses, lab coat).

Secondary Barriers

Facility or Laboratory Design and Construction are used to protect from exposure to infectious materials.
Biosafety Level 1 (BSL-1)

Introduction

Suitable for work involving well characterized agents *not known to cause disease in healthy adult humans* and of *minimal potential hazard* to laboratory personnel and the environment.

Examples:

- *Bacillus subtilis*
- *Infectious canine hepatitis virus*
- *E. coli* (non-infectious common lab strains, i.e. DH5alpha, K-12)
Biosafety Level 1 (BSL-1) Standard Work Practices

- Limited or restricted access when experiments are in progress
- Decontaminate work surfaces daily and after a spill
- Use mechanical pipetting devices
- No eating or drinking in work area
- Wash hands frequently
- Minimize splashes and aerosols
- Written procedures for safe handling of sharps (i.e. needles, scalpels, razor blades, broken glass, pipettes)
Biosafety Level 1 (BSL-1) Standard Work Practices

- Posted Biohazard Notification sign on lab entry door(s).
  http://www.colorado.edu/ehs/pdf/BiohazardLabEntry.pdf
- PI must ensure lab personnel receive appropriate training regarding their duties, precautions to prevent exposures, and exposure evaluation procedures
- Follow the EHS Biowaste Policy
- Contact CU Integrated Pest Management Program for insect or rodent issues – (303) 492-5522
Biosafety Level 1 (BSL-1)
Safety Equipment (Primary Barrier)

- Laboratory coats
- Disposable gloves
- Safety glasses
- Face protection as needed
Biosafety Level 1 (BSL-1)
Laboratory Design (Secondary Barrier)

Requirements:
- Laboratories have doors
- Sink for hand washing
- Work surfaces & floors easily cleaned and decontaminated
- Bench tops are impervious to water
- Sturdy furniture
- Windows fitted with fly screens
- No special ventilation
- Normal building construction
Biosafety Level 2 (BSL-2)

Introduction

Suitable for work involving well characterized agents that are associated with human disease that is rarely serious and for which preventative or therapeutic interventions are often available to laboratory personnel and the environment.

Examples:

- Schistosoma - including S. haematobium, S. intercalatum, S. japonicum, S. mansoni, S. mekongi
- Salmonella – all species
- Human cells or cell lines, bodily fluids, tissue
Biosafety Level 2 (BSL-2)
Standard Work Practices

Same as BSL-1 Practices

Special Work Practices

- Restricted lab access
  - limited or no access if immunocompromised
  - medical surveillance of lab personnel as appropriate
- Lab personnel
  - aware of potential hazards
  - proficient in practices/techniques
Biosafety Level 2 (BSL-2)

Special Work Practices

- Biosafety manual specific to lab and biological research
  - Biohazard Control Plan (part of IBC application)
  - Post Exposure Plan (may be required as part of IBC application depending on biological agents and materials used)

- Report accidents immediately
  - seek medical attention
  - notify supervisor or Principal Investigator
  - notify Biosafety Officer at EH&S (492-6025)
  - complete Risk Management Employee Injury Report
    https://urm.cu.edu/docs/forms/incident_report_form.asp
    or Needle Stick or Bodily Fluid Exposure Form
    https://urm.cu.edu/docs/forms/needlestick_incident_report_form.asp
Biosafety Level 2 (BSL-2) Safety Equipment (Primary Barrier)

Same as BSL-1 Safety Equipment PLUS

- Biosafety Cabinet
  - cabinet must be certified annually
  - UV light is strongly discouraged
  - properly disinfect before and after each use

- Eye, face, and respiratory protection used in rooms containing infected animals as determined by risk assessment
Biosafety Level 2 (BSL-2) Laboratory Design (Secondary Barrier)

- Self-closing doors with locks
- Sink for hand washing located in lab
- Spaces between benches, cabinets, and equipment must be accessible for cleaning
- Bench tops, work surfaces, chairs must be non-porous and can be decontaminated
- All operable windows must remain closed or fitted with screens when work is performed
- Emergency eye/face wash unit must be available
- Vacuum lines with liquid disinfectant traps or in-line filter
- Available autoclave for disinfection
- Trash can that is hard sided with foot operated lid and a biohazard label
What Do I Do if I’m Exposed to a Biohazard?

Work-related injury or illness. Administer first aid (e.g., thoroughly wash affected area with soap and water), if appropriate.

If non-emergent incident, seek care at DMP’s during M-F, regular business hours (or at own HCP if UCB WC ineligible). If out of town, go to nearest urgent care. Report to supervisor. Supervisor or patient to fill out URM* incident form and report to Biosafety Officer.

If life or limb threatening, call 911 or go directly to nearest ER or Urg Care. Report to supervisor. Any sharps exposures to human blood, tissues, body fluids, be evaluated immediately. Supervisor or patient to fill out URM* incident form and report to Biosafety Officer.

Follow-up with UCB DMP’s. Submit any bills to URM* if eligible for UCB WC**. If not eligible for UCB WC**, submit bills to parent institution or have personal insurance cover costs.

DMP: Designated Medical Provider
URM: University Risk Management
UCB WC: University of Colorado Boulder, Worker’s Compensation
Safe Use of Centrifuges

- **Before use, check to see if:**
  - balanced – may need a “placeholder tube”
  - overfilled
  - caps or stoppers properly in place
  - run conditions (rpm – revolutions per minute vs. rcf - relative centrifugal force)

- **Use sealable buckets (safety cups) or sealed rotors**

- **After run, check to see if:**
  - centrifuge completely stopped
  - spills or leaks (clean immediately with appropriate disinfectant)
  - allow aerosols to settle (30 minutes) or open in a Biosafety Cabinet
Ultra Violet Light

UV lights are strongly discouraged:

- Should never replace chemical surface disinfection
- Dust and debris collected on UV bulb will inhibit effectiveness
- They provide a false sense of security - not giving off UV light (just prior to the end life of the bulb)
- Only disinfects the surface – could contact a layer of media protein covering the pathogen
- Casts a shadow, leaving areas that remain unexposed to the UV wavelengths
- Could cause burns / damage eyes – turn off when working in the room
Needles and Syringes

- Avoid use whenever possible
- Use a biosafety cabinet for all operations with infectious agents or materials that may cause disease
- Fill syringes carefully
- Shield needles when withdrawing from stoppers
- Do not bend, shear or recap needles
- Dispose of all used needles and syringes in a sharps container
Pipettes

- Mouth pipetting is prohibited
- All biohazardous materials should be pipetted in a biosafety cabinet
- Never ‘blowout’ (force) fluids out, use ‘to deliver’ pipettes (pipette held vertically with tip against side of receiving vessel to drain completely)
- To avoid splashes, allow discharge to run down the receiving container wall
- Never mix material by suction and expulsion
- Reusable pipettes should be placed horizontally in a disinfectant filled pan and autoclaved before reuse
Blenders, Grinders, Sonicators and Lyophilizers

- Operate in a biosafety cabinet (except lyophilizer) whenever possible. Allow aerosols to settle for 5 minutes before opening.
- Safety blender
  - do not use glass blender jars
  - decontaminate immediately after use with appropriate disinfectant
- Lyophilizers
  - use glassware designed for vacuum work
  - ensure there is no damage before using
  - disinfected all surfaces immediate after use with appropriate disinfectant
  - use vapor traps whenever possible
Inoculation Loop

- Sterilization in an open flame may create aerosols which may contain viable microorganisms
- Use a shielded electric incinerator
- Shorter handles minimize vibrations
- Disposable plastic loops are good alternatives
Cryostats

- Wear gloves during preparation of frozen sections and heavy gloves when accessing the cryostat.
- Decontaminate frequently or immediately after use with biohazardous agent or material. Use an appropriate disinfectant that is specifically effective for the agent.
Export Controls

- Does your research involve...

- Export or transport of material outside the US?
- Working with foreign nationals, institutions, or students?

- If you answered “yes” to either of these questions, contact Linda Morris at 303-492-2889 or linda.morris@colorado.edu
QUESTIONS?

Contact Information

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THANK YOU

You are now ready to take the required Biosafety Quiz