Biosafety Level 2
Standard Practices and Containment
Office of Research Safety Affairs

Purpose and Objectives

Purpose

• To promote the safe conduct of research with biohazardous materials known to present a moderate threat to human health.
• Comply with training requirements established by the CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) and the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines).

Objectives

Upon completing this course you should be able to:

• Obtain hazard information and the exposure response procedure for the BSL2 agents that you handle.
• Identify containment requirements necessary for work at BSL2.
• Perform work practices required for work at BSL2.
• Describe the role of the Institutional Biosafety Committee (IBC).
Hazard Specific Checklist

- Guides supervisors through BSL2 agent specific training.
- Ensures communication of hazards, precautions and response activities.
- Provides researchers and opportunity to ask questions.
- Documents that training occurred.

BMBL

International Biohazard Symbol

CDC Biosafety in Medical and Biological Laboratories (BMBL)
### Pathogen Risk Groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Risk Group 1</th>
<th>Risk Group 2</th>
<th>Risk Group 3</th>
<th>Risk Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogen</td>
<td>Does not cause disease in healthy adults</td>
<td>Can cause infection of varying severity; Rarely lethal</td>
<td>Agents associated with moderate to severe disease outcome; Can be lethal</td>
<td>Capable of causing severe disease with lethal outcome</td>
</tr>
<tr>
<td>Availability of Treatment</td>
<td>Not applicable</td>
<td>Treatment usually available or host immune system is capable of controlling the infection</td>
<td>Treatment may not be available</td>
<td>Treatment is generally not available; Experimental treatment regimens possible</td>
</tr>
<tr>
<td>Routes of Transmission</td>
<td>Not applicable</td>
<td>Ingestion, through the skin and via facial mucous membranes</td>
<td>Same as Risk Group 2 plus inhalation</td>
<td>Same as Risk Group 3</td>
</tr>
<tr>
<td>Disease Severity to Individual</td>
<td>None in healthy adults</td>
<td>Low to moderate</td>
<td>Moderate to high; Higher mortality and morbidity</td>
<td>High; Highest mortality rates in this category</td>
</tr>
<tr>
<td>Community Risk</td>
<td>Low</td>
<td>Low</td>
<td>Low to moderate</td>
<td>High</td>
</tr>
<tr>
<td>Infectious Dose</td>
<td>Not applicable</td>
<td>Generally high (variable)</td>
<td>Lower doses capable of infection</td>
<td>Can be as low as 1 organism</td>
</tr>
<tr>
<td>Example Agents</td>
<td>Non-conjugal grains of E. coli; rodent cell lines; Saccharomyces cerevisiae</td>
<td>Parasites (Plasmodium, Trypanosomes, Leishmania); GI pathogens (Salmonella, Shigella); Bloodborne Pathogens (HBV, HCV, Borrelia)</td>
<td>Mycobacterium tuberculosis, West Nile Virus, Yellow Fever Virus, Rickettisia rickettsii</td>
<td>Ebola virus, Marburg virus, Saba virus, Equine Morbillivirus</td>
</tr>
</tbody>
</table>

### Biosafety Level 2

<table>
<thead>
<tr>
<th>Practice</th>
<th>Level 1 practices plus safe sharps work practices</th>
<th>Level 2 work practices, with all work performed inside primary containment devices.</th>
<th>Same as Level 3</th>
<th>Same as Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protective Clothing</td>
<td>Gloves and lab coat recommended</td>
<td>Same as Level 2 plus additional respiratory protection.</td>
<td>Supply air respirator and fully encapsulating protective suit (SIP laboratories)</td>
<td>Configuration of BSL4 is referred to as a Cabinet lab. All work performed in sealed glove boxes</td>
</tr>
<tr>
<td>Containment Equipment</td>
<td>Gloves and lab coat required; Face protection added if potential for splash or splatter</td>
<td>Same as Level 2 plus with additional equipment of controlled airflow into the lab, dedicated HVAC system, no recirculation of exhaust, airflow monitoring devices at entry, two-door separation from general traffic, and fan failure alarms.</td>
<td>Same as Level 3, with many more advanced features; Level 4 is a building within a building approach; All systems for lab separated from non Level 4 areas</td>
<td>Same as Level 3, with many more advanced features; Level 4 is a building within a building approach; All systems for lab separated from non Level 4 areas</td>
</tr>
<tr>
<td>Lab Design Features</td>
<td>Same as Level 1, with possible requirements of controlled airflow into the lab and a biosafety cabinet for aerosol containment</td>
<td>Same as Level 2 with additional equipment of controlled airflow into the lab, dedicated HVAC system, no recirculation of exhaust, airflow monitoring devices at entry, two-door separation from general traffic, and fan failure alarms.</td>
<td>Double HEPA filtered exhaust air; HEPA filtered supply air; Effluent decontamination system</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>HEPA filters for exhaust air may be required.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
BSL2 FACILITY REQUIREMENTS

BSL2 Facility Requirements

Pathways for Biohazardous Agents:
- Transported with researchers
- Plumbing
- Air currents
Restricted Access

- Door should remain closed.
- Unauthorized personnel should not enter.
- Be aware of the risk of contamination within the room.

Warning Sign

- Must be posted at entrances to BSL2 work areas.
- Identifies the following:
  - Hazardous agents
  - Responsible individuals
  - Necessary precautions for entry
Building Vacuum Line Filters

• Use filters to protect building vacuum system.
• 1.0 um or smaller pore size filter.
• Available through General Stores in the GEB.

No plants or animals
Equipment

- Label equipment with biohazard symbol.
- Use equipment with safety cups or sealed rotors.
- In case of breakage or spill follow response procedure.

Biosafety cabinet for aerosols

- BSL2 work that may generate aerosols must take place in a biosafety cabinet (BSC).
- Disinfect surfaces before and after use.
- Must be certified annually.
BSL2 STANDARD PRACTICES

Review of Standard Microbiological Practices for BSL 1

- Proper technique for donning and doffing gloves.
- Wash hands after removing gloves.
- No eating, drinking of food stored in lab.
- No mouth pipetting.
- Use sharps handling precautions.

- Minimize splash and aerosol creation.
- Decontaminate work surfaces at least once a day or after spills.
- Decontaminate stocks and cultures before disposal.
- Complete training and immunizations if applicable.
**BSL2 Standard Practices**

**Training and Supervision**

- Complete training.
- Review Institutional Biosafety Committee (IBC) protocols for the work that you perform.
- Work must be supervised by competent scientists.

**BSL2 Standard Practices**

**Routine Disinfection**

- Decontaminate surfaces and equipment after completion of work.
- Equipment must be decontaminated before removal from the laboratory.
- Transport materials in durable, leakproof container.
- Disinfect liquids (30 min.) prior to drain disposal.
- Potentially contaminated materials discarded as infectious waste in red or orange bags. (only solids)
  - *Do not use ethanol to disinfect surfaces!*
Sharps Handling

- Do not use bent, sheared, or broken needles.
- Do not recap needles.
- Used needles discarded in puncture resistant, FDA approved sharps container.
- No direct handling of broken glass.
- Secure sharps including razor blades, scalpels and similar materials when not in use.

BSL2 Standard Practices

Aerosol Generating Procedures

- Aerosol or splash procedures must be conducted in BSCs or physical containment.
- This includes:
  - Pipetting,
  - Centrifuging,
  - Sonicating
  - Mixing
  - Shaking
  - Inoculating animals intranasally
  - Loading or using syringes
  - Opening containers of infectious materials
Using a Biosafety Cabinet

Biosafety Cabinet Performance

Effective performance depends on:

- Proper airflow.
- Integrity of High Efficiency Particulate Air (HEPA) filters.
- Work practices:

Using a Biosafety Cabinet

Proper Use

- Check sticker for certification (must be within last 12 months)
- Turn on blower and wait ≥5 minutes.
- Decontaminate the sides, sash, and work surface by wiping with a suitable decontaminant.
Using a Biosafety Cabinet

Biosafety Cabinet Work Practices

• *Do not block the front grill!*
• Work at least 4 inches from front grill.
• Enter BSC straight on using smooth hand and arm movements.
• Wear PPE while at the cabinet.
  – Gloves
  – Lab coat
  – Eye protection

As required by the CDC BMBL

**BSL2**

**PERSONAL PROTECTIVE EQUIPMENT**
Lab Coat

- Lab coats (or equivalent) must be worn.
- Lab coat must be closed (i.e. buttoned)
- Remove before leaving work area or dispose of appropriately
- Tucked sleeves into the top of gloves.

Gloves

- Gloves must be worn.
- Discard as infectious waste after use.
- Do not re-use gloves.
- Remove gloves before leaving the lab.
Eye Protection

- Eye protection always required when handling hazardous chemicals.
- Eye protection required when splash or spray may be reasonably anticipated.
- Face shield when handled large volumes (>4 liters)
- Decontaminate face and eye protection after use or discard as contaminated waste.

BIOHAZARDOUS WASTE DISPOSAL
Packaging Infectious Waste for Transport

Step 1: Get Stericycle box and tape the bottom closed. (Make sure arrows are pointing upwards)

Step 2: Place red liner bag into the box

Step 3: Place closed biohazard waste bag or closed sharp container into liner inside the box

Step 4: Once box is full close the box liner and tape the box closed

Step 5: Place the shipping label on the appropriate area on the box

Step 6: Place the box in the designated area for Stericycle to pickup

Note:
- All waste bags and sharps containers must have the PI name and room number written on them.
- Do not leave sharps containers on the floor.
- Do not obstruct the door, stack full boxes.

INCIDENT RESPONSE
Medical Surveillance

• Medical surveillance may be appropriate and available.
• Occupational Health is located in 910 Madison Ave.
• Immunizations may be available for some infectious agents.

Exposure Response

Be familiar with the following:
• Symptoms of exposure.
• First aid/exposure response practices.
• Incident reporting procedure.
• Where to obtain follow-up medical care.
Exposures

Incident Reporting

Exposure must be evaluated and treated immediately.
• Provide first aid or emergency treatment.
• All incidents must be reported to lab supervisor.
• Call Corvel 24/7 nurse line at 1-866-245-8588.

BSL2 Spill Response

In the event of a spill of a BSL2 material:
• Exit the spill area
• Close the door
• Post warning sign
• Wait 30 minutes for aerosols to settle
• Return wearing PPE
• Clean spill by:
  – Covering with paper towels or rags
  – Pouring 1-10% solution of household bleach.
  – Waiting 10 minutes for bleach to work.
  – Discard material as infectious waste.
  – Use tongs to handle broken glass and sharp materials.

https://www.youtube.com/watch?v=aGrPG3zla2I

(Click link to watch video)
Quiz

Questions?
Email labsafety@uthsc.edu or call 448-6114.