Laboratory Biosafety

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Laboratory Biosafety

- Development of Biosafety Practices
- Biological Risk Assessment
- Principles of Biosafety
- Biosafety Levels
- Principles of Biosecurity
Development of Biosafety Practices

- Landmark studies by Pike and Sulkin
  - 1930-1978: 4,079 lab-associated infections with 168 deaths
- Most common causative agents of overt infections:
  - Brucella spp.
  - Hepatitis B
  - F. tularensis
  - B. dermatitidis
  - C. psittaci
  - Coxiella burnetii
  - Salmonella typhi
  - M. tuberculosis
  - Venezuelan equine encephalitis
  - C. immitis
Development of Biosafety Practices

- Follow-up worldwide literature search by Harding and Byers
  - 1979-2004: 1,267 overt infections with 22 deaths
- Most common causative agents:
  - M. tuberculosis
  - Coxiella burnetii
  - Hantavirus
  - Arboviruses
  - HBV
  - Brucella spp.
Biohazard:

An agent 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 plants or animals.
Risk Assessment:

A process to identify the hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person’s exposure to an agent, the likelihood that such exposure will cause a LAI, and the probable consequences of such an infection.
Biological Risk Assessment

- Primary Factors to Consider
  - Agent Hazards
  - Laboratory Procedure Hazards
  - Capability of Lab Staff to Control Hazards
    - Training
    - Technical Proficiency
    - Good Habits
    - Operational Integrity of Containment Equipment and Facility Safeguards
### Biological Risk Assessment

**Table 1. Classification of infective microorganisms by risk group**

<table>
<thead>
<tr>
<th>Risk Group 1</th>
<th>(no or low individual and community risk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A microorganism that is unlikely to cause human or animal disease.</td>
<td></td>
</tr>
</tbody>
</table>

**Risk Group 2** *(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.

**Risk Group 3** *(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.

**Risk Group 4** *(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.
Biological Risk Assessment

Chain of Infection

- Reservoir of pathogen
- Portal of escape
- Transmission
- Route of entry/infectious dose
- Susceptible host
- Incubation period

Methods:
- Practices/Equipment
- PPE
- Immunization
- Surveillance

Risk Assessment
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Principles of Biosafety

**Biosafety:**

Applying a combination of laboratory practices and procedures, laboratory facilities, and safety equipment when working with potentially infectious microorganisms.
Principles of Biosafety

Containment

- A term used in describing safe methods, facilities and equipment for managing infectious materials in the laboratory environment where they are being handled or maintained.
- To reduce or eliminate exposure of lab workers, other persons and outside environment to potentially hazardous agents.
Principles of Biosafety

- Key elements of containment
  - Laboratory practice and technique
  - Safety equipment (primary barriers and PPE)
  - Facility design and construction (secondary barriers)
Principles of Biosafety

- Key elements of containment
  - Laboratory practice and technique
    - The most important element
    - Aware of potential hazards, trained and proficient in practices and techniques
    - Lab-specific biosafety manual
Principles of Biosafety

- Key elements of containment
  - Safety equipment (primary barriers and PPE)
    - Biological Safety Cabinets (BSCs)
      - Principal device to provide containment of infectious droplets or aerosols
      - Provide personnel, environment and product protection
      - Three types of BSCs: Class I, II and III
Principles of Biosafety

- Key elements of containment
  - Safety equipment (primary barriers and PPE)
    - BSC – HEPA Filter
      - High Efficiency Particulate Air filter
      - Traps particulates only; chemicals, fumes, vapors pass through
      - Traps particulates 0.3 µ (least effective)
Principles of Biosafety

- Key elements of containment
  - Safety equipment (primary barriers and PPE)
    - Centrifuge cup: an enclosed container to prevent aerosols from being released during centrifugation.

<table>
<thead>
<tr>
<th>Centrifuge Type</th>
<th>Speed Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcentrifuges</td>
<td>~15,000 rpm</td>
</tr>
<tr>
<td>Low/high speed</td>
<td>2,000 – 20,000 rpm</td>
</tr>
<tr>
<td>Ultracentrifuges</td>
<td>~ 120,000 rpm</td>
</tr>
</tbody>
</table>
Principles of Biosafety

- Key elements of containment
  - Safety equipment (primary barriers and PPE)
    - Centrifuge cup - operating procedure
      1. Check tubes for cracks/chips.
      2. Use matched sets of tubes, buckets etc.
      3. Tightly seal all tubes and safety cups.
      4. Ensure that rotor is locked to spindle and bucket seated.
      5. Close lid during operation.
      6. Allow to come to complete stop before opening.
Principles of Biosafety

- Key elements of containment
  - Safety equipment (primary barriers and PPE)
    - Centrifuge cup **safe operation**
      - Use safety cups whenever possible
      - Disinfect weekly and after all spills or breakage's
      - Lubricate O-rings and rotor threads weekly
      - Do not use rotors that have been dropped
      - Contact your centrifuge rep for specific information
Principles of Biosafety

- Key elements of containment
  - Safety equipment (primary barriers and PPE)
    - Personal protective equipment (PPE): gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses or goggles.
Principles of Biosafety

- Key elements of containment
  - Facility design and construction (secondary barriers)
    - Contributes to personnel safety
    - Provides a barrier to protect persons outside of the lab, and persons or animals in community from infectious agents
    - Depends on the risk of transmission of specific agents
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## Biosafety Levels

### 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. PPE: laboratory coats and gloves; eye, face protection, as needed</td>
<td>Laboratory bench and sink required</td>
</tr>
</tbody>
</table>
| 2   | Agents associated with human disease  
- Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure | BSL-1 practice plus:  
- Limited access  
- Biohazard warning signs  
- "Sharps" precautions  
- Biosafety manual defining any needed waste decontamination or medical surveillance policies | Primary barriers:  
- BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials  
- PPE: Laboratory coats, gloves, face and eye protection, as needed | BSL-1 plus:  
- Autoclave available |
| 3   | Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure | BSL-2 practice plus:  
- Controlled access  
- Decontamination of all waste  
- Decontamination of laboratory clothing before laundering | Primary barriers:  
- BSCs or other physical containment devices used for all open manipulations of agents  
- PPE: Protective laboratory clothing, gloves, face, eye and respiratory protection, as needed | BSL-2 plus:  
- Physical separation from access corridors  
- Self-closing, double-door access  
- Exhausted air not recirculated  
- Negative airflow into laboratory  
- Entry through airlock or anteroom  
- Hand washing sink near laboratory exit |
| 4   | Dangerous/exotic agents which pose high individual risk of aerosol transmitted laboratory infections that are frequently fatal, for which there are no vaccines or treatments  
- Agents with a close or identical antigenic relationship to an agent requiring BSL-4 until data are available to redesignate the level  
- Related agents with known risk of transmission | BSL-3 practices plus:  
- Clothing change before entering  
- Shower on exit  
- All material decontaminated on exit from facility | Primary barriers:  
- All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure suit | BSL-3 plus:  
- Separate building or isolated zone  
- Dedicated supply and exhaust, vacuum, and decontamination systems  
- Other requirements outlined in the text |
Biosafety Level 1

Standard Microbiological Practices

- Restrict or limit access when working
- Wash hands!
Biosafety Level 1

Standard Microbiological Practices

- Eating, drinking, smoking, handling contact lenses, applying cosmetics, storing food not permitted
- Prohibit mouth pipetting
Biosafety Level 1

Standard Microbiological Practices

- Use mechanical pipetting devices
Biosafety Level 1

Standard Microbiological Practices

- Safe handling of sharps
  - Don’t break, bend, recap or reuse needles and syringes
  - Use puncture-resistant containers for sharps disposal
Biosafety Level 1

Standard Microbiological Practices

- Safe handling of sharps (cont.)
  - Non-disposable sharps placed in hard walled container for decontamination
  - Don’t handle broken glassware directly. Use plastic ware whenever possible
Biosafety Level 1

Standard Microbiological Practices

- Minimize splashes and aerosols
- Decontaminate work surfaces
- Decontaminate wastes before disposal
- Maintain pest management program
- Lab personnel receive training and updates
Biosafety Level 1

**Standard Microbiological Practices**

- Post a sign with universal biohazard symbol at the entrance to the laboratory when infectious agents are present.
Biosafety Level 1

Special Practices

- None required
Biosafety Level 1

Safety Equipment (Primary Barriers & PPE)

- Special containment (BSC) not required
- Protective clothing
- Protective eyewear
Safety Equipment (Primary Barriers & PPE)

- Gloves
  - Alternatives to latex should be available
  - Gloves not worn outside lab
  - Changes gloves when contaminated
  - Remove gloves and wash hands after working with hazardous materials
  - Do not wash or reuse disposable gloves
Biosafety Level 1

Laboratory Facilities (Secondary Barriers)
Biosafety Level 1

Laboratory Facilities (Secondary Barriers)

- Laboratories have doors for access control
- Sink for hand washing
- Work surfaces easily cleaned
- Sturdy furniture
  - Bench tops impervious to water
  - Chairs covered with non-porous material
- Windows fitted with screens
Biosafety Level 1

Summary

- Agent not known to cause disease
- Minimal hazard to personnel and environment
- Work conducted on open bench tops using standard microbiological practices
- Special containment not required
Biosafety Level 2

Examples

- Measles virus
- Salmonellae
- Toxoplasma spp.
- HBV

*Immunization or antibiotic treatment available*

- Bloodborne pathogens
- Human body fluids when visibly contaminated with blood

*Extreme precaution with contaminated needles or sharp instruments*
Biosafety Level 2

Differs from BSL-1:

- Lab personnel have specific training in handling pathogenic agents
- Supervisor competent in handling infectious agents and associated procedures
- Restricted access to lab
- BSCs or other containment equipment required
Biosafety Level 2

Standard Microbiological Practices

- As in BSL-1
Biosafety Level 2

Special Practices

- Policies and procedures for entry/exit
- Medical surveillance and immunizations
- Baseline serum samples
Biosafety Level 2

Special Practices

- Biosafety manual specific to lab
- Lab personnel proficient in standard and special practices
- Use leak-proof container
Biosafety Level 2

Special Practices

- Lab equipment routinely decontaminated
  - Spills must be contained, decontaminated and cleaned
  - Equipment decontaminated before repair, maintenance or removal from lab
Special Practices

- Incidents and exposure must be reported and evaluated
- Animal and plants not permitted in lab
Biosafety Level 2

Special Practices

- Use biosafety cabinets for work with infectious agents involving
  - Aerosols and splashes
  - Large volumes
  - High concentrations
Biosafety Level 2

Safety Equipment (Primary Barriers & PPE)

- Biosafety Cabinet – typical Class II
  - A) front opening
  - B) sash
  - C) exhaust HEPA filter
  - D) rear plenum
  - E) supply HEPA filter
  - F) blower
Biosafety Level 2

Safety Equipment (Primary Barriers & PPE)

- Biosafety Cabinet – operating procedure:
  - Load BSC with all needed supplies
  - Turn on and allow to run for 10-15 minutes
  - Check inward airflow with a piece of tissue
  - Enter straight into cabinet and perform work in a slow, methodical manner
  - At end of work, decontaminate all items to be taken out of BSC and interior of BSC
  - Allow cabinet to run for 10-15 minutes and shut off
Biosafety Level 2

Safety Equipment (Primary Barriers & PPE)

- Biosafety Cabinet – equipment layout
Biosafety Level 2

Safety Equipment (Primary Barriers & PPE)

- Biosafety Cabinet – safe operation
  - Always enter straight into cabinet – no sweeping
  - Watch for disruptions of laminar airflow
  - Place all work materials into cabinet before starting
  - Place discard pan within cabinet
  - Ensure annual certification
Biosafety Level 2

Safety Equipment (Primary Barriers & PPE)

- Biosafety Cabinet – CAUTIONS:
  - Chemicals may damage HEPA filter
    - Exposure risk – chemical/infectious agents
  - Volatile chemicals NOT retained by HEPA filter
    - Exposes personnel if not exhausted
  - BSC fans not spark proof
    - Chemical use may result in fire/explosion
    - Never use flammables
Safety Equipment (Primary Barriers & PPE)

- Use eye and face protection when work outside BSC
- Gloves not be worn outside the lab
- Eye, face and respiratory protection should be used in rooms containing infected animals
Biosafety Level 2

Laboratory Facilities (Secondary Barriers)
Biosafety Level 2

Laboratory Facilities (Secondary Barriers)

BSL-1 facilities PLUS:

- BSCs must be installed
- Vacuum lines protected with liquid disinfectant traps
Laboratory Facilities (Secondary Barriers)

BSL-1 facilities PLUS (cont.):

- Eyewash station
- Ventilation system
- Decontamination method: autoclave
Key Resources

- Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Ed.