Biosafety Levels

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"Biosafety is an inexact science, and the interacting system of agents and activities and the people performing them are constantly changing."

Every etiologic agent is different.

Every laboratory is different.

Every person is different.

Principles of Biosafety

BIOSAFETY LEVEL	POTENTIAL HAZARD
BSL-4 Large research Labs – CDC, NIH	HIGHEST
BSL-3 University research laboratories	
BSL-2 Clinical laboratories	
BSL-1 High school laboratories	LOWEST

Primary Barriers Protect the Worker and the Lab Environment

- Safety equipment
  - Personal Protective Equipment (PPE)
  - Biological Safety Cabinets (BSC)
  - Mechanical pipetting device
  - Safety centrifuge cups
  - Removable rotors

Secondary Barriers Protect the Worker and the Lab Environment

- Facility design
  - Separation of lab from public access
  - Autoclave facilities
  - Handwashing and eyewash facilities
  - Specialized ventilation systems
  - Directional airflow
  - Restricted access zones

Biosafety Levels (BSL) 1-4

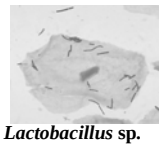
- Each level builds on previous levels
- Increasing emphasis on safety procedures and practices
- Increasing need for training, preparation and competent supervision
- Increasing requirements for PPE and facility containment

Biosafety Level 1 (BSL-1)

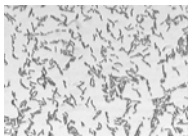
- Suitable for work involving well characterized agents not known to consistently cause disease in immunocompetent adult humans and present minimal potential hazard to laboratory personnel and the environment (most college training labs)
  - May cause opportunistic infection in the young, the elderly and the immunocompromised individuals

Biosafety Level 1 (BSL-1)

- Vaccine strains should not be considered avirulent simply because they are vaccine strains (RB51)



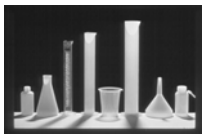
Lactobacillus sp.



Bacillus subtilis

Needles and Sharps Precautions

- Use sharps containers for disposal
- Never fill sharps container to the top
- Use plastic vs. glass
- Don't touch broken glass with bare hands



Safe Handling, Minimizing, and Disposal of Sharps

- Don't break, bend, re-sheath or reuse syringes or needles
- Use retractable or shielded needles
- Use shielded scalpels
- Collect reusable sharps in labeled, leak proof container

Minimize Aerosols

Aerosols

- Procedures that impart energy to a microbial suspension produce aerosols
- Ubiquitous in laboratory procedures
- Usually undetected
- Extremely pervasive, putting all at risk
- Likely to be the cause when other causes are ruled out and the person just "worked in the room" where the agent was

Procedures That Emit Aerosols

- Catalase
- Inoculating biochemicals or blood culture bottles
- Pipetting
- Mixing
- Centrifugation
- Grinding
- Vortexing
- Pouring
- Loading syringes
- Lasers, cell sorters
- Inoculating biochemicals or blood culture bottles
- Splashes
- Opening lyophilized cultures
- Flaming loops
- Entering or opening vessels at non- ambient pressures, fermenters, freezer vials

Aerosols from Laboratory Equipment

(10^{10}/ml culture - 10 min. use)0

Blender, open	10^6
Sonicator with bubbling	10^6
Pipetting, vigorous	10^6
Dropping culture	3×10^5
Splash on a centrifuge rotor	10^5
Blender, opened after 1 minute	2×10^4
Pipetting, carefully	10^4

Minimize Aerosols

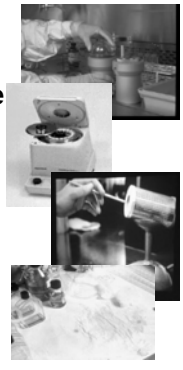
- Don't
  - Use Bunsen burners
  - Drop liquids onto hard surfaces
  - Blow out last drop in pipette
  - Mix by suction + expulsion
  - Open centrifuge before it stops

Minimize Aerosols

- Do
  - Discharge liquid down side of container
  - Deliver as close as possible to contents
  - Use capped tubes when mixing or vortexing
  - Use care with needles (gauze pad with alcohol on septum of blood culture bottle)

Minimize Aerosols

- Use pipette aids with filters
- Use horizontal pipette trays
- Use incinerators
- Pour liquids carefully
- Work over absorbent
- Use centrifuge safety cups
- Use sealed rotors



Protect Your Eyes and Mucous Membranes Against Splashes and Aerosols!

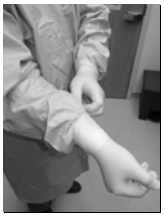
- Safety glasses?
- Plastic shields?

Training

- Biohazards
- Risks of different types of exposures
- Available vaccinations and side effects
- Post-incident first aid and remediation
- Signs and symptoms of infection
- Emergency response procedures
- Reporting procedures

Biosafety Training Should Be…

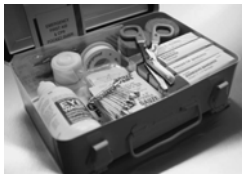
- Done routinely
- Made realistic
- Use proper equipment
- Be "Hands on"
- Practiced



What I hear, I forget
What I see, I remember
What I do, I understand

Importance of First Aid

Adequacy and timeliness of wound cleansing after an occupational exposure occurs may be the most critical determinant in preventing infection



First Aid Response for Exposures and Injuries

- Pre-defined
- Available and accessible immediately and 24/7
- Simple, easy to follow guidance
- Widely known about and reviewed often (are kit contents checked regularly?)
- Linked to further assessment and reporting
- Practiced!!

Emergency Procedures

- Written SOP's and *training* for:
  - Building exhaust failure
  - BSC failure
  - Spills
  - Loss of power
  - Evacuation
  - Medical injuries or exposure

BSL-1

- Facility Design (secondary barriers)

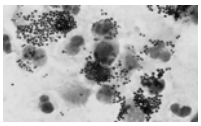
Requirements:

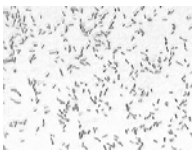
- Laboratories have doors
- Sink for hand washing
- Work surfaces easily cleaned
- Sturdy furniture
- Bench tops are impervious to water
- Windows fitted with flyscreens
- Insect and rodent control program
- Location: not separated
- Structure: normal construction
- Ventilation: none

Biosafety Level 2

Biosafety Level 2 (BSL-2)

- Suitable for work involving agents that pose moderate potential hazards to personnel and the environment (most clinical labs)





BSL-2

- Differs from BSL-1 in that
  - Laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures

BSL-2

- Access to the laboratory is restricted when work is being conducted
- All procedures in which infectious aerosols or splashes may be created are conducted in BSC's or other physical containment equipment

Biosafety Level 2

- Supervision
  - Supervisor is a competent scientist with increased responsibilities
    - Limits access if immunocompromised
    - Restricts access to immunized if necessary
- Lab Personnel
  - Aware of potential hazards
  - Proficient in practices/techniques

Biosafety Level 2: Special Practices

- Use biosafety cabinets (Class II) for work with infectious agents involving
  - Aerosols
  - Large volumes
  - High concentrations of organisms
  - Small Gram negative diplococci from spinal fluid or blood

Biosafety Level 2: Special Practices

- Small Gram negative or Gram variable rods, slow growth on BA, no growth on Mac

Personal Protective Equipment

- Act as a barrier to protect skin, mucous membrane or respiratory tract from exposure
- Prevent spread of contamination
- Protect the worker from splash and splatter
- Protect product from contamination

BSL-2 PPE

- Lab coat-long sleeved and buttoned
- Eye and face protection
- Gloves

Personal Protective Equipment

- Is appropriate PPE available?
- Separate waste stream for used PPE?
- Is PPE being worn outside the laboratory?
- Are personnel *trained?*

Personal Protective Equipment

- Is appropriate PPE available?
- Separate waste stream for used PPE?
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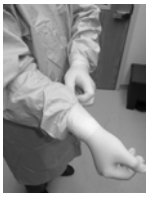
Personal Protective Equipment Gloves

- Check integrity before use
- Do not wash or reuse
- Disinfectants or chemicals enhance permeation
- Change often - Integrity decreases with use
- Do not touch "clean" surfaces



Does not eliminate the hazard!

When Do You Wear Gloves in the General Micro Lab?

- **"Gloves should be worn at the specimen receiving and set-up areas, and in TB/virology labs, and when hands may contact potentially infectious material, contaminated surfaces or equipment." (CLSI M29-A3)**

When Do You Wear Gloves in the General Micro Lab?

- **"Gloves must be worn to protect hands from exposure to hazardous materials" (BMBL 5th edition)**
  - **Based on a lab-specific risk assessment, the Laboratory Director or supervisor determines laboratory hazards and when to wear gloves**

BSL-2

- **Facility Design (secondary barriers)**

Requirements (BSL-1 Facilities PLUS)

- **Self-closing and lockable doors (restricted access)**
- **Air flow into lab without re-circulation to non-lab areas (for new construction)**
- **Easily cleanable-no rugs or cloth covered chairs**
- **Location: separated from public areas**

Biosafety Level 2 Facility Design

- **(Secondary Barriers)**
  - **Automatic hand washing sinks (preferably located near exit door)**
  - **Autoclave in or near lab (not required)**
  - **Eye wash**

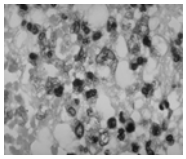
Biosafety Level 2 Facility Design

- **Vacuum system protection**
- **BSC's installed so that fluctuations in the air supply or exhaust do not interfere with proper operation**

Biosafety Level-3

Biosafety Level 3 (BSL-3)

- Applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or lethal disease through inhalation route exposure



Mycobacterium tuberculosis

Biosafety Level 3

- Differs from BSL-2 in that
  - Personnel have specific training to handle particular pathogens
  - Supervised by scientists experienced with these agents
  - All manipulations of infectious material carried out in BSCs
  - Laboratory has special engineering and design features
  - Supervisors evaluate effectiveness of training

What are BSL-3 Practices?

- All procedures involving the manipulation of infectious materials MUST be conducted within a BSC or other physical containment device
  - No work with open vessels is conducted on the bench

What are BSL-3 Practices?

- When a procedure cannot be performed within a BSC, a combination of PPE and other containment devices, such as centrifuge safety cups or sealed rotors, must be used
- Restricted access to the laboratory

What are BSL-3 Practices?

- Additional PPE (solid-front gown, gloves and eye protection as a minimum) are worn in the lab
  - Respiratory protection may be indicated
- Lab personnel must demonstrate proficiency prior to BSL-3 work
- Potentially infectious waste materials should be decontaminated before removal from the facility

When Do You Use BSL-3 Practices In A BSL-2 Lab?

- When working with agents that are normally handled under BSL-3 conditions, and a BSL-3 laboratory is not available
- When determined by the laboratory director based on their risk assessment

When Do You Use BSL-3 Practices In A BSL-2 Lab?

- When specific high-risk pathogenic organisms are suspected (such as *Brucella* spp., *Coccidioides*, *Blastomyces dermatitidis*, *Francisella tularensis*, *Histoplasma capsulatum*, Mtb, etc)

Biosafety Level 3: *Special Practices*

- Doors are kept closed
- Persons at increased risk of infection are not allowed in lab
- Only those advised of hazards and meet requirements can enter
- Entry requirements and contacts posted on door

Biosafety Level 3: Special Practices

- All cultures, stocks and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving
  - Preferably within the laboratory

Biosafety Level 3: Special Practices

- Use bioaerosol-containing equipment
- NO work in open vessels is conducted on the bench!
- Load/unload centrifuge rotors in BSC

Biosafety Level 3: Special Practices

- Additional Personal Protective Equipment
  - Minimum of solid-front wrap around gowns (preferably with tight cuffs), gloves, eye and face protection
  - Other PPE based on risk assessment

Biosafety Level 3: Special Practices

- Other PPE based on risk assessment
  - Coveralls
  - Booties, head covers
  - Double gloves
  - Respirators
  - Disposable sleeves
  - Scrubs

Personnel must be trained!

Biosafety Level 3 Personal Protective Equipment

- Respiratory protection may be indicated
  - Based on risk assessment
  - Personnel must be fit tested and trained (OSHA 29 CFR 1910.134)
  - Respirators must be maintained
  - Facial hair interferes with N95 seal

Biosafety Level 3 Personal Protective Equipment

- REDUCE exposure, do NOT eliminate exposure-risk is never zero
- Surgical masks are NOT respirators!

BSL-3

- Laboratory Facilities (secondary barriers)
- BSL-1 and BSL-2 Facilities PLUS:
  - Separate building or isolated zone
  - Directional inward airflow (verified)
  - Laboratory air is single-pass
  - Room penetrations and seams are sealed

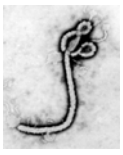
BSL-3

- Enclosures for aerosol generating equipment
- Double door entry (self-closing)
- Hands-free handwashing sinks
- Restricted access (locked doors, biometrics)
- Vacuum lines protected
- Pass-thru autoclaves (or other means of decon)

Biosafety Level 4 (BSL-4)

Required for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, aerosol transmission, or related agent with unknown risk of transmission.

- Cabinet laboratory
- Suit laboratory



Laboratory Biosafety Level Criteria

SUMMARY OF RECOMMENDED BIOSAFETY LEVELS FOR INFECTIOUS AGENTS

BSL	AGENTS	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Not known to consistently cause diseases in healthy adults	Standard Microbiological Practices	None required	Laboratory bench and sink required
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: • Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials PPEs: • Laboratory coats; gloves; face protection as needed	BSL-1 plus: • Autoclave available
3	• Indigenous or exotic agents with potential for aerosol transmission • Disease may have serious or lethal consequences	BSL-2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of laboratory clothing before laundering • Baseline serum	Primary barriers: • Class I or II BSCs or other physical containment devices used for all open manipulation of agents PPEs: • Protective laboratory clothing; gloves; respiratory protection as needed	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhaust air not recirculated • Negative airflow into laboratory

See supplemental handout in notebook

CLSI Resources

- M29-A3
  - Protection of Laboratory Workers From Occupationally Acquired Infections-Third Edition
- GP17-A2
  - Clinical Laboratory Safety-Second Edition
- GP18-A2
  - Laboratory Design-Second Edition

CLSI Resources

- GP5-A2
  - Clinical Laboratory Waste Management-Second Edition
- NCCLS Vol. 22 No. 4
  - Implementing a Needlestick and Sharps Injury Prevention Program in the Clinical Laboratory

Material Safety Data Sheet (MSDS)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Office of Laboratory Security

MSDS

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: ***Brucella*** **spp. (*B. abortus, B. canis, B. melitensis, B. suis*)**

SYNONYM OR CROSS REFERENCE: Brucellosis, Undulant fever, Bang's disease, Malta fever, Mediterranean fever

CHARACTERISTICS: Gram negative cocci or small rods, aerobic, non-motile, urease +

SECTION II - HEALTH HAZARD

PATHOGENICITY: All *Brucella* isolates are potentially pathogenic to humans; systemic bacterial disease with acute or insidious onset; intermittent fever, headache, weakness, profuse sweating, chills, arthralgia; localized suppurative infections; subclinical infections are frequent; <2% case fatality rate for untreated cases; may have long recovery period

EPIDEMIOLOGY: Worldwide, especially in Mediterranean countries of Europe and Africa; Middle East, India, central Asia, Mexico, Central and South America; common in those who eat raw caribou; occurrence often depends on extent of animal *Brucellosis*, predominantly an occupational disease of those who work with infected animals or their tissues

http://www.phac-aspc.gc.ca/msds-ftss/index.html#menu?

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