Biosafety Manual – Wilson/Erickson laboratories

1. Introduction

Laboratory-acquired infections are very rare. However, they are an important public health issue since an infected worker could be a transmission risk to other people. Laboratory workers are at greater risk of infection when working with high concentrations of pathogens and performing operations such as sonication, centrifugation, and vortexing that can produce large numbers of droplet nuclei.

The most common routes of laboratory infection are inhalation of aerosols, percutaneous inoculation (needlestick injuries, broken glass), direct contact with contaminated surfaces, and ingestion. Among bacteria, the pathogens most likely to cause laboratory infections are *Brucella*, *Mycobacterium tuberculosis*, and *Rickettsia*. Less frequent causes include *Francisella*, *Salmonella*, *Clostridium difficile* and *Shigella.* Reports of laboratory-acquired infection due to other bacterial species are extremely rare.

2. Occupational Safety and Health Program

All students enrolled in an academic laboratory course or working in a research lab are automatically subject to the requirements of BYU's occupational safety and health programs. These programs are based on applicable health and safety laboratory standards promulgated by Federal and State agencies including OSHA's Laboratory Standard (29 C.F.R. § 1910.1450), Bloodborne Pathogens Standard (29 C.F.R. § 1910.1030), Hazard Communications Standard (29 C.F.R. § 1910.1200), Personal Protective Equipment Standards (29 C.F.R. § 1910.132, 29 C.F.R. § 1910.133, 29 C.F.R. § 1910.134), and other specific standards where applicable. Personnel working in laboratories are expected to conduct themselves in a responsible manner that will uphold these guidelines. Effective laboratory safety depends on each individual's effort in working to eliminate unsafe acts and conditions. These written programs can be found on the College of Life Sciences and BYU Risk Management websites.

2.1. Student Responsibilities

2.1.1. Read, understand, and follow safety and health guidelines as outlined by the University, the College, the Department, and the Laboratory Supervisor.
2.1.2. Attend or complete all required health and safety training sessions conducted online or in-person.
2.1.3. Utilize all hazard controls/safety procedures provided by the Lab Supervisor.
2.1.4. Ask the appropriate resource if they are unsure of any safety or compliance rule or procedure.
2.1.5. Understand how to respond to an emergency situation.
2.1.6. Understand that they are ultimately responsible for their own safety.
2.1.7. Report accidents, unhealthy, and unsafe conditions to the faculty supervisor, Department Safety Coordinator, College Safety Coordinator and/or RM&S.
2.1.8. Notify the faculty supervisor of any preexisting health conditions that could lead to serious health situations in the laboratory or during fieldwork / class trips.

2.2. Safety Equipment

2.2.1. Eye Wash and Safety Shower: To operate either the eye wash or safety shower simply pull the black handle associated with the desired device. The shower has a modesty curtain if doffing of street clothing is required. When using the eye wash a more thorough rinse will involve pulling the eye lid back to allow rinse water to cover a larger area of the eye and prevent sequestered hazardous material.

2.2.2. Fire Extinguisher: To operate pull the pin, direct the nozzle at the base of the fire and pull the lever until it releases a powder spray. The fire extinguisher is a dry powder system that is good for all classes of fire except metals such as magnesium.

2.2.3. Biosafety Cabinets: Biosafety cabinets (BSC) are required for use with all risk group 2 agents and higher. All procedures that may create aerosols should be performed in a certified BSC.

2.2.4. PPE: Minimum PPE required for handling risk group 2 microorganisms includes: long pants, closed toe & heal shoes, lab coat, and latex or nitrile gloves. Safety glasses are recommended for any procedures where aerosol generation can occur.

3. **Standard Practices Biosafety Level 2**

Biosafety level 2 (BSL-2) is suitable for work involving risk group 2 agents that pose moderate hazards to personnel and the environment. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory students and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the Lab Supervisor, College Safety Officer, or Risk Management for appropriate counseling and guidance.

BSL-2 standard practice requirements include:
3.1. All laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures.

3.2. Persons working with infectious agents should receive initial and annual training on disease signs and symptoms and all exposure procedures including the location of the organism data sheets (4.3).

3.3. Access to the laboratory is restricted when work is being conducted. The door should remain closed at all times. Do not prop the door open!

3.4. Properly maintained BSCs, or other approved physical containment equipment, must be used for all procedures in which infectious aerosols or splashes may be created. These activities may include, but are not limited to:

- 3.4.1. Pipetting
- 3.4.2. Centrifuging
- 3.4.3. Grinding
- 3.4.4. Blending
- 3.4.5. Shaking
- 3.4.6. Mixing
- 3.4.7. Sonicating
- 3.4.8. Opening containers of infectious materials
- 3.4.9. Intranasal inoculation of animals
- 3.4.10. Harvesting infected tissues from animals or eggs.
- 3.4.11. Whenever high concentrations or large volumes of infectious agents are used

3.5 BSCs will not protect the operator from spillage, breakage or poor technique. The following principles should be followed when using BSCs:

- 3.5.1 The cabinet must not be used unless it is working properly.
- 3.5.2. The glass viewing panel must not be opened when the cabinet is in use.
- 3.5.3. Apparatus and materials in the cabinet must be kept to a minimum.
- 3.5.4. Air circulation at the rear plenum must not be blocked.
- 3.5.5. Bunsen burners must not be used in the cabinet. The heat produced will distort the airflow and may damage the filters. An electric microincinerator is permissible but sterile disposable transfer loops are better.
- 3.5.6. All work must be carried out in the middle or rear part of the working surface and be visible through the viewing panel.
- 3.5.7. Traffic behind the operator should be minimized.

3.5.8. The operator should not disturb the airflow by repeated removal and reintroduction of his or her arms.
3.5.9. Air grills must not be blocked with notes, pipettes or other materials, as this will disrupt the airflow causing potential contamination of the material and exposure of the operator.
3.5.10. The surface of the biological safety cabinet should be wiped using an appropriate disinfectant after work is completed and at the end of the day.
3.5.11. The cabinet fan should be run for at least 5 min before beginning work and after completion of work in the cabinet.
3.5.12. Paperwork should never be placed inside biological safety cabinets

3.6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling processing, storage, or transport within the facility. Do not transport open cultures without secondary containment.

3.7. Lab personnel will wash their hands with soap and water after handling viable materials, removing gloves, and before leaving the laboratory.

3.8. Eating, drinking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in lab areas.

3.9. Mouth pipetting is prohibited; mechanical pipetting devices are used at all times.

3.10. Laboratory equipment and work surfaces should be decontaminated at the end of every lab class, as well as, after spills, splashes, or other potential contamination.

3.11. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.

3.12. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor and/or the college safety officer. Medical evaluation, surveillance, and treatment may be required.

3.13. Laboratory coats designated for laboratory use must be worn while working with hazardous materials. They should be removed before leaving for non-laboratory areas (e.g. hallways, faculty offices, restrooms.)

3.14. Eye and face protection (goggles, face shield, masks, etc.) is used for anticipated splashes or sprays of infectious or other hazardous materials.

3.15. A spill kit containing a method for decontamination must be maintained and available in the laboratory area.

4. Infectious Agent Exposure Protocol

Exposures to infectious agents in the laboratory involve both qualitative and often quantitative differences from exposures in the community. Laboratorians are often working with high concentrations of the agent under study. Laboratory workers typically have significantly more information concerning the organism, specific virulence factors, and concentration of possible inoculum than someone with non-laboratory exposure.

4.1. **Exposure Incident**: The following will be considered an exposure incident:
4.1.1. Inhalation of aerosol droplets – failure of aerosol containment during energetic processes such as centrifugation, sonication, spilling, splashing etc. outside the biosafety cabinet.

4.1.2. Mucous membrane contact
4.1.3. Contact with non-intact skin
4.1.4. Injection

4.2. **Immediate Response Procedures**:

4.2.1. **Wash it off, rinse it out**: Use eyewash, sink, drench hose or shower to remove the infectious material as soon as possible following an exposure incident involving contact with skin or mucous membrane surfaces.

4.2.2. **Notify principal investigator (PI) of the accident and/or College Safety Officer (if applicable)**

4.2.3. **Notify Risk Management –** The PI or the College Safety Officer should notify BYU Risk Management

4.2.4. **Seek medical attention** - Go to or call the Student Health Center during normal working hours. After normal working hours go to the Utah Valley Regional Medical Center emergency room.

4.2.5. **HIV exposures** (exposure to human blood or viral isolate or culture)- Brigham Young University expects the exposed person to **immediately** go to the Student Health Center during normal working hours, or to the Utah Valley Regional Medical Center emergency room when the student health center is closed. **Time is critical**. Time from initial exposure to completion of medical consultation and initiation of post exposure prophylaxis (if warranted) should be no more than 2 hours.

4.3. **Organism Data Sheet** (Provided to Medical Care Personnel) – Each infectious or toxic organism should have a prepared organism data sheet as part of this biosafety manual. The organism data sheet is to be provided to medical care personnel in the event of an exposure.

Not all the information asked for in the organism data sheet may be known; however, prior to work involving an infectious pathogen or toxin, each PI or student working in the laboratory should review literature to learn as much as possible about organisms used in the laboratory. Information for the organism data sheet should come from peer reviewed sources, public health databases (CDC, Public Health Agency of Canada, etc.) or other accredited sources.

5. Sharps

Whenever practical, adoption of work practices that reduce the risk of sharps injuries should be implemented. Policies for the safe handling of sharps (needles, scalpels, pipettes, broken glassware, etc.) can include, but are not limited to:

5.1. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated.

5.2. Used disposable needles and syringes must be carefully placed in puncture-resistant containers used for sharps disposal.

5.3. Non-disposable sharps must be placed in a hard-walled container for transport to a processing or decontamination area.

5.4. Broken glassware must not be handled directly. Instead, remove broken glassware using a brush and dustpan, tongs, or forceps.

5.5. Plastic ware should be substituted for glassware whenever possible.

Appendix A: Organism-specific Information

Agent identification: *Escherichia coli* isolates from humans or from agricultural environments, excluding strains known to cause diarrheal disease

Agent characteristics: These Gram-negative bacteria live asymptomatically in the digestive tracts of animals, including humans.

Pathogenicity/toxicity: May cause disease when they colonize other tissues. They do not produce the virulence factors necessary to cause gastrointestinal disease. Some may be able to cause extraintestinal diseases such as urinary tract and bloodstream infections. They usually must gain entrance to the urinary tract or bloodstream through disruptions of the gastrointestinal barrier or by colonizing the urethra from the GI tract.

Median infectious dose: Unknown. If ingested, these strains may colonize the GI tract of a laboratory worker without causing disease. If accidentally injected via a needle or through contact with an open wound, the infectious dose required through this route would likely be between 10,000 and 1,000,000 organisms, but most strains have not been characterized for virulence in any animal model of infection. It is reasonable to suspect that ingesting even a very large dose (> 10 billion organisms) would be unlikely to cause disease in a laboratory worker.

Incubation period: Unknown. If infection were to occur via the bloodstream, the time required for disease to manifest would likely be 6-72 hours.

Known virulence factors: many, varies with strain

Because we cannot know in advance which *E. coli* strains are "pathogens" and which are not, all uncharacterized strains must be considered potentially pathogenic and handled using BSL2 precautions.

Host range: warm-blooded animals, reptiles

Zoonosis: yes, likely for some strains

Antimicrobial resistance patterns: varies, some strains are pan-resistant, most are susceptible to commonly prescribed antibiotics

Host immune status: *E. coli* are opportunistic pathogens, requiring breaks in first line defenses to cause disease. Females are more susceptible to urinary tract infection than males, and pregnancy may increase risk.

Concentration of the culture: overnight cultures typically reach 10^9 CFU/ml

Record of laboratory infections: unknown, humans are all colonized by *E. coli* so establishing LAI is difficult

Susceptibility to Disinfectants: susceptible to solutions of hydrogen peroxide, rubbing alcohol, ethanol, hypochlorite, iodines, etc.

Agent identification: *Staphylococcus aureus* isolates from humans or from agricultural environments

Agent characteristics: Gram-positive bacteria that live in the mucus membranes, digestive tract, and sometimes the skin of animals and humans

Pathogenicity/toxicity: Wide range of virulence, based on their variable carriage of toxin genes and colonization factors. *S. aureus* can cause a wide range of diseases, including pneumonia, bloodstream infections, boils, soft-tissue infections, and toxic shock syndrome.

Median infectious dose: Unknown, varies with strain. If injected accidentally via a needle or through an open wound, the dose required to initiate disease would fall between 1,000 and 10,000,000 organisms. If a worker were to inhale the bacteria through aspiration or aerosols, the infectious dose would likely be between 10,000 and 1,000,0000 organisms.

Incubation period: Unknown. If infection were to occur via the bloodstream, the time required for disease to manifest would likely be 6-72 hours.

Known virulence factors: Many, varies with strain. Several toxins that kill or interfere with the functions of neutrophils or other innate immune cells.

Host range: Warm-blooded animals. There is some species-specificity, meaning that livestock-associated strains are better at colonizing these animals than they are at infecting humans, and vice versa.

Zoonosis: Yes, demonstrated for some strains.

Antimicrobial resistance patterns: Varies, some strains are pan-resistant, most are susceptible to commonly prescribed antibiotics.

Host immune status: *O*pportunistic pathogens, requiring breaks in first line defenses to cause disease. Neutrophil deficiencies are a risk factor for invasive *S. aureus* disease.

Concentration of the culture: overnight cultures typically reach 10^8 CFU/ml

Record of laboratory infections: There are 3 reported cases of methicillin-resistant *Staphylococcus aureus* laboratory-acquired infections. Likely many more infections with *S. aureus* occur, but since it is normal flora for up to 50% of the population this is difficult to establish.

Susceptibility to Disinfectants: susceptible to solutions of hydrogen peroxide, rubbing alcohol, ethanol, hypochlorite, iodines, etc.

Agent identification: *Yersinia pseudotuberculosis*

Agent characteristics: Gram-negative bacteria that live in soil and water, digestive tract of some animals, occasional food-borne pathogen.

Pathogenicity/toxicity: May cause intestinal disease in humans that is frequently mild/asymptomatic. Bacteria colonize the local (intestinal) lymph nodes. Symptoms of disease include low-grade fever, pain, and diarrhea. In rare cases the bacteria may spread from the GI tract through the blood stream and/or colonize other organs including the liver and spleen. This would result in severe disease with high fever and signs of sepsis (low blood pressure, chills, fatigue, rapid breathing).

Median infectious dose: >10 billion organisms orally, ~10,000 organisms via needlestick injury

Incubation period: Via the bloodstream, the time required for disease to manifest would likely be 6-72 hours. Via ingestion the incubation period could be up to 2 weeks.

Known virulence factors: Surface proteins that enable penetration of intestinal epithelial barrier, injection system to kill/disable innate immune cells.

Host range: Warm-blooded animals. Frequently isolated from pigs.

Zoonosis: Yes.

Antimicrobial resistance patterns: Drug resistance in *Y. pseudotuberculosis* is extremely rare.

Host immune status: Persons with diabetes, pregnancy, cell-mediated immune defects are known to be at greater risk of developing disease.

Concentration of the culture: overnight cultures typically reach 10^9 CFU/ml

Record of laboratory infections: None reported.

Susceptibility to Disinfectants: susceptible to solutions of hydrogen peroxide, rubbing alcohol, ethanol, hypochlorite, iodines, etc.