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FOCUS on Field Epidemiology

Laboratory Biosafety Levels

Introduction

Some people are so involved in their work that they take it home with them. Teachers bring home papers, business people make phone calls and put together proposals, epidemiologists read all the latest reports of outbreaks....

But what are laboratorians bringing home with them? Are they bringing unwelcome infectious diseases?

Laboratorians have long recognized the hazards of processing infectious agents. In response to these hazards, guidelines have been developed to protect workers in microbiological and medical labs through a combination of safeguards including engineering controls, management policies and work practices. (1)

Biosafety Levels

In any laboratory, precautions must be taken so the people researching or trying to identify organisms do not become infected themselves. According to the Centers for Disease Control and Prevention (CDC), scientists and lab technicians have to be very aware of microorganisms; while handling or testing clinical specimens, they could accidentally infect themselves or their coworkers. Because of this danger, labs must adhere to very specific safety regulations to work with organisms that pose a threat to human health. (1)

The regulations outline precautions, special practices, and decontamination procedures for labs that work with infectious agents. Based on the degree of hazard posed by these agents, labs are divided into 4 biosafety levels, and mandated protective practices increase with each level. Biosafety Level 1 labs work with the least dangerous agents and require the fewest precautions; Biosafety Level 4 labs have the strictest methods for handling organisms because they deal with agents that are most dangerous to human health.

In this issue of FOCUS, we describe some of the differences between the biosafety levels, with examples of organisms studied and the precautions that must be taken in laboratories at each level. This will help you understand the process that state, federal, and private labs may have to undertake to identify a microorganism involved in an outbreak, and why every lab cannot test for every organism.

The information summarized here should not be used as a guide for establishing laboratory safety protocols.

Complete information and recommendations can be found in *Biosafety in Microbiological and Biomedical Laboratories 5th Edition,* available online at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s6.htm.

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Barriers

Each biosafety level has prescribed barriers to protect against microorganisms. *Primary barriers* are physical barriers or personal protective equipment between the lab worker and the pathogen, such as gloves, masks, or special breathing apparatuses. Laboratorians use these types of safety equipment to protect themselves directly when working with organisms.

Secondary barriers are structural aspects of the laboratory itself that make the working environment safer against the risk of infection; these include sinks for handwashing, special containment areas for working directly with organisms, and special air ventilation patterns designed to prevent contamination of other rooms and other workers in the building.

Biosafety Level 1 (BSL-1)

Biosafety Level 1 agents pose no threat to human health; that is, they are not known to cause disease in healthy adults. Some of these organisms may be known to cause disease in immunocompromised individuals.

Agents studied in BSL-1 labs include *Bacillus subtilis, Naegleria gruberi,* infectious canine hepatitis virus, and non-pathogenic *E. coli* species (see Figure 1). (2)

Figure 1. A transmission electron micrograph of *E. coli*; most *E. coli* variants are BSL-1 agents

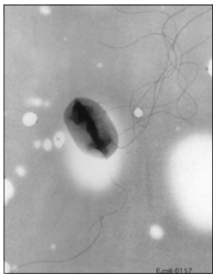


Photo courtesy CDC image library

Only standard practices are required for laboratory work at this level. Standard practices include:

- frequent handwashing, especially after removing gloves and before leaving the laboratory;
- a door that can be kept closed when working;
- limits on access to the lab space when working;
- no smoking, eating, drinking, or storage of food in the laboratory;
- care to minimize splashes and actions that may create aerosols (tiny droplets);
- decontamination of work surfaces after every use and after any spills;
- decontamination of laboratory wastes;
- use of mechanical pipettes only (no mouth pipetting);
- "sharps" precautions, including special containers for disposing of needles and other sharp objects;
- maintenance of an insect and rodent control program; and
- use of personal protective equipment (such as lab coats, latex gloves, and eye protection or face shields as needed depending on the type of work being done).

An open bench top sink is required for hand washing. No other major facility structures are required for BSL-1 labs.

Other Precautions

Biosafety precautions are important outside the lab also. Universal precautions have been developed to protect health professionals. While these precautions most often apply in a clinical setting such as a hospital or doctor's office, they may also be important for field epidemiology practices during an outbreak investigation, particularly when collecting lab specimens.

Universal precautions include hand hygiene, use of gloves, gown, masks, eye protection, and face shields, and safe injection practices. Universal precautions also require that all equipment or items likely to have been contaminated with infectious fluids are handled in a manner that prevents transmission of any infectious agents.

Special circumstances such as the decontamination of methamphetamine labs may require additional precautions such as protective clothing and special site decontamination.

Biosafety Level 2 (BSL-2)

Agents associated with human disease are studied in BSL-2 laboratories. A BSL-2 lab is generally required for working with any human-derived blood, other bodily fluids (particularly when visibly contaminated with blood), or tissues in which the presence of an infectious agent may be unknown.

In working with BSL-2 agents, the primary hazards to personnel are accidental needle sticks, potential infection through exposure to the eyes and nose (mucous membranes), and ingestion of infectious materials.

BSL-2 labs work with organisms such as the measles virus, many *Salmonella* species, pathogenic *Toxoplasma* species, *Clostridium botulinum*, hepatitis B virus (see Figure 2), and other bloodborne pathogens.

Figure 2. A transmission electron micrograph of hepatitis B virus, a BSL-2 agent

Photo courtesy CDC image library

BSL-2 agents do not cause lethal infections and are not transmissible via the airborne route. This means that they do not cause infection if tiny droplets of the material become airborne (i.e., aerosolized) and are inhaled, which might occur if the material were spattered. In addition, agents studied in a BSL-2 lab are pathogens for which immunization or antibiotic treatment is available. However, extreme care should still be taken with needles and sharp lab instruments when they are contaminated with agents.

To reduce accidental infection, special procedures for BSL-2 labs include all standard practices for BSL-1 labs, plus a few others:

- special policies and procedures to restrict access to the lab when work is being conducted;
- biohazard warning signs posted outside the lab (see Figure 3);
- surveillance of laboratory personnel with appropriate immunizations offered;
- a biosafety manual that includes definitions of any needed waste decontamination or medical surveillance policies specific to the activities and agents in that lab; and
- supervisory staff who have experience in working with infectious agents and specific training for laboratory personnel in handling these agents.

Some primary barriers in BSL-2 labs are biosafety cabinets or other approved containment devices. These areas minimize potential contamination while working with an agent, particularly if there may be splashes or aerosolization of infectious materials.

Personal protective equipment includes lab coats, gloves, and face protection as needed when working with infectious agents. Protective clothing must be removed when personnel leave the laboratory area.

Cabinets should be thoroughly decontaminated daily and if radioactive materials are used, monitored for radiation as a method of personal protection.

Secondary barriers include all BSL-1 barriers, plus an autoclave (sterilization machine) for lab glassware.

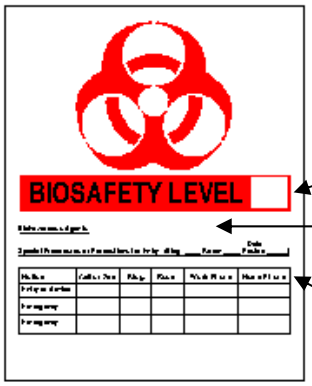
Biosafety Level 3 (BSL-3)

A BSL-3 laboratory must be used when work is done with indigenous or exotic agents that have the potential for respiratory (aerosol) transmission and may cause serious and potentially lethal infection. Materials potentially infected with these agents may be studied at a BSL-2 level for diagnostic purposes only, but further manipulation and experimentation require BSL-3 conditions.

The primary hazard for personnel working with these agents is risk of infection from needle sticks, ingestion, or exposure to infectious aerosols.

- For example, part of public health surveillance for West Nile virus (WNV) is testing birds for the presence of the virus since birds often serve as the first indicator of the virus in a geographic region or in a season. In August of 2002, a state laboratory worker accidentally cut his finger while dissecting a bird to test for WNV. Four days later, this worker had symptoms of fever, myalgia, recurring sweats, and hot flashes. The worker and the bird he was working with were both eventually diagnosed with WNV. (3) There were 2 lab-acquired cases of WNV in 2002.

Figure 3. A biosafety sign that would be posted outside a lab working with infectious agents



The sign contains the following information:

- The lab's biosafety level
- The infectious agents under study
- Contact information for the responsible person and 2 emergency contacts

Agents studied in a BSL-3 lab include *Mycobacterium tuberculosis* (tuberculosis), St. Louis encephalitis virus, *Francisella tularensis* (tularemia), and *Coxiella burnetii.*

Tularemia (see Figure 4) is a relatively common source of laboratory-acquired infection. Most infections occur while handling animals infected with the bacteria (such as rabbits, a natural reservoir), or while experimenting with tularemia cultures. Laboratory-acquired infections of tularemia were not reportable before 9/11/2001, but were known to occur. (1) Tularemia is now classified as a potential biological weapon and is a reportable disease.

Figure 4. A photograph of *F. tularensis* under a direct fluorescent antibody stain; this bacterium is a BSL-3 agent

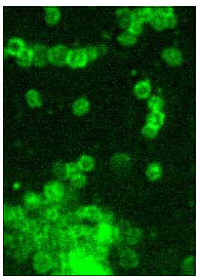


Photo courtesy CDC image library

Established lab practices that must be in place in BSL-3 laboratories include all BSL-2 practices, plus:

- strictly controlled access to the lab;
- specific training for lab personnel in handling potentially lethal agents;
- decontaminating all waste;
- changing contaminated protective lab clothing and decontaminating all lab clothing before laundering; and
- institutional policies regarding specimen (serum) collection and storage from lab workers to establish exposure to infectious agents.

Primary and secondary protective barriers in the BSL-3 lab emphasize protecting lab personnel, as well as personnel in nearby lab areas, the community, and the environment from exposure to potentially infectious aerosols. Primary barriers are similar to BSL-2 personal protective equipment, but may also include respiratory equipment if there is a risk of infection through inhalation.

Secondary barriers at BSL-3 labs include all BSL-2 barriers, plus a few more sophisticated barriers. Corridors must be separated from direct access to the laboratory. Access must be through self-closing double doors. Air handling systems must be designed to ensure negative air flow, so that air around doors and windows flows *into* the laboratory rather than out of the laboratory. Air pumped into the laboratory is not re-circulated in the building. This measure is to prevent infectious aerosols from being carried outside the lab through the air.

Biosafety Level 4 (BSL-4)

Dangerous and exotic agents that pose high risk of life-threatening disease and aerosol-transmitted infections are found in BSL-4 labs. Related agents with an unknown risk of transmission are also studied in these labs. These agents pose a high risk of life-threatening disease, can be transmitted via the aerosol (respiratory) route, and have no available vaccine or therapy.

All BSL-4 agents are viruses. Examples are Marburg virus, Ebola virus (see Figure 5), and viruses that cause Congo-Crimean hemorrhagic fever and Lassa fever.

Figure 5. A transmission electron micrograph of the Ebola virus, a BSL-4 agent

Photo courtesy CDC image library

Laboratory work with materials potentially infected with these agents, such as diagnostic samples or naturally and experimentally infected animals, pose a high risk of exposure and infection to laboratory personnel, and possibly even to the community and the environment.

Personnel working with BSL-4 agents may be at risk of respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets, and accidental needle sticks with needles or other sharp objects contaminated with infectious material.

- For example, in the late 1960s, there were 25 laboratory-acquired Marburg infections, including 5 deaths. These workers had been studying infected monkeys from Uganda. The first documented naturally-occurring human case occurred in 1975. (1)

Because of these risks, all lab personnel must receive specialized training in handling extremely dangerous infec-

tious agents and in containment equipment and functions.

Furthermore, access to the lab is restricted. Immunocompromised persons (including children and pregnant women) are never allowed to enter the lab.

Laboratory practices for the BSL-4 include all BSL-3 practices, plus:

- strictly controlled access to the laboratory;
- changing clothing before entering and exiting the lab (showering upon exiting the lab is recommended); and
- decontaminating all material exiting the facility.

Primary barriers include conducting procedures in the biosafety cabinets used at the other biosafety levels in combination with a full-body, air-supplied, positive pressure personnel suit. Thus BSL-4 lab workers do not enter the lab unless they are wearing a "space suit" (see Figure 6).

Figure 6. A BSL-4 personnel suit, with air supply provided through the coil coming from the top of the helmet

Photo courtesy CDC image library

Secondary barriers in BSL-4 facilities include all the physical barriers at BSL-3 labs, plus:

- an isolated zone or a separate building;
- dedicated supply and exhaust, vacuum, and decontamination systems; and
- a recommended absence of windows—any windows must be sealed and resistant to breakage.

Laboratory Locations

BSL-1 laboratories are fairly ubiquitous. Many are located in high schools, community colleges, and municipal drinking water treatment facilities. (1)

BSL-2 facilities may be found in local health departments, universities, state laboratories, private laboratories (e.g., hospitals or health care systems), and industrial laboratories (e.g., clinical diagnostic companies).

Most facilities that conduct infectious disease research have BSL-3 laboratories. They may be located in state health departments, universities, private companies, industry, and the federal government (e.g., the National Institutes of Health [NIH] and the Centers for Disease Control and Prevention [CDC]).

In 2007, there were only 15 BSL-4 facilities in the United States: 9 federal facilities (including the CDC in Atlanta, GA, and the National Institutes of Health in Bethesda, MD), 4 university facilities (including Georgia State University in Atlanta, GA, and the University of Texas Medical Branch in Galveston, TX), 1 state facility, and 1 private facility. Renovations are underway at several of these labs, and new BSL-4 facilities are being proposed at additional sites. (4)

Glossary

Microbiology lab: A laboratory that studies microorganisms, such as bacteria and viruses, from people, animals, plants, or the larger environment.

Transmission electron micrograph: A picture taken from a transmission electron microscope (TEM). The microscope shoots a ray of electrons instead of light to create a picture of the item under view.

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