Chapter 12
Standard Safety Practices in the Microbiology Laboratory

Laboratorians working with infectious agents are subject to laboratory-acquired infections through accidents or unrecognized incidents. The degree of hazard depends upon the virulence of the biological agent concerned and host resistance. Laboratory-acquired infections occur when microorganisms are inadvertently ingested, inhaled, or introduced into the tissues. The primary laboratory hazard associated with enteric pathogens such as *Shigella* and *E. coli* O157:H7 is accidental ingestion. Biosafety Level 2 (BSL-2) practices are suitable for work involving these agents, which are a moderate potential hazard to personnel and the environment. BSL-2 requirements:

- Laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists;
- Access to the laboratory is limited when work is being conducted;
- Extreme precautions are taken with contaminated sharp items;
- Certain procedures in which infectious aerosols or splashes may be created are conducted using protective clothing and equipment.

A. Standard Microbiological Safety Practices

The safety guidelines listed below apply to all microbiology laboratories regardless of biosafety level.

Limiting access to laboratory

Biohazard signs or stickers should be posted near all laboratory doors and on all equipment (incubators, hoods, refrigerators, freezers) used for laboratory work. Children under 12 years of age and pets are not allowed in laboratory areas. All laboratories should be locked when not in use. All freezers and refrigerators located in corridors should be locked.

Handwashing

Each laboratory should contain a sink for handwashing. Frequent handwashing is one of the most effective procedures for avoiding laboratory-acquired infections. Hands should be washed with an appropriate germicidal soap before exiting the laboratory or after handling infectious materials.

Eating

Eating, drinking, and smoking are not permitted in the work areas. Food must be stored and eaten outside of the work area in designated areas used for that purpose only. Do not lay personal articles such as handbags or eyeglasses on the workstations.

Mouth pipetting

Mouth pipetting should be strictly prohibited in the laboratory. Rubber bulbs or mechanical devices should be used.

Sharps

A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Dispose of sharps in designated containers. To minimize finger sticks, used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Nondisposable sharps, including syringes, should be placed in a labeled discard pan for decontamination before cleaning. Broken glassware should not be handled directly by hand but should be removed by mechanical means such as a brush and dustpan, tongs, or forceps.

Aerosols

Perform all procedures carefully to minimize the creation of splashes or aerosols. Techniques that tend to produce aerosols should be avoided. Cool inoculating wires and loops by holding them still in the air for 5 to 10 seconds before touching colonies or clinical material. Loops containing infectious material should be dried in the hot air above the burner before flaming. Vortexing and centrifugation should be done in closed containers. Gauze should be used to remove the tops on blood specimens and should be placed around the top of blood culture bottles to minimize aerosol production during removal of the needle. Needles should never be cut or removed from the syringe before autoclaving. All body fluids should be centrifuged in carriers with safety caps only.

When procedures with a high potential for creating infectious aerosols are conducted or when there is a risk of splashing or spraying the face with infectious or other hazardous materials, laboratory work should be conducted in a safety cabinet or with face protection (goggles, mask, face shield or other splatter guards). Procedures that pose a risk may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or eggs. Face protection should also be used when working with high concentrations or large volumes of infectious agents.

Decontaminating bench tops and other surfaces

Bench tops should be wiped with a disinfectant (a phenolic disinfectant, 1% sodium hypochlorite, or 70% alcohol) routinely after working with infectious agents or clinical specimens or after spills, splashes, or other contamination by infectious materials. Solutions of disinfectants should be maintained at the work station (see *Disinfectants* below).

Disposal of contaminated materials

All discarded plates, tubes, clinical samples or other contaminated materials are to be placed in disposal containers at each bench. Special disposal boxes must be used for sharps such as syringes or broken glass to minimize the risk of injury. Avoid overfilling such containers. Containers of contaminated material should be carefully transported to the autoclave room and autoclaved before disposal.

Autoclaving

An autoclave must be available for the BSL-2/3 laboratory and must be operated only by personnel who have been properly trained in its use. To verify that each autoclave is working properly, spore strips or other biological indicators designed to test for efficiency of sterilization should be included in autoclave loads on a regular basis. Each autoclave load should be monitored with temperature-sensitive tape, thermograph, or other means (e.g., biological indicators).

General laboratory policies

All areas of the laboratory must be kept clean and orderly. Dirt, dust, crowding, or clutter is a safety hazard and is not consistent with acceptable biological research. Floors should be kept clean and free of unnecessary clutter. They should be washed with a germicidal solution on a regular basis and after any spills of infectious material have occurred.

Refrigerators and freezers

Refrigerators and freezers should be regularly inspected for the presence of broken vials or tubes containing infectious agents. Wear gloves and proper attire when removing and discarding broken material. Refrigerators and freezers should be regularly cleaned with a disinfectant and defrosted to prevent possible contamination and temperature failure.

Fire prevention

Keep burners away from lamps and flammable materials. Bulk flammable material must be stored in the safety cabinet. Small amounts of these materials, such as ethyl acetate, ethyl alcohol, and methanol, can be stored in safety containers. Turn off burners when not in use. Know the location of fire extinguishers, fire blankets, and showers. Fire safety instructions and evacuation routes should be posted.

B. Special Practices

Transport of biohazardous materials

Transport of biohazardous materials from one building to another increases the risk of breakage and spills. If transport is necessary, the primary infectious agent container (regardless of size) must be placed in an unbreakable second container that can be sealed (e.g., screw-top tube, plastic bag).

Disinfectants

Organisms may have different susceptibilities to various disinfectants. As a surface disinfectant, 70% alcohol is generally effective for the *Enterobacteriaceae*, but other organisms are more resistant. However, 70% alcohol is not the disinfectant of choice for decontaminating spills. Phenolic disinfectants, although expensive, are usually effective against many organisms. Always read disinfectant labels for manufacturers' recommendations for dilution and for exposure times for efficacy, especially before use on BSL-3 organisms such as *Mycobacterium tuberculosis*. A good general disinfectant is a 1:100 (1%) dilution of household bleach in water; at this dilution, bleach can be used for wiping surfaces of benches, hoods and other equipment. A 1:10 (10%) dilution of bleach is corrosive and will pit stainless steel and should not be used routinely; however, it may be used to clean up spills of cultured or concentrated infectious material where heavy contamination has occurred. **Dilutions of sodium hypochlorite should be made daily from a stock solution.**

Decontamination of spills

The following procedure is recommended for decontaminating spills. Isolate the area to prevent anyone from entering. Wear gloves and protective clothing (gown or lab coat; mask if the spill may contain a respiratory agent or if the agent is unknown). Absorb or cover the spill with disposable towels. Saturate the towels with an appropriately diluted intermediate or high level disinfectant (e.g., a phenolic formulation or household bleach). Place disinfectant-soaked towels over the area and leave them in place for at least 15 minutes before removing and discarding them. Wipe area using clean disinfectant-soaked towels and allow area to air dry. Place all disposable materials used to decontaminate the spill into a biohazard container. Handle the material in the same manner as other infectious waste.

Accidents

All injuries or unusual incidents should be reported immediately to the supervisor. When cuts or puncture wounds from potentially infected needles or glassware occur, the affected area should be promptly washed with disinfectant soap and water. In the event of a centrifuge accident in which safety carriers have not been used, other personnel in the area should be warned immediately and the area isolated to prevent anyone from entering.

C. Protective Clothing and Equipment

Laboratory coats

Protective coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working in the laboratory. This protective clothing should be removed and left in the laboratory before leaving for non-laboratory areas. All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.

Gloves

Regardless of the type of infectious material, gloves should be worn when performing potentially hazardous procedures (e.g., slide agglutination) in which there is a risk of splashing or skin contamination or when the laboratory worker has cuts or broken skin on his or her hands. Gloves should always be worn when handling clinical specimens, body fluids, and tissues from humans and animals. These tissues should be assumed to be positive for hepatitis B virus, HIV, other bloodborne pathogens, or *Mycobacterium tuberculosis*. Gloves must be removed when contaminated by splashing or spills or when work with infectious materials is completed. Gloves should not be worn outside the laboratory. Do not use the telephone or open doors with gloves that have been used in laboratory procedures. Dispose of all used gloves by discarding them with other disposable materials and autoclaving. Hands should be washed immediately after removing gloves.

Barrier precautions

Clinical specimens, body fluids, and tissues from humans and animals should be assumed to be positive for hepatitis B virus, HIV, other bloodborne pathogens, or *Mycobacterium tuberculosis*. These materials should be handled in a safety cabinet or using other barrier precautions such as goggles, mask, face shield or other splatter guards whenever there is a potential for creating an aerosol.

References

Centers for Disease Control and Prevention, National Institutes of Health. Biosafety in microbiological and biomedical laboratories. Washington, DC: U.S. Government Printing Office; 1999: stock no. 017-040-00547-4.

World Health Organization. Laboratory biosafety manual, 2nd edition. Geneva: WHO; 1993: ISBN 92 4 154450 3.