

## CHAPTER 4

### Biosafety

Laboratorians working with infectious agents are at risk of laboratory-acquired infections as a result of accidents or unrecognized incidents. The degree of hazard depends upon the virulence and dose of the biological agent, route of exposure, host resistance, proper biosafety training, and experience with biohazards. Laboratory-acquired infections occur when microorganisms are inadvertently ingested, inhaled, or introduced into tissues. Multiple instances of laboratory-acquired meningococcal infection have been reported with a case fatality rate of 50% (1, 2). While laboratory-acquired *H. influenzae* and *S. pneumoniae* infections are not as extensively reported, deadly infections with any of these organisms are possible if appropriate biosafety procedures are not strictly followed in a properly equipped laboratory. Biosafety Level 2 (BSL-2) practices are required for work involving these agents as they present a potential hazard to personnel and the environment. The following requirements have been established for laboratorians working in BSL-2 facilities:

- Laboratory personnel must receive specific training in handling pathogenic agents and be directed by fully trained and experienced scientists.
- Access to the laboratory must be limited to personnel who have a need to be in the laboratory and have undergone proper training when work is being conducted.
- Extreme precautions must be taken with contaminated sharp items and sharps must be disposed of in labeled appropriate hardened plastic containers.
- Personal protective equipment (PPE) must be worn at all times, and particular care must be taken when performing procedures that have the potential to create aerosols.

#### I. Protective clothing and equipment

##### A. Laboratory coats

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

## **B. Gloves**

Regardless of the type of infectious material, gloves should be worn when performing potentially hazardous procedures involving infectious materials 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 specimens should be handled as if they are positive for hepatitis B virus, human immunodeficiency virus (HIV), or any bloodborne pathogens. Gloves must be removed when contaminated by splashing or spills or when work with infectious materials is completed. When removing gloves, avoid touching any areas of the gloves that may have come in contact with infectious material.

Gloves should not be worn outside the laboratory. Personnel should not use the telephone, computer, or open doors with gloves that have been used in laboratory procedures. All used gloves should be disposed of by discarding them with other disposable materials and autoclaving. Hands should be washed immediately after removing gloves.

## **C. Barrier precautions**

Clinical specimens, body fluids, and tissues from humans and animals should be assumed to be positive for human pathogens. These materials should be handled in a biosafety cabinet (BSC) or using other barrier precautions (e.g., goggles, mask, face shield, or other splatter guards) whenever a procedure is performed that can potentially create an aerosol. However, manipulating suspensions of *N. meningitidis* outside of a biosafety cabinet is associated with a high risk for contracting meningococcal disease (2) and using only a splatter guard on the bench top does not provide adequate protection (1).

## **D. Foot Protection**

Closed-toe comfortable shoes that have low heels should be worn in the laboratory or other areas where chemicals are present. This will reduce injuries that may occur from spills, splashes, falling objects, slipping, and broken glass.

## **II. Standard microbiological safety practices**

The following safety guidelines listed below apply to all microbiology laboratories, regardless of biosafety level. All procedures requiring handling of infectious materials, potentially infectious materials, or clinical specimens should be performed while wearing appropriate PPE.

## **A. Limiting access to laboratory**

Sometimes non-laboratorians attempt to enter the laboratory to obtain test results. Although this occurs more frequently in clinical laboratories, access to the laboratory should be limited to trained personnel with a need to work in the laboratory, regardless of the setting.

Biohazard signs or stickers should be posted near or on all laboratory doors and on all equipment used for laboratory work (e.g., incubators, hoods, microwaves, ice machines, refrigerators, and freezers). Children who have not reached the age of adulthood and pets are not allowed in laboratory areas. All laboratories should be locked when not in use. In addition, all freezers and refrigerators located in corridors should be locked, especially those that contain infectious organisms or other hazardous materials.

## **B. Disinfectants**

Organisms may have different susceptibilities to various disinfectants. As a surface disinfectant, 70% isopropyl alcohol is generally effective. However, 70% alcohol is not the disinfectant of choice for decontaminating spills. It should be noted that 100% alcohol is not as effective a disinfectant as 70% alcohol. Phenolic disinfectants, although expensive, are effective against many organisms. Always read disinfectant labels for manufacturers' recommendations for dilution and for exposure times for efficacy. An effective general disinfectant is a 1:100 (1%) dilution of household bleach (sodium hypochlorite) 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 should be used to clean up spills of cultured or concentrated infectious material where heavy contamination has occurred; however, it is more corrosive, will pit stainless steel, and should not be used routinely. If bleach is used, wipe down the area with 70% alcohol to inactivate the bleach. If bleach is used as a disinfectant, the diluted solutions should be made weekly from a concentrated stock solution.

## **C. Decontamination of spills**

The following procedure is recommended for decontaminating spills:

- Isolate the area to prevent anyone from entering.
- Wear gloves and protective clothing such as a gown or lab coat, shoes, and a mask (if the spill may contain a respiratory agent or if the agent is unknown).
- Absorb or cover the spill with disposable towels, but do not wipe up the spill or remove the towels.
- Saturate the towels and the affected area with an appropriately diluted intermediate or high-level disinfectant (e.g., a phenolic formulation or household bleach) and leave them in place for at least 15 minutes.
- 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. If broken glassware is involved, use mechanical means to dispose of it.
- Handle the material in the same manner as other infectious waste.

#### **D. Hand washing**

All laboratories should contain a sink with running water and soap for hand washing. Frequent hand washing is one of the most effective procedures for avoiding laboratory-acquired infections. Hands should be washed for at least one minute with an appropriate germicidal soap after infectious materials are handled and before exiting the laboratory. If germicidal soap is unavailable, then use 70% isopropyl or ethyl alcohol to cleanse hands.

#### **E. Eating**

Eating, drinking, and smoking are not permitted in laboratory work areas. Food must be stored and eaten outside of the laboratory in designated areas used for that purpose only. Personal articles (e.g., handbags, eyeglasses, or wallets) should not be placed on laboratory workstations.

#### **F. Mouth pipetting**

Mouth pipetting is strictly prohibited. Rubber bulbs or mechanical devices must be used.

#### **G. Sharps**

A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, glass pipettes, capillary tubes, broken glassware, and scalpels. Sharps should be disposed of in designated puncture-proof, leak-proof, and sealable sharps 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. Non-disposable sharps 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 (e.g., brush and dustpan, tongs, or forceps).

## H. Aerosols

All procedures must be carefully performed to minimize splashes or aerosolization. When procedures with a high potential for creating infectious aerosols are conducted or when a procedure that can result in splashing or spraying of the face with infectious or other hazardous materials is used, laboratory work should be conducted in a biosafety cabinet or by laboratorians wearing the appropriate face protection equipment (e.g., goggles, mask, face shield, or other splatter guards). Face protection should also be used when working with high concentrations or large volumes of infectious agents. Procedures that pose such a risk may include:

- Centrifugation, vortexing, and vigorous mixing: these procedures should be performed in closed containers. If safety capped tubes are not available, sealed tubes should be used. All body fluids and infectious materials should only be centrifuged in carriers with safety caps.
- Handling tissue specimens or bodily fluids: 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. Grinding of tissue specimens should be performed in a biosafety cabinet.
- Sonic disruption: infectious materials that undergo sonic disruption should be placed in a sealed container within the sonicator.
- Opening containers of infectious materials whose internal pressures or temperatures may be different from ambient pressures or temperatures.
- Loops containing infectious material should be dried in the hot air above a burner before flaming.
- Inoculating wires and loops should be cooled after flame sterilization by holding them still in the air for 5-10 seconds before they touch colonies or clinical material. Disposable loops are preferred if resources are available.

## **I. Decontaminating bench tops and other surfaces**

Bench tops and other potentially contaminated surfaces should be wiped with a phenolic disinfectant (10% bleach) routinely after working with infectious agents or clinical specimens or after spills, splashes, or other contamination by infectious materials. Following disinfection with 10% bleach, the surface must then be wiped down with 70% isopropyl or ethyl alcohol to inactivate the bleach and prevent corrosion of the work surface. Solutions of disinfectants should be maintained at each work station (see Disinfectants, Section II.B.).

## **J. Disposal of contaminated materials**

All discarded plates, tubes, clinical samples, pipettes, gloves, and other contaminated materials should be placed in disposal containers at each bench. Special disposal containers typically constructed of puncture-proof plastic must be used for sharps to minimize the risk of injury. Avoid overfilling disposal containers. The lids should rest flush with the top of the container. Containers of contaminated material should be carefully transported to the autoclave room and autoclaved before disposal. Water should be added to each container to be autoclaved for optimal sterilization. Waste disposal containers in the laboratory should be clearly labeled for disposal of infectious items or non-infectious items. Waste disposal containers for infectious or potentially infectious items should be lined with a plastic biohazard or otherwise specially marked bag.

## **K. Autoclaving**

An autoclave must be available for the BSL-2 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 (such as *Bacillus stearothermophilus*) or other biological indicators designed to test for efficiency of sterilization should be included in autoclave loads on a regular basis (i.e., monthly). Each autoclave load should be monitored with temperature-sensitive tape, thermograph, or by other means (i.e., biological indicators). A logbook should be maintained for each autoclave to record the date, times, and indicator of sterilization of each autoclave run.

## **L. General laboratory cleanliness**

All areas of the laboratory must be kept clean and orderly. Dirt, dust, crowding, or clutter is a safety hazard, may lead to contamination of specimens, isolates, and/or biological assays, and is not consistent with acceptable biological research. Floors should be kept clean and free of unnecessary clutter and should be washed with a germicidal solution on a regular basis and after any spill of infectious material.

## **M. Refrigerators and freezers**

The temperature of laboratory refrigerators and freezers should be monitored daily to ensure that they are functioning properly. They should also be regularly inspected for the presence of broken vials or tubes containing infectious agents. When removing and discarding broken material, laboratorians should wear gloves and PPE. If the broken material is suspected of being infectious, disinfectant should be applied to the affected area and kept in place for at least 15 minutes before removal of the broken material. Refrigerators and freezers should be regularly cleaned with a disinfectant and defrosted to prevent possible contamination or temperature failure.

## **N. Fire prevention**

Burners should be used away from light fixtures and flammable materials. Bulk flammable material must be stored in a safety cabinet. Small amounts of these flammable materials (e.g., ethyl acetate, ethyl alcohol, and methanol) can be stored in safety containers such as a safety bench can or dispenser can. Burners must be turned off when not in use. All laboratorians must know the location of fire extinguishers, fire blankets, alarms, and showers, and fire safety instructions and evacuation routes should be posted.

## **III. Special Practices**

### **A. 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 for 15 minutes. Report a needle-stick injury, any other skin puncture, to the supervisor and appropriate health officials immediately as prophylactic treatment of the personnel performing the procedure may be indicated. 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 should be isolated to prevent anyone from entering.

### **B. Laboratory design and equipment**

The laboratory should be designed to avoid conditions that pose biosafety problems. Ample space should be provided to allow for safe circulation of staff when working and cleaning. There should be clear separation of areas for infectious and non-infectious work. Illumination should be adequate. Walls, ceiling, floors, benches, and chairs must be easy to clean, impermeable to liquids, and resistant to chemicals and disinfectants. Hand-washing basins with running water and soap and disinfectant must be provided in each room. An autoclave or other means of decontamination must be available close to the laboratory. Adequate storage space for specimens, reagents, supplies, or personal items should be provided inside and outside the working area, as appropriate. Safety systems for fire, chemicals, electrical, or radiation emergencies, and an emergency shower and eyewash facilities should be in place. Security measures should also prevent theft, misuse, or deliberate release of the infectious materials.

### **C. Medical surveillance of laboratory workers**

The employing authority is responsible for providing adequate surveillance and management of occupationally acquired infections. Pre-employment and periodic health checks should be organized and performed. Prophylaxis or other specific protective measures may be applied after a risk assessment of possible exposure and a health check of the individual or individuals. Special attention should be paid to women of childbearing age and pregnant women as some microorganisms present a higher risk for the fetus (i.e., rubella virus).

Immunization of the laboratory workers can also be proposed taking into account the following criteria:

- Conclusion of the risk assessment.
- Verification by serology of the immunization status of the worker (some workers may be already immunized from prior vaccination or infection).
- The local availability, licensing state, and utility of vaccines (i.e., does the vaccine provide protection against the prevalent serogroups or serotypes circulating in the region?).
- The availability of therapeutic drugs (i.e., antibiotics) in case of accident.
- The existence of national regulations or recommendations.

A first-aid box containing basic medical supplies should be available along with a written emergency procedure to access a doctor for definitive treatment of the injury. First aid kits should be periodically checked to ensure contents are within the expiration date.

### **D. Biosafety management and implementation**

The laboratory director is responsible for implementation of biosafety measures. He or she can delegate tasks to a qualified individual or a group of individuals who perform them on a part-time basis, or even assign a biosafety officer with the appropriate background and knowledge.

### **E. Other sources of biosafety information**

For more information, please review:

Centers for Disease Control and Prevention. 1999. Biosafety in microbiological and biomedical laboratories, 5<sup>th</sup> ed. Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

Clinical and Laboratory Standards Institute. Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guidelines—Third Edition. CLSI document M29-A3. [ISBN 1-56238-567-4]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898. USA, 2005.

Laboratory Biosafety Manual, third edition. WHO/CDS/CSR/LYO/2004.11. World Health Organization, Geneva, 2004, p 178.



Laboratory Biosafety Guidelines, third edition. Public Health Agency of Canada, 2004, p 113.

Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world. Centers for Disease Control and Prevention and World Health Organization, 2003, p 359.

## **References**

1. **Sevjar, J.J., Johnson, D., Popovic, T., Miller, M. J., Downes, F., Somsel, P., Weyent, R., Stephens, D. S., Perkins, B. A., and Rosenstein, N. E.** 2005. Assessing the risk of laboratory-acquired meningococcal disease. *Journal of Clinical Microbiology*, **43**:4811-4813.
2. **Boutet, R., Stuart, J. M., Kaczmarek, E. B., Gray, S. J., Jones, D. M, and Andrews, N.** 2001. Risk of Laboratory-Acquired Meningococcal Disease. *Journal of Hospital Infection*, **49**:282-284.