

BIOSAFETY GUIDELINES FOR LABORATORIES ANALYZING ENVIRONMENTAL SAMPLES FOR *CRYPTOSPORIDIUM*

1.0 Introduction

Laboratory-related infections with *Cryptosporidium* can occur if personnel are not properly trained in biosafety techniques. In addition to waterborne, fecal-oral, person to person transmission, and animal to person, circumstantial evidence suggests that airborne transmission of oocysts may occur¹. Adoption of a biosafety policy by laboratory management that includes commitment to technician safety, training and supervision, as well as, rigid adherence to biosafety guidelines will prevent the occurrence of *Cryptosporidium* infection (cryptosporidiosis) in laboratory personnel.

Symptoms associated with cryptosporidiosis may include: watery diarrhea, abdominal cramps, nausea, low-grade fever, dehydration, weight loss, and loss of appetite. Symptoms may develop within 2 to 10 days after infection. There are no antibiotics or drug treatments that will cure cryptosporidiosis. For additional information : www.cdc.gov/ncidod/dpd/parasites/cryptosporidiosis/default.htm

Biosafety Level 2 (BSL 2) practices and facilities are recommended for activities with infective stages of *Cryptosporidium*. A BSL 2 facility is appropriate for agents known to cause disease in humans.

Biosafety Level 2 practices and facilities include the following 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
- Certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety II cabinets

2.0 Scope and Application

The biosafety guidelines described in this document are adapted from *Laboratory Safety: Principles and Practices, Second Edition* (Reference 8.1) and *Biosafety in Microbiological and Biomedical Laboratories (BMBL) Fourth Edition* (Reference 8.2). A readily available laboratory-specific biosafety manual should be developed, and maintained, to address the safety, handling, and laboratory practices described below. The manual should be distributed to all employees and available at all times. It should be reviewed annually, or as recommended, by the laboratory safety officer. Personnel are required to read and sign off on the document on a regular basis, as determined by the laboratory safety officer.

3.0 General Safety Practices

3.1 Basic Laboratory Safety

- 3.1.1** Closed-toed shoes shall be worn in the laboratory.
- 3.1.2** All work surfaces and floors should be cleaned regularly and free of clutter.
- 3.1.3** All emergency numbers should be posted in the laboratory.

¹Hojlyng, N., Holten-Andersen, W., and S. Jepsen. 1987. Cryptosporidiosis: a case of airborne transmission. *Lancet*. 2:271-272.

Blagburn, B.L., and W.L. Current. 1983. Accidental infection of a researcher with human *Cryptosporidium*. *J. Infect. Dis.* 142:772-773.

- 3.1.4 All employees should be trained in the use and location of all safety/emergency equipment in each work area.
- 3.1.5 Biological safety II cabinets must be tested and certified annually.
- 3.1.6 All laboratory personnel should be trained in the proper procedures to clean up biological spills.

4.0 Microbiological Practices

- 4.1 Access to the laboratory is limited or restricted at the discretion of the laboratory director or laboratory safety officer when experiments are in progress.
- 4.2 Persons wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory.
- 4.3 Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.
- 4.4 Mouth pipetting is prohibited.
- 4.5 All procedures are performed carefully to minimize the creation of splashes or aerosols. Ensure that lids are used during all centrifugation and vortexing. Any procedures with open containers should be performed inside a BSL 2 hood.
- 4.6 Work surfaces are decontaminated before and after each use and after any spill of viable material.
- 4.7 All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving.
 - 4.7.1 Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leakproof container and closed for transport from the laboratory.
 - 4.7.2 Materials to be decontaminated off-site from the laboratory are packaged in accordance with applicable local, state, and federal regulations before removal from the facility.

5.0 Special Practices when Processing *Cryptosporidium* Samples

- 5.1 In general, persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised may be at risk of acquiring infections.
- 5.2 The laboratory safety officer has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. The laboratory safety officer will report to the laboratory director on a regular basis on the status of safety in the laboratory, conduct training and maintain outside professional contacts to exchange safety information relevant to laboratory operations.
- 5.3 The laboratory director or laboratory safety officer, establishes policies and procedures, whereby only persons who have been advised of the potential hazard and meet specific entry requirements, enter the laboratory or animal rooms.
- 5.4 When the infectious agent(s) in use in the laboratory require special provisions or special training for entry, a hazard warning sign incorporating the universal biohazard symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the infectious agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

- 5.5** Laboratory personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary for procedural or policy changes.
- 5.6** Materials containing *Cryptosporidium* are placed in a container that prevents leakage during collection, handling, processing storage, transport, or shipping.
- 5.7** Laboratory equipment should be decontaminated with an appropriate disinfectant before and after the equipment is used, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations before removal from the facility.
- 5.8** Spills and accidents which result in overt exposures to infectious materials are immediately reported to the laboratory director or laboratory safety officer. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

6.0 Safety Equipment (Primary Barriers)

- 6.1** Properly maintained biological safety II cabinets, and other appropriate personal protective equipment or physical containment devices are used whenever:
- 6.1.1** Procedures with potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, or opening containers of infectious materials whose internal pressures may be different from ambient pressures.
- 6.1.2** High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.
- 6.2** Face protection (goggles, mask, faceshield, or other splatter guards) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face, when the microorganisms must be manipulated outside the biological safety cabinet.
- 6.3** Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.
- 6.4** Gloves are worn when handling infected animals and when hands may contact infectious materials, contaminated surfaces, or equipment.
- 6.4.1** Wearing two pairs of gloves may be appropriate; if a spill or splatter occurs, the hand will be protected after the contaminated glove is removed.
- 6.4.2** Gloves are disposed of when contaminated, removed when work with infectious materials is complete, and are not worn outside the laboratory.
- 6.4.3** Disposable gloves are not washed or reused.

7.0 Laboratory Facilities (Secondary Barriers)

- 7.1** Each laboratory contains a sink for handwashing.
- 7.2** The laboratory is designed so that it can be easily cleaned. Rugs in laboratories are not appropriate, and should not be used because proper decontamination following a spill is extremely difficult to achieve.
- 7.3** Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
- 7.4** Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning.
- 7.5** If the laboratory has windows that open, they are fitted with fly screens.
- 7.6** A method for decontamination of infectious or regulated laboratory wastes is available (e.g., autoclave, chemical disinfection, incinerator, or other approved decontamination system).
- 7.7** An eyewash facility is readily available.
- 7.8** The laboratory facilities should be clean, temperature and humidity controlled, and have adequate lighting at bench tops.

8.0 References

- 8.1** D. O. Fleming, J. H. Richardson, J. J. Tulis, and D. Vesley. *Laboratory Safety: Principles and Practices*. 1995. Second Edition. American Society for Microbiology, Washington, D.C., publisher.
- 8.2** US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 1999. Fourth Edition. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, and National Institutes of Health, US Government Printing Office, Washington, D.C., publisher.
- 8.3** Fayer, Ronald, Ed. 1997. *Cryptosporidium and Cryptosporidiosis*. CRC Press, Inc., Boca Raton.