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Biosafety

Biosafety work involves biological materials, agents, and other materials of biological origin (e.g., organisms, cells, viruses, and toxins) that pose varying levels of risk or disease to humans, animals, or plants when stored or used. Biosafety Level 2 (BSL2) applies to work with a broad spectrum of moderate risk agents that are generally present in the environment at large and are associated with human disease of varying severity.

Purpose

The purpose of these guidelines is to identify precautionary measures that employees should follow to protect their health when engaged in field or laboratory activities that pose a risk of exposure to toxic forms of organisms.

1. Background

Some toxic forms that have been researched here at UMCES include Pfiesteria piscicida and morphologically related organisms and Vibrio vulnificans.

2. Policy

Personnel who intend to study biological agents must first notify the Safety Officer well in advance of the proposed use. All biohazardous/potentially biohazardous materials are subject to prior review and approval. Protocol review and approval is required for all biological agents in Risk Group 2 and/or those that may require Biosafety Level 2 (BS2). Level 3 (BSL3) and Level 4 (BSL4) are currently prohibited at UMCES. Access to the laboratory must be limited or restricted when experiments or work with cultures or specimens is in progress. The PI must ensure that all laboratory personnel receive appropriate training on hazards associated with the agents/toxins involved, the precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive training before starting work with the agents/toxins.

Guidelines for determination of biosafety levels can be found in the Centers for Disease Control publication *Biosafety in Microbiological and Biomedical Laboratories.* The *NIH Guidelines* established a classification of human infectious agents into four “risk groups” on the basis of hazard. These descriptions generally correlate with, but do not equate to, biosafety levels. A risk assessment will determine an agent’s biosafety level. Risk Groups (from *NIH Guidelines)* and Biosafety Levels (from *CDC Biosafety*) are defined below:

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Risk Group 1	**Biosafety Level 1**
Agents not associated with disease in healthy adult humans.	Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a relate science.
Risk Group 2	**Biosafety Level 2**
Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available.	Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that: 1. Laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2. Access to the laboratory is restricted when work is being conducted; and 3. All procedures in which infectious aerosols or splashes may be created are conducted in Biosafety Cabinets (BSCs) or other physical containment equipment.
Risk Group 3	**Biosafety Level 3**
Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.	Facilities not available at UMCES.
Risk Group 4	**Biosafety Level 4**
Agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.	Facilities not available at UMCES.

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Routes of human exposure, in order of highest concern are:

- Respiratory - Inhalation hazard.
- Conjunctivae (eyes) - Contact between toxic material and the eyes.
- Skin - Skin contact with toxics water.

Personal Protective Equipment (PPE)

The minimum personal protective equipment for handling hazardous material includes lab coat, closed toed footwear, safety glasses and disposable gloves. Users of biohazardous agents must ensure fume hoods/biological safety cabinets are adjusted and functioning properly prior to their use.

- Protective lab coats are recommended to prevent contamination of personal clothing.
- Wear protective eyewear (goggles, mask, face shield or other splatter guard) when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials.
- Gloves must be work to protect hands from exposure to hazardous materials. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary. Remove gloves and wash hands when work has been completed and before leaving the laboratory. Do not wash or reuse disposable gloves.
- Closed toed shoes are necessary to protect the feet.

Medical

Primary concerns include accidental needle sticks or cuts or mucous membrane exposures, or ingestion of infectious materials. Even though organisms routinely manipulated at BSL2 are not known to be transmissible by aerosol route, procedures with aerosol or high splash potential may increase the risk of personnel exposure.

Exposure to certain infectious agents may adversely affect a fetus during pregnancy if the mother is infected with the agent. Women that are pregnant or become pregnant are encouraged to inform their supervisor or Safety Officer. If your immune system is compromised or suppressed it is also advised to notify your supervisor or Safety Officer. Your physician should be informed of you work if you are pregnant or immune compromised.

Emergency Procedures

In the event of an exposure to a biohazardous agent or material, the following guidelines should be used:

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Intact Skin

- Remove contaminated clothing. Clothing should not be pulled over the face as contact with eyes, nose, and mouth may occur.
- Vigorously wash contaminated skin with soap and water.
- Call 911 or seek medical attention, if necessary.
- Inform the PI and Safety Officer.

Broken, Cut or Damaged Skin or Puncture Wound

- Remove contaminated clothing. Clothing should not be pulled over the face as contact with eyes, nose, and mouth may occur.
- Vigorously wash contaminated skin for 5 minutes with soap and water.
- Call 911 or seek immediate medical attention, if necessary.
- Inform the PI and Safety Officer

Eye

- Immediately flush eyes for at least 15 minutes with water, using an eyewash. (Hold eyelids away from your eyeball and rotate your eyes so that all surfaces may be washed thoroughly.)
- Call 911 or seek immediate medical attention, if necessary.
- Inform PI and Safety Officer

Ingestion or Inhalation

- Move to fresh air immediately.
- Call 911 or seek immediate medical attention, if necessary.
- Do not induce vomiting unless advised to do so by a health care provider.
- Inform the PI and Safety Officer

In case of a serious accident and/or injury, involving biohazards dial 911 and tell the dispatcher your name, the building and location, and the seriousness of injury.

Spills

- In case of a small spill, notify others in the area of the spill, apply gloves, clean spill, disinfect and dispose of waste properly.
- If clothing is contaminated, remove it with gloved hands, folding area inward.
- Thoroughly wash all potentially contaminated areas with soap and water.
- In case of a large spill, notify others in the area and notify the supervisor and/or Safety Officer.

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- Cover the spill with absorbent pads, apply disinfectant as specified in product SDS or recommended by the CEC Disinfecting Guidelines onto adjacent surfaces working toward spill.
- Allow disinfectant to stand for at least 15 minutes, proceed with thorough wipe down of spill and adjacent surface areas. When sharps are involved, wipe down and collection of waste shall be conducted via mechanical means.
- If the floor and sink are affected by the spill, flush these areas with disinfectant.
- Autoclave all clean-up materials before disposal.

Standard Biosafety Procedures

- Avoid hand to mouth or hand to eye contact in the laboratory. Never eat, drink, apply cosmetics or lip balm, handle contact lenses or take medication in the laboratory.
- Mouth pipetting is forbidden; only mechanical pipetting devices are permitted.
- Wash hands after removing gloves and other personal protective equipment (PPE), and prior to leaving the laboratory.
- Use sharps only when no alternatives (e.g. safety devices or non-sharps) exist. Dispose of sharps in properly labeled, puncture resistant, autoclavable sharps containers.
- Conduct procedures likely to create splashes, sprays, or aerosols within a biological safety cabinet (BSC).
- Store and transport containers of biohazardous liquids in secondary containers that will hold the contents of the primary container in the event of breakage.
- Store all biohazardous materials securely in clearly labeled, sealed containers. Storage units, incubators, freezers or refrigerators should be labeled with the Universal Biohazard Sign when they contain infectious material.
- Never allow contaminated, infectious waste materials to leave the laboratory or to be put in the regular trash or sanitary sewer without being decontaminated or sterilized. When autoclaving use adequate temperature (121C), pressure (15psi) and time, based on the size of the load. Also use a sterile indicator strip to verify sterilization.
- Clean up work area after each experiment with an approved disinfectant and place disposable materials in appropriate waste container before removing gloves.
- Minimize the amount of airborne dust created during sweeping the floor.
    - Mist the area with Lysol IC Spray or similar product before sweeping or using a HEPA vacuum.

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  - Dust can be placed in the normal room waste receptacle.
  - Using the Lysol IC Spray decontaminate the broom, and allow the broom to sit for 20 minutes before using the broom in another location.
- Wash the floor of the laboratory with either a 1:6 bleach solution or Lysol IC and water.
  - Provided that one of the two solutions above are used, the mops may be used elsewhere.
  - After the floor has been completely washed, allow the mop to soak in the solution for 20 minutes.

Maintenance and Housekeeping Guidelines for BSL2 laboratories

In accordance with regulatory requirements of OSHA, CDC, NIH and UMCES the following guidelines and procedures shall be used by Maintenance, Housekeeping, and outside contractors before any initiatives are undertaken for BSL2.

- Before any work by Maintenance, Housekeeping, outside Contractors, or any other person not authorized for BSL2 the PI responsible for the laboratory or the Safety Officer must give them clearance to begin work.
- The PI and/or SO must identify hazards that may be present or encountered and shall describe the proper procedures and techniques for personnel to minimize risks.
- A sign can be attached to the door of the BSL2 lab to indicate that the room has been decontaminated and made ready for additional housekeeping or maintenance activities.
- To sweep the floor, minimize the amount of airborne dust created.
  - Mist the area with Lysol IC Spray or similar product before sweeping or using a HEPA vacuum.
  - Dust can be placed in the normal room waste receptacle.
  - Using the Lysol IC Spray decontaminate the broom, and allow the broom to sit for 20 minutes before using the broom in another location.
- Wash the floor of the laboratory with either a 1:6 bleach solution or Lysol IC and water.
  - Provided that one of the two solutions above are used, the mops may be used elsewhere.
  - After the floor has been completely washed, allow the mop to soak in the solution for 20 minutes.

Warning Signs and Labels

- The universal biohazard sign must be posted on all lab entrances, laboratory freezers/refrigerators or storage containers.
- Names of responsible individuals to be contacted in case of

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emergencies must be posted outside of entrance doors leading into each laboratory.

- The universal biohazard label must be placed on bags/containers of biohazardous waste; bags/ containers used to store, dispose of, transport, or ship biohazardous material; and on refrigerators and freezers used to store biohazardous material.
- A list of significant hazards and required PPE must be posted.

Biosafety Cabinets

Biological Safety Cabinets (BSCs) are used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. BSCs use High Efficiency Particulate Air (HEPA) filters to protect personnel and products inside the BSC from contamination from aerosols and particulates. Only those which are hard ducted to the outside and provide a face velocity of 80 to 125 feet per minute should be used when working with volatile chemicals.

- Never use chemicals with the potential to generate hazardous vapors inside a BSC. The HEPA filters are intended only to remove particulates and biological agents.
- Turn the cabinet on for at least 5 minutes prior to use to remove airborne contamination.
- Place items into cabinet so that they can be worked with efficiently without unnecessary disruption of airflow.
- Work with materials from the clean to the dirty side.
- Do not disturb airflow by covering any portion of the grillwork with materials.
- Work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after spills, splashes, or other contamination by infectious materials.
- Be careful not to let Kimwipes to be blown into the hood and disrupt the motor operations.

Decontamination

Three main categories for decontamination are heat, liquid decontaminants, and vapors and gases.

Heat

Moist or dry heat is recommended as the most effective method of sterilization. Steam at 121^{o}C under pressure in the autoclave is the most convenient method of achieving sterility.

- Autoclaves present the hazard of steam under pressure which can be a source of scalding liquids onto personnel handling the containers. Fluids treated by steam under pressure may be superheated if removed from the

sterilizer too soon after treatment causing a sudden and violent boiling of the contents. Follow the standard operating procedures for use of autoclaves.

Liquid Decontaminants

Liquid decontaminants are used in surface decontamination and decontamination of liquid wastes.

- Small variations in temperature, contact time, pH, penetrability, the presence and state of dispersion and reactivity of organic material at the site of application make large differences in the effectiveness of liquid decontaminants. Therefore, complete reliance should not be placed on them when the end result must be sterility.
- There are many liquid decontaminants available as halogens, acids, alkalis, heavy metal salts, quaternary ammonium compounds, phenols, aldehydes, ketones, alcohols, and amines. None are equally useful or effective under all conditions for infectious agents.
- Freshly diluted bleach is effective. Ethanol removes corrosive residue but evaporates too quickly to be an effective disinfectant.

Vapors and Gases

Decontamination or fumigation is used to decontaminate all areas of the Biological Safety Cabinet (BSC), including interior and plenums and components.

- Typically, the only time fumigation is required is at a major service interval, such as when HEPA filters are consumed and require replacement or when a non-functioning component located in a potentially contaminated plenum area requires replacement. Often a filter change is only required once during the cabinet's life cycle.
- The three most commonly used chemicals in BSC fumigation are formaldehyde, chlorine dioxide, and hydrogen peroxide.
- It is of paramount importance that both the front aperture and the exhaust HEPA filter are sealed prior to any fumigation process commencing.

References

1. "Biosafety Level 2 is the second of four levels of precautionary measures established by the Centers for Disease Control and Prevention, and the National Institutes of Health for working with potentially hazardous biological materials. The measures are described in detail in the manual entitled Biosafety in Microbiological and Biomedical Laboratories (HHS Publication No (CDC) 93-8395). Alternatively, the manual can be found on the Internet at the following address: https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF

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2. OSHA 29 CFR 1910.134 Respiratory Protection..
3. Maryland Guidelines for Protection of Workers Who May be Exposed to Estuarine Waters - Revised May, 1998.

For more information and/or suggestions, contact the Environmental Safety Compliance Officer (ESCO) at Ext. 8441 or Email umces-safety@umces.edu