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Biosafety Procedure Manual

April, 2011

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- Biosafety Level 3 (BSL-3) Requires that practices, safety equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. At BSL-3 more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. The BSL-3 requires that 1) access to the laboratory be restricted; 2) personnel must have specific training in laboratory procedures, the handling of infected animals and the manipulation of potentially lethal agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards and microbiological agents; and 4) procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, must be conducted in Biological Safety Cabinets (BSCs) or by use of other physical containment equipment.
- Biosafety Level 4 (BSL-4) Work at this level involves dangerous and exotic agents that pose a high individual risk of aerosol-transmitted infections and life-threatening disease. ***No*** research activity with Biosafety Level 4 is authorized within any laboratory, research facility, or by any researcher at Clark Atlanta University.

Risk Groups: *Infectious agents* may be classified into four risk groups (RG1, RG2, RG3, and RG4) as follows:

- RG1: Agents that are not associated with disease in healthy adult humans.
- RG2: Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.
- RG3: Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (*high individual risk but low community risk*).
- RG4: Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (*high individual risk and high community risk*).

A list of biohazard agents and classification is available at: http://www.absa.org/riskgroups/index.html.

Universal Precautions: CDC recommends that blood and body fluid precautions be consistently used for all patients regardless of their blood borne infection status. These precautions are referred to as "Universal Blood and Body Fluid Precautions" or "Universal Precautions". Under these precautions, blood and certain body fluids of all patients are considered potentially infectious for human immunodeficiency virus (HIV), hepatitis B (HBV) and blood borne pathogens.

PPE:

purpose of carrying any potentially hazardous material. If you are transporting materials requiring the use of two hands, then the recommendation is to use a clean, uncontaminated lab cart.

Engineering Controls:

- Primary Barriers: meant to use methods such as biological safety cabinets and their respective ventilation systems for containment of biohazards.
- Secondary Barriers: meant to use building design features including floor to ceiling walls,

Basic Laboratory Safety Practice Standards

The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or potentially infected materials must be aware of potential hazards, and must be trained and proficient in the practices and techniques required for handling such material safely. The PI, director, or person in charge of the laboratory is responsible for providing or arranging the appropriate training for personnel or arranging of appropriate training.

Each laboratory should develop or adopt a biosafety or operations manual that identifies the hazards that will or may be encountered, and that specifies practices and procedures designed to minimize or eliminate exposures to these hazards. Personnel should be advised of special hazards and should be required to read and follow the required practices and procedures. A scientist trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling infectious agents must be responsible for the conduct of work with any infectious agents or materials. This individual should consult with biosafety or other health and safety professionals with regard to risk assessment.

When standard laboratory practices are not sufficient to control the hazards associated with a particular agent or laboratory procedure, additional measures may be needed. The laboratory director is responsible for selecting additional safety practices, which must be in keeping with the hazards associated with the agent or procedure. Laboratory personnel, safety practices, and techniques must be supplemented by appropriate facility design and engineering features, safety equipment, and management practices.

The risks associated with hazards found in biosafety laboratories/facilities are greatly reduced or eliminated if proper precautions and practices are observed. To manage these risks, and in response to a heightened concern for safety in the workplace, Clark Atlanta University has developed these minimal laboratory safety guidelines from the example of Oklahoma State University. These are intended to be the

When the organisms containing recombinant DNA molecules or infectious agents in use in the laboratory require special provisions for entry (e.g., immunization), 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.

Safety Equipment and Facility Management

Biological Safety Cabinets or BioSafety Cabinets (BSC)

Prevention and containment of infectious splashes or aerosols are of paramount importance to ensure a safe working environment for microbial research. The primary safety equipment used when manipulating microorganisms in the laboratory is the biological safety cabinet (BSC). Biological safety cabinets are classified based on their construction, airflow velocities and patterns, and exhaust system.

1. An appropriate BSC should be used according to anticipated risk of biohazards.
2. The BSC and the working areas should be sterilized appropriately prior to and after use.
3. Allow enough time for disinfection to take place when a chemical disinfectant is used.
4. Cabinet blowers should be turned on for five minutes to purge the cabinet before use.
5. Proper airflow level should be maintained during the operation according to equipment specification.
6. All the agents should be safely transferred to and from the cabinet.
7. All the biohazards generated should be properly secured and handled.
8. Appropriate PPE should be worn by users during the use of the cabinet.
9. Open flames should not be used in a BSC.
10. Immediately notify the PI of any malfunction of the cabinet and any issue related to the use of it.

Important Notes: All **purchases** of BSCs must first be approved by the Biosafety Chair.

Centrifuges

Centrifuges have to be used properly to prevent biohazards from spewing to users and the environment, and to prevent hard objects inside from being released into other chemicals and biological agents to cause further spill and contamination. Centrifuges have to be properly maintained to prevent malfunction which would result in leak, tube breakage, and spill. Due to nature of high speed, the improper use and malfunction of centrifuges would lead to the generation and release of large amount of aerosols.

1. A close inspection should be conducted first of centrifuges which includes no-load test-run and blanket test-run to check working condition and balancing stability of centrifuges.
2. A close inspection should be conducted of accessories such as glass and plastic centrifuge tubes; hairline cracks, and chipped rims before use. Use unbreakable tubes whenever possible. Buckets should be kept clean and free of broken glass and plastic.
3. Avoid filling tubes to the rim.

4. Centrifuge tubes should be firmly capped. Avoid using lightweight materials such as aluminum foil as caps.
5. Aerosol-free (sealed) centrifuge buckets or rotors are required for all centrifuging of infectious specimens and bacteria. Only the correct size tubes should be used in any centrifuge bucket.
6. Follow manufactures recommendations for tube and rotor maximum safe operation speeds.
7. Once samples to be centrifuged are prepared, load tubes into buckets inside the biological safety cabinet and seal carefully before moving to centrifuge.
8. After centrifugation, buckets should be opened in a biological safety cabinet to prevent exposure from aerosolized particles. Always visually inspect rotor for signs of tube leakage prior to opening buckets.
9. Decontaminate the outside of the cups or buckets before and after centrifugation. Inspect o-rings regularly and replace if cracked or dry.

Additional steps should be taken when using high-speed or ultra centrifuges:

- Connect the vacuum pump exhaust to a disinfectant trap.
- Record each run in a log book: keep a record of speed and run time for each rotor.
- Install a HEPA filter between the centrifuge and the vacuum pump.
- Never exceed the specified speed limitations of the rotor.
- Regularly inspect the rotor for contamination, corrosion, or cracks.

The following tables list more detailed information on various recommended disinfectants.

Disinfectants	***Proprietarlb8***

temperature, presence of steam and pressure. It is imperative that guidelines be followed to prevent injury or damage to the autoclave. Familiarization with manufacturerôs instructions is imperative before operating the unit. The following guidelines are provided for safe and effective operation.

1. Autoclave bags must be properly packaged and labeled identifying materials and the generator before being brought to an autoclave room. Material to be autoclaved should not be taken to the autoclave room until it is ready to be loaded in the autoclave.
2. Autoclave bags shall be clear, red or orange in color labeled with the biohazard symbol and autoclave tape or equivalent, and capable of passing the ASTM 125 pound drop test for filled bags.
3. Wrap packages to allow for steam penetration; aluminum foil does not allow steam penetration, and should not be used for wrapping.
4. Do not seal bags or close bottles and other containers tightly.
5. Do not overload the chamber and avoid overpacking of autoclave bags.
6. Steam sterilization shall be evaluated periodically with spores of *Bacillus stearothermophilus*. Results of spore vial tests must be entered in the Autoclave Use Log.
7. The Autoclave Use Log must be completed each time the autoclave is used. The log contains the: 1) date, 2) time and operator name, 3) type and approximate weight of waste treated, 4) post sterilization reading of the temperature sensitive tape, and 5) results of the spore vial test.
8. An autoclave must be available for the **(BSL Level)** laboratory and must only be operated by personnel who have been properly trained in its use. Improper sterilization could result in laboratory personnel, other personnel involved in disposal of laboratory waste, or the community at large being exposed to potentially infectious agents.

Spill Control and Emergency Management

Safety is an intrinsic part of each laboratory operation; work is planned so that exposure to potentially hazardous material does not occur. In spite of this, accidents do occur. These may involve spills of potentially hazardous agents in the laboratory or failure of equipment and facility safeguards that may place the laboratory worker at higher risk of exposure. Likelihood of severe injury or infection can be significantly reduced if emergency plans are established and the elements of the plans are known by laboratory workers.

There is no a single plan of action that would be applicable in all situations. The following basic principles, however, may be useful in developing specific procedures for dealing with an accidental spill of potentially infectious material in the laboratory.

- Get everyone out of the affected area
- Notify Paul

1. **Spill in a Biological Safety Cabinet.**

A spill that is confined in a biological safety cabinet presents minimal hazard to personnel in the area. However, chemical disinfection procedures should be initiated immediately to prevent escape of contaminants from the cabinet or cross contamination of items within the cabinet. Wipe or spray walls, work surface and equipment with a disinfectant appropriate for the type of organism being used. A disinfectant with a detergent will help clean the surfaces by removing both dirt and the microorganism. A suitable disinfectant is 70% ethyl alcohol, a 3% solution

Biohazard Spill Cleanup Kits

Each laboratory using biohazardous materials must have appropriate equipment and supplies on hand for managing spills and incidents involving biohazardous materials. Permanent equipment should include a safety shower, eyewash, and a hand-washing sink and supplies. A Biohazard Spill Kit should also be kept on hand. The supplies available in a Biohazard Spill Kit should include and/or have immediately accessible, but are not limited to:

1. a copy of the following ***biohazard spill clean-up protocol***.
2. nitrile or latex disposable gloves (8 mil) (check for holes or deterioration; replace nitrile gloves every two years).
3. lab coat(s) or gowns.
4. goggles or safety glasses with side shields.
5. face masks.
6. disposable shoe covers (booties).
7. absorbent material, such as absorbent paper towels, granular absorbent material, etc. (a disposable or cleanable scoop will be needed for granular absorbent).
8. all-purpose disinfectant, such as normal household bleach (freshly diluted 1:10).
9. something disposable or easily disinfected such as tongs, forceps, manila folders, etc. for picking up broken glass, other contaminated sharps, or contaminated absorbent material.
10. autoclavable biohazard waste bags.
11. biohazard spill warning signs.
12. all non-

Biohazard Spill Clean-up Protocol

Spills inside of a Biosafety Cabinet

The occurrence of a spill in a biological safety cabinet poses less of a problem than a spill in an open laboratory as long as the spilled materials are contained in the biological safety cabinet. A Biosafety Cabinet is designed to contain spills and associated aerosols which are released during work within the cabinet. Provided that the Biosafety Cabinet is operating properly and has been inspected and certified, aerosols produced by a spill should be contained. A spill of a biohazardous material inside of a Biosafety Cabinet should be attended to immediately.

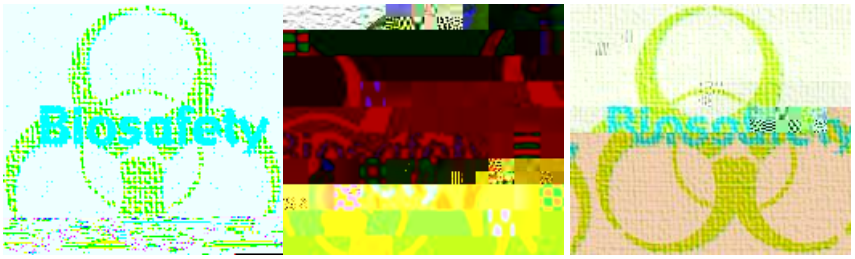
Decontamination of the work zone can usually be accomplished by direct application of concentrated liquid disinfectants along with a thorough wipe down procedure. Formaldehyde gas decontamination or vaporized hydrogen peroxide decontamination may be required to treat inaccessible sections of the cabinet interior following a spill.

1. Alert people in immediate area of spill.
2. Chemical decontamination procedures should be initiated immediately while the biological safety cabinet continues to operate.
3. Contain the spill and decontaminate as described in the following steps. All workers using the Biosafety Cabinets should have a supply of absorbent materials and decontaminating agent within the cabinet. This avoids the need to withdraw your arms from within the cabinet should a spill occur and allows you to decontaminate yourself prior to leaving the cabinet.
4. Wear gloves during decontamination procedure.
5. The spill should be covered with paper towels or other absorbent materials and the area soaked with a proven decontamination agent (e.g., 1:10 dilution of Clorox containing sodium hypochloride [NaOCl] or 70% ethanol) for 15 to 20 minutes.
6. Use paper towels to wipe up the spill, working from the edges into the center.
7. Decontaminate equipment and utensils. Items that are not readily or easily surface decontaminated should be carefully placed into autoclave bags and removed for further treatment (e.g., decontamination by autoclaving or other approved methods).
8. Contaminated gloves and clothes (your sleeves are most likely contaminated and, if disposable sleeves are used; they should be removed after decontamination is complete, or remove and decontaminate the lab coat by autoclaving or soaking in decontaminant).
9. Remove protective gear. Individuals involved in the spill and clean-up should remove protective clothing (either disposing as biohazardous waste or decontaminating), wash their hands and face with an appropriate decontamination soap, and report to the Biological Safety Officer if containment level is BSL-

11. Remove gloves and other contaminated garments and place them in an autoclave container for autoclaving.
12. Thoroughly wash hands, face, and other apparently contaminated areas.

Personal Exposure to Infectious Material

In the event that a substance enters the mouth, eyes, lungs, or penetrates/comes in contact with the skin follo



Biohazardous Waste Disposal Procedure

All biohazardous waste *must* be decontaminated before disposal. Common decontamination methods include heat sterilization (e.g. autoclaving), chemical disinfection, and tissue digestion.

1. Liquids

Decontaminate all liquid biohazardous materials (such as human blood, bacterial cultures in liquid media, body fluids from animals experimentally infected with pathogens, etc.) by autoclaving or treatment with the appropriate chemical disinfectant. After decontamination, liquids may be disposed of by pouring them down the drai(dow)5(n sde(i)-41[(e)s)4(a)n(qu)11(i)(ont)6(er)1(b

Appendices

Appendix 1: Agency Guidelines

For Biosafety:

Biosafety in Microbiological and Biomedical Laboratories (BMBL), Centers for Disease Control and Prevention and National Institutes of Health, U.S. Department of Health and Human Services, 5th Edition, February 2007. http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm

For Recombinant DNA:

NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines), National Institutes of Health, U.S. Department of Health and Human Services, April 2002. http://www4.od.nih.gov/oba/rac/guidelines_02/NIH_Guidelines_Apr_02.htm#_Toc7261560

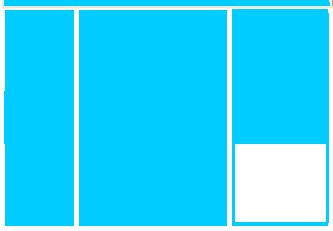
For Biosecurity:

Uniting and Strengthening America by Providing Appropriate Tools Required to Intercept and Obstruct Terrorism (USA PATRIOT ACT) Act of 2001: http://www.epic.org/privacy/terrorism/hr3162.html

Public Health Security and Bioterrorism Preparedness and Response Act of 2002: http://www.fda.gov/oc/bioterrorism/PL107-188.html

Appendix 2: List of Disinfectants and Properties

Disinfectants	*Practical requirements*					*Inactivates*
						–

Disinfectants	*Characteristics*
	

References

1. "**Biosafety in Microbiological and Biomedical Laboratories** ", 5th Edition, CDC/NIH, 2007.
2. "**NIH Guidelines for Research Involving Recombinant DNA Molecules**: Standards guiding containment and safe research practices"; and relevant regulations from The Occupational Safety and Health Administration (OSHA).
3. Oklahoma State University, Biological Research Safety Plan (URL?)
4. EVMS Biosafety Procedure Manual (more detail?)