C A U

Biosafety Procedure Manual

April, 2011

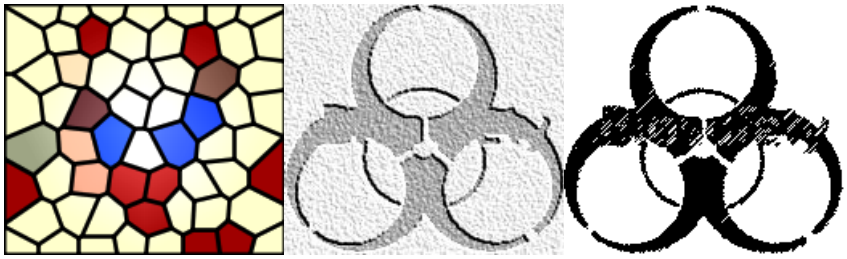
(Revised November 2011)

Table of Contents

About This Manual

This manual, referred to as the CAU biosafety plan, is compiled by the Biosafety Committee (BC) of Clark Atlanta University (CAU) as a guideline for the purposes of (1) promoting safe scientific research and safe working conditions; (2) safeguarding the health of the research personnel and the environment from the biological hazards in the laboratory, (2) Providing standardized laboratory operating procedures, and (3) Providing information about safe work practices, safety equipment, and personal protective equipment.

The CAU biosafety plan addresses the general research and teaching activities currently conducted on the CAU sites. For specialized projects/activities, the principle investigators should follow recommendations from (1) "**Biosafety in Microbiological and Biomedical Laboratories** ", 5th Edition, CDC/NIH, 2007"; and (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).



Definitions and Scopes

Biological Material refers to "all prokaryotic and eukaryotic organisms (and their components), viruses, subviral agents, recombinant DNA, and biologically-derived toxins used in research and instructional laboratories."

Biohazardous Material includes "all infectious agents, vectors known to carry and transmit infectious agents, infected or potentially infected animals, infectious material, recombinant DNA, and biologically-derived toxins" that are capable of producing deleterious effects upon humans, animals, plants, or the environment "either directly through infection or indirectly through damage to the environment".

Biologically-derived Toxins include "all molecules produced by animals, plants, microorganisms or other agents that have a median lethal dose (LD50) value of <50 mg/kg (oral administration in rats). Examples are bacterial exotoxin, plant lectins (ricin), and mycotoxins (aflatoxins, sterigmatocystin, luteoxkyrin, rugulosin, patulin, etc.)".

Biosafety: Development and implementation of administrative policies, work practices, facility design, and safety equipment to prevent transmission of biological agents to workers, other persons, and the environment.

Biosecurity: Protection of high-consequence microbial agents and toxins, or critical relevant information, against theft or diversion by those who intend to pursue intentional misuse.

Recombinant DNA molecules: are molecules constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or molecules that result from their replication (National Institutes of Health, 2002). Recombinant DNA molecules are considered biohazardous if they are not exempt from the NIH Guidelines. Examples include recombinant DNA that is formed by the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally (if that transfer could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture); is designed for use in human gene transfer experiments; contains genes for the biosynthesis of toxic molecules lethal for vertebrates at a median lethal dose (LD50) of less than 100 ng/kg body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin); is designed for the generation of transgenic plants or animals; or contains infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus.

Select Agent: Specifically regulated pathogens and toxins as defined in Title 42, CFR, Part 73, including pathogens and toxins regulated by both HHS and USDA (i.e., overlapping agents or toxins).

"Significant" reportable violation (incident): Any research related spill, accident, or problem that leads to personal injury or illness or to a breach of containment. Failure by an investigator to adhere to the containment and biosafety practices outlined in the NIH Guidelines must be reported. Minor spills of low-risk agents not involving a breach of containment that were properly cleaned and decontaminated generally will not require reporting to a federal agency but is at the discretion of the IBC.

Containment: the safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained.

Primary Containment: the protection of personnel and the immediate laboratory environment from exposure to infectious agents. It is attained by good microbiological technique and the use of appropriate safety equipment. The use of vaccines may provide an increased level of personal protection.

Secondary Containment: the protection of the environment external to the laboratory from exposure to infectious materials. It is attained by a combination of facility design and operational practices.

Biological Safety Levels: Biosafety Level (BSL) is a description of the degree of physical containment being employed to confine biohazardous material and to reduce the potential for exposure of laboratory workers, persons outside of the laboratory, and the environment. Four biosafety levels (BSL) are specified in the CDC BMBL and the NIH Guidelines. Additionally, the USDA published a paper describing four biosafety levels for arthropod containment. Details on Animal Biosafety Level and Plant Biosafety Level as well as Arthropod Biosafety Level are available at the CDC and NIH Guidelines website. The four Biosafety Levels are defined as follows:

- Biosafety Level 1 (BSL-1)→ Require the use of standard laboratory practices and equipment found in most research and teaching institutions. Such a laboratory provides areas of open bench space, with no special containment equipment, and is generally not well separated from the general traffic of the building. This type of laboratory is suitable for experiments involving agents and strains of viable microorganisms not known to consistently cause disease in healthy adult humans. In general, within a BSL-1 laboratory there is no known hazard, or minimal potential hazard, to laboratory personnel or the environment.

- Biosafety Level 2 (BSL-2)→ Require the use of standard laboratory practices and equipment designed for experiments with agents of moderate hazard to personnel and the environment. The BSL-2 differs from BSL-1 mainly in providing areas of containment (e.g., a biological safety container, class 2, type A-1 or A-2) to carry out certain laboratory experiments and operations in which aerosols could be created. Work is done with a broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human diseases of varying severity. Biosafety Level 2 is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or in devices such as a biosafety container or safety centrifuge cups.

- 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: personal protective equipment. Appropriate PPE may include lab coats, safety goggles, gloves, face shields, and safety glasses. Gloves should be worn in the laboratory when working with hazardous agents, but preferably not in the hallways since as contaminated surfaces they represent a hazard to others. The recommended method of transporting hazardous material within CAU is to utilize secondary containment. Secondary containment must be clean and uncontaminated and can range from plastic tubs, containers, or lab carts. This method allows your hands to be free from exposure to any hazardous material thus eliminating your need to wear gloves. In cases where secondary containment cannot be used, you may feel more safe wearing gloves. If this is the case, we recommend that you keep one hand un-gloved for the purpose of opening doors, entering and exiting elevators. The other hand can then be gloved for the

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, operating areas under negative pressure, and closed doors to affect containment.

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 cornerstone of a safety program designed to aid faculty, staff, and students in maintaining a safe environment in which to teach, conduct research, and learn.

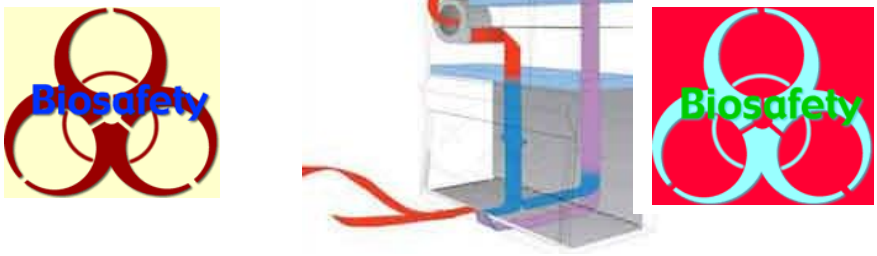
1. All the labs are considered to be restricted areas. Access to the laboratory is limited or restricted at the discretion of the Principal Investigator when experiments are in progress. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. The PI must identify and inform lab personnel potential risk of agents to be used.
3. The laboratory supervisor must *ensure* that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures.
4. Appropriate *signage* must be displayed indicating the biosafety level of the laboratory, the biohazard symbol, and the proper PPE for working in the laboratory.

5. Work surfaces are decontaminated once a day and after any spill of viable material.
6. All contaminated liquid or solid wastes are decontaminated before disposal and labeled appropriately for disposal.
7. Mechanical pipetting devices are used; mouth pipetting is prohibited.
8. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.
9. Persons wash their hands: (i) after they handle materials involving organisms containing recombinant DNA molecules and animals, and (ii) before exiting the laboratory.
10. People who wear contact lenses in laboratories should wear goggles or a face shield.
11. All procedures are performed carefully to minimize the creation of aerosols.
12. Policies for safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Items to be disposed must be stored in appropriate containers which have to be labeled accordingly.
13. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferable by autoclaving.
14. Broken glassware must not be handled directly by hand, but must be removed by mechanical means, such as a brush and dustpan, tongs, or forceps.
15. Laboratory coats and protective gloves are worn when manipulating viable materials.
16. The 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.
17. Facilities (e.g., hand washing sink, shower, and changing room) shall be used when applicable.
18. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.
19. The laboratory is organized so that it can be easily cleaned. Spaces between benches, cabinets, and equipment are accessible for cleaning.
20. Use bench tops that are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
21. Use sturdy laboratory furniture. Each laboratory contains a sink for hand washing.
22. An insect and rodent control program is in effect.
23. If the laboratory has windows that are open, they are fitted with fly screens.

For Research Involving Biohazardous Materials

The BC requires the review and approval of all research and teaching activities that involve the use of animal, plant, or human pathogens; recombinant DNA molecules; prions; and biological toxins. Each of these research/teaching areas has a unique set of risk associated with that type of work. Additional biosafety practices/procedures/standards must be developed based on the particular risk associated with a specific activity.

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.

Disinfection and Sterilization

Standard microbiological procedures require work surface decontamination and proper destruction of cultures, stocks and biological waste. Selection of a disinfectant or sterilization process is dependent on the sensitivity of the organism to its action; therefore, knowledge of characteristics of the organism is essential to ensure adequate decontamination.

The term **disinfectant** is most commonly used to designate chemicals that kill growing forms of microorganisms, but not necessarily resistant spore forms of bacteria, except when the intended use is specifically against an organism forming spores or a virus. Proper use of a disinfectant is contingent upon the purpose for which it is employed. A disinfectant (1) removes infection, (2) kills, not just inhibits microorganisms in the vegetative state, (3) does not necessarily kill spores, (4) is ordinarily a chemical but could be a physical process, and (5) is used only on inanimate objects.

Typical disinfectants used in labs: ammonium salts, chlorine compounds, iodine compounds, phenolics, alcohols and aldehydes, as well as ethylene oxide as summarized in the following and Appendix 2:

A Concise Summary of Practical Disinfectants

Disinfectant	Dilution	Contact time (minutes)		Irritant type		
		Lipovirus	Broad-Spectrum	Skin	Eye	Respiratory
Quaternary ammonium (L)	0.1-2.0%	10	Not effective	Yes	Yes	No
Phenolics (L)	1.0-5.0%	10	Not effective	Yes	Yes	No
Chlorine cpds. (L)*	500ppm*	10	30	Yes	Yes	Yes
Iodophor cpds. (L)	25-1600ppm	10	30	Yes	Yes	No
Ethyl alcohol (L)	70-85%	10	Not effective	No	Yes	No
Isopropyl alcohol (L)	70-85%	10	Not effective	No	Yes	No
Formaldehyde (L)	0.2-8.0%	10	30	Yes	Yes	No
Glutaraldehyde (L)	2%	10	30	Yes	Yes	No
Ethylene oxide (G)	8-23g/ft^3	60	60	Yes	Yes	Yes
Paraformaldehyde (G)	0.3g/ft^3	60	60	Yes	Yes	Yes

L = liquid; G = gas; *Commercially available chlorine bleach is 5.25% chlorine (52,200 ppm). A dilution of 1 to 100 will yield a 525 ppm solution, which is suitable for disinfecting purposes.

A physical or chemical process that destroys all forms of life, especially microorganisms is **sterilization**. To achieve sterilization, destruction must be adequate to ensure the organism is no longer detectable in standard culture media in which it had been previously found to proliferate. Physical processes include steam, heat, ultraviolet radiation, and ionizing radiation, whereas chemicals that sterilize are ethylene oxide and glutaraldehyde.

Chlorine compounds: Chlorine in an aqueous solution exhibits fast bactericidal action. The mechanism of the action however hasn't been fully elucidated. Chlorine is readily available in sodium hypochlorite and calcium hypochlorite which contain 95.8 and 99.2 weight percent available chlorine respectively. Household bleach contains 5.25% (52,500 parts per million) available chlorine and in a solution of 1 part bleach to 10 parts water, 5250 parts per million chlorine is available for bactericidal activity. pH of the solution containing the organisms will greatly influence the antimicrobial activity. An increase in pH substantially decreases the microbial activity of chlorine and a decrease in pH increases its activity.

To assure adequate biocidal activity, organisms must be in contact with the chlorine solution for an extended period. Contact time varies by type of organism, but at least 10 minutes contact time in 1:10 bleach to chlorine solution is recommended for most vegetative bacteria. Chlorine solutions gradually lose strength so solutions should be made fresh at least weekly. At this concentration the mixture may be corrosive to some materials and equipment, so be cautious with its application.

Various types of bacteria, viruses, fungi and algae exhibit resistance to hypochlorite. This selective resistance of organisms to chlorine may be compensated either by increasing the chlorine concentration, by lowering the pH or by raising the temperature. Spore forming organisms are about 10 to 1000 times more resistant to chlorine than vegetative forms.

Iodine and iodine compounds. Iodine is able to penetrate the cell wall of microorganisms rapidly and attack various amino acids and nucleotide bonds. Iodine and iodophors, a complex of elemental iodine and a carrier, vary in the percentage of iodine in solution. A topical iodine solution contains 2% iodine and 2.4% sodium iodide while Lugol's solution contains 5% iodine and 10% potassium iodide. As with other disinfectants, adequate contact time must be allowed to kill or inactivate the organism.

Phenolics. A number of commercially available disinfectants contain phenol for bactericide activity. Phenols act as protoplasmic poisons at high concentration and inactivate essential enzyme systems at low concentration. Phenolic compounds are considered to be low to intermediate level disinfectants that are appropriate for general disinfection of noncritical and semicritical areas. The Environmental Protection Agency regulates phenolic compounds which must be registered to claim various bactericidal, tuberculocidal, fungicidal and virucidal activity. Lists of registered compounds may be viewed at the EPA web site (http://www.ace.orst.edu) under "Antimicrobial Information". Phenol is a Resource Conservation and Recovery Act (RCRA) hazardous chemical which means that it must be handled and disposed properly. A few milliliters of phenol in a waste solution makes the entire solution subject to special handling and increased disposal costs.

Alcohol. Alcohol acts by denaturing protein in microorganisms. In the absence of water, proteins are not denatured as readily as when water is present. This is why absolute ethyl alcohol is less bactericidal than mixtures of alcohol and water. Like chemical disinfectants in general, their destructive action against spore forms is much less than against vegetative forms. Both ethyl and isopropyl alcohol are excellent disinfectants for vegetative organism and have been shown to vary in effectiveness against fungi and viruses. The important criteria are contact time and concentration, with contact time of at least ten minutes at a 70% concentration appearing most effective.

Glutaraldahyde. Glutaraldehyde displays a broad spectrum of activity, including sporocidal activity, and rapid rate of kill against the majority of microorganisms. An exception is activity against mycobacteria which requires adequate (increased) time for decontamination. Applications of glutaraldehyde as a sterilizer are best suited for instruments and equipment that cannot be sterilized by heat or other chemicals. Glutaraldehyde is also a RCRA hazardous chemical that requires special handling.

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

Disinfectants		***Proprietary disinfectants***
Type	**Category**	
Liquid	Quaternary ammonium compounds	A-33, CDQ, End-Bac, Hi-Tor, Mikro-Quat
	Phenolic compounds	Hil-Phene, Matar, Midro-Bac, O-Syl
	Chlorine compounds	Cholramine T, Chlorox, Purex
	Iodophor	Hy-sine, Ioprep, Mikroklene, Wescodyne
	Ethyl alcohol	N/A
	Isoprophyl alcohol	N/A
	Formaldehyde	Sterac
	Glutaraldehyde	Cidex
Gas	Ethylene oxide	Carboxide, Cryoxicide, Steroxicide
	Parafomaldehyde	N/A

Choice of a chemical disinfectant for recombinant DNA wastes must be preceded by consideration of the purpose of the decontamination and organisms in use. Consideration of the following will assist you to determine what destruction process is appropriate.

1. What organisms or forms are involved, i.e., host, vector, donor organism from which the DNA segment is obtained?
2. What disinfectants are known to inactivate the organism?
3. What degree of inactivation is required?
4. What is the highest concentration of cells anticipated to be encountered?
5. Can the disinfectant contact the organism and can an effective period of contact be maintained?
6. Will carrier materials interact with the disinfectant to decrease its effectiveness?

The principal investigator must make these determinations based on the product used in the research. Once a disinfectant or process is selected, its effectiveness should be evaluated against products and mixtures generated by the research.

Sterilization: Steam sterilization in an autoclave is a common process to destroy wastes and sterilize instruments in microbiological laboratories and clinics. The variables of temperature, time and pressure are used to achieve adequate sterilization. Operating a steam sterilizer is potentially hazardous due to the high

temperature, presence of steam and pressure. It is imperative that guidelines be followed to prevent injury or damage to the autoclave. Familiarization with manufacturer's 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 Abrahams, Laboratory Safety and Chemical Hygiene, 404.880.6974, and the Biosafety Chair, Dr. Myron Williams, 404.880.6856; in the order listed.
- Do not reenter until the extent of the hazard is determined
- Determine the need to treat persons exposed to the hazardous agent
- Decontaminate the affected area

For emergencies involving failure of equipment or facility safeguards, the most important action should be to stop work with the hazardous agent and safely contain the material until corrective action has been taken. In cases of serious injury or sudden illness, the principal investigator or laboratory supervisor should determine whether to override containment. Emergency response personnel should also be alerted to the possibility of potentially infectious agents.

General procedures for situations involving laboratory spills follow. Where a "spill kit" is indicated, this consists of a combination of personal protective equipment, disinfectants appropriate for the microorganism is use, absorbent material (paper towels), forceps, waste containers and warning signs.

A**. Laboratory Spills.**

These are situations where a culture, specimen or container with microorganisms or blood or other potentially infectious material has breached its containment on a bench, in a biological safety cabinet, on the floor, or in an animal cage. This could be associated with manipulations in a centrifuge, grinder, or shaker or simply losing control of the container. The primary step in any loss of control incident is to contain the material to prevent its spread. More specific procedures will be presented in the following sections.

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 of an iodophor such as Wescodyne or a 1 to 10 dilution of household bleach. The operator should wear gloves during the disinfecting procedure. Use sufficient disinfectant solution to ensure that drain pans and catch basins below the work surface are exposed to the disinfectant. Wipe the catch basin and drain the disinfectant into a container. The disinfectant, gloves, wiping clothes and sponges should be discarded into an autoclave bag and sterilized. This procedure does not disinfect the filters, blower, air ducts or other interior parts of the cabinet.

2**. Spill in the Laboratory.**

If potentially infectious biological material is spilled in the laboratory, the first essential is to avoid inhaling any airborne material by holding the breath and leaving the laboratory. Warn others in the area and move a safe distance away from the area. If outer clothing, such as a lab coat is known or suspected of being contaminated, remove it with care, folding inward as it is removed. Wash all potentially contaminated areas of the skin thoroughly. Delay reentry into the lab for about 30 minutes to allow reduction of aerosols generated by the spill. Advance preparation for management of a spill is essential. A "spill kit" should be readily available.

Protective clothing should be worn when entering the laboratory to clean the spill area. Rubber gloves, autoclavable or disposable shoe covers, an outer garment and respirator should be worn. If the spill was on the floor, do not use a surgical gown that may trail on the floor when bending down. Take the "spill kit" into the laboratory room, place a waste container near the spill, and transfer large fragments of material into it then replace the cover. Using sodium hypochlorite solution containing 5000 ppm (1:10 dilution of household bleach) available chlorine, or other appropriate disinfectant, carefully pour or spray the disinfectant around and into the visible spill. These concentrations of disinfectants are higher than those normally employed in the laboratory because the volume of spill may significantly reduce the concentration of active ingredient in the disinfectant. Avoid splashing. Allow at least 15 minutes contact time then use paper or cloth towels to wipe up the disinfectant and spill, working toward the center of the spill. Discard towels into a biohazard waste container for either autoclaving or disposal via commercial vendor. Remove shoe covers, outer protective clothing, respirator and gloves and either disinfect or dispose of these items.

3. **Biohazard Spill Containing Radioactive Material.**

In the event that a biohazardous spill also contains radioactive material, the decontamination procedure may have to be modified, depending on the type and quantity of radioactivity involved. Personnel in laboratories using radioactive materials have received training in spill response; therefore, decontamination (clean-up) is the responsibility of the laboratory staff with assistance and consultation from Radiation Safety. Radiation Safety (Dale Mack, Radiation Safety Officer, MSM, 404.756.6686) should be notified immediately to assist in determining the relative degree of risk from external or internal exposure prior to decontamination procedures commencing. The spill should be cleaned up as with other spills in the laboratory, with the exception that the waste will be placed in biohazard bags or containers with radioactive material labels attached. The decision on the method to inactivate the microorganism will be made by the Radiation Safety Officer in consultation with the principal investigator. A radiation wipe survey of the area should be performed, and documented, by the laboratory staff to confirm that the area was properly decontaminated.

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-disposable items should either be autoclavable or compatible with the disinfectant to be used.

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-2/ABSL-2 or BSL-3/ABSL-3.

Spills outside of a Biosafety Cabinet

Biological spills outside biological safety cabinets will generate aerosols that can be dispersed in the air throughout the laboratory. Appropriate protective equipment is particularly important in decontaminating spills involving infectious microorganisms or recombinant materials. This equipment at a minimum includes lab coat with long sleeves, back-fastening gown or coveralls, disposable gloves, disposable shoe covers, and safety goggles and mask or full-face shield. Use of this equipment will prevent contact with contaminated surfaces and protect eyes and mucous membranes from exposure to splattered materials.

Minor Spills

-less than 10 ml and generating little aerosol on equipment, laboratory benches, walls, or floors:

1. Ensure laboratory doors are closed and if possible post warning signs to prevent others from entering the laboratory.
2. If you are not already wearing gloves, thoroughly wash hands and other apparently contaminated areas with soap and water and don clean disposable gloves.
3. Cover the spill area with paper towels soaked in appropriate decontamination solution such as 1:10 dilution of bleach or 70% ethanol solution, or cover spill with paper towels and then pour decontamination solution around and on the area of the spill.
4. Let stand for 20 minutes then wipe up with paper towels. Place gloves and paper towels in autoclave pan and autoclave.
5. Wash hands and other apparently contaminated areas again with soap and water.
6. Remove all PPE immediately upon leaving the work area and as soon as possible if overtly contaminated. Contaminated PPE will be DISPOSED of as biohazardous waste or decontaminated.

Major Spills

-more than 10 ml or with considerable aerosol:

1. Leave the biological safety cabinet operating and if possible place cultures inside cabinet.
2. If personal clothing is contaminated, remove all outer clothing and place it in the autoclave or container for autoclaving.
3. Leave the laboratory for 30 minutes to allow dissipation of aerosols created by the spill.
4. Properly doff any remaining PPE and wash hands with soap and water.
5. Report the accident to the Principal Investigator and to the Biosafety Chair.
6. Ensure laboratory doors are closed and if possible post warning signs to prevent others from entering the laboratory.
7. Upon returning to the laboratory to start decontamination, check to see if laboratory doors are closed and appropriate signs are displayed. Put on gloves and other protective equipment.
8. Pour a decontamination solution around the spill and allow this solution to flow into the spill. Paper towels soaked with decontamination solution may be used to cover the area. Do not pour decontamination solution directly onto the spill in order to avoid additional release of aerosols.
9. Let decontamination solution – microorganism mixture stand for 20 minutes or longer to allow adequate contact time.
10. Transfer all contaminated materials to autoclave pan, cover with suitable cover, and autoclave according to standard directions.

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 follow the instructions below and seek immediate medical attention.

1. Remove all contaminated clothing and place it in a biohazard bag, the biological safety cabinet, or a garbage bag if neither of the above is available or convenient.
2. Vigorously wash exposed area (if possible) with soap and water or disinfectant for one minute. If mouth or eyes are exposed flush with water for one minute.
3. If others are present in the laboratory warn them of the biohazard.
4. Report the spill to the Principal Investigator or Lab Manager.
5. If an individual is injured during work: call 911 to obtain emergency assistance. Persons requiring immediate emergency care should seek it. Preparation of paperwork will be secondary to obtaining prompt medical attention. The Principal Investigator or Lab Manager will accompany injured personnel to receive a medical evaluation and complete an incident form. Personnel working in the Biosafety Level 2 or 3 laboratory, or who have performed duties in the past 6 months in an area containing infectious materials, will notify their supervisors and seek medical attention if they:
   • Develop a fever greater than 100.4 ºF
   • Display initial onset symptoms consistent with contraction of the infectious agent used in the laboratory.
6. If a biohazardous spill also includes radioactive material, the clean-up procedure may need to be modified. For these situations, the Radiation Safety Officer (Dale Mack, Radiation Safety Officer, MSM, 404.756.6686) must also be notified immediately.

Shipment of Hazardous Agent

Recombinant DNA molecules contained in an organism or in a viral genome shall be shipped under the applicable regulations of the U.S. Postal Service (39 Code of Federal Regulations, Part 3); the Public Health Service (42 Code of Federal Regulations, Part 72); the U.S. Department of Agriculture (9 Code of Federal Regulations, Subchapters D and E; 7 CFR, Part 340); and/or the U.S. Department of Transportation (49 Code of Federal Regulations, Parts 171-179).

Host organisms or viruses will be shipped as etiologic agents, regardless of whether they contain recombinant DNA, if they are regulated as human pathogens by the Public Health Service (42 Code of Federal Regulations, Part 72) or as animal pathogens or plant pests under the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (Titles 9 and 7 Code of Federal Regulations, respectively).

Host organisms and viruses will be shipped as etiologic agents if they contain recombinant DNA when: **(1)** the recombinant DNA includes the complete genome of a host organism or virus regulated as a human or animal pathogen or a plant pest; or **(2)** the recombinant DNA codes for a toxin or other factor directly involved in eliciting human, animal, or plant disease or inhibiting plant growth, and is carried on an expression vector or within the host chromosome and/or when the host organism contains a conjugation proficient plasmid or a generalized transducing phage; or **(3)** the recombinant DNA comes from a host organism or virus regulated as a human or animal pathogen or as a plant pest and has not been adequately characterized to demonstrate that it does not code for a factor involved in eliciting human, animal, or plant disease.

For further information on shipping etiologic agents contact: **(1)** The Centers for Disease Control and Prevention, ATTN: Biohazards Control Office, 1600 Clifton Road, Atlanta, Georgia 30333, (404) 639-3883, FTS 236-3883; **(2)** The U.S. Department of Transportation, ATTN: Office of Hazardous Materials Transportation, 400 7th Street, S.W., Washington, DC 20590, (202) 366-4545; or **(3)** U.S. Department of Agriculture, ATTN: Animal and Plant Health Inspection Service (APHIS), Veterinary Services, National Center for Import-Export, Products Program, 4700 River Road, Unit 40, Riverdale, Maryland 20737. Phone: (301) 734-8499; Fax: (301) 734-8226.

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 drain to the sanitary sewer with cold running water.

2. Disposable solid items

Collect all non-sharp disposable items (such as gloves, plasticware, Kimwipes, etc.) contaminated with biohazardous materials in leakproof autoclavable biohazard bags. Decontaminate the bags by autoclaving when they are ready for disposal. After autoclaving, place the now decontaminated biohazard bag inside dark garbage bag, seal, and place in regular trash. It is the responsibility of each user to ensure that bags are transported to the dumpster behind the Research Center via the freight elevator in a timely fashion

3. Sharps and broken glass

Collect all sharps in an approved, rigid, leakproof, puncture-resistant, autoclavable, plastic sharps containers. Decontaminate the containers by autoclaving when they are ready for disposal. Broken glassware must be collected in a similar type of container that is autoclavable if contaminated with biohazardous material. After decontamination, the sharps containers will be picked up on request by the designated environmental and safety coordinator. For broken glassware that is not contaminated with biohazardous material, collect in a rigid, leakproof container labeled broken glass. Package in cardboard box that has been sealed and labeled "broken glass", and discard in regular trash.

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

42 CFR Part 73 "Possession, Use, and Transfer of Select Agents and Toxins, Final Rule": http://www.selectagents.gov/resources/42_cfr_73_final_rule.pdf

Appendix 2: List of Disinfectants and Properties

Disinfectants	*Practical requirements*						*Inactivates*			
Type	**Category**	**Use dilution**	**Contact time**		**Temp (C)**	**Relative humidity**	**Vege–tative bacteria**	**Lipovirus**	**Nonlipid viruses**	**Bacterial spores**
			Lipovirus	**Broad spectrum**						
Liquid	Quaternary ammonium compounds	0.1%–2.0%	10	Not effective			+	+		
	Phenolics	1.0%–5.0%	10	Not effective			+	+	Variable results dependent on virus	
	Chlorine compounds	500 ppm	10	30			+	+	+	+
	Iodphor	25–1600 ppm (available halogen)	10	30			+	+	+	+
	Alcohol, Ethyl	70%–85%	10	Not effective			+	+	Variable results dependent on virus	
	Alcohol, isopropyl	70%–85%	10	Not effective			+	+	Variable results dependent on virus	
	Formaldehyde	0.2%–8.0%	10	30			+	+	+	+
	Glutaraldehyde	2.0%	10	30			+	+	+	+
Gas	Ethylene oxide	8–23 g/cubic foot	60	60	37	30	+	+	+	+
	Para–formaldehyde	0.3 g/cubic foot	60	60	gt;23	gt;60	+	+	+	+

Disinfectants			*Characteristics*									
Type	**Category**	**Effective shelf life** >1 week Protected from light and air	**Corrosive**	**Flammable**	**Explosion Potential**	**Residue**	**Inacti–vated by organic matter**	**Compatible for optics* (Read special consider–ations).**	**Compatible for electronics**	**Skin irritant**	**Eye irritant**	**Respiratory irritant**
Liquid	Quaternary ammonium compounds	+					+	+		+	+	
	Phenolic compounds	+	+			+				+	+	
	Chlorine		+			+	+			+	+	+
	Iodophor	+	+			+	+			+	+	
	Alcohol, ethyl	+		+							+	
	Alcohol, isoprophyl	+		+							+	
	Formaldehyde	+				+				+	+	
	Glutaraldehyde	+				+		+		+	+	
Gas	Ethylene Oxide	N/A		+ Neither flammable nor explosive in 90% CO2, or fluorinated hydrocarbon, the usual form	+ Neither flammable nor explosive in 90% CO2, or fluorinated hydrocarbon, the usual form			+	+	+	+	+
	Parafor-maldehyde	N/A		+ At concentrations of 7%-73% by volume in air, solid-exposure to open flame	+ At concentrations of 7%-73% by volume in air, solid-exposure to open flame			+	+	+	+	+

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?)