



Institutional Biosafety Manual

Office of Environmental Health, Occupational Safety and Risk Management

This manual was adapted from materials provided by the Georgia University and State College

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I. CONTACT INFORMATION

DSC Public Safety (Police Department):	706-272-4461
Environmental Health and Occupational Safety:	706-272-4463
Fire:	9-911
Emergency Medical Services:	9-911
Poison Control Center:	9-1-800-222-1222

II. MISSION

This manual outlines appropriate practices, College policies and regulatory requirements for working safely in the research laboratory with biohazardous materials at Dalton State College facilities.

The mission is to provide guidance and assistance to faculty, staff and students in order to protect them from exposure to biohazardous materials in the research setting and to guard against the accidental release of such materials that may be harmful to humans, animals, plants or the environment. In doing so, the Office of Environmental Health, Occupational Safety and Risk Management provides guidance and assistance to the College's research community on regulatory needs associated with the receipt and shipment of infectious agents, administrative support to the Institutional Biosafety Committee (IBC), advising staff who work with bio-hazardous materials on safe use, movement and disposal, and providing assistance with obtaining regulatory permits for facilities on the College's campus.

III. BIOLOGICAL RISK ASSESSMENT

Performance of a risk assessment is the primary responsibility of the Principle Investigator (PI) but it is shared with the Institutional Biosafety Committee (IBC), biosafety professionals, and laboratory staff. Three broad categories to consider in a risk assessment are agent hazards, lab procedure hazards and the capabilities of the staff to control hazards. Biosafety in the laboratory, therefore, strongly depends on training, technical proficiencies and good work habits of the lab staff. Operational integrity of equipment and the facilities are important as well.

1. Risk assessments should identify:
 - ☒ The hazardous characteristics of an agent
 - ☒ Ability to infect and cause disease, infective dose, host range
 - ☒ Virulence (as measured by severity of disease)
 - ☒ Availability of preventative measures and effective treatments for disease
 - ☒ Probable routes of transmission
 - ☒ Stability in the environment
 - ☒ Endemic nature of the organism
2. Lab procedure hazards
 - ☒ Use of needles or other sharps
 - ☒ Spills or splashes
 - ☒ Pipetting
 - ☒ Aerosol generation-pipetting, blenders, centrifugation, sonicators, vortex mixers
3. Likelihood that the agent can cause laboratory-acquired infections
4. Consequences of an infection

Information identified in risk assessments should provide guidance for the selection of the biosafety level (BSL) for work and establish the four primary controls necessary for safe work:

- ☒ Work Place Practices
- ☒ Personal Protective Equipment (PPE)
- ☒ Administrative Controls
- ☒ Engineering Controls

V. ROLES AND RESPONSIBILITIES

A. Office of Environmental Health, Occupational Safety and Risk Management:

The Office of Environmental Health, Occupational Safety and Risk Management (EH&OS) is responsible for the development and oversight of proper management practices for biohazardous materials in the research setting at Dalton State College, including developing and implementing policies supporting their mission. Staff within the office provides support for health and safety, biosecurity and compliance involving biohazardous materials.

B. Biosafety Officer (BSO):

The Biosafety Officer is appointed by the institution in compliance with the NIH Guidelines and ensures that periodic inspections are performed to ensure laboratory standards are rigorously followed. The BSO also reports to the IBC on any significant problems or violations with the NIH Guidelines or on any significant research-related accidents or illnesses, develops emergency plans for handling accidental spills and personnel contamination, and investigates laboratory accidents involving biohazardous materials. The BSO is a voting member of the IBC following the NIH Guidelines.

C. Individual Personnel:

Individuals who work with biohazardous materials have a responsibility to follow the guidelines presented in this manual and to consult with their supervisors regarding the safe handling and proper disposal of specific biohazardous materials used in their work area.

D. Institutional Biosafety Committee (IBC):

The IBC serves as review board of all research activities involving recombinant DNA studies, as required by the NIH Guidelines. Additionally, the IBC reviews and approves research projects involving human, animal or plant pathogens, and select agents and toxins (as defined by CDC/USDA Select Agent Programs). The IBC establishes compliance policies for the NIH Guidelines (integrated within this manual). The IBC has the authority to require operational changes in the event of noncompliance with required conditions.

On behalf of the institution, the IBC notifies PIs of results of IBC protocol reviews and approvals, adopts emergency plans covering accidental spills and personal contamination, and reports to the NIH OBA and UGA IO significant problems or violations of the NIH Guidelines or any significant-research related accidents and illnesses on projects involving rDNA research.

E. Supervisors:

Principal Investigators (PIs), instructors and supervisors are primarily responsible for ensuring that all personnel under their jurisdiction, including collaborating researchers, strictly follow the policies and guidelines established in this manual. To ensure biosafety, a trained scientist, knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling infectious agents is responsible for the conduct of work with any biohazardous materials used in their lab. Therefore, the lab supervisor or PI is responsible for enforcing institutional policies that restrict access to the lab and support safety for work in the lab.

V. INSTITUTIONAL BIOSAFETY COMMITTEE (IBC)

Prior to commencement of work, the DSC IBC must approve any teaching or research projects that involve the use of:

- Recombinant DNA, including transgenic animals or plants BSL-2 or higher human or zoonotic pathogens;
- Animal pathogens that cause diseases reportable to the State Veterinarian;
- Plant pathogens that have not been established in the State;
- Any select agent or toxin

The IBC will provide full committee review for all recombinant DNA work that is not exempt from the NIH Guidelines, for any work involving the creation of transgenic animals or plants, for all BSL-2 or higher human or zoonotic pathogens, or for any select agent or toxin.

Research projects falling under these guidelines will receive IBC review and approval prior to initiation of work.

A. Submitting an IBC Application:

The PI must complete the IBC Recombinant DNA Registration Form and submit it to the IBC Chair. Registration forms can be submitted via email, fax or campus mail. The IBC Chair will provide an initial review to ensure all appropriate information is provided. Once the initial review is completed, the IBC Coordinator will submit the registration form to the entire IBC via email if the project requires full committee review.

B. IBC Review Process:

The IBC members generally reviews applications when a registration form is submitted. If an application is returned to the PI because it is incomplete or the committee has questions, the PI has 45 days to respond. If the IBC does not receive a response within 45 days of the initial request, the application will be administratively closed and the PI must resubmit the application and signature page.

A quorum of 50% of the existing IBC membership must review and approve the request. This can be done either electronically or through a scheduled meeting of the IBC.

Ad hoc members will be employed to assist in the primary review of protocols in their area of expertise. They may attend meetings and review documents, but do not have voting rights. Alternate members may also serve in an ad hoc capacity.

C. IBC Authorization:

When a project is approved by the IBC, the PI will be notified via e-mail. An authorization form, specifying any special conditions under which authorization is granted, will be forwarded to the PI by email. If approval is denied, a written notification will be sent to the PI. This notification will explain the decision and will identify possible modifications to the project that would allow approval.

Additional personnel that may receive a copy of a PI's IBC authorization include animal care and use, sponsored programs, internal grants, and co-PIs. In most cases, IBC authorizations are valid for 12 months.

D. IBC Renewal:

IBC authorizations must be renewed. Every five years the application shall be reviewed by the IBC for any project (including exempt NIH protocols) in which work is ongoing. If the renewal is not submitted by the expiration date, the project will be considered terminated and will be administratively closed. If closed, according to federal regulations and College policy, all work on the project must stop. A new application must be completed and submitted to re-establish approval in this scenario. If the project is still ongoing a new submission may be required at which time the IBC will notify the project lead of their requirements.

E. IBC Modification Procedure:

If any changes are planned for an approved project before the authorization expiration date, such as changes in the research scope, personnel or facility location changes, the PI must notify the IBC. IBC approval must be obtained prior to implementation of the modifications. Modification requests can be submitted by e-mail. The IBC will determine whether proposed changes are major and require a new protocol application. Approval of a modification does not alter the date by which continuing review must occur.

F. Evaluating Laboratory Safety through the IBC Protocol Submittal:

The IBC Recombinant DNA Registration Form includes all criteria necessary to perform a risk assessment for work with biohazardous materials and for work with recombinant DNA. Completing the IBC Recombinant DNA Registration Form will help the PI, lab personnel and the IBC ensure that good laboratory safety practices are being used. More than one related research project or grant can be managed on a single IBC Form.

The IBC maintains the right to increase safety practices or containment requirements as charged by the NIH Guidelines.

VI. BIOSAFETY PRACTICES AND PROCEDURES

A. Laboratory Biosafety Level Criteria:

The essential elements of the four biosafety levels for activities involving infectious microorganisms and laboratory animals are summarized in Table 1.

The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community. Standard microbiological practices are common to all laboratories.

Special microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increasing levels of containment.

Table 1. The Four Biosafety Levels

BSL	Agents	Practices	Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
1	Not known to consistently cause diseases in healthy adults	Standard microbiological practices	! No primary barriers required. ! PPE: laboratory coats and gloves; eye, face protection, as needed	Laboratory bench and sink required
2	! Agents associated with human disease ! Routes of transmission include per- cutaneous injury, ingestion, mucous membrane exposure.	BSL-1 practice plus: ! Limited access ! Biohazard warning signs ! “Sharps” precautions ! Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers: ! BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials ! PPE: Laboratory coats, gloves, face and eye protection, as needed	BSL-1 plus: ! Autoclave available
3 DALTON STATE DOES NOT HAVE A BSL-3 LAB	Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure.	BSL-2 practice plus: ! Controlled access ! Decontamination of all waste ! Decontamination of laboratory clothing before laundering	Primary barriers: ! BSCs or other physical containment devices used for all open manipulations of agents ! PPE: Protective laboratory clothing, gloves, face, eye and respiratory protection, as needed	BSL-2 plus: ! Physical separation from access corridors ! Self-closing, double-door access ! Exhausted air not recirculated ! Negative airflow into laboratory ! Entry through airlock or anteroom ! Hand washing sink near laboratory exit
4 DALTON STATE DOES NOT HAVE A BSL-4 LAB	! Dangerous/exotic agents which post high individual risk of aerosol transmitted laboratory infections that are frequently fatal, for which there are no vaccines or treatments ! Agents with a close or identical anti-genic relationship to an agent requiring BSL-4 until data are available to re-designate the level ! Related agents with unknown risk of transmission.	BSL-3 practices plus: ! Clothing change before entering ! Shower on exit ! All material decontaminated on exit from facility	Primary barriers: ! All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure suit	BSL-3 plus: ! Separate building or isolated zone ! Dedicated supply and exhaust, vacuum, and decontamination systems ! Other requirements outlined in the text

B. Biosafety Level 1:

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.

BSL-1 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.

The following standard practices, safety equipment, and facility requirements apply to BSL-1.

i. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area.
4. Mouth pipetting is prohibited; mechanical pipetting devices **must** be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.

These include:

- a. Careful management of needles and other sharps are of primary importance.
 - b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for Sharps disposal.
 - c. Non-disposable Sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
 7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
 8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method.
 9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present.
 10. An effective integrated pest management program is required.
 11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures.

ii. Special Practices

None required.

iii. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. Special containment devices or equipment, such as BSCs (Biological Safety Cabinets), are not generally required.
2. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
3. Protective eyewear should be available for use when conducting procedures

that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.

4. Gloves must be worn to protect hands from exposure to hazardous materials, where appropriate. Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition, BS-1 workers should:
 - a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
 - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
 - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste.
 5. Hand washing protocols must be rigorously followed.
- iv. Laboratory Facilities (Secondary Barriers)
1. Laboratories should have doors for access control.
 2. Laboratories must have a sink for hand washing.
 3. The laboratory should be designed so that it can be easily cleaned.
 - a. Carpets and rugs in laboratories are not appropriate.
 4. Laboratory furniture must be capable of supporting anticipated loads and uses.
 5. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
 - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
 - b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
 6. Laboratories windows that open to the exterior should not be opened.

C. Biosafety Level 2:

Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. The following standard and special practices, safety equipment, and facility requirements apply to BSL-2.

i. Standard Microbiological Practices

Same as described for BSL-1

ii. Special Practices

1. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
2. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
3. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
4. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
 - a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

- b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
 5. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor.
 6. Animal and plants not associated with the work being performed must not be permitted in the laboratory.
 7. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.
- iii. Safety Equipment (Primary Barriers and Personal Protective Equipment)
 1. Properly maintained BSCs, other appropriate personal protective equipment, or other physical containment devices must be used whenever:
 - a. Procedures with a potential for creating infectious aerosols or splashes are conducted.
 - b. High concentrations or large volumes of infectious agents are used.
 2. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
 3. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials.
 4. Remove protective clothing before leaving for non-laboratory areas, e.g., cafeteria, library, and administrative offices). Laboratory clothing is not be taken home.
 5. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device.
 6. Gloves must be worn to protect hands from exposure to hazardous materials.
 7. Alternatives to latex glove should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:
 - a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
 - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
 - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
- iv. Laboratory Facilities (Secondary Barriers)
 1. Same as described in BSL-1, plus the following:
 2. BSCs' must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSC's should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
 3. Vacuum lines should be protected with liquid disinfectant traps.
 4. An eyewash station must be readily available.
 5. There are no specific requirements for ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
 6. HEPA filtered exhaust air from a Class II BSC can be safely recirculated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected

to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.

7. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

VII. Occupational Health and Safety Program (OH&SP)

The goal of an Occupational Health and Safety Program is to promote a safe and healthy workplace through medical support services, limit opportunities for personnel exposure, promptly detect and treat any exposures and use information gained from work related incidents that can result in injury to enhance the existing safety program. The OHSP in the research setting is a shared responsibility of personnel, safety specialists, and medical service providers. Workers must be fully informed of their work place hazards and available medical services.

Employee supervisors will inform laboratory personnel of available medical support services and encourage an appropriate level of participation.

When appropriate and available, personnel who work with or have the potential to be exposed to human pathogens will be offered the opportunity to receive vaccinations and will be informed of the risks associated with the vaccine. Agent-specific medical surveillance plans may be required and will include protocols for employees who are ill with signs and symptoms of the human pathogens they work with (but without any known exposure incident), as well as protocols for employees suspected of having been exposed to a live pathogen during work activities. Costs of occupational health needs will be billed to the PI or department, as applicable.

Employees working with human pathogens will receive orientation training prior to beginning work and annually thereafter as part of their occupational health program. This orientation training will include the following:

- Description of the signs and symptoms commonly associated with agents of concern;
- Appropriate PPE and training including information on the proper fitting, donning, positioning, adjustment, and removal of PPE;
- Training on work related techniques designed to minimize exposure to pathogens, as well as potential routes of exposure;
- Training on use of incident and potential exposure reporting protocols;
- Training on standard contact and airborne precautions;
- Training on spill cleanups and emergency procedures

Information about specific vaccines and exposure tests commonly given to personnel working with Biohazard will be provided to all individuals working in biohazard labs.

VIII. Biohazardous Materials Laboratory Incidents or Accidents

A biohazardous laboratory incident or accident involves:

- Any potential or known exposure to Biosafety Level 2 (BSL-2) agents or higher;
- Any potential or known system failure that could result in the release of a BSL-2 or higher organism from primary containment;
- Any potential breach in biosecurity in containment facilities

A. Chain-of-notification

All College employees are responsible for reporting potential biohazardous incidents to their immediate supervisor, the Biosafety Officer (BSO) or the Office of Environmental Health, Occupational Safety and Risk Management (EH&OS).

Supervisors are responsible for reporting biohazardous incidents to EH&OS. PIs are responsible for immediate reporting of a theft, loss or release of a select agent or toxin to the EH&OS. Select agent and toxin labs will have lab-specific incident response plans that describe their site and agent specific protocols.

The BSO is responsible for reporting to the IBC any significant problems, violations, research-related accidents or illnesses involving work described under the NIH Guidelines for Recombinant DNA Research. The Office of Environmental Health, Occupational Safety and Risk Management is responsible for reporting any reported theft, loss or release of a select agent or toxin to their federal reporting agency utilizing CDC/USDA Form 3. Records related to biohazardous incidents or accidents will be maintained for a minimum of three years.

The IBC is responsible for reporting significant problems, violations or significant research-related accidents or illnesses to the Institutional Official and EH&OS within 30 days of the incident.

B. Reporting an incident

Incident reports are initiated with an employee report to a supervisor or directly to EH&OS. Personnel will be able to report an incident or seek medical attention without fear of reprisal. Medical evaluation, surveillance and treatment will be provided as necessary. If a medical emergency exists, personnel must first seek medical attention. The biosafety occupational health provider will review any medical incident involving potential exposure to a biohazardous substance that poses a human health threat.

The employee's supervisor must contact the BSO and EH&OS to report the biohazardous Incident/accident as soon as reasonably possible. PI's of containment and select agent labs will report immediately to EH&OS any incident resulting in the potential release of an agent outside of primary containment and as established in their written plan.

C. Investigation and Review of Incidents

Following the reporting of a biohazardous incident/accident, a risk assessment and incident review will be performed with the employee's supervisor, PI (if different), biosafety professional and any other necessary personnel (ex. medical consult). The Office of Environmental Health, Occupational Safety and Risk Management will utilize the Biohazardous Incident/Accident Reporting and Response form as a record. PIs, safety professionals and medical doctors will serve as subject matter experts. Employee supervisors will provide EH&OS with protocol modifications and SOPs following an incident or accident.

D. Training Personnel

Supervisors will formally review incident reporting protocols with personnel upon hire and then annually at a minimum. If an incident involving potential exposure or release of a biohazardous agent does occur, the supervisor will provide a follow up meeting with personnel to discuss the scenario and any program modifications or changes that apply following review of the incident. This follow-up meeting will be documented. Related to human health hazards, it is well documented that laboratory-acquired infections (LAIs) are often not associated with a single known incident. Therefore, lab personnel will be encouraged by their supervisor to seek medical evaluation for signs and symptoms that they suspect may be related to possible biohazardous agent exposure in the lab. A diagnosed LAI without a known incident is considered reportable to EH&OS.

E. Spill Response

When accidents occur that involve the release of biohazardous agents, the PI should be notified as soon as possible. Trained lab staff working with these agents will be responsible for mitigation. The Office of EH&OS is available for assistance and should be contacted as soon as possible (following incident reporting protocols).

Spills of biohazardous materials must be first contained, decontaminated and further cleaned up by staff properly trained and equipped to work with infectious materials. Each lab using biohazardous materials must have appropriate equipment and supplies on hand for managing spills and accidents involving biohazardous materials. Permanent equipment should include a safety shower, eyewash, a hand-washing sink, and disinfection and cleanup supplies. Spill protocols should be posted in areas where agents are handled and a biohazard spill kit should be readily available. Examples of biohazard spill kit supplies:

- Nitrile or other appropriate disposable gloves;
 - Lab coats, disposable gowns, disposable Tyvek-like suits;
 - Goggles, safety glasses, or disposable face shield;
 - Disposable shoe covers (booties);
 - Absorbent material- paper towels, absorbent pads;
 - Appropriate disinfectant (should be freshly prepared with available materials on hand);
 - Tools to aid in collecting material- tongs, forceps, dustpan
 - Biohazard bags and sharps waste containers;
 - Warning sign to post for restricted entry
- i. Managing a Biohazardous Spill INSIDE a Biosafety Cabinet (BSC)
1. Keep the BSC running.
 2. Immediately cover with absorbent material.
 3. Soak absorbent material with freshly prepared disinfectant. Work from the outside of the absorbent material to the center. Allow for appropriate contact time.
 4. Remove gloves and other contaminated clothing, according to standard procedures.
 5. Place in biohazard bag(s) for autoclaving.
 6. Wash hands and arms thoroughly. Don a new pair of gloves and additional PPE as needed.
 7. After appropriate contact time, collect disinfected materials placed on the spill area in a biohazard bag. If tubes or solid materials are involved, utilize tools such as tongs to pick up those materials. Broken glass and sharps should be placed in a Sharps container rather than in a biohazard bag.
 8. Wipe up spill area with disinfectant soaked paper towels.
 9. Wipe down walls, work surfaces, and equipment in BSC with disinfectant.

10. If leaked through the BSC grille
 - a. Wipe down all items within the cabinet and remove
 - b. Ensure drain valve is closed
 - c. Flood tray top, drain pans, and catch basins with disinfectant
 - d. Allow to stand for the appropriate contact time
 - e. Lift out tray and remove exhaust grille work
 - f. Clean top and bottom surfaces with sponge/cloth soaked in decontaminating solution
 - g. Replace grille tray and grille work
 - h. If applicable, drain decontaminating solution from cabinet base into a collection vessel containing additional decontaminating solution. A flexible tube should be attached to the drain valve and be of sufficient length to allow the open end to be submerged in the disinfectant within the collection vehicle. The drain pan should be flushed with water and drain tube removed.
 - i. Remove gloves and other contaminated clothing, according to standard operating procedures. Place in biohazard bag for autoclaving.
 11. Place all contaminated materials within a biohazard bag. Autoclave all contaminated material.
 12. Follow incident reporting protocols for notifying lab supervisor (PI) and the Biosafety Officer.
 13. For BSL-2 labs, work may not resume until the PI or lab supervisor agrees that the clean-up is complete.
 14. Record spill clean-up on the Laboratory Decontamination Log sheet.
- ii. Managing a Biohazardous Spill OUTSIDE of a Biosafety Cabinet (BSC)
1. Quickly place absorbent pads on the spilled area and carefully flood with liquid disinfectant.
 2. Remove PPE and potentially contaminated clothing, place in biohazard bag and wash any apparently contaminated body parts with soap and water before leaving the laboratory.
 3. Post warning signs and/or a guard to keep anyone from entering the spill area. Do not allow anyone entry to the area unless cleared to do so by the PI or BSO. Leave the area as necessary following standard exit protocols.
 4. Report the incident to the lab supervisor, PI or BSO as needed.
 5. Allow 20 minutes for any potential aerosols to settle and appropriate contact time for liquid disinfectant to work.
 6. Once any potential aerosol has settled, don appropriate PPE for entry (double gloves are recommended).
 7. Further soak absorbent material with freshly prepared disinfectant. Work from outside the absorbent material to the center being careful to minimize splashing or potential formation of aerosols. Allow for appropriate contact time.
 8. Collect disinfected materials placed on the spill area in a biohazard bag. Utilize tools such as tongs to pick up those materials. Broken glass and sharps should be placed in a Sharps container.
 9. Wipe up the general area surrounding the spill with disinfectant soaked paper towels - including walls, work surfaces, and equipment.
 10. Remove gloves and other contaminated clothing, according to standard operating procedures. Place in biohazard bag for autoclaving. Place all contaminated PPE in

biohazard bag and autoclave with all contaminated material.

11. Record spill clean-up on the Laboratory Decontamination Log sheet.

IX. Transportation and Shipping

This section of the manual only accounts for shipping need of biological or biohazardous materials. The Dalton State College Institutional Biosafety Manual was referenced in the establishment of this section for parts applicable to the US Department of Transportation (DOT) and International Air Transport Association (IATA) guidelines.

Various regulations provide for oversight of movement of biohazardous materials. The Federal Aviation Association (FAA) and the DOT monitor the shipping of Dangerous Goods. There are 9 categories of dangerous goods and this manual only provide guidance for biologicals, infectious substances and either of those two shipped on dry ice. Failure to comply with federal shipping requirements for any dangerous good can result in fines:

- Up to \$250,000 and 1 year in jail for individuals
- Up to \$500,000 per incident for an organization

Other federal agencies providing requirements and oversight for shipping of Dangerous Goods:

- International Air Transport Association (IATA)
- US Department of Transportation (DOT)
- US Public Health Service (PHS)
- Occupational Health and Safety Administration (OSHA) United State Postal Service (USPS)
- United States Department of Agriculture (USDA)
- US Department of Health and Human Services, Centers for Disease Control (DHHS, CDC)

Biohazardous materials must always be transported following applicable regulations. Never move a biohazardous material in a vial in your pocket. Each regulator will have necessary training and protocols involved that must be followed.

A. On-Campus (Intra-entity) Transport of Biohazardous Materials:

Any biohazardous materials transported between laboratories on campus must be contained, as it would be within the laboratory to prevent a release to the environment. Secondary containers should be utilized and labeled with the biohazard symbol and the identity of the material inside.

Example: Transport of a rack of test tubes containing serum samples from pigs infected with *Salmonella* spp. from an animal facility to a laboratory building:

- Tubes will be tightly capped, placed in secondary container such as a PP or HDPE bin container with a biohazard label indicating *Salmonella* spp.
- The secondary container used must contain the samples in case the person carrying the container drops it. Adequate absorbent material must be placed between the two containers in case any of the tubes break.

Intra-entity movement of materials classified as Category A or B, or those transported on dry ice via a vehicle on public roadways must follow IATA/DOT regulations for packaging and shipping dangerous goods, including filling out a shipper's declaration which can be obtained from EHOS. Only College vehicles shall be used for these intra- campus transports. Privately owned vehicles (POV's) are not to be used.

Intra-entity movement of animal and plant pathogens subject to a USDA permit can be performed only in accordance with the permit conditions. For example, a researcher has a permit to work with a plant virus in growth chambers. This virus cannot be transported to a greenhouse on campus without the written permission of the USDA.

Or a researcher may have an Animal and Plant Health Inspection Service (APHIS) permit for an animal pathogen in their lab only for in vitro work. If that researcher or another researcher wants to work with that pathogen elsewhere or in vivo, they would need to either amend an existing permit or apply for a permit to move it. Plant GMOs or GMO plant pests are subject to USDA regulations (7 CFR 340.8) and labeling must be according to 7 CFR 340.7. Contact the Office of EH&OS for questions or assistance.

B. Off-Campus (Inter---entity) Transport of Biohazardous Materials:

All off-campus transport of biohazardous materials must comply with federal and state shipping and permitting requirements, as described in the following sections. Off-campus may include across town to a collaborative research facility, out of town within the state, out of state in the United States, and out of the country.

C. Transportation Permit Requirements:

Special federal permits may be required for importing, exporting and/or transporting human pathogens, animal pathogens, animals or animal products, plant pathogens or plant pests, and plants or plant products. Permit requirements should be verified well in advance of needing the material in question, because some permits can take 60-180 days to receive. In general, the receiver is required to be approved and hold a permit for the material before it is shipped. The Office of Biosafety can provide assistance with any questions about shipping and/or required permits for biological materials.

- **Animals, Plants, Introduction of Genetically Modified Organisms**

The USDA, through its Animal and Plant Health Inspection Service (APHIS), regulates transport of materials that could potentially harm U.S. agricultural products, such as livestock or crops. For this reason, APHIS permits may be required for import, export and/or transport of animal or plant pathogens, soil samples, insects, import or export of animals, animal products, plants or plant products, or introduction of genetically modified organisms into the environment. A quick reference guide to regulations including permit oversight and a web resource page are available within this manual. The Office of Environmental Health and Occupational Safety can help determine if a permit is required and can assist with the application process.

- **Human Pathogens or Biological Toxins**

The Department of Health and Human Services (DHHS), through the CDC, regulates the import and transport of biological materials that could cause illness in humans. These regulated biological materials include pathogenic bacteria or viruses, toxins from biological sources (i.e. tetanus toxin), blood or tissues capable of containing pathogens transmissible to humans and certain animals, and insects that may harbor disease-causing organisms. The information contained on the CDC Etiologic Agent website and the Office of Biosafety can help determine if a permit is required and can assist with the application process.

Following DOT regulations and IATA guidelines, biological materials, when shipped, will fall under any of the following categories:

- ☒ Unregulated biological material
- ☒ Category A infectious substances

- ☒ Category B infectious substances
- ☒ Patient specimens
- ☒ Genetically modified organisms

D. Category A Infectious Substances

(See IATA packaging requirement 602)

Agents capable of causing permanent disability, life threatening or fatal disease in humans or animals when exposure occurs.

- ☒ UN2814 – infectious substances, affecting humans, *OR* UN2900 – infectious substances affecting animals
- ☒ Must be triple packaged
- ☒ Specify if shipment is a refrigerated sample
- ☒ Maximum quantity that can be shipped by air in one package (over-pack) is 4L or 4kg
- ☒ Maximum quantity that can be shipped via passenger aircraft is 50mL or 50g
- ☒ Labeling of outer container:
 - Sender and recipient's full name and address
 - Infectious substance label; UN2814 and net quantity *OR* UN2900 and net quantity
 - Text – "Person Responsible" with their name and phone number (24-hr. contact person while shipment is in progress)
 - Class 9 label including UN1845 and net weight if packaged with dry ice
 - Cargo Aircraft label when shipping over 50mL or 50g
- ☒ Dengue virus (cultures only) are classified as Category A infectious substances

E. Category B Infectious Substances:

(IATA packaging requirement 650)

Infectious agents that do not meet the standard for inclusion in Category A.

- ☒ UN3373
- ☒ No shipper's declaration necessary
- ☒ Must be triple packaged
- ☒ Specify if shipment is a refrigerated sample
- ☒ Maximum quantity of liquid per primary receptacle is 1L and outer package must not contain more than 4L or 4 kg
- ☒ Labeling of outer container:
 - Sender and recipient's full name and address
 - Text – "Biological Substance, Category B"
 - UN 3373 label
 - Text – "Person Responsible" with their name and phone number (24-hr contact person while shipment is in progress)
 - Class 9 label including UN1845 and net weight if packaged with dry ice

F. Genetically Modified Organisms (GMO's):

GMO's are organisms that have been purposefully altered through genetics in a way that does not occur naturally. GMO's that are not infectious but that can alter microorganisms, animals or plants in a way that is not normal are assigned to UN3245. GMO's that are infectious are assigned to UN2814, UN2900 or UN3373.

- ☒ Packaging
 - Ship same as Category A
 - Maximum allowable quantity for primary containers is 100mL or 100g.

- There is no maximum net quantity per package
- Outer container labeling for GMO assigned UN3245
- Sender's full name and address
- Class 9 label
- UN3245, Genetically Modified Organisms and net quantity.

G. Unregulated biological materials:

Not subject to IATA or DOT shipping regulations but may require a federal permit before shipping and include:

- ☒ Materials that do not contain infectious substances or are unlikely to cause disease in humans or animals;
- ☒ Non-infectious biological materials from humans, animals or plants (i.e. non-infectious cells, tissue cultures, blood or plasma from individuals not suspected of having infectious disease, DNA, RNA, genetic elements);
- ☒ Substances containing microorganisms not suspected of causing disease in humans, animals or plants;
- ☒ Inactivated substances or environmental samples not considered a significant risk for infection;
- ☒ Dried blood spots or fecal occult blood screening tests (note: must following packaging guidelines when shipping through USPS);
- ☒ Samples (other than Category A) contained in a patient sample transported for research, diagnosis, investigational activities, disease treatment and prevention, or a biological product, when being transported by a private or contract carrier in a motor vehicle used exclusively to transport such materials;
- ☒ Blood or blood components collected for transfusion or preparation of blood products to be used for transfusion or transplantation;
- ☒ Material with low probability of containing infectious disease or where the concentration is at a level naturally occurring in the environment so it cannot cause disease with exposure (foodstuff, environmental samples like water, dust, mold);
- ☒ Biological product, including an experimental or investigational product or component of a product, subject to federal approval, permit, review or licensing requirements such as those required by the FDA or USDA.

H. Packaging & Paperwork Requirements for Shipping Biohazardous Materials & Dry Ice:

Biohazardous materials must be transported in accordance with Department of Transportation (DOT) requirements, 49 CFR, and the International Air Transport Association (IATA) Hazardous Materials Regulations. All dangerous goods, including biohazardous agents and dry ice, under DOT/IATA regulations, must be properly classified, packaged, documented and handled by trained personnel. A trained point of contact must be available 24 hours a day from the time the shipment leaves until it arrives at its location. A specialized dangerous goods training is required of anyone who directly affects hazardous materials transportation (including people who package, manage shipping papers, maintain emergency response information, ship or transfer and receive a dangerous goods package). Training must include general awareness and hazard familiarization, job function---specific training, and safety and security training. Training records must be maintained for a minimum of five years and training must be provided every three years for ground transportation (DOT) and every two years for air and international transportation (IATA includes air, rail, sea, road transportation). Non-compliance can result in federal citations, fines and suspension of shipping privileges.

When shipping a biohazardous agent:

- o Determine what type(s) of materials are being shipped – classify them into an
- o appropriate shipping category so to determine packaging, labeling, etc.
- o Obtain a copy of any required permits from the permit holders (package
- o receiver) to enclose within the shipped package.
- o Package materials in approved containers and under the supervision of a DOT/IATA Dangerous Goods (DG) trained individual.
- o An individual trained to package dangerous goods will properly prepare Dangerous Goods Bill of Laden or Shipper's Declaration of Dangerous Goods form and packaging labels.
- o Ship through a commercial company that allows for tracking capabilities. Maintain copies of all shipping records including tracking of materials to arrival on file with PI for a minimum of five years.

Commercial Shippers include:

- o DHL – will accept all shipments made according to DOT/IATA regulations
- o FedEx – will accept all shipments except BSL4 agents
- o World Carrier will accept all shipments made according to DOT/IATA regulations
- o UPS – will not accept Category A materials but will accept Category B shipments and exempt patient specimens
- o United States Postal Service (USPS) – is highly restrictive for shipments of hazardous materials by mail. They will not accept Category A materials but will accept Category B and exempt patient specimens. Category B shipments and exempt patient specimens will require more strict packaging and labeling requirements than the standards have established. Contact USPS for requirements before shipping through them.

I. Packaging Materials

Ship in packaging that can withstand breakage and leakage of contents, dramatic changes in temperature, pressure changes and other potential conditions ordinary when handling packages.

Purchase certified packaging materials (see appendix for list of companies) that are specific to your shipping category (Category A, Category B, etc.). All biological materials should be triple packed. Those three elements consist of a primary container, a leak-proof secondary container, and the final, durable outer container.

Note: purchased packaging products do not contain primary containers. Over-packs can be used to combine several triple packed items into one large package.

Primary Container:

This container holds the actual biological material(s). It must be leak-proof and labeled with the name of contents. Use a leak-proof seal such as a heat seal, skirted stopper or metal crimp. For a threaded lid, utilize waterproof tape such as Parafilm to secure it in position. Petri dishes are not suitable as primary receptacles. Lyophilized substances can only be shipped in flame sealed glass ampoules or rubber stopped glass vials with metal seals.

Secondary Containers:

This container holds the primary container and is leak-proof. For Category A and B shipments that include liquids, secondary containers must meet federal standards for pressure. When shipping liquids, provide for enough absorbent material to be placed within the secondary container that can absorb all of the liquid in the primary receptacle in the event of a leak from the primary container. Wrap multiple containers in a cushioning material to reduce the likelihood of one breaking or cracking during transport.

Outer Containers

This is a rigid container with at least one side being 100 mm X 100 mm in size in order to allow for labels and shipping markings to be placed on the package. It must have strength to provide support for its capacity, mass and intended use. An itemized list of the package contents, a copy of any required federal permit, and three copies of the shipper's declaration or Bill of Lading must be included and located between the secondary and outer container. Markings on the outer contents must identify the hazards of contents, proper shipping name, UN number and net quantity of each substance shipped within the package, if required.

Over-packs:

If over-packing several triple packed items, each triple pack must be individually, appropriately marked and labeled as if they were being shipped alone. The outside of the over-pack must have the same markings and labels as a standard outer container. This includes identification of hazardous contents, proper shipping name(s), UN number and net quantity for each substance, if required. The over-pack must also be labeled with the word "Over-pack".

Dry Ice:

When shipping biologicals with dry ice, triple packaging applies and the outer package must allow for the release of carbon dioxide. Dry ice must be placed within the secondary container and that secondary container must provide for interior supports to secure the container as the refrigerant sublimates. Do not tape the secondary container closed. Dry ice is categorized as a Miscellaneous Hazardous Substance (Class 9). Outer packages for dry ice must have a Class 9 label on it and be marked with the proper shipping name (dry ice), UN number (UN1845) and net quantity. Commercial packaging designed for dry ice are typically pre-labeled and marked as such. A Shipper's Declaration for Dangerous Goods is not required for shipments in which the only hazard is dry ice.

J. Shippers Declaration of Dangerous Goods:

A Shipper's Declaration of Dangerous Goods is required when shipping a Category A substance with UN2814 or UN2900 OR when shipping a GMO assigned UN3245. A declaration is not required for Category B or shipments only containing dry ice as its hazardous substance.

The Shipper's Declaration must be typed or computer generated; handwritten declarations will not be accepted by any carrier. Declarations must be printed in color to display the red-stripped border. Always print at least four original copies, providing three to the carrier and keeping one for your records. Sign and date each copy – do not photocopy any Shipper's Declaration supplied to the carrier. Maintain shipping records for five years.

Sections within the Shipper's Declaration of Dangerous Goods:

- Shipper – enter full name, address and telephone number
- Consignee – enter full name and address of recipient
- Transport Details: if shipping more than 50ml or 50g of an infectious substance, indicate here that shipment is restricted to cargo aircraft only.
Note: the carrier will fill out airport of departure and destination.
- Shipment Type: cross out "radioactive" when non-radioactive substance is being shipped.
- UN or ID Number: enter appropriate UN number (see Table 2).
- Proper Shipping Name: Enter proper shipping name exactly as it appears in Table 2 Class or Division: Enter as found in Table 2
- Packaging Group: for dry ice, enter "III" in this column. Biologicals are not assigned packaging groups.

- Quantity and Type of Packaging: Enter the net quantity of each material here. Use only metric units. Indicate the number and type of packages used (usually “all packed in one fiberboard box). If using an over-pack, indicate “Over-pack Used” here.
- Packaging Instructions: Enter appropriate packaging instruction number (refer to Table 2)
- Authorization: leave this blank
- Additional Handling Instructions: indicate here the 24hr emergency contact person and number. This must be an individual with knowledge of the hazards of the package content.
- Signature section: self---explanatory

K. Off-Campus Transport of Biohazardous Materials by Non-Commercial Routes

Dalton State College personnel may not transport Category A infectious substances in personal vehicles.

Table 2. Summary Shipping Information

Shipment Type	Proper Shipping Name	UN Number	Hazard Class	Packing Group (PG)	Packing Instruction (PI)	Max. Qty. per Primary Container	Max. Net. Qty/Pkg for Passenger Aircraft	Max. Net. Qty/Pkg for Cargo Aircraft
Category A infectious substances, affecting humans and possibly animals	Infectious substances, affecting humans	UN2814	6.2	---	602	Liquids: 4L Solids: 4kg	50ml or 50g	4L or 4kg
Category A infectious substances, affecting animals (not humans)	Infectious substance, affecting animals	UN2900	6.2	---	602	Liquids: 4L Solids: 4kg	50ml or 50g	4L or 4kg
Category B infectious substance	Biological substance, Category B	UN3373	6.2	---	650	Liquids: 1L Solids: 4kg	4L or 4kg	4L or 4kg
Dry Ice	Dry Ice or Carbon Dioxide, solid	UN1845	9	III	904	N/A	2000kg	2000kg
Non-infectious transducing genetically modified organisms or microorganisms	Genetically modified microorganisms	UN3245	9	---	913	No limit	No limit	No limit

X. Disposal and Disinfection of Biohazardous Materials

Biohazardous materials used in teaching and research laboratories at the College fall under the State of Georgia Biomedical Waste Rules (391-3-4-.15)

A. Biomedical waste means:

- ☒ Pathological waste;
- ☒ Biological waste;
- ☒ Sharps;
- ☒ Chemotherapy waste;

- ☣ Contaminated, discarded equipment that was in contact with infectious agents, contaminated animal carcasses, body parts, bedding or wastes from infected animals
- ☣ Cultures and stocks of infectious agents and associated biologicals from medical, pathological, research and industrial laboratories;
- ☣ Waste from production of biologicals;
- ☣ Discarded live and attenuated vaccines; or
- ☣ Culture dishes and devices used to transfer, inoculate and mix cultures.

Storage and containment of biomedical waste will be in a manner and location that protects materials from animals, rain and wind, does not provide a breeding place or a food source for insects and rodents, and minimizes exposure to the public.

Biomedical waste, except for sharps, must be placed in containers that are impervious to moisture and have sufficient strength to preclude ripping, tearing, or bursting under normal conditions of use. Sharps shall be contained for storage, transport, treatment and subsequent disposal in leak-proof, rigid, puncture-resistant containers that are taped closed or tightly lidded to prevent loss of contents. Containers will be securely closed so as to prevent leakage or expulsion of contents during storage, handling, or transport. All containers used for contaminated biological waste will be red or orange or clearly identified with the universal biohazard symbol or clearly marked with the word "Biohazard". Biomedical waste placed in storage for handling or transport must be placed in secondary containers as well, either disposable or reusable pails, cartons, boxes, drums, dumpsters, or portable bins. These secondary containers may be of any color and shall be conspicuously labeled with the universal biohazard symbol and the word "Biohazard" on the sides so as to be readily visible from any lateral direction when the container is upright.

All cultures, stocks, and other potentially infectious materials should be decontaminated before disposal using an effective method. If treated in accordance with methods as described within the State of Georgia biomedical waste regulations, the waste shall no longer be considered biomedical waste and may be combined and handled as regular solid waste.

B. Treatment methods described within the state biomedical waste regulations:

Heating with Steam Under Pressure:

Autoclaves and effluent decontamination systems provide decontamination by heating with steam under pressure (typically an autoclave) so as to render the biomedical waste noninfectious. A recording thermometer must be used during each cycle to ensure the attainment of a temperature of 121°C (250°F) for 30 minutes or longer to achieve decontamination of the entire load.

Elements Required for Effective Autoclave Use:

Autoclaves must be properly used to effectively sterilize their contents. Autoclave use for microbiological media preparation requires various time and temperature settings for sterilization; therefore, individual trials should be done to determine the proper loading and time settings to determine adequate sterilization.

When autoclaving biohazardous waste, take into account the volume of waste and the ability of steam to penetrate the load. Vials of biological indicators can be placed inside of a load to determine if lab specific settings are appropriate. Minimum autoclave cycle time for a light load of biohazardous waste is 30 minutes at 121°C, 15psi. The following parameters contribute to autoclave effectiveness:

- ☣ Temperature: unless specifically instructed by media manufacturers' directions, autoclave chamber temperature should be at least 121°C (250°F). Prions require higher autoclave temperatures but alternate disposal methods for prions should be considered in the risk assessment.

- ☣ Time: cycle time will vary according to the contents of the autoclave. If media is to be prepared, the manufacturers' instructions should be followed. An adequate autoclave time for biohazardous waste is a minimum of 30 minutes, measured after the temperature of the material being sterilized reaches 121°C and 15 psi pressure. The tighter the autoclave is packed, the longer it will take to reach 121°C in the center of the load.
- ☣ Steam Contact: steam saturation of the load is essential for effective decontamination. Air pockets or insufficient steam supply will prevent adequate saturation. To ensure adequate steam contact, leave autoclave bags partially open during autoclaving to allow steam to penetrate into the bag. The addition of a small amount of water inside the bag before autoclaving will help ensure heat transfer to the items being decontaminated (do not add water if it will cause biohazardous materials to splash out of the bag).
- ☣ Containers: use leak-proof autoclavable containers only. Always consider substitutes for glassware when selecting containers. Plastics such as polypropylene, polypropylene copolymer or fluoro-polymer products are capable of being autoclaved repeatedly. Place non-borosilicate glass bottles in a tray of water to help prevent heat shock. Place plastic bags inside a secondary container in the autoclave in case liquids leak out. Autoclavable plastic or stainless steel containers are appropriate secondary containers. Make sure plastic bags and pans are autoclavable to avoid melting.

Validation of load is required through the use of an autoclave log and an indicator of some type. Various indicators can be used with loads or separately to indicate that various test parameters have been met. With each load, chemical indicators will be used that test for the presence of heat, pressure and the presence of steam be utilized and attached to the daily use log. Chemical indicators are available through most scientific vendors and a sample autoclave log is available through the EHOS. Biological indicators (i.e. *Geobacillus stearothermophilus*) and certain chemical indicators (i.e. Sterigage) verify that the autoclave reached adequate temperature for a long enough time to kill microorganisms. Biological indicators (SporAmpule Steam BI, SPS Medical)) should be used annually at a minimum for performance verification. The BI should be placed in one or more points within autoclave in a simulated load for quality assurances. For larger autoclaves, we recommend placing on BI in the center of the load and another near the drain as the weakest point in the system. Document the biological indicator results in a logbook or other suitable form and maintain those records with the autoclave log in the lab. All autoclave records should be maintained for a minimum of five years.

C. Types of Chemical Disinfectants:

Chemical Treatment:

Items that cannot be autoclaved can generally be decontaminated using a chemical disinfectant. The choice of chemical disinfectant depends on the surface or item needing decontamination, as well as the particular organism requiring inactivation. When choosing a chemical disinfectant, refer to the agent summary sheet, if available, for information. The categories of disinfectants listed in this section and the disinfectant product label must be reviewed. Contact the EH&OS for assistance in a waste solution assessment. The following are outlines of the basic properties and examples of the most common categories of chemical disinfectants, including alcohols, chlorine compounds, liquid formaldehyde, glutaraldehyde, iodophors, peracetic acid, phenolic compounds, and quaternary ammonium compounds. Adequate contact time is very important to ensure complete disinfection. Contact time varies with the type of material being disinfected.

Chlorine compounds:

Solutions of 50 – 500 ppm available chlorine are effective against vegetative bacteria and most viruses. Bacterial spores require concentrations of 2,500 ppm with extended exposure time. Prions require 20,000 ppm with extended exposure time. A 5,000-ppm available chlorine solution is preferred for general use since excess organic materials inactivate chlorine compounds. Diluting household bleach 1:10 with water makes this type of solution. Shelf life for diluted bleach is approximately seven days. Air and light inactivate diluted solutions, so solutions must be freshly made in order to maintain adequate available chlorine concentrations. These solutions should be stored in an airtight, opaque container out of the light. Strong oxidizers are very corrosive to metal surfaces, as well as to skin, eyes and respiratory tract.

D. Prion Inactivation

Prions are resistant to conventional inactivation protocols such as irradiation, dry heat, boiling and chemical treatment. The use of conventional autoclave usage for waste management has not resulted in complete inactivation of prions. According to the 5th edition of the BMBL, the safest and most unambiguous method for ensuring that there is no risk of residual infectivity on contaminated instruments or other materials containing prions is to discard and destroy through incineration. Current recommendations for inactivation of prions on instrumentation or other materials is based on use of sodium hypochlorite, sodium hydroxide, Environ LpH and the moist heat of autoclaving with combinations of heat and chemical being most effective.

Five Options for Prion Inactivation for Reusable Instruments and Surfaces:

- ☣ Immerse in 1N NaOH, and heat in a gravity displacement autoclave at 121°C for 30 minutes. Clean and sterilize by conventional means.
- ☣ Immerse in 1N NaOH or sodium hypochlorite (20,000ppm) for 1 hour. Transfer into water and autoclave (gravity displaced) at 121°C for 1 hour. Clean and sterilize by conventional means.
- ☣ Immerse in 1N NaOH or sodium hypochlorite (20,000ppm) for 1 hour. Rinse instruments with water, transfer to open pan and autoclave at 121°C (gravity displacement) or 134°C (porous load) for 1 hour. Clean and sterilize by conventional means.
- ☣ Surfaces or heat-sensitive instruments can be treated with 2N NaOH or sodium hypochlorite (20,000ppm) for 1 hour. Ensure surfaces remain wet for entire time period, and then rinse well with water. Before chemical treatment, it is strongly recommended that gross contamination of surfaces be reduced because the presence of excess organic material will reduce the strength of either NaOH or sodium hypochlorite solutions.
- ☣ Environ LpH (EPA Reg. No. 1043-118) may be used on washable, hard, non-porous surfaces (such as floors, tables, equipment, and counters), items (such as non-disposable instruments, sharps, and sharp containers), and/or laboratory waste solutions (such as formalin and other liquids). This product is currently being used under FIFRA Section 18 exemptions in a number of States. Users should consult with the Environmental Safety Division prior to use.

Working solutions of 1N NaOH equals 40grams per liter of water. Solutions should be prepared daily. A stock solution of 10N NaOH can be prepared and fresh 1:10 dilutions (1 part 10N NaOH plus 9 parts water) used daily.

20,000ppm sodium hypochlorite equals a 2% solution. Most commercial household bleach contains 5.25% sodium hypochlorite, therefore, makes a 1:2.5 dilution (1 part 5.25% bleach plus 1.5 parts water)

to produce 20,000ppm solution. This ratio can be stated as two parts 5.25% bleach to three parts water. Working solutions should be prepared daily.

Such solutions described above are corrosive and require suitable PPE and secondary containment. Consult with the BioSafety Officer or EH&OS for assistance. Appropriate disposal of corrosive solutions requires coordination with EH&OS.

Precautions in Using NaOH or Sodium Hypochlorite Solutions in Autoclaves:

NaOH spills or gas may damage the autoclave if proper containers are not used. The use of containers with a rim and lid designed for condensation to collect and drip back into the pan is recommended. Caution must be taken when handling hot NaOH solutions (post-autoclave) and in avoiding potential exposure to gaseous NaOH. Exercise caution during all sterilization steps and allow autoclave, instruments and solutions to cool down before removal. Immersion of sodium hypochlorite bleach can cause severe damage to some instruments.

USDA Recommendations for Inactivation of Prions Affecting Livestock:

Use porous load autoclaving at 134°C-138°C at 30psi for 18 minutes holding time at temperature (does not include warm-up and cool-down). (Please note that this practice is consistent with USDA requirements for prions affecting animals, but not BMBL recommendations for prions affecting humans.)

Soak ground samples in 40% household bleach (5.25% sodium hypochlorite) to provide 20,000 ppm available chlorine (prepared freshly at time of use). Soak for minimum of one hour at 20°C.

Non-disposable instruments should be soaked in 40% household bleach for one hour, then rinsed with water and autoclaved (porous load) at 134°C for one hour.

Wash all surfaces with 40% household bleach, soaking for 60 minutes, then rinse with water.

Note: Some surfaces are prone to corrosion from prolonged exposure to these chemicals, so rinsing is very important.

XI. APPENDICES

A. Biosafety Level 2 (BSL-2) Safety Sign

The sign is available as a PDF fillable form through EH&OS



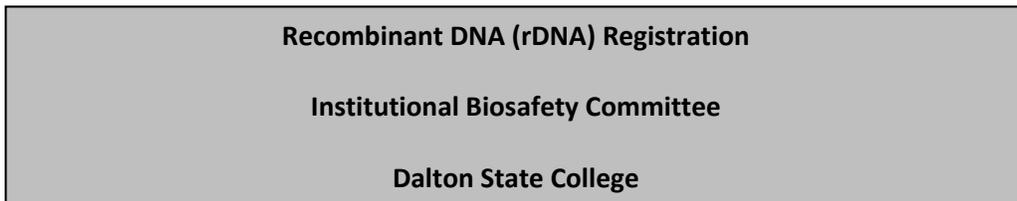
BIOSAFETY LEVEL

Biohazardous Agents:

Special Procedures or Precautions for Entry: [Bldg. _____ Room _____ Date Posted _____]

Notice	Call or See	Bldg.	Room	Work Phone	Home Phone
Entry or Advice					()
Emergency					()
Emergency					()

B. Recombinant DNA Registration Form



Instructions:

Investigators must use this form to register all rDNA research covered by the NIH Guidelines for Research Involving rDNA Molecules. The NIH Guidelines are available at: http://oba.od.nih.gov/rdna/nih_guidelines_oba.html

Registration is not required for rDNA research that is exempt from the NIH Guidelines.

This completed registration form must be submitted by e-mail to: jpepper1@daltonstate.edu Microsoft Word (*.doc) or Rich Text (*.rtf) format.

Section A. Project summary

Date: _____

Project Title: _____

Principal Investigator: _____
 (Full Name)

Department: _____

Email: _____

Telephone: _____

Contact person: _____

Email: _____

Co-Investigators: _____

List buildings and room numbers for ALL locations where rDNA research will occur under this registration: _____

Laboratories: _____

Shared resources (e.g., flow cytometry): _____

Vertebrate animal housing: _____

Other: _____

Is this application an amendment to a previously approved rDNA registration?

Indicate Yes or No by marking (X). If yes, provide original project title and registration number.

___ Yes ___ No Registration #: _____

Title: _____

Project Description: Provide a brief description (less than 250 words) of your project in laymen's terms, including the broad goals and potential benefits of the research. Describe the specific procedures conducted with the recombinants to meet these goals (ex. cloned in E. coli, transfected into cells, sorted in flow cytometry, injected into animals). If this application is an amendment, describe the original project and the additions or changes for this amendment.

Section B. Description of recombinant DNA (rDNA):

[Use this Section to describe your use of recombinant DNA. Please be sure to provide enough information to allow the Georgia College Institutional Biosafety Committee to assess the hazards potential risks to research staff or the community at large. If convenient, the information requested in this Section can be provided by attaching a table or spreadsheet to your registration, and referring to the spreadsheet in your responses. For amendments, clarify what is new in comparison to the original protocol.]

- 1) List the recombinant gene inserts to be used. Please include the source species (mouse, human, bacterial species, etc.), and the function of the insert if known. Please mark by an * all inserts that may pose a specific hazard or risk. Include oncogenes, toxin genes, inhibitors of tumor suppression genes, other proteins that may alter the mammalian cell cycle, and RNA molecules used to inhibit gene expression.

- 2) Describe in detail the function and potential hazards from the gene inserts marked by * in Section B1. For inhibitory RNA molecules, discuss consequences of the loss of the targeted gene product and potential off---target effect.

- 3) List all vector/host systems to be used in the research (e.g., bacterial expression plasmid cloned in lab strains of E. Coli, mammalian expression plasmid transfected into cell culture, replication--- deficient adenovirus infecting mouse neurons):

4) Will the research involve the use of antibiotic selection markers? If yes, list the markers and the microbial agents used (e.g., kanamycin resistance marker in E. coli). ____Yes ____No

5) List all cell lines to be used in the research, including the source species (Note that work with human or primate cell lines requires BSL2 containment.):

6) List all animals (vertebrate or invertebrate) or plants to be used in the research:

Section C. General Queries.

1) Will you conduct large-scale growth experiments in excess of 10 liters? If yes, explain:
____Yes ____No

- 2) Does this research involve “the deliberate transfer of a drug resistance trait to microorganisms if such acquisition could compromise the ability to treat or manage disease agents in human and veterinary medicine, or agriculture?” If yes, explain:

Yes No

- 3) Are you working with genetic material coding for a vertebrate toxin as defined in the NIH Guidelines. If yes, explain:

Yes No

- 4) Does this work involve a Select Agent (see [Select Agent Web Site](#))? If yes, explain:

Yes No

In reviewing registrations, Dalton State College IBC considers "dual use" potential, namely the potential for research projects with a beneficial purpose to provide knowledge, products or technologies that could be directly misapplied to pose a threat to public health and safety, agricultural crops and other plants, animals, the environment, or material. For a full discussion of this topic, consult the [NSABB web site](#). Consider whether your research is reasonably anticipated to do any of the following based on current understanding:

- Enhance the harmful consequences of a biological agent or toxin.
- Disrupt immunity or the effectiveness of an immunization without clinical and/or agricultural justification.

- (B) Expression of the gene insert product, and
- (C) Integration of the viral vector into the host genome leading to insertional mutagenesis.

Please mark by (X) your approach to replication competence for this vector.

This vector is capable of replication. Appropriate precautions for safe lab and/or animal work are described in my safety SOP.

The vector is intended to be replication deficient. However, the research will be conducted under conditions that would be appropriate for a replication-competent vector (RCV) that could arise as a result of a recombination event. Appropriate precautions for safe lab and/or animal work are described in my safety SOP.

The vector is intended to be replication-deficient. Every vector stock will be screened for the presence of RCV prior to use in the lab or injection in animals. I have attached a detailed SOP describing the screening assay, including the target level of detection for RCV in virus stock, the quantity of virus stock to be screened, and the positive control to be used to demonstrate sensitivity of the assay.

Section E. Recombinant DNA in animals or plants.

Complete this section ONLY if you are working with rDNA in any animals (vertebrates or invertebrates) or plants, or with genetically modified animals or plants (knockouts, transgenics, etc.).

- 1) List all species of plants or animals (vertebrate or invertebrate) that will be involved in the rDNA research, including genetically-modified plants or animals:

2) Will attempts be made to insert recombinant DNA into the germ line in order to establish a genetically modified animal or plant? If yes, explain:

Yes No

3) Will there be an attempt to crossbreed two or more genetically modified animals or plants? If yes, explain:

Yes No

4) Do these experiments involve the use of existing genetically modified animals or plants? If yes, explain:

Yes No

5) Where will the animals or plants be housed?

6) For vertebrate animals only, has the work been submitted to the IACUC for approval?

Yes No

Section F. Biosafety Level(s)

1) Does the research include work with an agent in Risk Group 2, 3, or 4, as defined in the NIH Guidelines? Do not list viral vectors from Section D or cell lines from Section B5.

Yes No

If yes, please describe the risks associated with the agent briefly here and in detail in a biosafety SOP.

2) Indicate the biosafety level(s) to be used for each category of work included in this registration. If research will be conducted at multiple biosafety levels, identify the components of the work conducted at each level (e.g., BSL1: Plasmids in E. coli, BSL2: viral vector in mammalian cells, ABSL2: viral vector in mice.)

- See Section IV of <BMBL5> and Appendix G of <NIH Guidelines> for laboratory biosafety levels (BSL1, BSL2, BSL3).
- See Section V of <BMBL5> for lab animal safety levels (ABSL1, ABSL2, ABSL3).
- See Appendix Q of <NIH Guidelines> for large animal containment (BL1---N, BL2---N, BL3---N).
- See Appendix P of <NIH Guidelines> for greenhouse plant containment levels (BL1---P, BL2---P, BL3---P).

Work at BSL1: _____

Work at BSL2: _____

Work at ABSL1: _____

Work at ABSL2: _____

Work at other containment level (specify): _____

3) Will this work be conducted with specific enhancements of biosafety levels (e.g., BSL2 PLUS all open container work confined to a biological safety cabinet)? If yes, summarize here, and describe in safety SOP.

Yes No

Summarize Enhancements:

Section G. Additional comments (optional).

Provide any additional information or clarifications that will assist the Dalton State College Institutional Biosafety Committee in assessment of the hazards and potential risks to research staff or the community at large:

Section H. Principal Investigator Assurances

Please mark each statement by (X).

NIH Guidelines compliance. I will comply with all requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules, available at <NIH Guidelines>.

Complete rDNA registration. The information above regarding this project is accurate and complete. I will maintain IBC registration(s) that accurately describe my current rDNA research. I will amend my registration or submit a new registration prior to beginning any new rDNA research.

Shipping and other compliance. I will comply with all federal transport regulations and NIH requirements pertaining to shipment and transfer of biohazardous and/or rDNA materials. I will comply with all CDC, OSHA, IBC, and other federal, state, local, and institutional regulations and policies relevant to this research.

Facilities. My laboratory has appropriate facilities, equipment, and work practices to conduct this work safely.

Training. I will assure the adequate training of all personnel associated with this project, including the following online training (check all training that applies to this protocol):

Lab Safety-General (all lab staff)

Biosafety Level 2- (all staff working at BSL-2)

Laboratory-specific Standard Operating Procedures applicable to this project

Bloodborne Pathogens (all staff handling human blood, body fluids, or cell lines)

Incident reporting.

The NIH requires institutions to report incidents involving rDNA materials including loss, theft, release, or human exposure. Examples of reportable events include the escape of a transgenic mouse or a needle-stick with a plasmid preparation.

____ Responsible for all investigators and locations: I will be responsible for assuring compliance for all rDNA work conducted under this registration, including all investigators and locations listed in Section I.

Principal Investigator

Date

This registration form can be submitted by e-mail to: jpeppers1@daltonstate.edu.
If the PI sends the form directly by e-mail, then the PI signature is not required.