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Last update 3/9/2021

Biological Safety Manual & Exposure Control Plan

1.0 INTRODUCTION

The MSU Biosafety Manual outlines the policies and procedures for the handling of and research involving biological materials, including recombinant DNA (rDNA) and human source materials. At the present time, no biological containment greater than biosafety level 2, as outlined by the Center for Disease Control (CDC) or the National Institute of Health (NIH) is permitted. This manual is intended for use as a supplement to the NIH Guidelines for Research Involving rDNA Molecules and the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories. These resources should be referred to should any questions or clarifications be required for applicability to the work being proposed.

This manual serves as both the written program and as a biosafety training document. Copies of these documents and other pertinent information can be found with the Facility Biosafety Officer. All laboratory personnel working with biological agents/materials must read and be familiar with the contents of this manual.

2.1 ROLES AND RESPONSIBILITIES

2.2 Morgan State University

MSU is responsible for ensuring that the handling of and research involving biological agents/material is carried out in a safe and efficient manner in compliance with the provisions of the NIH Guidelines and the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) practices. In order to provide for the safe conduct of rDNA and/or biological research, BioLabs has established policies to ensure compliance with federal, state and local regulations. BioLabs, through its Institutional Biosafety Committee (IBC), may establish additional procedures to assist researchers and employees in the safe conduct of their work.

2.3 Institutional Biosafety Committee (IBC)

The IBC has been established by MSU to review potential projects involving rDNA and biological agents classified as risk group 3 or risk group 4. The IBC is composed of no fewer than five members so selected that they collectively have experience and expertise in rDNA technology and the capability to assess the safety of rDNA research experiments and any potential risk to public health or the environment. At least one member is a non-doctorial person from the laboratory technical staff and two members are local community representatives not affiliated with MSU.

The IBC is responsible for the following:

- Monitoring and guiding rDNA projects, ensuring that rDNA research activities at MSU comply with the NIH Guidelines and internal policies.
- Performing risk assessments and making recommendations to the safety committee with regards to appropriate laboratory practices and protection based on information contained in the MSU Biological Research Project Registration (Appendix 1) which has been completed and submitted by the project's principal investigator.

- ❑ Determining the need for medical surveillance for personnel on a project-by-project basis and ensuring an appropriate medical surveillance program is in place as needed.

The IBC project registration review and approval includes:

- ❑ An assessment of the appropriate containment level.
- ❑ An annual assessment/review of registered projects
- ❑ An annual review of the facilities, procedures, and practices, and of the training and expertise of the project personnel.
- ❑ Notification to the Principle Investigator (PI) of the results of the review.
- ❑ Development of emergency plans covering accidental spills, injuries and exposures.
- ❑ A report to the appropriate company officials regarding any significant problems or violations, and any significant research-related accidents or illnesses.
- ❑ The IBC may not authorize initiation of experiments not explicitly covered under current NIH or CDC guidelines until the appropriate containment requirements have been established.

2.4 Laboratory Principle Investigator(s)

The principal investigator (PI) in each laboratory has primary responsibility for complying fully with the requirements as set forth in this manual.

As part of this general responsibility, investigators must not initiate a research project involving rDNA or biological agents without IBC approval. The PI must complete a Biological Project Registration (Appendix 1) and submit it to the IBC for approval. If a modification is made to a protocol the investigator should inform the IBC before initiating the change.

The PI is responsible for:

- ❑ Reporting to the IBC and Biosafety Officer, within 10 days, all significant problems with and violations of the guidelines and all research-related accidents and illnesses.
- ❑ Reporting to the IBC any proposed modifications or changes to the project prior to implementation.
- ❑ Training laboratory personnel on the potential hazards of the biological agents with which they are working and the appropriate safe work practices, techniques and equipment.
- ❑ Knowing and understanding the approved emergency plans for dealing with accidental spills and personnel contamination.
- ❑ Complying with shipping requirements for recombinant DNA molecules, infectious agents, and microorganisms.
- ❑ Maintaining an inventory of biological agents and rDNA molecules in their possession
- ❑ Reporting to the BSO any changes in the health status of research personnel or illness which lasts four days or longer.

As part of the protocol submission process the investigator should:

- ❑ Make the initial determination of the required levels of physical and biological containment in accordance with NIH and/or CDC guidelines.

- ❑ Select appropriate microbiological practices and laboratory techniques to be used in the research.
- ❑ Submit the initial research protocol and subsequent changes to the IBC for review and approval.

Once the protocol is approved by the IBC, the PI should:

- ❑ Discuss and show staff copies of the approved protocols and describe the potential biohazards and the precautions to be taken.
- ❑ Provide training in the appropriate safe practices and techniques and be sure that the staff knows what to do and how to deal with an accident or incident.
- ❑ Be sure the staff understands the reasons and provisions for any precautionary medical practices advised or requested, such as vaccinations.

During the conduct of approved research the PI should:

- ❑ Supervise the performance of the staff to ensure that the required safety practices and techniques are employed
- ❑ Investigate any significant problems pertaining to the operation and implementation of containment practices and procedures; report any problems, violations, or issues to the IBC and Biological Safety Officer.
- ❑ Ensure the integrity of equipment used for physical containment and host-vector systems used for biological containment (e.g., genotypic and phenotypic characteristics that reduce virulence, pathogenicity, infectivity, or the ability to survive outside the laboratory or in the environment)

The principal investigator is responsible for the safe conduct of the research on a day-to-day basis.

2.5 Biosafety Officer

The BioLabs facility Biosafety Officer (BSO) shall be responsible for:

- ❑ Conducting periodic laboratory inspections to ensure standards are rigorously followed
- ❑ Reporting to the IBC all significant problems with and violations of BioLabs biosafety policies and procedures and all research-related accidents and illnesses of which the BSO becomes aware unless the BSO determines that the Principal Investigator has done so
- ❑ Ensuring that each laboratory has emergency plans for dealing with accidental spills and personnel contamination
- ❑ Investigating research laboratory accidents.
- ❑ Providing advice on laboratory security;
- ❑ Providing technical advice to the Principal Investigator and the IBC on research safety procedures

2.6 Technical Research Staff

All laboratory personnel must be adequately trained prior to beginning any work with biological, chemical, or radioactive material. General safety training will be provided by the BSO and Department CHO. Training on the specific techniques required to safely perform the work will be provided by the PI. Each staff member will demonstrate in the training sessions a good working knowledge of all protocols used in research. On an annual basis, retraining sessions will be held to provide a review and summary of all safety procedures.

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Last update 3/9/2021
Training for research personnel will include:

- ❑ Biological safety including rDNA research safety.
- ❑ OSHA Bloodborne Pathogen training.
- ❑ Waste management and decontamination/disinfection.
- ❑ Emergency procedures.
- ❑ Laboratory safety.
- ❑

Before research personnel can begin work they must:

- ❑ Read and understand the Biosafety Manual and Chemical Hygiene Plan.
- ❑ Complete applicable sections of the Medical Surveillance Program if appropriate for the work being conducted.
- ❑ Demonstrate working knowledge of all relevant safety practices, an understanding of the research they will perform and any potential hazards, and all information contained in the Health and Safety Manual.

2.7 Non-Technical Staff

All non-technical staff members are to be familiarized with the potential hazards associated with research in general. In general, all non-technical staff members must not enter the laboratory research area unless properly supervised by research personnel.

3.1 GOVERNANCE OF BIOLOGICAL RESEARCH PROJECTS

In order, to ensure that local requirements for the use of rDNA and/or biological agents are met, all research projects involving either rDNA or biological agents must be reviewed prior to initiation.
MSU has implemented the following process for governance of all biological research projects.

3.2 Project Registration

Upon project set up PI are required to complete a Biological Research Project Registration (Appendix 1) to describe work to be conducted, techniques to be used, containment procedures and equipment etc. This is required for all new projects as well as projects that have scientifically changed. If their science evolves from the original plan, PI is required to fill out a new project registration if they change their procedures and/or employ new vectors, cell lines etc.:

- ❑ Project registrations are reviewed by the MSU BSO and members of the IBC.
- ❑ New project registration documents will be sent by email to community IBC members for opportunity to review and comment.
- ❑ Any concerns expressed by reviewers initiate scheduling of an ad hoc IBC meeting to formally review project.
- ❑ If no concerns are identified, project will be given tentative approval and allowed to proceed.
- ❑ Copies of all correspondence to be maintained on file at MSU.
- ❑ Project registration documents will be formally reviewed at the next regularly scheduled IBC meeting.
- ❑ Resident companies are required to purchase all biological and chemical agents through state approved vendors to provides a mechanism for control over agents brought into the campus.

4.1 BIOLOGICAL CONTAINMENT

Created 2018

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Four levels of biosafety have been defined by the Center for Disease Control (CDC) and the National Institutes of Health (NIH). They are combinations of lab practices & techniques, safety equipment and lab facilities. Experience has shown that strict adherence to these guidelines contributes to a healthier and safer environment, both in the work place and in the surrounding community. Generally, Biosafety Level 1 represents basic good microbiological practices. Biosafety Level 2 is used where potentially infectious material is handled and where the transmission route is parenteral or by skin/mucous membrane contact. Biosafety Level 3 and Biosafety Level 4 present higher individual and community risk and require increased levels of physical containment, personal protection and access restrictions.

- ❑ Biosafety Level 1 (BSL1) Least restrictive, low risk biohazards. Good microbiological practices.
- ❑ Biosafety Level 2 (BSL2) Contact Exposure risk. Potentially infectious agents for which treatment or prevention is available; e.g. human clinical specimens, some virus, some human cell lines.

Personal protection and engineering controls required.

- ❑ Biosafety Level 2+ (BSL2+)Hybrid level (BL2/BL3). Aerosol exposure risk; non-serious (and not fatal) disease risk. Risk is suspected due to the inert location, vector or cell line. Risk can be controlled by strict adherence to enhanced BL2 practices and other specific lab practices and experimental procedures as defined by a Standard Operating Procedure (SOP) or Operating Method (OM).
- ❑ Biosafety Level 3 (BSL3) Aerosol exposure risk. Highly infectious agents for which treatment may not be available; e.g. tuberculosis, working with HIV.
- ❑ Biosafety Level 4 (BSL4) Extremely infectious. Not permitted.

5.1 HUMAN SOURCE MATERIALS

5.2 Bloodborne Pathogens

The Occupational Safety and Health Administration (OSHA) created the Occupational Exposure to Bloodborne Pathogens Standard, 29 CFR Part 1910.1030 to minimize or eliminate exposure to infectious agents that may be present in human blood, tissues or certain body fluids. The Bloodborne Pathogens Standard applies to all employers having employees that are “occupationally exposed” to human blood or other potentially infectious materials (OPIM). An employee is considered occupationally exposed if there is “reasonably anticipated skin, eye, mucous membrane, or parenteral contact with human blood or other potentially infectious materials in the performance of an employee’s duties.” Other potentially infectious materials include:

- ❑ Human cell or tissue cultures.
- ❑ Organ cultures.
- ❑ Any unfixed tissue or organ, other than intact skin, from a human being (living or dead).
- ❑ HIV- or HBV- containing culture media or other solutions.
- ❑ Human body fluids, except urine, feces, saliva or tears unless visibly contaminated with blood.
- ❑ Blood, organs, or other tissues from experimental animals infected with HIV, HBV, or other bloodborne pathogens.

An individual is also considered occupationally exposed if they do not have direct contact with blood or OPIM but uses equipment that is used to process or store blood, OPIM, or bloodborne pathogens. All

occupationally exposed employees are required to attend a bloodborne pathogens training session prior to beginning work and annually thereafter. There are additional requirements for research laboratories and production facilities engaged in the culture, production, concentration and manipulation of HIV and HBV.

OSHA has determined that occupational exposure to human blood, tissues and body fluids poses a significant health risk because these may contain bloodborne pathogens including, but not limited to:

- ❑ Human Immunodeficiency virus (HIV)
- ❑ Hepatitis B virus (HBV)
- ❑ Hepatitis D virus
- ❑ Hepatitis C virus
- ❑ Human T-lymphotropic Virus Type I
- ❑ Hemorrhagic Fever viruses
- ❑ Creutzfeldt-Jakob virus

The OSHA Bloodborne Pathogen Standard defines safety requirements for working with human blood and other clinical materials, human immunodeficiency virus, and the bloodborne hepatitis viruses. Those safety requirements are described in this Biosafety Manual. Before working with any human materials, contact the Biosafety Officer for guidance and to schedule appropriate training.

Materials other than those mentioned above which should also be handled at BL2 containment using universal precautions are:

- ❑ Human derived cell lines
- ❑ Human cell strains
- ❑ Human serum derived reagents
- ❑ Non-human primate blood, tissues and cells

5.3 Human Cell Lines

Characterization of human cells, for determination of compliance with the Bloodborne Pathogens Standard, would include screening of the cells lines for viruses characterized as bloodborne pathogens including human immunodeficiency viruses, hepatitis viruses, and EBV. Most cell lines are screened for human mycoplasmas and are free of bacterial and mycotic contaminants. Testing may include antigenic screening for viral or agent markers, co-cultivation with various indicator cells that allow contaminants to grow or using molecular technology to identify latent viruses capable of infecting humans. Cell lines that are procured from commercial vendors or other sources with documented testing that they are free of human bloodborne pathogens and which have been protected by the researcher from environmental contamination may be excluded from the Bloodborne Pathogens Standard.

It should be noted that human cells or other transformed human cell lines are sometimes adulterated with laboratory pathogens accidentally introduced by cultivation with other cell cultures, or physically contaminated by other cell cultures handled in the same lab. In order, to handle human cells, without having to comply with the requirements of the Bloodborne Pathogens Standard, human cells should be documented to be pure cells and shown to be free of bloodborne pathogens by testing.

When cell cultures are known to contain an etiologic agent, an oncogenic virus, or amphotropic packaging system the cell line must be classified at the same level as that recommended for the agent. This is the same for all cell cultures purposely inoculated with an infectious agent. Hybridoma cell lines are immortalized cell lines created by fusion of primary cells with a continuous cell line. In general,

primary cell cultures are less characterized than permanent cell lines and are not typically tested for contaminating pathogens. The tumorigenic potential is a risk to consider with permanent cell lines. The following must be handled at Biosafety Level 2, within a Class II Biosafety Cabinet:

1) All cell lines (primary and established) of human/primate origin. If cell lines are classified by other organizations as downgraded to BL1, documentation indicating the line is bloodborne pathogen free is required, as described above.
2) All cell lines from tumor tissue or transformed by any oncogenic virus.
3) All cell lines exposed to or transformed by amphotropic packaging systems.
4) All human clinical material (such as samples of human tissues and fluids obtained after surgical resection or autopsy).
5) All hybridoma use.
6) Unknown environmental samples.

5.4 Human Cell Strains

All primary human cell explants from tissues and subsequent in vitro passages of human tissue explant cultures, also known as human cell strains, must be regarded as containing potential bloodborne pathogens and should be handled in accordance with the Bloodborne Pathogens Standard. Non- transformed, human cell strains, characterized by documented, reasonable laboratory testing as described for human cell lines, to be free of human immunodeficiency virus, hepatitis viruses, or other bloodborne pathogens may be exempted from the standard's requirements. However, if such tissue explants or subsequent cultures are derived from human subjects known to carry bloodborne pathogens, such as hepatitis viruses or human immunodeficiency viruses or are deliberately infected with bloodborne pathogens, they must be handled in accordance with the precautions noted in the Bloodborne Pathogens Standard. Likewise, animal tissues, explants or cell cultures known to be contaminated by deliberate infection with human immunodeficiency virus or Hepatitis B virus are also subject to the Standard.

5.5 Human Derived Reagents

The Centers for Disease Control cautions that all human-serum-derived reagents used in the lab, such as Human Serum Albumin (HSA), be handled at BL2 levels with universal precautions because no test method can offer complete assurance that laboratory specimens do not contain HIV, hepatitis B virus, or other infectious agents

5.6 Exposure Determination

The OSHA Bloodborne Pathogens Standard requires that an exposure determination be performed in laboratories where human source materials are used. Appendix 2 provides a list of laboratories, job classifications, tasks and responsibilities where personnel may have exposure to human materials.

6.2 LABELING

All areas and equipment that contain biohazards agents must be marked with a biohazard warning label. It must be red or orange in color with a universal biohazard symbol and lettering in black as illustrated below.

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Last update 3/9/2021

The following are examples of biohazards and are required to be labeled with a Universal Biohazard symbol:

- ❑ Human blood, blood products, bodily fluids, tissues
- ❑ Biotechnology by-product effluent from living organisms
- ❑ Recombinant DNA materials
- ❑ Biologic agents some of which may harbor pathogenic agents
- ❑ Equipment which is used with any of the above
- ❑ Equipment in which any of the above are stored

7.1 MEDICAL SURVEILLANCE & VACCINATIONS

7.2 Medical Surveillance and Consultation

All tenants are responsible for making their own arrangements with a local occupational health company for their employees. The BioLabs IBC, in consultation with the contracted occupational health physician, will be responsible for recommending appropriate medical surveillance for the agents handled and type of work being conducted.

Tenants will offer medical consultation at no cost to their personnel under the following circumstances:

- ❑ Whenever an employee develops signs or symptoms associated with a hazardous chemical or biological agent which the employee may have been exposed to in the lab.
- ❑ Following a report of an exposure to human source material.
- ❑ Where chemical exposure monitoring reveals an exposure level routinely above OSHA's Action Level or Permissible Exposure Limit for an OSHA regulated substance which requires such monitoring or medical surveillance.
- ❑ Whenever an event occurs such as a spill, leak, explosion, which results in the

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likelihood of a hazardous exposure to chemical or biological material.

7.3 Hepatitis B and Other Vaccinations

The OSHA Blood-borne Pathogen Standard requires that all personnel with the potential for occupational exposure to blood borne pathogens and other human source potentially infectious materials be offered the Hepatitis B vaccine. Tenants of BioLabs PA shall offer the Hepatitis B vaccination series available to all personnel who have the potential for occupational exposure, and extend post-exposure evaluation and follow-up to all personnel who have had an exposure incident.

The Hepatitis B vaccination form is given in Appendix 3. All employees with anticipated exposure to blood, tissue or OPIM must accept or decline the vaccine and sign the form.

8.1 BIOSAFETY PROCEDURES & PRACTICES

Good microbiological practice is important not only for safe handling of biological material but also for ensuring good experimental results. This section outlines laboratory practices to be used for Biosafety Level One (BSL 1) and Biosafety Level Two (BSL 2) laboratories.

The objective of physical containment is to confine the organisms containing rDNA molecules and to reduce the potential for exposure to the laboratory worker, to persons outside the laboratory, and to the environment. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design. The primary means of physical containment is provided by laboratory practices and containment equipment. Special laboratory design provides a secondary means of protection against the accidental release of organisms outside the laboratory or to the environment.

8.2 Biosafety Level 1

Biosafety Level 1 is suitable for work involving well-characterized agents not known to cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required nor generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science. Laboratory facilities for BSL1 must include:

- ❑ A sign is posted on the door to the laboratory stating “BSL1”. It is recommended that the Universal biohazard symbol be posted as well.
- ❑ The door to the lab must stay closed with limited access.
- ❑ The laboratory contains a sink for hand washing.
- ❑ The laboratory is designed so that it can be easily cleaned. Rugs in laboratories are not appropriate, and are not used because proper decontamination following a spill is extremely difficult to achieve.
- ❑ Bench tops are impervious to water and resistant to acids, alkalis, organic

solvents, and moderate heat.

- ❑ Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.

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Last update 3/9/2021

8.3 Biosafety Level 2

Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazards to personnel and the environment. It differs in the following ways:

- ❑ Laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists,
- ❑ Access to the laboratory is limited when work is being conducted,
- ❑ Extreme precautions are taken with contaminated sharp items, and
- ❑ Certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.
- ❑
- ❑ BSL 2 laboratory facilities are fitted with everything in a BSL 1 lab as well as the following:
- ❑
- ❑ A sign posted on the door with the universal biohazard symbol and designating the lab as a BSL2 lab.
- ❑ An entry sign stating: AUTHORIZED ENTRY ONLY; Requirements for entry (depending on the type of biohazard); the contact name for entry; and the agents in use.
- ❑ Doors to the lab must be kept closed and access is restricted.
- ❑ A method for decontamination of infectious or regulated laboratory wastes is available (i.e. autoclave, chemical disinfection, incinerator, or other approved decontamination system).
- ❑ An eyewash facility is readily available.

8.4 Standard Microbiological Practices

The following standard microbiological practices shall be practiced in both BSL1 and BSL2:

- ❑ Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures and specimens are in progress. A BSL 1 sign or BSL 2 sign, as appropriate for the work being conducted, is posted on the doors to the laboratory. Laboratory doors are to remain closed at all times.
- ❑ Personnel wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory. Sinks are available in the laboratory for this purpose.
- ❑ Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
- ❑ Mouth pipetting is prohibited; mechanical pipetting devices are used.

- ❑ All procedures are performed carefully to minimize the creation of splashes or aerosols.
- ❑ Work surfaces and equipment are decontaminated at least once a day with an appropriate disinfectant and after any spill of viable material.
- ❑ Avoid contact with your mouth, eyes and nose.
- ❑ Wounds/dermatitis should be protected with a bandage then gloves
- ❑ Substitute plastic for glass where possible.

The following containment procedures are required in BSL 2 (and recommended for BSL 1 labs):

- ❑ Work in a biosafety cabinet as much as possible. Minimize movements inside the biosafety cabinet.
- ❑ Keep tubes capped as much as possible.
- ❑ Use shields when at the lab bench (or goggles and a mask).
- ❑ Use good techniques including wrapping tube cap with gauze or using filter tops tips for pipetting.
- ❑ BL2 materials are placed in a closed primary container which is placed within a closed secondary container preventing leakage during storage and transport through corridors; and when possible during handling and processing. Examples of secondary containers include biosafety cabinets, centrifuge safety cups, and Tupperware/zip-lock bags.

8.5 Personal Protection

- ❑ The following are personal protective equipment (PPE) guidelines for all BioLabs laboratories:
- ❑ Lab coats are required at all times. Buttoned lab coats are required in BL2 areas.
- ❑ Safety glasses are required at all times.
- ❑ Nitrile or latex gloves are strongly recommended in BSL1 laboratories and are required in BSL2 labs. Gloves should be changed frequently, and disposable gloves must not be reused.
- ❑ In BSL2 labs, open operations should be performed in the Biosafety Cabinet (BSC).
- ❑ Lab coats must be removed, and hands washed prior to entering a non-lab area.

8.6 Sharps

Sharps are any of the following: needles, scalpels, razors, glass Pasteur pipettes, serological pipettes, slides, cover slips, syringes, plastic tips, or anything that can easily puncture or scrape the skin. When working with hazardous materials (e.g. BL2, acutely hazardous chemicals, radiation), use of needles, scalpels and other “sharps” are restricted to only be used when there is no alternative.General Sharps Guidelines:

- ❑ Place the "sharps" waste container next to work area.

- ❑ Do not overfill the container and do not force sharps into a full container as this action often results in puncture injuries.
- ❑ Needles/Syringes should be disposed of immediately after use.
- ❑ Do NOT remove needle from syringe and dispose of as a single unit.
- ❑ Do NOT recap needles
- ❑ Do NOT bend or break needles.
- ❑ Substitute plastic for glass whenever possible.

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Last update 3/9/2021

8.7 Insect/Rodent Control

A monthly insect and rodent control program is in place and coordinated by the building owner.

9.1 EQUIPMENT PROCEDURES

9.2 Biosafety Cabinets

Biosafety cabinets are primary containment devices that are designed to provide protection for the worker and the environment, as well as provide a work environment free of contaminants. The effectiveness of the biosafety cabinet is directly dependent on the manner in which users perform their work. The effectiveness of the cabinet is a function of three separate directional airflows:

- ❑ Inward from the room through the front grille
- ❑ Downward through a HEPA filter onto the work surface
- ❑ Out of the cabinet through an exhaust HEPA filter.

Prior to working in the BSC follow these procedures to ensure proper effectiveness:

- ❑ Wipe the cabinet down with an appropriate disinfectant.
- ❑ Place necessary supplies.
- ❑ If the BSC was not already on, run the blower for 10 -15 min. prior to beginning work.
- ❑ Wear gloves.
- ❑ Keep the vertical sash below indicated height.
- ❑ Avoid creating disrupting the air flow of the BSC.

- ❑ Set-up equipment and workflow pattern; dirty to clean.
- ❑ Keep sides and grilles clear.
- ❑ When finished, wipe down the BSC surfaces with an appropriate disinfectant and leave the blower running for at least 10 to 15 minutes.
- ❑ UV light should not be used for decontamination purposes
- ❑ When the hood is not being used the sash should be lowered but not completely closed and the blower can be left on.

Turbulence in a biosafety cabinet may cause aerosols to cross-contaminate open vessels and/or escape the hood. Turbulence may be caused by:

- ❑ Blocking air flow grilles.
- ❑ Heat from Bunsen burners cause air current eddies.
- ❑ Rapid movement of arms in/out of cabinet.
- ❑ People moving rapidly behind worker, across the face of the cabinet.
- ❑ Down drafts from ventilation systems.
- ❑ Cross drafts from doors.

The HEPA filter within the BSC only protects against particulates. The pressure gauge on the side of the cabinet indicates the performance of the filter. This gauge should be monitored daily. Contact the Biosafety Officer or if the needle deflects over to one side or the other.
Some additional comments on use of BSCs:

- ❑ Do not store anything on top of the cabinet.
- ❑ The filter gauge reading decreases as the filter clogs.
- ❑ Hazardous chemicals shall not to be used in the re-circulating air BSCs.
- ❑ Post biohazard labels; and label the biosafety level as appropriate.
- ❑ Biosafety cabinets are tested and certified annually. If a BSC is moved it needs to be tested and recertified.
- ❑ If the BSC alarm goes off, cap tubes, close sash, and put a “Do Not Enter” sign on the door. Contact the laboratory PI and the facility Biosafety Officer.

9.3 Centrifugation

Centrifugation of materials shall be done in screw cap or pressure seal tubes/bottles. These should be inverted carefully after filling to check the seal. If there has been any possibility of leakage, the inner walls of the centrifuge chamber and the rotor are to be immediately decontaminated with an appropriate disinfectant.

9.4 Growth Chambers and Shakers

All growth chambers and shakers are to be covered. If shaking water baths are used, use copper sulfate (enough to give blue color) to prevent growth of microorganisms and mold. NEVER USE SODIUM AZIDE for this purpose! Plastic flasks and bottles should be used whenever possible to avoid breakage. Cotton

plugged flasks are not considered open vessels as long as the plugs fit tightly, with no tendency to pop out.

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Last update 3/9/2021

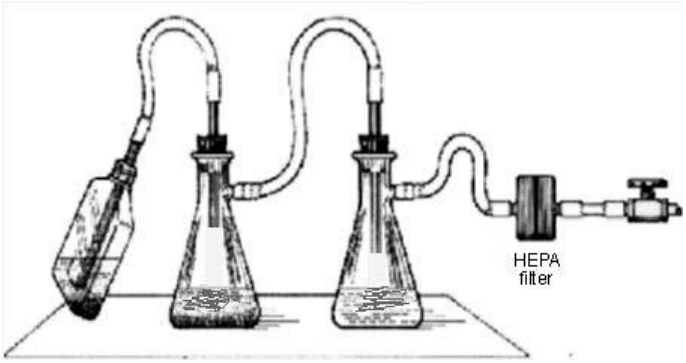
9.5 Blenders

Safety blenders, which have been designed to contain aerosols, are available and should be used whenever possible. A biological safety cabinet also serves as a primary barrier.

9.6 Aspiration

During aspiration, protect the vacuum lines by setting up a collection flask followed by an overflow flask if set-up is not at eye level. A vacushield or alternate should also be placed in the line to reduce the risk of contaminating the in-house vacuum.

The aspiration set-up should have a premeasured disinfectant in the flask, which should be labeled accordingly. If the flask is on the floor, protect against breakage with the use of secondary containment. Work in the Biosafety Cabinet when removing the stopper from the flask.
Proper set-up for an aspiration process is illustrated below. Also see Appendix V.



10.1 STERILIZATON AND DISINFECTION

Laboratories are subject to contamination by infectious and non-infectious biological material.

Frequent decontamination is necessary to provide a work area that is suitable for good microbiological practices and to render contaminated material safe for handling.

10.2 Sterilization

Sterilization is used to decontaminate items with steam or gas. Examples of these sterilization methods are steam sterilizers or ethylene oxide autoclaves. Sterilization is used to process clean, pre-wrapped items in which the steam or gas can penetrate to reach all areas within the packaging. Sterilization is also used for liquids, like culture media, to ensure biological experiments are accurate. The use of sterile equipment, media, and techniques prevents unwanted microorganisms from contaminating cultures.

Most equipment, media, and sometimes waste materials are sterilized in the steam autoclave. The autoclave can be used at various cycle lengths for different purposes. For example, the cycle time for dry goods sterilization will be shorter than for a liquid with a high protein load. As protein load increases, so does the cycle time for sterilization.

10.3 Disinfection

Disinfection is the process of using antimicrobial agents on inanimate objects to destroy all non-spore forming organisms that could pose a hazard to humans or compromise an experiment. Usually disinfection is performed with a chemical agent, but heat can also be a type of disinfection treatment for liquid materials.

There are many types of chemical disinfectants used in laboratories:

- ❑ Chlorine based compounds, usually sodium hypochlorite solution (Bleach)
- ❑ Alcohols, typically ethanol or isopropanol
- ❑ Glutaraldehyde solutions
- ❑ Iodophors, such as iodine
- ❑ Phenol based solutions
- ❑ Quaternary ammonium compounds

There is no universal disinfectant for all microbial agents. Some disinfectants are useful against many different types of microbes, others are used for very specific situations and agents. Contact time is another factor that plays a role in how effective decontamination methods are. Various hazards exist for each type of chemical disinfectant. A risk assessment is performed for all agents in use to determine which disinfectant is effective against the agent in question,

10.4 Disinfectants for Work Surfaces and Reusable Items

The following disinfectants are acceptable for work surfaces and reusable items at the prescribed concentrations:

- ❑ 10% solution of bleach. The shelf life of diluted bleach is only a few hours, so bleach should be diluted fresh immediately before use, or daily at a minimum. Allow a contact time of at least 10 minutes.
- ❑ 70% solution of ethyl alcohol & isopropyl alcohol. The shelf life of diluted alcohol is one month. Allow a contact time of at least 10 minutes.
- ❑ Wescodyne solution made in accordance with product label. Allow a contact time of at least 10 minutes or more.

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The following disinfectants are approved for liquid waste decontamination at the prescribed concentrations:

- ❑ Bleach to a final concentration of 10% in liquid waste. This disinfectant is best for human source materials. If the protein load is high in the liquid waste that is being disinfected, a 20% final concentration is necessary. Allow a contact time of at least 20 minutes.
- ❑ Wescodyne to a 1.0% final concentration in liquid waste. Wescodyne is an iodofor disinfectant/detergent. This is acceptable for tissue culture media and other such solutions but not for any human source materials. Allow a contact time of at least 20 minutes.
- ❑ The following Disinfectants are approved for spill clean-up
- ❑ Bleach concentration of 10 to 20% bleach. A 70% ethanol rinse solution can follow bleach when cleaning up a spill. Approximately twice the volume of disinfectant to the volume of the spill should be used. Allow a contact time of at least 30 minutes.

11.1 EMERGENCY SPILL PROCEDURES

11.2 Emergency Response

- ❑ Call for help.
- ❑ Contain the spill if it is safe to do so.
- ❑ Evacuate yourself and others from the area.
- ❑ Restrict access to the spill. Immediately contact the laboratory PI and/or Emergency Coordinator and the facility BSO.
- ❑ Report all accidents.
- ❑ Follow the emergency response procedures as outlined in the Emergency Action Plan.

11.3 Spill Supplies

Spill supplies available in the BioLabs laboratories include: gloves, absorbent pads, absorbent gel, splash goggles, biohazard bags, forceps, scraper, and scoop. Additionally, disinfectant and paper towels are located throughout the labs for use in case of a biological spill incident.

11.4 Exposure Response

Wounds and skin sites that have been in contact with blood or body fluids should be washed with soap and water; mucous membranes should be flushed with water. Promptly seek medical attention in the case of any exposure. Seek counseling regarding the risk of infection. In case of any of the following exposures, follow these decontamination procedures

Exposure Decontamination Method

- ❑ Eye Splash: Hold eye open while using eyewash station for 15 min.
- ❑ Mucous Membrane: Wash well with soap and water for 15 minutes
- ❑ Exposed Skin: Antimicrobial or nonabrasive soap; Sink or drench shower

- ❑ Clothing: Remove clothing; wash exposed skin.

11.5 Biohazard Spill in a Biological Safety Cabinet

The laboratory PI and facility BSO are to be notified immediately when there is a spill of more than 250 ml. of viable BSL 1 organisms or more than 100 ml. of viable BSL 2 organisms.

Chemical decontamination procedures should be initiated at once while the cabinet continues to operate to prevent escape of contaminants from the cabinet.

- ❑ Spray or wipe walls, work surfaces and equipment with an approved disinfectant, as noted above in Section 8.0.
- ❑ Flood the top work surface tray, and the drain pans and catch basins below the work surface, with disinfectant and allow to stand for 30 minutes.
- ❑ Remove excess disinfectant from the tray by wiping with a sponge or cloth soaked in disinfectant. For Class II cabinets, drain the tray into the cabinet base, lift out tray and removable exhaust grille, and wipe off top and bottom (underside) surfaces with a sponge or cloth soaked in disinfectant. Then replace in position and drain the disinfectant from the cabinet base into an appropriate container and dispose of according to liquid waste procedures. Gloves, cloth, sponge, and other cleanup materials should be discarded as biohazardous waste.

11.6 Biohazards Spill Outside of a Biological Safety Cabinet

The laboratory PI and facility BSO are to be notified immediately when there is a spill of more than 250 ml. of viable BSL 1 organisms or more than 100 ml. of viable BSL 2 organisms. In case this volume of material is spilled outside a BSC, take the following measures:

- ❑ Leave the room immediately, making sure to evacuate all others from the room as well, and close the door.
- ❑ Warn others not to enter the contaminated area.
- ❑ Remove and put any contaminated garments into a container for biohazardous waste disposal. Thoroughly wash hands and face.
- ❑ Wait 30 minutes to allow dissipation of aerosols created by the spill.
- ❑ Put on a long-sleeved gown, surgical mask and rubber gloves before reentering the room.
- ❑ Pour a decontaminant solution around the spill and allow to flow into the spill. Paper towels soaded in the decontaminant may be used to cover the area. To minimize the generation of aerosols, avoid pouring the decontaminant solution directly onto the spill.

- ❑ Let stand for 30 minutes to allow for an adequate contact time.
- ❑ Using a dustpan and squeegee, transfer all contaminated materials into a biohazard bag. Handle per biohazard waste procedures.

12.1 WASTE MANAGEMENT

12.2 Liquid Biological Waste

Decontamination of liquid biological waste takes place in the biological safety cabinet. Cell culture waste and other liquid waste are emptied into a container which contains disinfectant. If using bleach as the disinfectant, use 10% final concentration in the liquid volume. The waste must remain in contact with the disinfectant for at least 30 minutes. The disinfected liquid may then be carefully transported to the appropriate sink for disposal into the sink drain. See Appendix IV for specific steps for the bleach inactivation of liquid biological waste.

12.3 **Solid Biological Waste** (Non Sharps)

All non-sharps equipment and supplies, which have been in direct contact with biological materials, such as gloves, bench paper, plastic ware, and culture plates should be placed in a leak proof, labeled, covered container that is lined with a biohazard bag. Typically this would be a pre labeled, cardboard box that is provided by the waste vendor. When either 3/4 full or at least weekly, the containers must be sealed and moved to the biological waste storage area within the BioLabs's facility. The biological waste is picked up by a licensed biological waste hauler. Biological waste boxes will not be picked up if they are leaking, has materials poking out of the sides, or are not properly sealed.

12.4 "Sharps" Waste and Physically Dangerous Waste

Dispose of sharps in a puncture resistant, rigid "sharps container". Sharps containers are typically red plastic with a plastic cover available in different sizes. Sharps containers are to be closed after each work period. Full containers will be brought to the biohazardous waste storage area for pickup by the biohazards waste hauler.

Typical Sharps Containers

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Last update 3/9/2021

13.1 SELECT AGENTS

Select Agents are materials that have been identified by the U.S. Government as agents that have potential use in biological terrorism or warfare. The Department of Health and Human Services (DHHS), through the U.S. Centers for Disease Control and Prevention (CDC), and the Animal Plant Health Inspection Service (APHIS), through the United States Department of Agriculture (USDA) regulate Select Agents in the United States and its territories. Each agency has developed and will maintain a list of Select Agents, including human, animal and plant pathogens, high-risk toxins of biological origin, and prions. The current list of Select Agents can be accessed at the CDC and USDA web sites.

A permit must be obtained from the CDC or APHIS before Select Agents may be possessed, shipped, or received. In order to obtain a permit for Select Agents, the facility must be registered and create a Select Agents program. This manual does not cover a Select Agents program.
For more details on Select Agents go to http://www.cdc.gov/od/sap/. Contact the facility Biosafety Officer before you have a need to work with any agents identified as a select agent or toxin.

Created 2018

Last update 3/9/2021

Appendix I: Recombinant and Biological Research Project Registration

Company Name:	
Project Title:	
Principle Investigator:	
Telephone:	Email:

The purpose of this form is to inform the BioLabs Institutional Biosafety Committee (IBC) of the types of work that you will be conducting and the risks they may or may not present to you and those around you. Please answer all questions as specifically as you can without divulging proprietary information.

A. Brief summary of project goals stated in non-technical terminology:

B. Technical description of experiments (information provided must be sufficient to eliminate the need to reference to other documents or scientific papers):

1. The source of DNA/RNA and nature of inserted DNA/RNA segments (e.g. toxic products, DNA percentage of viral genome etc.)

2. Identify the hosts and vectors used (e.g. bacterial strains, cell lines, plasmids, phage, viruses, etc.). Be specific:

3. Describe the highest level of risk to those who may come in contact with the rDNA or DNA constructs, hosts, and/or vectors:

C. Identify biological material to be used other than recombinant source materials (e.g. etiologic agents, human source materials, animal source materials):

D. Specialized equipment needed or used routinely (e.g. centrifuges, biosafety cabinets). Identify the shared equipment to be used:

E. Describe chemical aspects of the project which may present a risk of exposure due to the hazardous or toxic properties of the chemicals involved (e.g. cyanide compounds, acutely toxic substances, mutagens, etc.):

F. Room numbers where project will be conducted:

G. List all associated investigators and personnel conducting the work. If any are new to the project, please note their experience in handling these or related materials.

A. Please identify and discuss the health and safety risks associated with the proposed research use of these materials.

B. Describe the signs and symptoms of infection, the mode(s) of transmission, availability of vaccine or therapeutic treatment.

C. Identify the procedures that will create the greatest risk of exposure or infection (e.g. generation of aerosols, injection from sharps) during the course of the research.

D. Describe the engineering controls and personal protective equipment required to minimize exposure of laboratory personnel during procedures requiring handling or manipulation of these materials (e.g. biosafety cabinets, gloves, lab coats, safety glasses, etc.).

E. Identify decontamination procedures and disinfectant(s) to be used for work surfaces, instruments, equipment, liquid containing biological materials and glassware.

F. Identify disposal and/or decontamination procedures for contaminated sharps, contaminated solid waste, tissues, pipette tips, etc.

As Principal Scientist, and on behalf of _____
(Company Name)

I certify that the attached application is accurate and complete. I agree to abide by the following requirements:

- ❑ I will assure that personnel have received appropriate information about the biological hazards of the research outlined in this registration by making available copies of approved protocols, Biosafety Manuals, and Research Project Registrations that describe the potential biohazards and precautions to be taken to prevent exposures or release to the laboratory or the environment.
- ❑ I am familiar with and will ensure use of appropriate biosafety level laboratory practices and procedures in the conduct of this research.
- ❑ I certify that laboratory personnel have appropriate technical expertise.
- ❑ I will ensure that laboratory personnel know the procedures for dealing with incidents and spills of biological materials and know the appropriate waste

management procedures.

- ❑ I will ensure that all laboratory personnel working with biological materials are listed on this registration.
- ❑ I will assure that all laboratory personnel have completed all necessary training and that their training records are up to date.
- ❑ I certify that all laboratory spaces associated with the research described in this registration are listed.
- ❑ If this research involves recombinant DNA technologies, I am familiar with and understand my responsibilities as a Principal Investigator as outlined in Section IV-B-7 of the "NIH Guidelines for Research Involving Recombinant DNA Molecules"
- ❑ I will assure adequate supervision of personnel and will correct work errors and conditions that could result in breaches of the guidelines and regulations pertaining to this research as listed above.
- ❑ I will inform the BioLabs Biosafety Officer of any serious spills, potential exposures or breaches of the guidelines and regulations listed above.

_____

Name (Print) Sign Date

APPENDIX II: BIOLABS BLOODBORNE PATHOGENS EXPOSURE DETERMINATION

The OSHA Bloodborne Pathogens Standard requires that an exposure determination be performed in laboratories where human source materials are used. A list of laboratories, job classifications, tasks and responsibilities at BioLabs that may have exposure to human materials must be made.

Laboratories

Employees working in the following laboratories have exposure or potential exposures to human source materials not limited to:

- ❑ AQD
- ❑ Invisible Sentinel

Job Classifications

The potential for occupational exposure to blood and other potentially infectious material may occur with these job classifications:

- ❑ All employees of the laboratories identified above

Tasks and Procedures

The following tasks or procedures may cause potential exposures to personnel listed in the above job classifications:

- ❑ Receiving materials/samples
- ❑ Handling and manipulation of materials including pipetting, centrifuging, vortexing, sonication, etc.

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Last update 3/9/2021

Other Potential Exposures to Human Source Materials

- ❑ Contract cleaning employees who enter the laboratories identified above

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Last update 3/9/2021

APPENDIX III: BIOLABS HEPATITIS B ACCEPTANCE/DECLINATION

Company:_____

DECLINATION

Please sign below.

I understand that due to my occupational exposure to blood or other potentially infectious materials I may be at risk of acquiring a hepatitis B virus (HBV) infection. I have been given the opportunity to be vaccinated with the hepatitis B vaccine, at no charge to myself. However, I decline hepatitis B vaccination at this time. I understand that by declining this vaccine, I continue to be at risk of acquiring hepatitis B, a serious disease. If in the future, I continue to have occupational exposure to blood or other potentially infectious materials and I want to be vaccinated with the hepatitis B vaccine, I can receive the vaccination series at no charge to me.

_____

Name (Print) Sign Date

Created 2018

Last update 3/9/2021

CONSENT FOR TITER ONLY

I have previously received the hepatitis B vaccination or have acquired immunity and would like to have my titer evaluated and a booster administered if necessary.

Print Name **Signature** **Date**

Created 2018

Last update 3/9/2021

CONSENT FOR VACCINE AND TITER

I understand that due to my occupational exposure to blood or other potentially infectious materials I may be at risk of acquiring a hepatitis B virus (HBV) infection. I understand the risks and benefits of the hepatitis B vaccine and that I will need to receive a series of three shots followed by a scheduled titer to complete the vaccine. I would like to participate in the hepatitis B vaccination program as offered through BioLabs. The vaccination series and titer are offered at no cost to me.

Print Name **Signature** **Date**

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Last update 3/9/2021

APPENDIX IV: BLEACH DISINFECTION OF LIQUID BIOLOGICAL WASTE

1. Effectiveness

Bleach, a sodium hypochlorite solution, is a broad-spectrum disinfectant that is effective for enveloped viruses (e.g. HIV, HBV, HSV), vegetative bacteria (e.g. *Pseudomonas, Staphylococcus, and Salmonella*), fungi (e.g. *Candida*), mycobacterium (e.g. *M. tuberculosis, M. bovis*), and non-enveloped viruses (e.g. Adenovirus, Parvovirus).

Clorox bleach EPA registration number is 5813-50

2. Recommended Personal Protective Equipment for Handling Bleach and Solutions

- ❑ Lab coat
- ❑ Nitrile gloves (latex is effective as well)
- ❑ Safety Glasses
- ❑ Closed toe shoes

3. Disinfectant Concentration

The appropriate concentration of sodium hypochlorite for disinfecting liquid biological waste is 5,000 parts per million or approximately 0.5%. Household bleach is 5.2 – 6.1% sodium hypochlorite; therefore a 1:10 (V/V) dilution of bleach to liquid biological waste is appropriate.

Using Clorox (5.25% hypochlorite) in a 1:10 dilution (1 part Clorox and 9 parts liquid) yields 5,250 ppm or a 0.53% hypochlorite solution for use within 24 hours,

4. Contact Time

An appropriate contact time of sodium hypochlorite with liquid waste is 20 minutes. After a minimum of 20 minutes of contact time, disinfected liquid biological waste can be poured down the drain and flushed with water.

5. Stability and Storage

Bleach should be stored between 50 and 70o F. According to Clorox, in-diluted household bleach has a shelf life of six months to one year from date of manufacture after which bleach degrades at a rate of 20% per year.

A 1:10 bleach solution has a shelf life of 24 hours.

Created 2018

Last update 3/9/2021

APPENDIX V: COLLECTION OF ASPIRATION WASTE

1. Protect the vacuum lines by setting up a collection flask followed by an overflow flask. If the flasks are on the floor, protect against breakage with the use of secondary containment.
2. A vacushield or alternate should also be placed in the line to reduce the risk of contaminating the in-house vacuum or vacuum pump.
3. Fill the first flask with household bleach to ~10% of the flask's volume. If a different EPA- approved disinfectant is utilized, add the volume of disinfectant required to achieve the manufacturer's recommended final concentration.
4. Aspirate the tissue culture waste into the first flask containing disinfectant. Discontinue use when the vacuum flask is 75% full.
5. Leave at room temperature for minimum of 20 minutes or let sit overnight to ensure sufficient contact time with disinfectant.
6. Following minimum contact time remove stopper from flask(s) inside a biosafety cabinet and dispose of contents into a laboratory sink.