NORTH SOUTH UNIVERSITY

Center of Excellence in Higher Education

Biological Safety Manual

Office of Research
NORTH SOUTH UNIVERSITY

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FOREWORD

The safety of all members of the campus community is a primary concern of the North South University (NSU). The university demonstrates this concern through compliance and enforcement of government and NSU-promulgated policies, regulations, and procedures to which university researchers are subject. The purpose of this manual is to further promote safety in campus facilities through the proper management of potentially hazardous biological materials. This manual contains helpful information for the day-to-day management of university laboratories. For additional information or clarification of the contents of this manual, please contact the NSU Institutional Biosafety Officer (IBO) and/or the NSU Office of Research (OR-NSU).

HELPFUL TELEPHONE NUMBERS AND CONTACT INFORMATION

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I. POLICIES AND RESPONSIBILITIES

A. Campus Biosafety Policy

1. Biological agents used in teaching and research shall be used in a manner that will ensure the safety, health, and well-being of faculty, staff, students, visitors, neighboring populations, wild and domestic animals and the environment.

2. Biosafety in Microbiological and Biomedical Laboratories (BMBL) (published by the U.S. Public Health Service) current edition and Biosafety Guidelines Bangladesh (approved and published by Ministry of Environment, Forest and Climate Change, Govt. of Bangladesh) current edition, has been adapted as the NSU standard for the use of biological agents. This adaptation is approved by the NSU Institutional Biosafety Committee (NSU-IBC) and the Director, Office of Research-NSU (OR-NSU).

3. All projects and laboratory courses involving biological agents must follow guidelines in the BMBL and the Biosafety Guidelines Bangladesh current edition. Approval for the use of such agents is required by either the Institutional Biosafety Officer (IBO) or Institutional Biosafety Committee (IBC), based on the nature of the project. Contact the IBO for any queries and to submit forms for project registration and IBO review.

4. All research and teaching involving recombinant or synthetic nucleic acid molecules shall be treated as prescribed by the most recent edition of NIH's Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (copies are available from Centers for Disease Control and Prevention (CDC) website) and as directed by this NSU Biological Safety Manual. All projects involving recombinant or synthetic nucleic acid molecules require prior approval by the Institutional Biosafety Committee (IBC). A Biosafety Registration Form must be submitted to OR-NSU.

5. Research involving organisms in **Risk Groups 3 and 4 is NOT permitted at NSU**. Biosafety Level 3 (BSL-3_ or 4 (BSL-4) facilities are NOT available on campus.

6. All research and laboratory courses involving human blood, cell lines, body fluids, and/or unfixed human tissue, and other primate cells must be conducted at Biosafety Level 2 (BSL-2) according to the guidelines in Appendix G of the BMBL. BSL-2 research and laboratory courses may be conducted at NSU only with prior IBC review and approval when BSL-2 level containment is available and required procedures may be followed without exception.

7. Newly isolated or recognized infectious agents of unknown pathogenicity shall be treated as Biosafety Level 2 (BSL-2) infectious agents. The IBO must be duly informed of all research activities involving such agents whenever a research project either isolates or recognizes such agents. Continued research with such agents may occur only with IBC approval.

8. All Visiting Researchers working in a laboratory must be registered with the Office of Research, North South University (OR-NSU) and, prior to initiating laboratory work, must complete all applicable training stipulated by the IBO. Contact the IBO to register Visiting Researchers working in a laboratory.

9. The NSU Biological Safety Manual will be the basis for general safety guidelines in all laboratories on campus and facilities otherwise operated under NSU authority. Laboratory personnel are expected to follow practices outlined in this manual, the BMBL publication, as well as the prudent practices specific to the project(s) in which they are involved.

B. Responsibilities

1. The Principal Investigator (PI) of a research project or faculty member supervising a teaching laboratory is responsible for the following:

- Being adequately trained in good microbiological & proper animal handling techniques.
- Ensuring the integrity of physical containment (i.e., biosafety cabinets) and biological containment (i.e., purity and genotypic and phenotypic characteristics).
- Obtaining approval from the different committees relevant to the project (for example, the NSU Institutional Biosafety Committee (NSU IBC), the NSU Institutional Animal Care and Use Committee (NSU IACUC), the NSU Institutional Review Board/Ethical Review Committee (NSU IRB/ERC) if the project involves the use of biological agents, recombinant or synthetic nucleic acid molecules, animals, human subjects/samples and radioactive materials.
- Developing and implementing specific protocols to ensure the safe use of biological agents and recombinant or synthetic nucleic acid molecules. The protocols must outline potential biohazards, necessary precautions, and proper emergency procedures in the case of an accidental exposure of students in a teaching laboratory and/or research personnel in a research project.
- Informing the laboratory staff of the reasons and provision for any precautionary medical practices advised or requested (i.e., vaccinations or serum collection).
- Complying with the safety protocol, this manual, campus policy, and any applicable government laws and regulations.
- Registering Visiting Researchers working in the laboratory with the Office of Research-NSU through the IBO.
- Training all personnel involved in a research project so that they have a complete understanding of the hazards involved, safety procedures/practices/techniques required, and the emergency protocols in place for managing accidents. This includes animal care personnel not directly supervised by the PI, who provide care for infected animals. Documentation of training must be kept on file with the OR-NSU and be subject to routine IBO review.
- Supervising laboratory staff and laboratory officers to ensure that the required safety practices and techniques are employed.
- Correcting work errors and conditions that may result in the release of biohazardous agents and recombinant or synthetic nucleic acid molecules.
- Notifying the IBO of any changes in biological agents, procedures, personnel or protocols stated in the approved Biosafety Registration Form (BRF) by submitting an amendment to the BRF (submitted to the OR-NSU).
- Monitoring the access of laboratory visitors and assuring their safety and the

security of biological agents and toxins.

- Complying with proper handling of biological waste by following recommendations in this manual, campus policy, and any applicable government laws and regulations.
- Adhering to IBC-approved emergency plans for handling accidental spills and personnel contamination. Consult the IBO for any questions on such plans.
- Complying with proper permit and shipping of infectious and diagnostic material by following recommendations in this manual, campus policy, and any applicable government laws and regulations.
- Reporting any accidents or adverse events immediately to the IBO so that the IBO may complete and submit to the Chair of the IBC and OR-NSU an adverse event report as soon as reasonable after mitigating any such adverse event.

2. Laboratory staff, graduate and undergraduate students and research fellows who work in the laboratory are responsible for the following:

- Being familiar with all protocols and having at least a basic operating knowledge of organisms being used in the laboratory, regardless if the organism is handled directly.
- Knowing all emergency procedures established by the Principal Investigator in the case of a research project or those established by the faculty supervising a teaching laboratory.
- Completing training and verifying documentation of required laboratory safety training as arranged by the IBO or otherwise as provided by the Principal Investigator of a research project or by a supervising faculty member in a teaching laboratory.
- Following all appropriate laboratory practices as outlined in this manual, the BMBL publication, and all additional practices outlined in the laboratory safety protocol.

3. The Department Chair or Director is responsible for the following:

- Ensuring the health and safety of personnel, visitors, students and research fellows, while in departmental facilities.
- Ensuring departmental compliance with applicable laws, regulations, and guidelines covering the use of biological agents in research within laboratory facilities falling under the operational purview of the department.
- As supervised by the Dean of the School of Health and Life Sciences and as recommended by the IBO, assuring proper pick-up and disposal of hazardous/biological waste materials from laboratories.

4. Office of Research-NSU (OR-NSU) is responsible for the following:

- Providing information to the NSU community regarding policy, procedures, and regulations for the safe use of biological agents, bloodborne pathogens, and recombinant or synthetic nucleic acid molecules in research.
- Providing consultation through the IBO and IBC in the development of safety

protocols as requested by the Principal Investigators or Department Chairs.

- Providing application materials (templates) for working with biological agents upon request.
- Supervising the review of all applications (new, renewal and amendment) for the use of biological agents and submission of all Biosafety Registration Forms to the Institutional Biosafety Committee (IBC) for approval.
- Through the day-to-day operational oversight of the IBO, assuring department and user compliance with the IBC's requirements.
- Through the IBO, scheduling and performing periodic laboratory assessments or audits of facilities in use..
- Monitoring completion of projects through updates of protocols.
- Through the IBO and as supplemented by internal and/or external experts, providing training materials and short-term classes on biosafety as needed.
- Through the IBO, advising researchers who generate biohazardous waste on proper biohazardous waste handling, treatment, and disposal methods in accordance with government and University Hazardous Waste Standards (consult the NSU IBO for the latter).
- Providing assistance and training for the proper shipment of biohazardous material.
- Providing biosafety consultations and technical assistance through the IBO and IBC as warranted.
- Record-keeping and retention/archiving in accordance with applicable NSU policy, regulation, and government laws or regulations

5. The Institutional Biosafety Committee (IBC) is responsible for the following:

- Assuring the safe use of recombinant or synthetic nucleic acid molecules, biological agents, and bloodborne pathogens at the NSU campus and/or other facilities under NSU authority.
- Reviewing and recommending acceptance or rejection of all proposed projects requiring registration and authorization through the stipulated biosafety registration process.
- Formulating and recommending changes in campus policy for the safe use of biological agents and complying with government laws, regulations, and best practice standards.
- Authorizing the IBO to terminate or curtail any project or any teaching program involving the use of biological agents when it is in the best interest of the health and safety of the NSU community.

II. Requirements for working with Biological Materials

A. Introduction and general requirements:

1. Registration of Biological Agents: Projects involving material(s) included in any of these categories must submit a Biosafety Registration Form (BRF) (see Appendix A) for

Institutional Biosafety Committee (IBC) review and approval.

- Potentially biohazardous biological agents.
- Recombinant or synthetic nucleic acid molecules, human blood and blood products, human body fluids, human cell cultures, and/or human tissue.
- Biological toxins.
- Pathogenic organisms carried by experimental animals that may pose significant risk to human health.
- Whenever a contractual agreement or grant proposal requires IBC and/or the IBO's approval for the safe handling of a biological material.
- When it is unclear as to whether a material constitutes a potential biohazard, the IBO should be consulted in advance of any use.

2. Approvals, renewals and amendments: Projects evaluated by the Institutional Biosafety Committee (IBC) and or the Institutional Biosafety Officer (IBO) receive approval as follows:

- BRFs at Biosafety Level 1 & 2 are approved for three (3) years, but otherwise for less time if the research project has a termination date prior to three years.
- PIs must submit a renewal BRF (see Appendix A of this manual) at least three months prior to expiration to OR-NSU and the IBO.
- If any changes or additions are proposed to revise prior approved protocols, an BRF amendment must be submitted to OR-NSU and the IBO for review and approval prior to implementation.

3. Biosafety Level 1 (BSL-1): All staff and students working at BSL-1 must complete OR-NSU arranged Biosafety Training. Refresher Biosafety training will be required whenever biosafety protocols are revised and/or renewed.

Laboratory classes must be conducted by trained lab officers under supervision of the course faculty.

4. Biosafety Level 2 (BSL-2): Research projects approved at Biosafety Level 2 (BSL-2) must comply with the following requirements:

- Biosafety Cabinets: Research that has the potential for the production of aerosols must be conducted in a certified biosafety cabinet.
- Training: All staff and students working on a Biosafety Level 2 (BSL-2) project must complete the Biosafety Training with Bloodborne Pathogens arranged by OR-NSU or have equivalent certified training recognized and authorized by the IBC.

Laboratory classes must be conducted by trained lab officers under supervision of the course faculty.

5. Inspections: Laboratories with research involving the use of all biological agents will be inspected periodically for compliance with general laboratory practices, as well as specific biological safety practices and procedures. PIs and trained lab officers are

expected to comply with all statements of the safety plan as approved by the IBC in the submitted BRFs.

B. Requirements and procedures for the safe use of biological agents

1. Classification of the biological agents:

Biological agents are those pathogenic bacteria, viruses (including viral vectors), fungi, and parasites that can be transmitted to a person or animal, directly or indirectly, and are capable of causing disease in the new host. Biological agents classified according to risk are listed in NSU Biological Safety Manual (Appendix-B). If the agent in use or planned for use is not listed, contact the IBO.

*****NOTICE: Biological agents classified as Risk Groups 3 and 4 (BSL-3 & BSL-4) are currently *prohibited* and may not be used or planned for use in research or teaching laboratories at NSU.**

2. Registration through the Biosafety Registration Form (BRF):

A BRF includes information regarding personnel, biological agent(s), project protocol, and safety procedures. This form, found in Appendix A, must be submitted to the IBO for distribution to and review by the NSU Institutional Biosafety Committee (IBC). The research protocol must be approved by the IBC prior to introducing the organism into any laboratory operated under NSU authority.

3. Written Standard Operating Procedures (SOP), including a Safety Plan:

Written laboratory safety procedures must be prepared by the PI or laboratory officer for each laboratory in which biological agents are used for teaching or research purposes. These procedures must be included in the safety plan of the BRF. Research conducted at Biosafety Level 2 (BSL-2) containment procedures that has the potential for the production of aerosols must be conducted in a certified biosafety cabinet with all personnel complying with BSL-2 safety precautions while entering, working within, and exiting the research laboratory. The PI and lab officers must ensure all laboratory personnel comply with laboratory standard operating procedures and the safety plan. The individual laboratory safety plan must be based on actual laboratory safety practices. Suggested reference material for laboratory safety plans are: this NSU Biological Safety Manual, Biosafety Guidelines Bangladesh, CDC/NIH's Biosafety in Microbiological and Biomedical Laboratories (BMBL), IATA's Shipping Infectious Substances/Diagnostic Specimens Regulations (49 CFR Part 173).

4. Medical Surveillance related to work with biological agents:

Research personnel and students must be familiar with signs and symptoms of illnesses known to be caused by the agent(s) used in the laboratory. A person who develops symptoms or manifest illness that could be of laboratory origin must inform his/her supervisor at the time of onset of symptoms or illness and report this to the IBO, who will report such incidents to the Director, OR-NSU. All personnel and students who will work

with, or will otherwise be present in a laboratory where biological agents are in use, should be immunized against those agents if a vaccine is available.

5. Restrict access to the biological agents:

To ensure the safety and security of the NSU community, short-term students and visitors must not be exposed to biological agents in laboratory facilities unless they are trained in safe operating procedures and familiarized with the safety plan of the laboratory in use. ***At all times, non-essential visitors and children are not allowed access to laboratories where infectious agents may be present.*** Persons working in the laboratory who are not formally affiliated with the NSU must be registered with OR-NSU by completing either the Visiting Research in Laboratories application prior to performing lab activities.

6. Report any injuries, overt exposures or adverse events:

In case of any accidents, injuries, overt exposures or adverse events that occur within the research laboratory, the PI and the designated laboratory officers must complete an Incident Report (on the requisite template) and provide the details of the incident, submitting the report to the IBO, who will report to the Director, OR-NSU. At all times the PI and the designated lab officer is responsible for mitigating any such incident according to safety procedures adopted for the laboratory in use. If needed, the IBO will conduct an assessment to identify the reasons of such incident and to take requisite action designed to prevent future accidents. Related reports will be preserved by OR-NSU for improvement of the biosafety practices at NSU.

C. Requirements and procedures for the safe use of Recombinant or Synthetic Nucleic Acid Molecules

1. Procedures for the classification of the biological material and the Recombinant or Synthetic Nucleic Acid Molecules:

In the context of the US NIH Guidelines, recombinant or synthetic nucleic acids are defined as: (i) molecules that (a) are constructed by joining nucleic acid molecules and (b) that can replicate in a living cell, i.e., recombinant or synthetic nucleic acid molecules; (ii) nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids; or (iii) molecules that result from the replication of those described in (i) or (ii) above.

NSU biosafety procedures require that research and teaching programs utilizing recombinant or synthetic nucleic acid molecules are conducted in full compliance with government promulgated laws and regulations, regardless of the source of funding for the research. Classification and containment requirements for use of recombinant or synthetic nucleic acid molecules can be found in the latest edition of the US NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. Please consult the NIH guidelines and the Biosafety Guidelines Bangladesh to classify the etiological agents on the basis of hazard, as well as to determine the section under which your specific experiments are described and covered by the guidelines.

2. Registration through the Biosafety Registration (BRF) Form:

All use of recombinant or synthetic nucleic acid molecules requires the submission of a completed BRF, which is reviewed and approved by the IBC and remains on file for reference at OR-NSU. You can find the form in Appendix A of the NSU Biological Safety Manual. The BRF contains information regarding personnel, host organisms and cell lines, vectors, DNA inserts, experimental protocols, and safety procedures. The PI is encouraged to remain in communication with OR-NSU throughout the review process and the duration of the project. The IBC will review containment levels required by the guidelines and will assess facilities, procedures, practices, personnel training, and personnel expertise, as appropriate. The IBO will perform containment assessments as directed by the IBC.

3. Written Standard Operating Procedures including a Safety Plan:

The PI or designated laboratory officer must prepare written laboratory safety procedures for each laboratory in which recombinant or synthetic nucleic acid molecules are used for teaching or research purposes. Research conducted at Biosafety Level 2 (BSL-2) that has the potential for the production of aerosols must be conducted in a certified biosafety cabinet. Please maintain proper cabinet certification through regular IBO inspection and OR-NSU administrative oversight. The PI must ensure compliance by all staff and students working within the laboratory.

Prior to initiation of the research project, the PI must provide the laboratory staff copies of written safety protocols that describe potential hazards and precautions to be taken routinely and in the event of an accident. Ensure that a copy of the safety protocol is maintained in the laboratory in a visibly accessible location. Significant safety issues with recombinant or synthetic nucleic acid molecules projects must be reported immediately to the IBO, who will report to the chairperson of the IBC and Director, OR-NSU. The IBO will then be responsible for investigating the incidents, assuring all pertinent mitigation measures have been implemented, and reporting appropriate details to the OR-NSU and the IBC within 30 days.

4. Training of Laboratory Staff and Students:

Training of personnel and students is an extremely important safety factor in the laboratory. The institution is responsible for ensuring that the PI is adequately trained in conducting research safely. It is assumed the department chairperson and school dean are satisfied that a PI has such adequate training as part of the general course of the PI's approved research workload. PIs are responsible for ensuring that laboratory personnel attend the required OR-NSU sponsored laboratory safety training, in addition to providing protocol-specific training and closely supervising the laboratory personnel to ensure that procedures are being properly conducted. Personnel conducting research in biological laboratories must attend the OR-NSU Biosafety course when offered and scheduled according to IBO recommendation. All training must be documented through training log-books that maintain a record of course modules completed.

5. Illness prevention related to work with Recombinant or Synthetic Nucleic Acid Molecules

Personnel and students must be familiar with signs and symptoms of illnesses known to be caused by the agents and recombinant or synthetic nucleic acid molecules technologies used in the laboratory. If a person who develops symptoms or a manifest illness that could be of laboratory origin, he/she must inform his/her supervisor and, if at the campus, report to the

NSU Medical Center for medical assessment. If symptoms or illness occur while off-campus, the personnel should inform the supervising PI and seek medical assessment privately as appropriate and update the PI as to any medical diagnosis or treatment. The PI must inform the staff and students of reasons and provisions for precautionary medical practices (e.g. vaccinations, serum collection) advised or requested. Primary consideration must be given to the protection of the health of personnel, students, and the public, the protection of animal populations, and the protection of the environment. PIs are to adopt emergency plans covering accidental spills and personnel contamination. Maintain copies of these plans in a visible and readily accessible location in the laboratory in the event of an accident.

6. Restriction of access to the Recombinant or Synthetic Nucleic Acid Molecules:

To ensure the safety and security of the NSU community, short-term students and visitors to the laboratory must not be exposed to potentially infectious biological material resulting from work with recombinant or synthetic nucleic acid molecules unless they are trained in safe procedures and familiarized with the safety plan of the laboratory. ***At all times non-essential visitors and children must not be allowed access to a laboratory where infectious biological agents and recombinant or synthetic nucleic acid molecules may be present.*** Persons working in the laboratory who are not formally affiliated with NSU must be registered with OR-NSU by completing either the Visiting Researcher in Laboratories application prior to performing lab activities.

D. Requirements and procedures for the safe use of bloodborne pathogens: human cells, tissue, and body fluids

1. Determine if you are working with a bloodborne pathogen—***follow BSL-2 practices***:

According to the current edition of the BMBL, "The potential laboratory hazards associated with human cells and tissue include the bloodborne pathogens HBV and HIV, as well as agents such as *Mycobacterium tuberculosis* that may be present in human lung tissue. Other primate cells and tissue also present risks to laboratory workers. Potential hazards to laboratory workers are presented by cells transformed with viral agents, such as SV-40, EBV, or HBV, as well as cells carrying viral genomic material. Tumorigenic human cells also are potential hazards as a result of self-inoculation."

The current edition of the BMBL recommended practices are **mandatory** at NSU for all research undertaken with bloodborne pathogens:

- Human and other primate cells (commercial lines as well as patient isolates) must be handled using Biosafety Level 2 (BSL-2) practices and containment.
- All procedures that can result in the production of aerosols must be performed in a certified biosafety cabinet.
- All material must be decontaminated by autoclaving or chemical disinfection before discarding.
- All personnel working with human cells and tissue must be registered with OR-NSU and must work under the policies and guidelines established by the University's

Exposure Control Plan (see Appendix C).

- All personnel and students working with bloodborne pathogens must complete the OR-NSU sponsored Bloodborne Pathogens Training annually, to be conducted as and when recommended by the IBO.

2. Registration through the Biosafety Registration (BRF) Form:

All research involving human cell lines, body fluids, and unfixed human tissue, must be conducted at Biosafety Level 2 (BSL-2). The use of biological material that may cause potential bloodborne pathogens requires the submission of a completed BRF which is reviewed and approved by the IBC and remains on file at OR-NSU for reference. You can find the form in Appendix A. The BRF contains information regarding personnel, cell lines, tissue, body fluids, experimental protocols, and safety procedures. The IBC and OR-NSU will review containment levels required by the operative biosafety guidelines and will assess facilities, procedures, practices, personnel training, and personnel expertise, as appropriate.

3. Exposure Control Plan - Written Standard Operating Procedures and Safety Plan:

NSU adopts best practices as available and implemented in research universities. Hence, as approved by the NSU IBC, universal precautions and guidelines set by the US Public Health Service & US Centers for Disease Control and Prevention as well as national guidelines such as Biosafety Guidelines Bangladesh must be followed at NSU. NSU has formulated an Exposure Control Plan (ECP) that must be followed by all personnel potentially exposed to bloodborne pathogens in the work place. It is a requirement that PIs read, instruct staff and students, and follow the ECP. You can find the NSU Exposure Control Plan on the NSU-OR website and in Appendix C of this manual.

The PI must prepare written laboratory safety procedures for each laboratory in which bloodborne pathogens are used for teaching or research purposes. The PI must ensure compliance by all staff and students. Prior to initiation of the research project, provide the laboratory staff and students with copies of written safety protocols that describe potential hazards and precautions to be taken routinely and in the event of an accident. Ensure that a copy of the safety protocol is maintained in a visible and readily accessible location in the laboratory. Significant safety problems with bloodborne pathogens must be reported immediately to the IBO and the PI is responsible for undertaking all requisite mitigating actions according to approved safety protocol. The IBO will report any such incident to OR-NSU and the IBC as appropriate.

4. Training of Laboratory Staff and Students:

All personnel and students working with bloodborne pathogens are required to complete the OR-NSU sponsored online bloodborne pathogens refresher training module annually. The training of personnel and students is an extremely important safety factor in the laboratory. The PI has primary responsibility for training laboratory staff in project-specific safety procedures, and such training may be carried out by the PI (and is to be documented in writing with review by the IBO). The responsible faculty member or PI will provide protocol-specific training and then closely supervise the laboratory staff and students to ensure that procedures are being properly conducted. All training must be documented

through training log-books of course module completion.

5. Illness prevention related to work with bloodborne pathogens:

Personnel and students must be familiar with signs and symptoms of illnesses known to be caused by the agents present in human products used in the laboratory. The PI must follow the exposure control plan (ECP) as approved by the IBC and ensure that all personnel who have been identified as having occupational exposure to blood or other potentially infectious materials have taken the hepatitis B vaccine as soon as possible. When personnel incur an exposure incident, the PI or supervising faculty member must report the incident to the IBO, who will report to Director, OR-NSU and the IBC.

6. Restriction of access to the bloodborne pathogens:

For the safety and security of the NSU community, short-term students and visitors to the laboratory must not be exposed to bloodborne pathogens unless they are trained in safe procedures and familiarized with the safety plan of the laboratory. ***At all times non-essential visitors and children must not be allowed access to a laboratory where bloodborne pathogens may be present.*** Biosafety Level 2 (BSL-2) practices require that the PI set restriction standards for the laboratory. Persons working in the laboratory who are not formally affiliated with NSU must be registered with OR-NSU by completing the Visiting Researcher application prior to performing lab activities.

E. Projects Using Non-Hazardous Animal Samples

An Animal Sample Registration Form must be completed for all projects involving animal samples that are not registered by the IACUC or the IBC (cell lines, body fluids and tissues) that neither are infected nor involve the use of recombinant or synthetic nucleic acid molecules. To register your project with the OR-NSU and the IBO, please submit the Animal Sample Registration form (provided in Appendix D of this manual).

F. Projects Using Human Products

A Human Products Registration Form must be completed for all projects that utilize only human products (no recombinant or synthetic nucleic acid molecules or other biological material are in use). To register your project with the OR-NSU and the IBO, please submit the Human Products Experiments form (provided in Appendix E of this manual).

G. Projects Using Experimental Animals

1. Registration of projects using experimental animals and biohazards:

All projects involving the use of animals in conjunction with microbial agents, biological toxins, bloodborne pathogens, and/or recombinant or synthetic nucleic acid molecules must be registered with the Institutional Biosafety Committee (IBC) as well as the Institutional Animal Care and Use Committee (IACUC). To register with the IBC please submit the BRF form as provided in Appendix A of this manual. All projects involving animal experimentation at NSU must be conducted according to the requirements for Animal Biosafety (see Appendix-H).

2. Written Standard Operating Procedures and Safety Plan:

Laboratory animals have been shown to carry agents infectious for humans and, therefore, laboratory safety plans must be developed for all projects that use animals. It is recommended researchers using animal models seek the assistance of the NSU IACUC concerning animal care and use operations. The following types of work must be addressed in the safety plan:

- Transplantation or injection of human tissue into animals.
- The use of non-human primates.
- The use of non-human primate tissue.
- The use of retroviruses and other infectious organisms from any species.
- The use of any dangerous chemical such as carcinogens in the animal facility.

3. Training of Laboratory Staff and Students:

Training of personnel and students is an extremely important safety factor in the laboratory. The PI has primary responsibility for training laboratory staff, and such training may be carried out through the PI. The PI or responsible faculty member will provide protocol-specific training and then closely supervise the laboratory staff and students to ensure that procedures are being properly conducted.

Personnel conducting animal research must:

- Take the appropriate institutional animal research training courses (species-specific) as provided and documented by the PI or supervising faculty member
- Receive additional training as needed to conduct animal manipulations (as determined by the PI or supervising faculty member in consultation with the IBO)
- If working with human products, complete the Bloodborne Pathogens course module as sponsored by OR-NSU
- Complete the Biosafety course module as sponsored by OR-NSU
- Be aware of the occupational hazards associated with the animals in use and the research protocol.
- Take proper precautions to minimize hazards in the laboratory and the animal facility and in all handling of animals in use.
- Follow all safety precautions and occupational health programs required by animal care operations.

4. Illness prevention related to work with animals and biohazard

The IBC requires that all personnel working at BSL-2 containment be familiar with signs and symptoms of illnesses known to be caused by the agents and animals. A person that develops an illness that could be of laboratory origin must inform his/her supervisor and should seek medical care as appropriate. PIs must inform the personnel of reasons and provisions for precautionary medical practices (e.g., vaccinations, serum collection) advised or requested. Primary consideration must be given to the protection of the health of personnel, students, the public, animal populations, and the environment.

5. Restriction of access to the laboratory and animal facility:

For the safety and security of the NSU community, short-term students and visitors to the laboratory must not be exposed to experimental animals and biohazards unless they are trained in safe procedures and familiarized with the safety plan of the laboratory and animal facility. ***At all times non-essential visitors and children must not be allowed access to the animal facility or a laboratory where animals are present***. Biosafety Level 2 (BSL-2) practices require that the PI set restriction standards for the laboratory. Persons working in the laboratory who are not affiliated with NSU must be registered with OR-NSU by completing either the Visiting Researcher in Laboratories application prior to performing lab activities.

H. Projects Using Select Agents and Toxins

A Principal Investigator (PI) may not possess, use, receive (from outside Bangladesh), or transfer (within Bangladesh) biological agents or toxins until they have been approved to use such biological agents or toxins by the IBC.

- A list of Select Agents can be accessed by visiting http://www.selectagents.gov/. Please contact OR-NSU Biosafety for Select Agent and Toxins when considering potential research in this category.
- It is the responsibility of the Principal Investigator to identify his or her research involving one or more of the agents or toxins listed on the Select Agent Registry and notify the NSU IBC through the NSU IBO.
- National guidelines such as Biosafety Guidelines Bangladesh should be consulted for regulations related to such agents. If any regulations cannot be found in national guidelines, international guidelines such as that of NIH can be followed upon consultation with the IBO and the IBC (if needed).

I. Projects Involving Agents and/or Toxins of Dual Use Research of Concern

Dual Use Research of Concern (DURC) is life sciences research which, based on current understanding, can reasonably be anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, material, or national security.

Currently, there are 15 non-attenuated agents and toxins listed here, and all are ***prohibited*** from use at research and/or teaching facilities operating under NSU authority:

- **Agents/Toxins-**

- Avian influenza virus (highly pathogenic)
- *Bacillus anthracis*
- Botulinum neurotoxin
- *Burkholderia mallei*

- *Burkholderia pseudomallei*
- Ebola virus
- Foot-and-mouth disease virus
- *Francisella tularensis*
- Marburg virus
- Reconstructed 1918 Influenza virus
- Rinderpest virus
- Toxin-producing strains of *Clostridium botulinum*
- Variola major virus
- Variola minor virus
- *Yersinia pestis*

Other agents not listed above are subject to review and explicit approval by the IBC prior to any planned use of such agents and will not be approved in the absence of the appropriate level of biosafety containment.

➢ **Categories of prohibited experiments-**

- Enhances the harmful consequences of the agent or toxin
- Disrupts immunity or the effectiveness of an immunization against the agent or toxin without clinical or agricultural justification
- Confers to the agent or toxin resistance to clinically or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies
- Increases the stability, transmissibility, or the ability to disseminate the agent or toxin
- Alters the host range or tropism of the agent or toxin
- Enhances the susceptibility of a host population to the agent or toxin
- Generates or reconstitutes an eradicated or extinct agent or toxin listed above

J. Transportation and Shipping of Infectious Substances and Exempt Specimens

- Any movement or transport of biological hazards within laboratories or between buildings must be performed in a manner such as to prevent any spills and/or leakage.
- Use primary containers with closed lids, closed plastic secondary containers, and carts to prevent spills and accidents when transporting biohazards in the laboratory.
- Materials must be transported in containers that can be sealed. If the outside of the primary container is contaminated, a secondary container must be used. If the transported material could puncture the primary container, a secondary, puncture-resistant container must be used.
- Any contaminated equipment must be contained or decontaminated prior to movement maintenance, and/or repair.
- Laboratory personnel must be trained and certified on transportation regulations prior to transporting any infectious substance or diagnostic specimen. Such training and certification are the responsibility of the Principal Investigator supervising the research project.
- Infectious Substances are substances that are known to contain, or reasonably expected to contain, pathogens. For shipping purposes, Infectious Substances are divided into two categories:
    - Category A Infectious Substances—infectious substances in a form that, when

exposure occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals. They are assigned the following UN numbers and proper shipping names:

UN2814 – Infectious Substance, affecting humans

UN2900 – Infectious Substance, affecting animals

- Category B Infectious Substances—infectious substances that do not meet criteria for inclusion in Category A. They are assigned the proper shipping name:

UN3373 – Biological Substance, Category B

- Infectious Substances that are to be shipped must have triple layer packaging:
  - Primary container—must be leak proof (test tube)
  - Secondary container—must contain absorbent material capable of absorbing fully and contain a lid for complete seal of the volume of liquid contents
  - Rigid outer packaging— must have proper labeling

K. Principal Investigator Check-Out Procedure

In the event a PI moves from a laboratory or leaves employment the NSU, a check out must be completed by the IBO through arrangement with OR-NSU. Contact OR-NSU to schedule a laboratory check out at least two weeks prior to the planned move. Please refer to Principal Investigator Checkout Procedure in Appendix I.

III. BIOLOGICAL SAFETY GUIDELINES AND PROCEDURES

A. General Information

1. Risk Assessment:

An infectious agent is considered to be a biological hazard if exposure may result in risk to the well being of humans, animals, or plants. Infectious agents include, but are not limited to, conventional pathogens, recombinant or synthetic nucleic acid molecules, research involving pathogenic vectors, agents carried in human tissue, and inherent and experimental infections of laboratory animals.

Molecular biology and microbiology laboratories are often unique work environments that may pose identifiable infectious disease risks to persons in or near them. To prevent infection, PIs must make an initial risk assessment based on the Risk Group (RG), followed by a thorough consideration of the agent itself and how it is to be manipulated.

Factors to be considered in determining the level of containment, include agent factors, such as:

- virulence
- pathogenicity
- infectious dose
- environmental stability

- potential routes of exposure
- communicability
- laboratory procedures
- quantity
- availability of vaccine or treatment
- gene product toxicity
- physiological activity
- allergenicity

Any strain that is known to be more hazardous than the parent (wild-type) strain should be considered for handling at a higher containment level. (Note: Research requiring BSL-3 or above containment measures is not permitted at NSU research facilities.) Certain attenuated strains or strains that have been demonstrated to have irreversibly lost known virulence factors may qualify for a reduction of the containment level compared to the Risk Group assigned to the parent strain. The containment level required may be equivalent to the Risk Group classification of the agent or it may be raised or lowered as a result of the above considerations.

The IBC must approve the risk assessment and the biosafety containment level designated by the PI in the BRF. No such proposed research is to be performed without advance explicit written approval of the IBC.

2. Infectious Agent Risk Group Classification:

Four Risk Groups of biological agents have been established by the US Centers for Disease Control and Prevention (CDC)/National Institute of Health (NIH): Risk Group (RG) 1, 2, 3, and 4, with RG1 being the least hazardous.

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

The US NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (Appendix B) contains a comprehensive list of agents classified by Risk Group. For additional information, consult the Biosafety Guidelines Bangladesh and the

BMBL and the American Biological Safety Association (ABSA) website. For a list of organisms and their Risk Group, please visit http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines .

3. Summary of Biological Safety Levels of Practices and Containment:

There are four Biosafety Levels (BSL) that consist of combinations of laboratory safety practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed, for the documented or suspected routes of transmission of the infectious agents, and for the laboratory function or activity. The recommended Biosafety Level for an organism represents the conditions under which the agent can be ordinarily handled safely. Please consult this link for recommended biosafety levels: http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf

4. Summary of the NIH Guidelines for the Use of Recombinant or Synthetic Nucleic Acid Molecules technology

This summary serves only as a guide to the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines. It is the responsibility of each Principal Investigator (PI) to make sure that his/her laboratory is in compliance. At NSU all recombinant or synthetic research requires registration. Check the Guidelines for the appropriate biosafety level and relevant section. All PIs engaging in research with human pathogenic material or potentially pathogenic material (human tissue and blood products) must also register with the Institutional Biosafety Committee by filing the Human Products Registration Form (Appendix-E).

Please consult this link for guidelines for research involving recombinant or synthetic nucleic acid molecules research: http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

5. Bloodborne Pathogens (BBP)

Hazardous body fluids include blood and other potentially infectious materials (OPIM) such as human and nonhuman primate organs, tissue, cell cultures, etc., which are known or assumed to be associated with the transmission of bloodborne pathogens. Please consult the Exposure Control Plan (Appendix C) for specific information regarding prevention and exposure control procedures.

Direct contact with someone's blood or OPIM carrying a bloodborne pathogen is not necessary for an exposure. Staff or students who perform tasks such as handling clinical specimens, biohazardous trash, laundry soaked with blood or body fluid, or needles or other sharps should be aware and be careful of exposure to bloodborne pathogens.

a. Potential Hazards:

In the laboratory, the potential hazards associated with human blood, cells, and tissue include the following bloodborne pathogens:

- Hepatitis B virus (HBV): This virus causes Hepatitis B and has been found in all body secretions and excretions, with blood and semen being the most infectious. HBV infections are a major cause of liver damage, cirrhosis, and liver cancer. Routine vaccinations have reduced the number of HBV infections significantly. HBV can cause acute or chronic infections depending on the body's response to the virus. Those who develop chronic HBV infections do not develop antibodies and carry the virus with the potential to infect others for decades. The HBV vaccine is the best protection against this disease.
- Hepatitis C virus (HCV): This virus causes Hepatitis C and is found in blood. HCV does not always cause serious health problems. Many carriers may present liver damage with no symptoms. In others, cirrhosis of the liver may develop, resulting in eventual liver failure. In the laboratory, the primary risk of HCV infection is via direct contact with infectious blood through an accidental needle-stick or injury with other sharps. Currently there is no vaccine available for HCV. However, there is a cure approved by the US Food and Drug Administration (FDA) that may or may not be available in Bangladesh. Therefore, preventive measures are very important.
- Human immunodeficiency virus (HIV): This virus causes acquired immunodeficiency syndrome (AIDS) and is found in blood and OPIM. Often HIV infected persons are asymptomatic. AIDS damages cells that are essential for immune functions, causing susceptibility to opportunistic infections that might become fatal. No vaccine is currently available for HIV, and there is no cure for AIDS. Therefore, preventive measures are very important.

Primate cells and tissue also present other risks to laboratory workers. Some examples are:

- *Mycobacterium tuberculosis* that may be present in human lung tissue.
- Cells transformed with viral agents, such as SV-40, EBV, or HBV.
- Cells carrying viral genomic material.
- Tumorigenic human cells as a result of self-inoculation.

b. Risks of Infection:

The risk of infection following an exposure to blood or other potentially infectious material (OPIM) depends on many factors, including these:

- whether the pathogens are present in the source blood or OPIM
- the number of pathogens present
- the type of injury or exposure (how the infectious material gets into the body)
- the current health and immunization status of the exposed person

This means that even if the source blood or OPIM do contain pathogens, you are not necessarily infected. To be safe, however, always assume an exposure is potentially infectious and follow all recommended measures to prevent exposures from occurring, such as working in a certified biosafety cabinet.

c. Prevention of BBP Infections:

Occupational exposure to blood borne pathogens (BBP) can be reduced by following the

Exposure Control Plan and using the following four strategies:

- Engineering controls: devices that isolate or remove the BBP hazard from the workplace. These devices include needleless systems, eye wash stations, handwashing facilities, biohazard labels, and biosafety cabinets.
- Work practice controls: controls to reduce the likelihood of exposure by altering the manner in which a task is performed. Depending upon the environment, the controls might include the use of personal protective equipment (PPE), hand washing, decontaminating and sterilizing equipment and areas, safely handling sharps, correctly disposing of wastes, safely handling laundry, and good personal hygiene habits.
- Personal protective equipment: consists of barriers such as gloves, scrubs, aprons, gowns, eye shields or goggles, face masks or shield, caps and booties that can be worn to prevent exposure to blood and OPIM.
- Universal precautions: safety guidelines in which all blood and OPIM are handled as if they are contaminated. Under universal precautions, you treat all materials as if they are infected with bloodborne pathogens. Following universal precautions means using PPE and following all the safe work practice controls described in this manual.

B. Laboratory practices and containment

1. Laboratory Safety Procedures

Principal Investigators must maintain written laboratory safety procedures for each research and teaching laboratory where personnel and students may be exposed to biological hazards such as infectious microorganisms, recombinant or synthetic nucleic acid molecules, human tissue and body fluids, and experimental animals. In addition, all personnel and students working in a research or teaching laboratory with potential exposure to biological hazards must be appropriately trained in biosafety and laboratory techniques. Records of the training must be available for review by the NSU IBO.

The following list of safety procedures can serve as a guide to develop and implement a safety plan. Please use the BMBL, NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules governing exposures to bloodborne pathogens as reference for your specific plan. Consult the NSU IBO as appropriate.

a. Set requirements for access to the laboratory

- Only persons who have been advised of potential hazards and who meet specific entry requirements (e.g., training, occupational medical clearance, immunization) must be allowed to enter the laboratory working area.
- Minors under the age of 18 are not permitted in laboratory work areas unless registered and approved by the Director, OR-NSU, as recommended by the NSU IBO.
- Laboratory doors must be kept fully closed when research work is in progress.
- Access to laboratories where animals are present must be restricted to

authorized personnel only.

- Animals not involved in the research work being performed shall not be in the laboratory.

b. Set standards for appropriate behavior in the laboratory

- Eating, drinking, smoking, storing food, applying cosmetics, and handling contact lenses are not permitted in the laboratory or the animal work area.
- Personnel must not use work surfaces as seats.
- Personnel with wounds that are weeping or purulent (pus-exuding) must not work in the laboratory or animal care areas whenever infectious agents might be present.
- Personnel are required to keep their hair at an appropriate length, covered, or tied in a manner such that it does not become contaminated.
- Personnel must wash their hands thoroughly after all procedures involving animals and potentially contaminated materials.

c. Set standards for minimizing contamination with biohazardous materials when procedures are in progress.

- Determine the level of personal protective equipment (PPE) for your specific procedures. All laboratory personnel/students working with potentially infectious materials are required to wear personal protective equipment (e.g., lab coat, long pants/skirt, close-toed shoes, protective eyewear, respiratory protection, and gloves)
- All technical procedures must be performed in a manner that minimizes the creation of aerosols.
- Biosafety cabinets must be certified annually by the NSU IBO and certified biosafety cabinets must be used when working with bloodborne pathogens and when performing procedures with Risk Group 2 agents that might create aerosols.
- Specimens containing infectious materials to be centrifuged must be covered. A safety centrifuge cabinet or a safety centrifuge cup must be considered for infectious materials.
- Mouth pipetting is not permitted for any materials or reagents. Mechanical pipetting devices are to be utilized.

d. Enforce procedures to minimize the risk of sharps injuries.

- Standard procedures for needle stick and other injuries, animal bites/scratches, and occupational illness must be incorporated into individual procedures, as needed.
- Hypodermic needles and syringes must be used only for parenteral injection and aspiration of fluids from patients, laboratory animals, and bottles sealed with a diaphragm.
- Hypodermic needles and syringes must not be used as a substitute for automatic pipetting devices in the manipulation of potentially infectious fluids.
- Needles used in collection of potentially infectious material must not be recapped after use.
- All syringes, needles, and other sharps must be placed into red plastic puncture

resistant containers labeled as containing "sharps" and "infectious material."

e. Set procedures for routine decontamination, accidental spill cleanup, disposal of contaminated materials, and emergencies.

- All liquid or solid materials containing potentially infectious material must be decontaminated before disposal.
- Work surfaces, which may have contact with potentially infectious material, must be decontaminated with a disinfectant at the beginning and end of the day and after any spill of potentially dangerous material. Soak up the disinfectant and contaminated material with an absorbent material (such as paper towels) and dispose of these materials in a double plastic bag or sealed container. Gloves must be worn for cleanup.
- All spills and other accidents, with overt or potential exposure to infectious materials, must be reported immediately to the laboratory supervisor and laboratory officers and the NSU IBO, who will report to OR-NSU.
- A written record of such incidents (Incident Report) must be maintained in the laboratory or department. The report should be prepared by the PI in consultation with the laboratory officer(s) and be subject to review by the NSU IBO as a measure of biosafety compliance.

2. Warning Signs and Postings

The universally accepted biological hazard-warning symbol must be used in all locations engaged in research with such hazardous/infectious agents to notify workers about the presence of such agents. The warning symbol must be removed when the hazardous agent is no longer in use or present in the research/teaching facility.

- The location of the posting is determined by the access to the area where biological hazards are used.
- Doors to any laboratory containing a designated infectious agent must be have the hazard-warning posted.
- Postings must be displayed in other areas such as biosafety cabinets, freezers, or other specially designated work and storage areas or equipment where biological hazards are used.
- All individual containers of biological hazards must be labeled to identify the content and any special precautionary measures that must be taken.
- Universal biohazard labels must be affixed to containers of regulated waste and to refrigerators and freezers containing blood or other infectious materials.
- Labels must be affixed to other containers used to store, transport, or ship blood or other potentially infectious materials.
- Acceptable color-coded (red or orange) bags or containers may be substituted for the labeling requirement.

3. Safety Equipment

Safety equipment includes biosafety cabinets, enclosed containers, and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The biosafety cabinet (BSC) is the principal engineering control used to provide containment of infectious splashes or aerosols generated by many microbiological procedures.

Safety equipment also may include items for personal protection, such as personal protective clothing, respirators, face shields, safety glasses or goggles. Personal Protective Equipment (PPE) is often used in combination with other safety equipment when working with biohazardous agents. In some situations, personal protective clothing may form the primary barrier between personnel and the biohazardous agents.

a. Biosafety Cabinets

Biosafety cabinets are used to provide primary containment in the laboratory when using potentially infectious materials and can be used for manipulation of sterile cultures. BSCs must be used in Biosafety Level 2 (BSL-2) laboratories if aerosol-generating procedures are conducted, a high concentration of infectious agents is used, or if large volumes of infectious agents are used.

Biosafety cabinets must be decontaminated before the following can take place:

- Any maintenance work requiring disassembly of the air plenum, including filter replacement
- Cabinet recertification
- Movement of the cabinet to a new laboratory
- Discarding or salvaging

There are three types of BSCs as defined by CDC/NIH's Biosafety in Microbiological and Biomedical Laboratories. Types of biosafety cabinets:

- The Class I BSC provides personnel and environmental protection but no product protection. It is similar in function to a chemical fume hood but has a HEPA filter in the exhaust system to protect the environment. The Class I BSC is not commonly used on the NSU campus.
- Class II BSC (Types A, B1, B2 and B3) are designed for work involving microorganisms assigned to Biosafety Levels 1, 2 and 3. These cabinets provide the microbe-free work environment necessary for cell culture propagation and may be used for nonvolatile chemotherapeutic drug preparation.
- The Class III BSC is designed for work with Biosafety Level 4 microbiological agents and provides maximum protection to the operator and the environment.

Horizontal and vertical laminar-flow clean-air benches are not BSCs and may not be used as substitutes for properly certified BSCs. They discharge HEPA-filtered air across the work surface and toward the user. These devices provide only product protection.

b. Personal Protective Equipment (PPE)

Personal protective equipment (PPE) shall be worn in instances where engineering controls are not feasible and must not be used as a substitute for engineering controls. Individuals will be encouraged to use appropriate personal protective equipment as indicated by the PI and/or the IBO. Adequate PPE is provided at no cost by the PI to the employee and must be readily accessible at the worksite. This includes, but is not limited to, the following: gloves, gowns, laboratory coats, face shields or masks, head covers and eye protection. Accommodations will be made for individuals determined to be unable to use certain protective devices.

- Gloves must be worn when it can be reasonably anticipated that the employee may have hand contact with blood, other potentially infectious materials, mucous membranes, and non-intact skin, and when handling or touching contaminated items or surfaces. In some instances, double gloves are required. Disposable single use gloves shall be replaced as soon as possible when visibly soiled, torn, punctured, or when their ability to function as a barrier is compromised. Hands must be washed thoroughly each time gloves are removed. Disposable gloves shall never be washed or disinfected for reuse. Utility gloves may be disinfected for reuse if the integrity of the glove is not compromised. However, they must be discarded if they are cracked, peeling, discolored, torn, punctured, or exhibiting any sign of deterioration.
- Safety goggles must be worn when it can be reasonably anticipated that the employee may perform tasks that could generate splashes or spatters and containment equipment is not required. Safety glasses must be worn when the anticipation of splashes and spatters have been eliminated by the use of containment equipment or tasks performed will not generate splashes or spatters.
- Masks and eye protection shall be worn whenever splashes, spray, droplets, or aerosols of blood or other potentially infectious materials may be generated and there is a potential for eye, nose, or mouth contamination.
- Laboratory coats, gowns, aprons, clinic jackets, or similar outer garment must be worn in situations where there is a potential for exposure to infectious agents.
- All PPE shall be removed immediately upon leaving the work area or as soon as possible if overtly contaminated and placed in an appropriately designated area for decontamination or disposal.
- The PI and lab officers are responsible for arranging and enforcing laundering and disposal procedures for PPE. When PPE is removed, it must be placed in an appropriately designated area or container for storage, washing, decontamination, or disposal.

4. Facility Design

The design of a facility is important in providing a barrier to protect people working inside and outside the laboratory and to protect people or animals in the community from infectious agents that may be released accidentally in the laboratory. Facilities must be commensurate with the laboratory's function and the recommended Biosafety Level for the agent being manipulated.

The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. For example, the exposure risks for most laboratory work in BSL-1 and BSL-2 facilities will be direct contact with the agents, or inadvertent contact exposures through contaminated work environments. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave), and hand washing facilities.

As the risk for aerosol transmission increases, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features could include specialized ventilation systems to assure directional airflow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks at laboratory entrances, or separate buildings or modules for isolation of the laboratory.

C. Waste management

The following concepts and procedures can help to handle the biological waste generated by laboratories in a safe and efficient way. Methods of how to process biological waste on-site are particularly useful to minimize waste pickups and to handle small quantities of biological waste generated during research.

1. Maintenance

- Areas where designated infectious agents are used should be cleaned on a regular basis by trained laboratory personnel with an appropriate disinfectant.
- Personal protective equipment such as gloves must be worn throughout the entire procedure.
- All equipment and working surfaces should be cleaned and decontaminated upon completion of procedures, spills, or after contact with blood or other potentially infectious materials.
- Decontamination must be performed using an appropriate disinfectant for the agent in use.
- If an area becomes contaminated with blood or biohazardous fluids, the fluid shall be absorbed with disposable absorbent material and placed in a biohazard container or bag.
- Protective coverings, such as absorbent paper, are to be removed and replaced when overtly contaminated or at completion of procedures.
- All receptacles intended for reuse, such as bins, pails, or cans that may be contaminated should be inspected and decontaminated on a regular basis.
- Broken glassware should be cleaned up using mechanical means, such as brush, broom, dust pans, tongs, forceps, etc. and placed in a sharp's container for later pickup.
- Equipment that may become contaminated with blood or other potentially infectious materials shall be checked routinely and prior to servicing or shipping and shall be

decontaminated as necessary.

2. Sterilization and Disinfection

a. Sterilization

- Sterilization is a method or process to remove all viable microorganisms from an object or material.
- The process must consistently produce objects that are negative to chemical and biological indicators of contamination.
- Achieving sterility of the finished product depends on the number and type of organism present, the temperature, and the length of contact time.
- Steam sterilization (autoclaving) will kill most microorganisms when steam under pressure is applied at 121°C for a minimum of 45 minutes.
- Sterilization will not be complete if steam does not reach all surfaces of the object, for example on items that have a high soil load and densely packed materials.
- Spore strips (*B. stearothermophilus*) can be placed at the center of the autoclave pack as a biological indicator of sterility.
- Autoclave tape is not an indicator of sterility; it simply indicates that the proper temperature has been achieved on the surface.
- Please follow the manufacturer's recommendation and make sure all items are autoclavable.

b. Disinfection

- Disinfection must be utilized where sterilization is not practical, for instance, on tables, cabinets, and some equipment.
- Disinfection is the use of antimicrobial chemicals on inanimate objects with the purpose of destroying all non-spore forming organisms of pathogenic nature or which would compromise the integrity of the experiment.
- Disinfection does not mean the destruction of all organisms.
- Disinfectants destroy microorganisms by coagulating or denaturing proteins, injuring the cell membrane, and stopping normal enzymatic reactions.
- The range of susceptibility of microorganisms to disinfectants is relatively broad.
- The vegetative bacteria, fungi, and lipid containing viruses are highly susceptible to disinfecting agents.
- Non-lipid containing viruses are moderately resistant to these disinfecting agents.
- Spore forms are the most resistant to disinfectants.
- Use only disinfectants approved for use with a particular organism.
- There are many chemical disinfectants on the market, with the main constituent being one of the following: chlorine, quaternary ammonium compounds, alcohol, formaldehyde, iodine, phenolics, or glutaraldehyde.

3. Biological waste disposal

a. Description of Biological Waste

Biological or infectious waste is waste that has pathogens or biologically active material present in sufficient concentration or quantity so that exposure of a susceptible host could result in disease. NSU biosafety guideline defines it as a solid waste which, if improperly treated or handled, may serve to transmit an infectious disease and is comprised of the following:

- Microbiological waste such as discarded cultures and stocks of infectious agents and associated biological materials, discarded cultures of specimens from medical, pathological, pharmaceutical, research, clinical, commercial and industrial laboratories, discarded live and attenuated vaccines, discarded used disposable culture dishes, discarded used disposable devices used to transfer, inoculate, or mix cultures.
- Sharps, defined as contaminated scalpel blades, razor blades, suture needles, disposable razors, disposable scissors, intravenous stylets and rigid intruders, glass Pasteur pipettes, specimen tubes; blood culture bottles, microscope slides, broken glass from laboratories.
- Bulk blood, bulk human blood products, and bulk human body fluids.
- Pathological waste such as body parts, tissue, recognizable human tissue, organs, bulk blood and body fluids.
- Animal Waste.

These types of waste should always be handled in accordance with practices that minimize exposure to waste handlers and to ensure that the waste will ultimately receive the proper treatment. This can be accomplished by adhering to the following general guidelines:

- Minimizing the potential number of persons exposed to the waste.
- Maintaining the integrity of the waste containers during handling and treatment.
- Using personal protective equipment as needed.
- Conducting waste management practices that will avoid spills and accidents.
- Biological waste pickups are scheduled through use of the online form (consult the NSU IBO as needed). Solid biological waste must be placed in leak proof receptacles containing a lid and labeled with the biohazard symbol. The receptacle must be lined with orange or red bags bearing the biohazard symbol.
- Liquid waste must be collected in an appropriate container that is leak proof, disinfected with 10% bleach solution/ dilution and allowed to stand for at least 15 minutes prior to disposal.
- All sharps must be placed in an approved puncture resistant “sharps” container. This container must have securely capped ends or a closable top or lid.
- Animal carcasses containing known biohazardous agents stored in the ACO freezer. Contact the IBO or NSU IACUC as needed for further information.
- Contact the NSU IBO for ways to reduce waste and for information on local

disposal of small quantities of biological waste.

b. On-Site Waste Treatment and Disposal

Infectious waste is treated so as to render it noninfectious. Treatment techniques approved by the NSU IBC are:

- Chemical disinfection
- Steam sterilization
- Incineration
- Thermal inactivation
- Chlorine disinfection maceration
- Encapsulation (only for sharps in containers)
- Moist heat disinfection

The two most common methods utilized at NSU are steam sterilization and chemical disinfection. Each method requires strict adherence to government rules and regulations in order to be an effective means of treating the waste.

- Steam Sterilization (Autoclave): Steam sterilization utilizes pressurized steam at 250 to 270 °F (121 to 132 °C) to kill pathogenic organisms that are present in the infectious waste. Steam sterilization process does not destroy the waste. Instead, it renders it noninfectious. **Properly sterilized waste can be disposed of in the regular trash after placing the autoclaved bag containing the waste in a regular black household garbage bag (Appendix-F).**

  Standard operating procedures must include the following criteria:

  - The proper bags must be utilized.
  - The temperature of the autoclave must be at least 121°C (250°F).
  - The pressure must be at least 15 psi.
  - Waste must be treated for a minimum of 45 minutes.
  - A sterilization indicator strip that changes color when operating parameters are achieved should be run with every cycle.
  - Routine biological monitoring using the appropriate *Bacillus* species should be conducted.
  - Biological indicators can be in the form of either an ampule or strip containing the spore *Bacillus stearothermophilus*.
  - All autoclaves should be tested at least once a month. Compliance is reviewed regularly by the NSU IBO.
  - For those autoclaves in which a continuous readout of operating procedures is available, routine parameter monitoring can be substituted for biological monitoring.
  - Once the waste has been treated, it should be double bagged in 2 miL thick

black liners and placed in designated garbage containers.

- Treated waste can then be disposed of into a municipal solid waste landfill.

- Chemical Disinfection: Aqueous or solid biohazard waste that does not contain hazardous materials can be disposed of through the sanitary sewer provided it is treated prior to doing so. In order for this waste to be disposed on in the proper manner, the following criteria must be met:
  - The waste must be treated with a chemical agent as a disinfectant and in accordance with the manufacturer's instructions.
  - Disinfectants used must have been shown to be effective against the microorganisms present.
  - The waste must be immersed for a minimum of ten minutes in a freshly prepared solution of 10% bleach solution, 70% isopropanol solution, or other acceptable disinfection methods.

- Records: Records are an essential part of a waste management program. All departments that treat waste are required by government regulations and OR-NSU to keep records that include the following:
  - Date of treatment
  - Method/Conditions of treatment
  - Quantity of waste treated (pounds)
  - Verification of operating parameters or biological monitoring
  - Written procedures for the operation and testing of equipment used
  - Printed name and initials of person treating the waste

  All such records are subject to regular review by the NSU IBO and reported regularly to the Director, OR-NSU, and the IBC.

c. Off-Site Waste Treatment and Disposal

For those departments that do not have the proper equipment to effectively treat waste, an offsite treatment option is available. Please fill out the on-line form for biological waste pick up. The Department of concern shall assure that:

- Waste is collected and a commercial firm is in charge of incineration or land disposal.
- Infectious waste should be handled as little as possible.
- When the waste must incur additional handling therefore special care should be taken when packaging it for pickup.
- Waste must be placed in biohazard bags and containers.
- If the storage of infectious material is necessary, it should be stored in a rigid, leak-proof container and bear the universal biohazard symbol.

- Infectious waste may be stored at room temperature until the storage container is full, but no longer than 30 days from the date of generation.
- Frozen waste may be kept up to 90 days from the date of generation.
- If infectious waste becomes putrescent during storage, it should be pre-treated within 24 hours.
- Storage of waste should be in a manner that affords protection from theft, vandalism, human or animal exposure, rain, water, and wind.
- Infectious waste should be stored separate from chemical and radioactive waste.
- Transporting biohazard waste through the hallways or between buildings should be conducted with the use of secondary containment to prevent spills or exposure to other personnel.

4. Biological Spill Clean-Up Procedures

The following procedures are provided as a guideline to biological spill cleanup.

a. For hazardous biological spills inside the biosafety cabinet:

- Wear laboratory coat, eye protection, and gloves during clean-up.
- Allow the cabinet to run during clean-up.
- Apply disinfectant and allow a minimum of 15-20 minutes contact time. A list of recommended disinfectants can be viewed at http://www.epa.gov/oppad001/chemregindex.htm .
- Wipe up spillage with disposable disinfectant-soaked cloth or tissue.
- Wipe the walls, work surfaces, and any equipment in the cabinet with a disinfectant-soaked cloth.
- Discard contaminated disposable materials in appropriate biohazard waste container(s) and autoclave before discarding as waste.
- Place contaminated reusable items in biohazard bags or in autoclave pans with lids before autoclaving and cleanup.
- Expose non-autoclavable materials to disinfectant and allow 20 minutes contact time before removing from the biosafety cabinet.
- Remove protective clothing used during cleanup and place in a biohazard bag for autoclaving; if disposable, treat as biohazardous waste.
- Run the cabinet 15 minutes after cleanup before resuming work.

b. For hazardous biological spills in the laboratory, outside the biosafety cabinet:

- Clear the area of all personnel. Wait approximately 30 minutes for the aerosols to settle before entering the spill area.
- Remove any contaminated clothing and place in biohazard bag to be autoclaved; if disposable, treat as biohazardous waste

- Wear a disposable gown, shoe covers, eye protection, N95 respirator (if needed) and gloves.
- Initiate cleanup with disinfectant as follows:
  - Soak paper towels in disinfectant and place over spill.
  - Encircle the spill with additional disinfectant, being careful to minimize aerosols during pouring while assuring adequate contact. Start from the periphery and work toward the center.
  - Decontaminate all items within the spill the area.
  - Allow 20 minutes contact time to ensure germicidal action of disinfectant before passing items to clean area.
  - Wipe equipment with 1:10 bleach, followed by water, then 70% ethanol or isopropanol.
  - Place disposable contaminated spill materials in appropriate biohazardous waste container(s) for autoclaving.
  - Place contaminated reusable items in biohazard bags in autoclave pans with lids or wrap in newspaper before autoclaving and cleanup.

c. <u>For hazardous biological spills inside the centrifuge</u>:

- Clear the immediate area of all personnel. Wait 30 minutes for aerosol to settle before attempting to clean up spill. Keep centrifuge closed.
- Wear a laboratory coat, eye protection, N95 respirator, and gloves during cleanup.
- Remove rotors and buckets to nearest biosafety cabinet for clean-up.
- Thoroughly disinfect inside of centrifuge.
- After thorough disinfection of rotor or rotor cups, remove contaminated debris and place in appropriate biohazardous waste container(s) and autoclave before disposing as infectious waste.

d. <u>For hazardous biological spills outside laboratory, during transport</u>:

- Transport biohazardous materials in an unbreakable sealed primary container, placed inside a second unbreakable lidded container. Label the outer container with the biohazard symbol.
- Should a spill occur in a public area, do not attempt to clean it up without appropriate personal protective equipment. Call the PI and IBO for assistance.
- As an interim measure, wear gloves and place paper towels, preferably soaked in disinfectant, directly on spilled materials to prevent spread of contamination. To assure adequate contact, surround the spill with disinfectant, if available, taking care to minimize aerosols.

If you are not sure about the proper procedures or need assistance, call the IBO or OR-NSU for guidance and assistance.

D. Emergency Procedures

All biohazard laboratories must establish written emergency procedures based on the biohazardous agents used as well as other hazards that may be present. Emergency procedures must take into consideration the use of radioactive materials and chemicals. These procedures may be outlined in your approved BRF.

The following items should be noted for the type of biohazardous agent used in the laboratory in the event of an accident, exposure, and/or spill:

- Attend to any injured personnel.
- For spills in BSL-2 laboratories, evacuate the room and close the doors.
- After evacuating the area, wait to assist emergency responders.
- Notify the IBO about a spill or exposure to a biohazardous agent outside of containment.
- Report exposures and injuries to IBO within 24 hours when that time frame is reasonable, but otherwise assure that appropriate mitigation measures are undertaken.
- Report the accident to the IBO for a review of laboratory protocols and procedures. The IBO will recommend any and all changes to protocols reasonably deemed to enhance prevention of accidents, exposures, and/or spills.

IV. Training and Resources

A. Training

The PI and lab officers have primary responsibility for instruction concerning individual laboratory procedures and the development of a laboratory safety plan.

Training modules regarding basic and refresher training for laboratory safety are to be provided by OR-NSU as recommended by the IBO or IBC. Several such courses/modules are to be offered during the calendar year. Consult the IBO for required information on such training modules.

Courses:

- **Biological Safety with Bloodborne Pathogens Training**: This course is *mandatory* for all personnel and students working in biosafety designated laboratories. The content of the course provides an understanding of the principles of biological safety up to BSL-2 and the training required for working with bloodborne pathogens. This course is recommended for all PIs and their staff that have a biosafety registration for working with biohazards and/or recombinant or synthetic nucleic acid molecules. Refresher training may be required at the end of the 3-year BRF approval cycle.
- **Bloodborne Pathogens**: This is a one-hour course *mandatory* for all personnel who have exposures to blood or any other bodily fluids over the course of their job duties. The content of the course fulfills all the training requirements regarding work with bloodborne pathogens.

- **Bloodborne Pathogens Refresher Training**: This course must be completed every year by all personnel who handle bloodborne pathogens or may have occupational exposures to bodily fluids. The training can be completed online. The content of the course fulfills all the training requirements regarding work with bloodborne pathogens.

B. Online Biosafety Resources

- Biosafety Guidelines of Bangladesh (2008)
- Bangladesh Biosafety Rules (2012)
- Bangladesh Biosafety and Biosecurity Society (BBBS)
- NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Molecules
- Biosafety in Microbiological and Biomedical Laboratories, 5th ed. (BMBL - CDC)
- American Biological Safety Association (ABSA) Biosafety Links
- Importation Permits for Etiologic Agents (CDC)
- National Select Agent Registry (CDC/APHIS)
- Packaging, Labeling Shipping of Biological Substances (IATA)
- Risk Group Classification for Infectious Agents (ABSA)
- Selection, Installation and Use of Biosafety Cabinets (CDC)

APPENDIX A.

Biosafety Registration Form (BRF)

Download from the following link
(http://www.northsouth.edu/research-office/nsu-research-profile/nsu-research-policies.html)

Office of Research
NORTH SOUTH UNIVERSITY

BIOSAFETY REGISTRATION FORM

Biosafety Registration Code: **2022/OR-NSU/IBC/___**

Type of Application: ☐ **New** ☐ **Resubmission** ☐ **Renewal**

School SRC/ CTRG Review Code: ____

1. Title of Research Project:

2. Principal Investigator:

Name:	
Faculty Rank:	
Department:	
Email:	
PABX or Mobile number:	

3. Co-investigators:

Name	Faculty Rank	Email

4. Project Duration:
(Note: Biosafety registration is effective for a maximum of three calendar years from the date of commencement of research activity.)

Project Start Date:	
Project Completion Date:	

5. Does this project require approvals or permits from any of the following government authorities? *(Note: If answered "Yes" on any of the following, a copy of all relevant approvals must accompany this application for IBC review.)*

Department of Health	☐ Yes	☐ No
Department of Natural Resources	☐ Yes	☐ No
Fisheries Permit	☐ Yes	☐ No
Environment	☐ Yes	☐ No
Other (provide detail below)	☐ Yes	☐ No

6. Will the proposed research be involved with a Material Transfer Agreement (MTA)?

☐ Yes ☐ No

7. Is the proposed research funded by either internal or external grant in whole or in part?

☐ Yes ☐ No

[If "yes" identify the funding organization, grant ID (whether pending or approved).]

8. Does this project involve inter-institutional (including international) collaboration?

☐ Yes ☐ No

(If "yes," provide names, titles, roles of co-investigators along with identified institutional affiliations.)

Name	Faculty Rank / Title	Affiliated Institution/Location	Role/Function

9. Project Research Type: *(check one--☒-- or more as applicable)*

	In vitro experiments		
	Whole animals	IACUC No:	Approval date:
	Human subjects	IRB No:	Approval date:
	Human gene transfer		

10. Overview of the Research Activity

a. Specific aims of the research project

b. Abstract/Summary of the research project

c. Methodology *(Briefly describe the methodologies employed in the proposed research. If applicable, include all recombinant or synthetic nucleic acid constructs and their combinations, the targets for expression/transformation/transduction/gene editing (e.g.: CRISPR/Cas9) and if the resultant genetic manipulation is transient, stable, heritable and/or infectious.) If you are using flow cytometry please include a brief procedural description, instrument (type and location), and list the name of the user.*

11. Names of microorganisms (viruses, bacteria, etc.), recombinant DNA/Synthetic nucleic acids, human cell lines, and/or hazardous drugs used

12. Recombinant DNA or Synthetic Nucleic Acids

a. RECOMBINANT INSERT (TRANSGENE)			
(1) Specify the source of the DNA/RNA sequences (gene of interest, including genus, species, gene name(s) and specify oncc			
(2) If the recombinant DNA or synthetic nucleic acids contain viral DNA, does the insert represent more than 2/3 of the viral genome?	☐ N/A	☐ No	☐ Yes
(3) Will a deliberate attempt be made to obtain expression of the foreign structural or regulatory gene encoded in the recombinant DNA or synthetic nucleic acids?		☐ No	☐ Yes
(4) What is the biological activity of the gene product, sequence inserted or sequence knockdown?			
b. VECTOR			
(1) List the name(s) of the plasmid(s) for propagation of the recombinant DNA or synthetic nucleic acids (include genus, species and parent strain). Provide the genetic map of the plasmid(s).			
(2) Is the host strain prokaryotic (for example, use *E. Coli* amplify the plasmid(s))? If yes, complete the following.		☐ No	☐ Yes

(a) Is it a plasmid, phage, or other? List the strains of *E. Coli*.

(b) Is a packaging cell lines or transfected plasmids with helper functions required? ☐ No ☐ Yes

Check the box if applicable

☐ Use of two or three plasmids lentivirus expression system (BSL2)

☐ Use of four plasmids lentivirus expression system (BSL2)

(b) If yes, list the name of packaging cell lines and specify the expression system:

(3) Is the host strain eukaryotic (for example, use adenoviral vector or mammalian vector)? ☐ No ☐ Yes

If yes, complete the following.

(a) Is the strain a virus, clone viral genome, pro-virus, or other? If other, specify:

(b) Can it infect human cells? ☐ No ☐ Yes

(c) Is a helper virus required? ☐ No ☐ Yes List the name of the helper virus/helper plasmids

(d) If a viral vector, what % of the viral genome remains? ☐ N/A or %

c. TARGET RECIPIENT

(1) Specify the target recipient of the vector-recombinant DNA or vector-synthetic nucleic acids combination (indicate animal species or Cell lines):

d. SYNTHETIC NUCLEIC ACIDS

(1) Research with genetically modified virus or a vector derived solely by synthetic nucleic acid techniques

☐ No ☐ Yes

(2) Synthetic nucleic acids that can replicate or generate nucleic acids in any living cell

☐ No ☐ Yes

(3) Synthetic nucleic acids are designed to integrate into DNA

☐ No ☐ Yes

(4) Synthetic nucleic acids produce a toxin that is lethal for vertebrates at an LD50 of <100 ng/kg body wt

☐ No ☐ Yes

(5) Clinical Research involves the transfer of synthetic nucleic acids (> 100 nucleotides) into human subject

☐ No ☐ Yes

13. Safety and Protection

a. SOP

SOP # is followed. Submit a new SOP if there is any deviation from the standard SOP.

b. BIOHAZARDS/ HAZARDOUS DRUGS	BUILDING	ROOM	Biosafety Level	QTY/VOL	Biosafety Cabinet/Fume Hoo		USES ("x" all that apply)		
					Type	Date Certified	Stored	Prep'd	Used

(1)										
(2)										
(3)										
(4)										
(5)										

c. SHIPPING

Will hazardous materials be shipped, transferred or transported? ☐ No ☐ Yes

Dangerous goods shipment training, IBO notification and IBC approval are required prior to any shipment, transfer and transportation.

d. HAZARDOUS DRUG(S), example chemotherapeutic drugs

Will hazardous drugs be used in this protocol? ☐ No ☐ Yes

Hazardous drugs safety training is required for PI, Co-I, and lab workers.

e. PI has trained all workers and animal care providers (if applicable) in SOP# Date: ☐ No

f. OCCUPATIONAL HEALTH REQUIREMENT

(1)	Are there any special groups of workers at risk of infection or disease from the use of the biohazard(s)/ ☐ No ☐ Yes hazardous drug(s) (e.g. pregnant, immuno-compromised, allergic, etc.)? If yes, describe below.
(2)	Are any special immunizations necessary for personnel involved in the research (e.g. Hepatitis B, Tetanus/Tdap, etc.)? If yes, describe below. ☐ No ☐ Yes

14. Whole Animal Use

a. SUMMARY CHART

	SPECIES (include the names of transgenics and knockouts)	HAZARD (biological agent or hazardous drug)	BUILDING	ROOM (holding and procedure)	LOCATION TYPE (animal facility, lab, other)	ROUTE ADMINISTERED (ip, iv, etc.)
(1)						
(2)						
(3)						
(4)						

b. NARRATIVE AND CHECKLIST

(1)	Will the use of the hazard in animals be intermittent or one time only? If once, indicate how long the hazardous condition will last. If more than once, indicate the frequency of use and how long the hazardous condition will last when used. ☐ N/A ☐ Once ☐ >Once please explain

(2)	Will special signage indicating the hazards be needed for rooms/cages? Door signage is approved by IBO for use of BSL2 biological agents & animals.	☐No ☐ Yes

15. Any other relevant information

16. Declarations

- I certify the information provided in the *Biosafety Registration* form is complete and accurate and understand my responsibilities as noted in it. No changes will be made without advance approval from the Institutional Biosafety Officer.

- I acknowledge my responsibility for the safe conduct of this research in accordance with NSU Biosafety Guidelines. I will inform all associated personnel of the nature and risks of this work, as well as necessary precautions and safe practices. I also agree to comply with the requirements for the shipment and transfer of recombinant DNA materials.

- I further acknowledge my responsibility to ensure compliance with the following:

(1) Work surfaces will be appropriately decontaminated at least daily and immediately after working with biohazardous materials.

(2) All personnel involved will wash thoroughly with soap and water after working with biohazardous materials. Clothing will be changed as needed.

(3) All contaminated materials will be discarded appropriately according to guidelines (e.g. as Biohazard waste, as Hazardous drug waste, as Chemotherapeutic waste).

(4) The Institutional Biosafety Officer will be immediately notified of all spill or incidents occurring in lab spaces operating under Biosafety Level 2l containment.

(5) In the event of an incident where there is a risk of infection or other consequences to incident, affected personnel will be counseled to seek appropriate medical attention.

Name (PI/ Co-PI/ Co-I)	Signature	Date

Add additional cells above as needed for additional the research personnel and members of the research team as identified.

Submittal

When the foregoing proposal document is completed and signatures provided, the entirety of the proposal, including required supporting materials, is to be submitted to the Chairperson of the NSU Biosafety Committee through the Office of Research-NSU (Admin Building Rm 625). Provide one hard copy to the Office of Research-NSU and send the electronic copy to mostafizur.rahman09@northsouth.edu, CC to bio.safety@northsouth.edu

APPENDIX B.

List of Potential Pathogenic Agents and Toxins

Introduction and Scope

This appendix lists the following biological agents and toxins:

- Risk group classification of organisms from Annex-4 of Biosafety Guidelines Bangladesh.
- Human etiologic agents (pathogens) from Appendix B of the *NIH Guidelines*
- Select agents and toxins from the National Select Agent Registry (NSAR)
- Plant pathogens previously identified by U.S. Department of Agriculture (USDA)

These lists are provided for convenience in this manual, but may not reflect the actual regulatory list or applicable agents or materials. Regulatory sources, standards, and Web links noted in this appendix should be consulted to confirm applicable agents or toxins. For any query related to any agents, the NSU IBO should be consulted.

NIH Guidelines Human Etiologic Agents

This section provides a list of human pathogens and their Risk Group (RG) 2, RG3, and RG4 designations as excerpted from Appendix B, Classification of Human Etiologic Agents on the Basis of Hazard, of the *NIH Guidelines*.

Risk Group 1 Agents
RG1 agents are not associated with disease in healthy adult humans. Examples of RG1 agents include asporogenic *Bacillus subtilis* or *Bacillus licheniformis*; adeno-associated virus (AAV, all serotypes); and recombinant or synthetic AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and which are produced in the absence of a helper virus. A strain of *Escherichia coli* is an RG1 agent if it (a) does not possess a complete lipopolysaccharide (i.e., lacks the O antigen) and (b) does not carry any active virulence factors (e.g., toxins) or colonization factors and does not carry any genes encoding these factors.

Those agents not listed in RGs 2, 3, and 4 are not automatically or implicitly classified in RG1; a risk assessment must be conducted based on the known and potential properties of the unlisted agents and their relationship to the listed agents.

Risk Group 2 Agents
RG2 agents are associated with human disease that is rarely serious and for which preventive or therapeutic interventions are *often* available.

Risk Group 2 Bacterial Agents, Including Chlamydia

- *Acinetobacter baumannii* (formerly *Acinetobacter calcoaceticus*)

- *Actinobacillus*
- *Actinomyces pyogenes* (formerly *Corynebacterium pyogenes*)
- *Aeromonas hydrophila*
- *Amycolata autotrophica*
- *Archanobacterium haemolyticum* (formerly *Corynebacterium haemolyticum*)
- *Arizona hinshawii*: all serotypes
- *Bacillus anthracis*
- *Bartonella henselae, B. quintana, B. vinsonii*
- *Bordetella* including *B. pertussis*
- *Borrelia recurrentis, B. burgdorferi*
- *Burkholderia* (formerly *Pseudomonas* species)
- *Campylobacter coli, C. fetus, C. jejuni*
- *Chlamydia psittaci*, *C. trachomatis*, *C. pneumoniae*
- *Clostridium botulinum*, *C. chauvoei, C. haemolyticum, C. histolyticum, C. novyi, C. septicum, C. tetani*
- *Coxiella burnetii,* specifically the Phase II, Nine Mile strain, plaque purified, clone 4
- *Corynebacterium diphtheriae, C. pseudotuberculosis, C. renale*
- *Dermatophilus congolensis*
- *Edwardsiella tarda*
- *Erysipelothrix rhusiopathiae*
- *Escherichia coli*: all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including *E. coli* O157:H7
- **Francisella tularensis,* specifically**F. tularensis* subspecies *novicida* (aka *F. novicida*), strain Utah 112; **F.tularensis* subspecies *holarctica* LVS; **F tularensis* biovar *tularensis* strain ATCC 6223 (aka strain B38). **For research involving high concentrations, BL3 practices should be considered.*
- *Haemophilus ducreyi, H. influenzae*
- *Helicobacter pylori*
- *Klebsiella*: all species except *K. oxytoca* (RG1)
- *Legionella*, including *L. pneumophila*
- *Leptospira interrogans*: all serotypes
- *Listeria*
- *Moraxella*
- *Mycobacterium*, including *M. avium* complex, *M. asiaticum, M.bovis* BCG vaccine strain, *M. chelonei, M. fortuitum, M. kansasii, M. leprae, M. malmoense, M. marinum, M.paratuberculosis, M. scrofulaceum, M. simiae, M. szulgai, M. ulcerans, M. xenopi*
- *Mycoplasma*, except *M. mycoides* and *M. agalactiae*, which are restricted animal pathogens
- *Neisseria gonorrhoeae*, *N. meningitidis*
- *Nocardia asteroides, N. brasiliensis, N. otitidiscaviarum, N. Transvalensis*
- *Pseudomonas aeruginosa*
- *Rhodococcus equi*
- *Salmonella,* including *S. arizonae, S. cholerasuis, S. enteritidis, S. gallinarum-pullorum, S. meleagridis, S.paratyphi,* A, B, C, *S. typhi*, *S. typhimurium*
- *Shigella,* including *S. boydii, S. dysenteriae,* type 1, *S. flexneri, S. sonnei*
- *Sphaerophorus necrophorus*
- *Staphylococcus aureus*

- *Streptobacillus moniliformis*
- *Streptococcus,* including *S. pneumoniae, S. pyogenes*
- *Treponema pallidum, T. carateum*
- *Vibrio cholerae, V. parahemolyticus, V. vulnificus*
- *Yersinia enterocolitica,*
- *Yersinia pestis,* specifically $pgm^{(-)}$ strains (lacking the 102 kb pigmentation locus) and $lcr^{(-)}$ strains (lacking the LCR plasmid)

Risk Group 2 Fungal Agents

- *Blastomyces dermatitidis*
- *Cladosporium bantianum, C. (Xylohypha) trichoides*
- *Cryptococcus neoformans*
- *Dactylaria galopava (Ochroconis gallopavum)*
- *Epidermophyton*
- *Exophiala (Wangiella) dermatitidis*
- *Fonsecaea pedrosoi*
- *Microsporum*
- *Paracoccidioides braziliensis*
- *Penicillium marneffei*
- *Sporothrix schenckii*
- *Trichophyton*

Risk Group 2 Parasitic Agents

- *Ancylostoma* human hookworms, including *A. duodenale, A. ceylanicum*
- *Ascaris,* including *Ascaris lumbricoides suum*
- *Babesia,* including *B. divergens, B. microti*
- *Brugia* filaria worms, including *B. malayi, B. timori*
- *Coccidia*
- *Cryptosporidium,* including *C. parvum*
- *Cysticercus cellulosae* (hydatid cyst, larva of *T. solium*)
- *Echinococcus,* including *E. granulosis, E. multilocularis, E. vogeli*
- *Entamoeba histolytica*
- *Enterobius*
- *Fasciola,* including *F. gigantica, F. hepatica*
- *Giardia,* including *G. lamblia*
- *Heterophyes*
- *Hymenolepis,* including *H. diminuta, H. nana*
- *Isospora*
- *Leishmania,* including *L. braziliensis, L. donovani, L. ethiopia, L. major, L. mexicana, L. peruvania, L. tropica*
- *Loa loa* filaria worms
- *Microsporidium*
- *Naegleria fowleri*
- *Necator* human hookworms, including *N. americanus*
- *Onchocerca* filaria worms, including *O. volvulus*
- *Plasmodium,* including simian species, *P. cynomologi, P. falciparum, P. malariae, P. ovale,*

P. vivax
- *Sarcocystis,* including *S. sui hominis*
- *Schistosoma,* including *S. haematobium, S. intercalatum, S. japonicum, S. mansoni, S. mekongi*
- *Strongyloides,* including *S. stercoralis*
- *Taenia solium*
- *Toxocara,* including *T. canis*
- *Toxoplasma,* including *T. gondii*
- *Trichinella spiralis*
- *Trypanosoma,* including *T. brucei brucei, T. brucei gambiense, T. brucei rhodesiense, T. cruzi*
- *Wuchereria bancrofti* filaria worms

Risk Group 2 Viruses
- Adenoviruses, human: All types
- Alphaviruses (Togaviruses), group A Arboviruses:
    - Chikungunya vaccine strain 181/25
    - Eastern equine encephalomyelitis virus
    - Venezuelan equine encephalomyelitis vaccine strain TC-83
    - Western equine encephalomyelitis virus
- Arenaviruses:
    - Junin virus candid #1 vaccine strain
    - Lymphocytic choriomeningitis virus (non-neurotropic strains)
    - Tacaribe virus complex
    - Other viruses as listed in BMBL
- *Bunyaviruses:*
    - Bunyamwera virus
    - Rift Valley fever virus vaccine strain MP-12
    - Other viruses as listed in BMBL
- Calciviruses
- Coronaviruses
- Flaviviruses, Group B Arboviruses:
    - Dengue virus, serotypes 1, 2, 3, and 4
    - Japanese encephalitis virus strain SA 14-14-2
    - Yellow fever virus vaccine strain 17D
    - Other viruses as listed in BMBL
- Hepatitis A, B, C, D, and E viruses
- Herpesviruses, except *Herpesvirus simiae* (monkey B virus):
    - Cytomegalovirus
    - Epstein Barr virus
    - *Herpes simplex,* types 1 and 2
    - *Herpes zoster*
    - Human herpesvirus, types 6 and 7
- Orthomyxoviruses:
    - Influenza viruses, types A, B, and C
    - Tick-borne orthomyxoviruses
- Papilloma viruses: All human papilloma viruses
- Paramyxoviruses:
    - Newcastle disease virus

    - Measles virus
    - Mumps virus
    - Parainfluenza viruses, types 1, 2, 3, and 4
    - Respiratory syncytial virus
- Parvoviruses: Human parvovirus (B19)
- Picornaviruses:
    - Coxsackie viruses, types A and B
    - Echoviruses, all types
    - Polioviruses, all types, wild and attenuated
    - Rhinoviruses, all types
- Poxviruses: All types except monkeypox virus and restricted poxviruses including Alastrim, Smallpox, and Whitepox
- Reoviruses: All types including Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)
- Rhabdoviruses:
    - Rabies virus, all strains
    - Vesicular stomatitis virus non exotic strains: VSV-Indiana 1 serotype strains (e.g. Glasgow, Mudd-Summers, Orsay, San Juan) and VSV-New Jersey serotype strains (e.g. Ogden, Hazelhurst)
- Rubivirus (Togaviruses), Rubella virus

Risk Group 3 Agents

RG3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available.

Risk Group 3 Bacterial Agents Including Rickettsia

- *Bartonella*
- *Brucella* including *B. abortus, B. canis*, *B. suis*
- *Burkholderia (Pseudomonas) mallei, B. pseudomallei*
- *Coxiella burnetii (*except the Phase II, Nine Mile strain*)*
- *Francisella tularensis (*except those strains listed in *NIH Guidelines* Appendix B-II-A, *Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia)*
- *Mycobacterium bovis* (except BCG strain, see *NIH Guidelines* Appendix B-II-A, *Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia*), *M. tuberculosis*
- *Pasteurella multocida* type B: "Buffalo" and other virulent strains
- *Rickettsia akari, R. australis, R. canada, R. conorii, R. prowazekii, R. rickettsii, R, siberica, R. tsutsugamushi, R. typhi (R. mooseri)*
- *Yersinia pestis (*except those strains listed in *NIH Guidelines* Appendix B-II-A, *Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia)*

Risk Group 3 Fungal Agents

- *Coccidioides immitis* (sporulating cultures, contaminated soil)
- *Histoplasma capsulatum, H. capsulatum var. duboisii*

Risk Group 3 Parasitic Agents

None

Risk Group 3 Viruses and Prions

- Alphaviruses (Togaviruses), Group A Arboviruses:
  - Chikungunya virus (except the vaccine strain 181/25 listed in *NIH Guidelines* Appendix B-II-D Risk Group 2 (RG2) – Viruses)
  - Semliki Forest virus
  - St. Louis encephalitis virus
  - Venezuelan equine encephalomyelitis virus (except the vaccine strain TC-83, see Appendix B-II-D (RG2))
  - Other viruses as listed in BMBL
- Arenaviruses:
  - Flexal
  - Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)
- Bunyaviruses:
  - Hantaviruses, including Hantaan virus
  - Rift Valley fever virus
- Coronaviruses, SARS-associated coronavirus (SARS-CoV)
- Flaviviruses (togaviruses), group B arboviruses:
  - Japanese encephalitis virus (except those strains listed in *NIH Guidelines* Appendix B-II-D Risk Group 2 (RG2) - Viruses)
  - West Nile virus (WNV)
  - Yellow fever virus
  - Other viruses as listed in BMBL
- Middle East Respiratory Syndrome coronavirus (MERS-CoV)
- Orthomyxoviruses: Influenza viruses 1918–1919 H1N1 (1918 H1N1), human H2N2 (1957–1968), and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1)
- Poxviruses: Monkeypox virus
- Prions: Transmissible spongioform encephalopathy (TME) agents (Creutzfeldt-Jacob disease and kuru agents) (see BMBL, for containment instruction)
- Retroviruses:
  - Human immunodeficiency virus (HIV) types 1 and 2
  - Human T cell lymphotropic virus (HTLV) types 1 and 2
  - Simian immunodeficiency virus (SIV)
- Rhabdoviruses: Vesicular stomatitis virus (except those strains listed in *NIH Guidelines* Appendix B-II-D Risk Group 2 (RG2) - Viruses)

Risk Group 4 Agents

RG4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available.

Risk Group 4 Bacterial Agents

None

Risk Group 4 Fungal Agents

None

Risk Group 4 Parasitic Agents

None

Risk Group 4 Viral Agents

- Arenaviruses:
  - Guanarito virus
  - Lassa virus
  - Junin virus (except the candid #1 vaccine strain listed in Appendix B-II-D Risk Group2 (RG2) – Viruses)
  - Machupo virus
  - Sabia
  - Bunyaviruses (Nairovirus): Crimean-Congo hemorrhagic fever virus
- Filoviruses:
  - Ebola virus
  - Marburg virus
- Flaviruses (Togaviruses), Group B Arboviruses: Tick-borne encephalitis virus complex including Absetterov, Central European encephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses
- Herpesviruses (alpha): Herpesvirus simiae (Herpes B or Monkey B virus)
- Paramyxoviruses: Equine morbillivirus (Hendra virus), Nipah virus (Henipavirus)
- Hemorrhagic fever agents and viruses as yet undefined

Animal Viral Etiologic Agents in Common Use

The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents are associated with disease in healthy adult humans; however, they are commonly used in laboratory experimental work. A containment level appropriate for RG1 human agents is recommended for their use. For agents that are infectious to human cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.

- Baculoviruses
- Herpesviruses:
  - Herpesvirus ateles
  - Herpesvirus saimiri
  - Marek's disease virus
  - Murine cytomegalovirus
- Papilloma viruses:
  - Bovine papilloma virus
  - Shope papilloma virus
- Polyoma viruses:
  - Polyoma virus
  - Simian virus 40 (SV40)
- Retroviruses:
  - Avian leukosis virus
  - Avian sarcoma virus
  - Bovine leukemia virus
  - Feline leukemia virus
  - Feline sarcoma virus

- Gibbon leukemia virus
- Mason-Pfizer monkey virus
- Mouse mammary tumor virus
- Murine leukemia virus
- Murine sarcoma virus
- Rat leukemia virus

Murine Retroviral Vectors

Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication-competent retrovirus can be maintained, handled, and administered under Biosafety Level (BL) 1 containment.

APPENDIX C.

Exposure Control Plan- Bloodborne Pathogens

BLOODBORNE PATHOGENS EXPOSURE CONTROL PLAN

(as approved by the NSU IBC)

In accordance with US OSHA Bloodborne Pathogens Standard and as approved by the NSU IBC, OR-NSU has implemented the following Exposure Control Plan (adapted from the ECP of the University of Houston):

EXPOSURE DETERMINATION

The NSU Bloodborne Pathogens Exposure Control Plan requires employers to perform an exposure determination for personnel who have occupational exposure to blood or other potentially infectious materials. The exposure determination is made without regard to the use of personal protective equipment. Job classifications that include personnel who have potential occupational exposure risks are: laboratory personnel; custodial personnel; medical personnel; security and/or law enforcement personnel; operations and maaintenance personnel; solid waste management or waste disposal personnel; and fire and safety personnel.

IMPLEMENTATION AND METHODOLOGY

Compliance Methods

In all research and teaching laboratories operating under NSU authority, universal/standard precautions are observed to prevent contact with blood or other potentially infectious materials. All blood or other potentially infectious materials are considered infectious regardless of the perceived status of the source individual.

Engineering and work practice controls are used to eliminate or minimize exposure to personnel. Where occupational exposure remains after institution of these controls, personal protective equipment is used. Examples include safety design devices, sharps containers, needleless systems, sharps with engineered sharps injury protection for personnel, passing instruments in a neutral zone, etc.

Supervisors and workers examine and maintain engineering and work practice controls within the work environment on a regular schedule.

Handwashing facilities are available to the personnel who may incur exposure to blood or other potentially infectious materials.

If handwashing facilities are not available, the employer is required to provide either an antiseptic cleanser in conjunction with clean cloth/paper towels, antiseptic towelettes, or waterless disinfectant. If these alternatives are used, then the hands are to be washed with soap and running water as soon as possible.

After removal of personal protective gloves, personnel wash hands and any other potentially contaminated skin area immediately or as soon as feasible with soap and water. If personnel incur exposure to their skin or mucous membranes, then those areas are washed with soap and water or flushed with water as appropriate as soon as possible following contact.

Needles

Contaminated needles and other contaminated sharps are not bent, recapped, removed, sheared, or purposely broken. The exception to this is if no alternative is feasible and the action is required by a specific medical procedure. If such action is required, then the recapping or removal of the needle must be done by the use of a device or a one-handed technique.

Contaminated Sharps Discarding and Containment

Contaminated sharps are discarded immediately or as soon as feasible in containers that are closable, puncture resistant, leak-proof on sides and bottom, and biohazard labeled or color-coded.

During use, containers for contaminated sharps are easily accessible to personnel; located as close as is feasible to the immediate area where sharps are being used or can be reasonably anticipated to be found (e.g., laundries); maintained upright throughout use; are not allowed to overfill; and replaced routinely.

Work Area Restrictions

In work areas where there is a reasonable likelihood of exposure to blood or other potentially infectious materials, personnel are not to eat, drink, apply cosmetics or lip balm, smoke, or handle contact lenses. Food and beverages are not to be kept in refrigerators, freezers, shelves, cabinets, or on counter/bench tops where blood or other potentially infectious materials are present.

Mouth pipetting/suctioning of blood or other potentially infectious materials is prohibited.

All procedures are conducted in a manner to minimize splashing, spraying, splattering, and generation of droplets of blood or other potentially infectious materials.

Collection of Specimens

Specimens of blood or other potentially infectious materials are placed in a container, which prevents leakage during the collection, handling, processing, storage, transport, or shipping of the specimens. The container used for this purpose is labeled with a biohazard label or color-coded unless universal/standard precautions are used throughout the procedure and the specimens and containers remain in the facility. Specimens of blood and other potentially infectious body substances or fluids are usually collected within a hospital, doctor's office, clinic, or laboratory setting. Labeling of these specimens should be done according to the department's specimen collection procedure. This procedure should address placing the specimen in a container, which prevents leakage during the collection, handling, processing, storage, transport, or shipping of the specimens. In departments where specimen containers are sent to other institutions and/or universal precautions are not used

throughout the procedure, a biohazard or color-coded label should be affixed to the outside of the container.

If outside contamination of the primary container occurs, the primary container is placed within a secondary container, which prevents leakage during the handling, processing, storage, transport, or shipping of the specimen. The secondary container is labeled with a biohazard label or color-coded.

Any specimen that could puncture a primary container is to be placed within a secondary container, which is puncture proof.

Contaminated Equipment

Equipment that may become contaminated with blood or other potentially infectious materials is examined prior to servicing or shipping and decontaminated as necessary unless the decontamination of the equipment is not feasible. University personnel place a biohazard label on all portions of contaminated equipment that remain to inform personnel, service representatives, and/or the manufacturer, as appropriate.

Personal Protective Equipment

All personal protective equipment used is provided without cost to personnel. Personal protective equipment is chosen based on the anticipated exposure to blood or other potentially infectious materials. The protective equipment is considered appropriate only if it does not permit blood or other potentially infectious materials to pass through or reach the employee's clothing, skin, eyes, mouth, or other mucous membranes under normal conditions of use and for the duration of the time which the protective equipment is used. Examples of personal protective equipment include gloves, protective eyewear, gowns, lab coats, aprons, shoe covers, face shields, and masks.

All personal protective equipment is cleaned, laundered, and disposed of by the supervising department of the employer. All repairs and replacements are made by the employer upon recommendation by the supervising department.

All garments which are penetrated by blood are removed immediately or as soon as feasible and placed in the appropriate container. All personal protective equipment is removed prior to leaving the work area and placed in the designated receptacle.

Gloves are worn where it is reasonably anticipated that personnel will have hand contact with blood, other potentially infectious materials, non-intact skin, and mucous membranes. Latex sensitive personnel are provided with suitable alternative personal protective equipment.

Disposable gloves are not to be washed or decontaminated for re-use and are to be replaced as soon as practical when they become contaminated or as soon as feasible if they are torn, punctured, or when their ability to function as a barrier is compromised.

Utility gloves may be decontaminated for re-use provided that the integrity of the glove is not compromised. Utility gloves are discarded if they are cracked, peeling, torn, punctured, exhibit other signs of deterioration, or when their ability to function as a barrier is compromised.

Masks in combination with eye protection devices, such as goggles, or chin length face shields, are required to be worn whenever splashes, spray, splatter, or droplets of blood or other potentially infectious materials may be generated and eye, nose, or mouth contamination can reasonably be anticipated.

Surgical caps or hoods and/or fluid resistant shoe covers or boots are worn in instances when gross contamination can reasonably be anticipated.

Housekeeping

All contaminated work surfaces are decontaminated after completion of procedures, immediately or as soon as feasible after any spill of blood or other potentially infectious materials, and at the end of the work shift. This is the responsibility of the PI or faculty member supervising the laboratory research.

Protective coverings (e.g., plastic wrap, aluminum foil, etc.) used to cover equipment and environmental surfaces are removed and replaced as soon as feasible when they become contaminated or at the end of the work shift.

All bins, pails, cans, and similar receptacles are inspected and decontaminated on a regularly scheduled basis.

Any broken glassware that may be contaminated is not picked up directly with the hands.

Regulated Waste Disposal

All contaminated sharps are discarded as soon as feasible in sharps containers located as close to the point of use as feasible in each work area.

Regulated waste other than sharps is placed in appropriate containers that are closable, leak resistant, labeled with a biohazard label or color-coded, and closed prior to removal. If outside contamination of the regulated waste container occurs, it is placed in a second container that is also closable, leak proof, labeled with a biohazard label or color-coded, and closed prior to removal.

All regulated waste is properly disposed of in accordance with department-specified requirements. Consult the NSU IBO as needed.

Laundry Procedures

Although soiled linen may be contaminated with pathogenic microorganisms, the risk of disease transmission is negligible if it is handled, transported, and laundered in a manner that avoids transfer of microorganisms to personnel and environments. Rather than rigid rules and regulations, hygienic storage and processing of soiled linen is recommended.

Use of Biohazard Labels

These materials may include but are not limited to, regulated waste, refrigerators, and freezers containing blood or other potentially infectious materials. Other containers used to store, transport, or ship blood or other potentially infectious materials should have

biohazard-warning labels or be placed in color-coded bags.

Training

Training for all personnel is conducted prior to initial assignment to tasks where occupational exposure may occur. All personnel also receive annual online refresher training. Training is arranged by OR-NSU upon request from the department or NSU IBO.

Training for personnel includes an explanation of the following:

- Bloodborne Pathogen Control
- OSHA Bloodborne Pathogen Final Rule;
- Epidemiology and symptoms of bloodborne diseases;
- Modes of transmission of bloodborne pathogens;
- University's Exposure Control Plan (i.e., points of the plan, lines of responsibility, how the plan will be implemented, where to access plan, etc.);
- Procedures that might cause exposure to blood or other potentially infectious materials at the workplace;
- Control methods that are used at the NSU research and teaching laboratories to control exposure to blood or other potentially infectious materials;
- Personal protective equipment available at the NSU research and teaching laboratories (types, use, location, etc.);
- Procedures to follow in an emergency involving blood or other potentially infectious
- materials;
- Procedures to follow if an exposure incident occurs;
- Post exposure evaluation and follow up;
- Signs and labels used at the university research locations; and,
- An opportunity to ask questions with the individual conducting the training and additional resource personnel.

Pre-Exposure Hepatitis B Vaccine

- All personnel who have been identified as having occupational exposure to blood or other potentially infectious materials should have the hepatitis B vaccine as soon as possible.
- Personnel who decline the Hepatitis B vaccine sign a declination statement.

Post Exposure Evaluation and Follow up

When an employee incurs an exposure incident, the employee must consult a physician as soon as possible. The supervisor must complete the report of injury form (can be found at the end of this appendix):

All personnel who incur an exposure incident must complete a medical evaluation and follow up as follows:

- Documentation of the route(s) of exposure and the circumstances related to the incident.
- Identification and documentation of the source individual.
- The results of testing of the source individual are made available to the exposed employee with the employee informed about the applicable laws and regulations concerning disclosure of the identity and infectivity of the source individual.
- The physicians are expected to examine the employee and order blood collection for

testing of the employee's HIV/HBV serological status.

- The employee should have post exposure prophylaxis.
- The employee is given appropriate counseling concerning infection status, results and interpretations of tests, and precautions to take during the period after the exposure incident.
- The employee is informed about what potential illnesses can develop and to seek early medical evaluation and subsequent treatment.

Interaction with Healthcare Professionals

A written opinion is obtained from the healthcare professionals after an exposure incident. In order for the healthcare professional to adequately evaluate the employee, the supervising PI or supervising faculty member is to provide the healthcare professional with:

- a copy of the NSU Exposure Control Plan;
- a description of the exposed employee's duties as they relate to the exposure incident;
- documentation of the route(s) of exposure and circumstances under which the exposure occurred (consistent with the incident report);
- results of the source individual's blood tests (if available); and,
- medical records relevant to the appropriate treatment of the employee (if available).

Recordkeeping

OR-NSU will maintain training, vaccination, and occupational exposure records for the university. Individual medical records of occupational exposures are maintained by the respective medical personnel that provided post-exposure evaluation and follow-up.

Office of Research
NORTH SOUTH UNIVERSITY

INCIDENT REPORT FORM

This form must be completed and signed by the principal investigator (PI) or designated representative, not the employee, and must be submitted *within 7 days* to the Office of Research-NSU via email or by in-person delivery.

IBC Protocol #:		PI:	
Describe the experimentation (if applicable)			

Incident Date:		Incident Time:	
Incident Location(s):			

How many individuals were involved? _____

Did the incident involve any biological or recombinantly modified agents (if yes, complete section 2)?
☐ Yes ☐ No

Did the incident involve animals exposed to biological or recombinantly modified agents (if yes, complete section 3)? ☐ Yes ☐ No

Section 2- Incidents involving biological or recombinantly modified agents:

What agent(s) was in use at the time the incident occurred? _____

Was the agent(s) recombinantly modified? ☐ Yes ☐ No

Provide a detailed description of the incident (include a description of any injuries, routes of exposure, first aid administered, clean-up procedures, etc. Attach additional pages if necessary):

Section 3- Incidents involving animals exposed to biological or recombinantly modified agents:

Animal species: _____

What agent(s) was the animal exposed to? _____

Was the agent(s) recombinantly modified? ☐ Yes ☐ No

Provide a detailed description of the incident (include a description of any injuries, routes of exposure, first aid administered, clean-up procedures, etc. Attach additional pages if necessary):

Section 4- Root cause and corrective action:

Is there a Standard Operating Procedure (SOP) for the work being conducted at time of incident (if yes, attach copy)? ☐ Yes ☐ No

Was the SOP being followed at the time this incident occurred? ☐ Yes ☐ No
If no, specify:

Are engineering controls (e.g., biosafety cabinet) used for this work? ☐ Yes ☐ No
If yes, specify: _

Were ALL engineering controls used/working properly? ☐ Yes ☐ No
If no, specify: __

Is personal protective equipment required for this work? ☐ Yes ☐ No
If yes, specify: _

Was ALL personal protective equipment available/used during the work? ☐ Yes ☐ No
If no, specify: __

Has a cause for this incident been identified? ☐ Yes ☐ No
If yes, specify: (e.g., engineering controls or personal protective equipment failed or were not used properly

What changes do you believe will prevent this incident from happening again?

For any questions, email to bio.safety@northsouth.edu

Office of Research
NORTH SOUTH UNIVERSITY

SUPERVISOR'S FIRST REPORT OF INJURY OR ILLNESS

This form must be completed and signed by the supervisor or designated representative, not the employee/ students, and must be submitted *within 7 days* to OR-NSU via email or in-person delivery.

Name:	Employee ID:	Date of Birth:	Gender: Male☐ Female☐
Employee's Work Telephone Number/Department Name:	Home/Mailing Address:		
Marital Status: ☐ Married ☐ Widowed ☐ Separated ☐ Single ☐ Divorced	Number of Dependent Children:	Spouse's Name:	
Treating Doctor's Name (if medical treatment involved):	Clinic Address:	Telephone Number:	
Date of injury (m-d-y):	Time of injury: AM☐ PM☐	Date Lost Time Began (if applicable):	
Type of Injury: (example: sprain, burn, contusion, laceration, fracture, puncture)		Part of body injured or exposed: (Please be specific – e.g. right middle finger, left ankle, upper back)	
Describe in detail how the accident occurred: (Describe the work process the employee was engaged in. Give the purpose of the function or task, describe how the injury occurred, and explain the cause). Attach additional sheets if necessary:			
Was the employee doing his or her regular j☐? YE☐ NO	Location of accident: Building #:	Room No.:	Area: (hallway, office, parking lot, etc.)
Cause of injury: (fall, tool, machine, etc.)		List Witnesses: (Name/Phone #)	
Return to work date:	Did employee ☐ die? ☐	Supervisor's Name:	Phone #:
Length of service in current position: Years_____ Months ____	Length of Service in Occupation Years_____ Months _____		
Employee's Title:	Number of hours of sick/vacation accrued on date of injury: Sick hrs. Vacation hrs.		
Do you agree with the employee's description of the accident? YES☐ NO ☐			
If no, explain:			
Was safety equipment provided? (if applicable) YES☐ NO☐	Was safety equipment used (if applicable)? YES☐ NO ☐		
If no, explain:			
Action taken to prevent this type of accident from recurring **(must be completed):**			
Name of Supervisor:	Title:	Work phone number:	

For any questions, email to bio.safety@northsouth.edu

APPENDIX D.

Animal Samples Registration Form

Office of Research
NORTH SOUTH UNIVERSITY

Animal Sample Registration
(Animal Tissue, Fluids and Cell Lines Experiments)

SECTION A Principal Investigator and Personnel Information (please type)		
***P.I. Name*:**	***Title*:**	***Dept*:**
***Phone No*:**	***Lab Phone*:**	***Mail code:***
***Building and Lab Room No(s)*:**	**E-mail:**	
Title of the protocol:		

The Principal Investigator is responsible for: (please initial each statement)

____Training of personnel on how to correctly work with animal cell/tissue cultures.

____Limiting access to authorized users.

_____ Minimizing the possibility of inadvertent ingestion or inhalation and direct skin contact, eye contact, or accidental inoculation with the cells or tissue cultures.

____Reporting any adverse events, such as exposures or injuries, immediately to the Biosafety Office.

_____ _____
Principal Investigator (Signature) Date

_____ _____
Institutional Biosafety officer Date

Please send Registration to: Institutional Biosafety Officer (IBO), North South University, Office of Research-NSU.
Email- bio.safety@northsouth.edu

Section B Experimental Design

Briefly describe experimental design:

Types of Manipulations:

☐ Centrifugation ☐ Bleeding/Mixing ☐ Dissection ☐ Sonication ☐ Pipetting
☐ Other _____

Origin of samples: _____

If samples are purchased from ATCC provide ATCC number _____

Are the samples harvested or collected from animals infected with a pathogen?

☐ No ☐ Yes If yes, please list _____

Section D Use of live animals

Will live animals be used for this project? ☐ No ☐ Yes IACUC protocol #_____

Section E Safety Plan

Training Plan:

Personal Protective Equipment (PPE) Required:

☐ Lab coat Gloves ☐ Goggles ☐ Safety glasses ☐ Closed-toe shoes ☐ Long pants
☐ Respirator (specify) _____ ☐ Face mask
☐ Other _____

Containment Equipment:

Is containment equipment available in the laboratory? ☐ No ☐ Yes

Containment equipment used for this project:

☐ Biological Safety Cabinet Location:_____ Last Certified:_____
☐ Fume Hood ☐ Containment Centrifuge ☐ Other _____

Handling of Biohazardous Waste:

Liquid-

Solid-

Spill Cleanup Procedures:

Will the samples be shipped? ☐ No ☐ Yes

APPENDIX E.
Human Products Registration Form

Office of Research
NORTH SOUTH UNIVERSITY

Registration of Human Products Experiments

SECTION A Principal Investigator and Personnel Information (please type)		
***P.I. Name*:**	***Title*:**	***Dept*:**
***Phone No*:**	***Lab Phone*:**	***Mail code:***
***Building and Lab Room No(s)*:**	**E-mail:**	
Title of the protocol:		

Principal Investigator Acknowledgement:

I accept responsibility for: (please initial each statement)

____The safe use of human products
____All personnel have been informed of potential risks, and proper laboratory practices for working safely with human products and have had or have been given the opportunity for the Hepatitis B vaccination.
____Verification of medical insurance for laboratory personnel handling human products.
____Reporting any adverse events, such as exposures or injuries, immediately to the Biosafety Office.

The University's Biological Safety Manual is located at NSU-OR webpage. This manual must be supplemented with the laboratory's safety plan and must include special practices when working with human products. Also, all laboratory personnel must be familiar with safe handling practices (e.g., training with proof of training).

_____ _____
Principal Investigator (signature) Date

_____ _____
Institutional Biosafety Officer (signature) Date

Please send Registration to: Institutional Biosafety Officer (IBO), North South University, Office of Research-NSU.
Email- bio.safety@northsouth.edu

Section B Experimental Design
Briefly describe experimental design:
Types of Manipulations: ☐ Centrifugation ☐ Bleeding/Mixing ☐ Dissection ☐ Sonication ☐ Pipetting ☐ Other _____
Type of human products manipulated: ☐ Cell lines ☐ Blood ☐ Tissues ☐ Urine ☐ Feces ☐ Other -
Origin of samples: _____
How long will samples be maintained?_____ How much sample will be maintained at any given time?_____
~~Are samples infec~~ted with a pathogen? ☐ No ☐ Yes If yes, please list
Is the project registered with the Institutional Review Board (IRB)? ☐ No ☐ Yes IRB protocol# _____ IRB date of approval:_____

Section C Safety Plan
Training Plan:
Personal Protective Equipment (PPE) Required: ☐ Lab coat Gloves ☐ Goggles ☐ Safety glasses ☐ Closed-toe shoes ☐ Long pants ☐ Respirator (specify) _____ ☐ Face mask ☐ Other _____
Containment Equipment: *Is containment equipment available in the laboratory?* ☐ No ☐ Yes

Containment equipment used for this project: ☐ Biological Safety Cabinet Location:_____ Last Certified:_____ ☐ Fume Hood ☐ Containment Centrifuge ☐ Other _____
Handling of Biohazardous Waste: Liquid- Solid- Spill Cleanup Procedures:
Will the samples be shipped? ☐ No ☐ Yes
Will samples be transported between laboratories or outside University? ☐ No ☐ Yes Hepatitis B vaccination offered to laboratory personnel (if applicable)? ☐ No ☐ Yes

APPENDIX F.
Autoclave Quality Assurance Program

Autoclave Quality Assurance Program

Autoclaving is an accepted procedure for the decontamination of certain biohazardous waste. Biological cultures and stocks, contaminated solid waste, and liquid waste can be sterilized through autoclaving. After sterilization in a steam autoclave, these materials are considered non-infectious. All autoclaved waste is placed into the solid biohazard waste stream. Materials that contain hazardous chemicals or radioisotopes are not to be autoclaved.

To ensure that biohazardous waste is properly decontaminated during autoclaving, the following procedures should be followed by laboratory personnel.

1. Processing Time
Infectious waste must be treated in an autoclave for a **minimum of 30 minutes at 121° C (250° F);** however, the total processing time required to decontaminate infectious waste depends on the specific loading factors (container type, water content, quantity, etc.). A total processing time of 60 minutes is recommended for gravity displacement autoclaves and 10 minutes for vacuum-type autoclaves (132° C).

Sterilization by autoclaving is accomplished through exposure and penetration of the contaminated material by superheated steam for an adequate amount of time. Because steam will not penetrate a sealed plastic autoclave bag, bags containing dry loads must not be tightly sealed (rubber band closures will allow bags to "breathe") or adequate amounts of water must be added to the load.
Consult the manufacturer's instructions for sterilizing materials inside plastic autoclave bags. Liquid waste may also be autoclaved in lieu of adding an appropriate chemicals disinfectant, and disposed in the sink.

2. Steam Sterilization Indicator
All autoclaved waste must include a steam sterilization indicator (the use of biohazard bags with a "built-in" indicator is recommended).

3. Minimum Temperature
Steam autoclaves used to treat infectious waste must operate at a minimum temperature of 121° C. The operating temperature of the autoclave must be verified for each run by maintaining a record of the temperature either as a chart or paper tape recording or a manual recording in a logbook.

4. Confirm Adequate Sterilization Conditions
On a monthly basis, confirm that adequate sterilization conditions are being met through the use of ampoules containing heat-resistant spores (Geo*bacillus stearothermophilus*) placed in the center of an autoclave load. In conjunction with the *B. stearothermophilus* testing, measure and record the maximum temperature achieved during the autoclave cycle through the use of a maximum registering (or "holding") thermometer or calibrated data logger for full cycle.

5. Maintain Records
Maintain records of *B. stearothermophilus* testing and maximum autoclave temperature recordings for a minimum of one year.

Monthly Spore Testing Procedure
Place ampoule of *B. stearothermophilus* spores and holding thermometer or data logger in the center of an autoclave load.

Process the load under normal operating procedures.

The highest temperature indicated on the holding thermometer is entered on the Autoclave QC Log.

If this temperature is less than 121° C, the autoclave is not to be used to treat infectious waste until it has been repaired and passes retesting. In the interim, tag the autoclave as "Not Approved for Infectious Waste."

Incubate the autoclaved ampoule and a non-autoclaved, control ampoule according to the manufacturer's instructions (normally 55-60°C for 24 to 48 hours).

If a color change occurs, the sterilization process was unsuccessful. Discontinue use of the autoclave until it is repaired and passes retesting. Tag the autoclave as "Not Approved for Infectious Waste" until the autoclave passes retesting.

Autoclave QC data should be recorded in log book and will be retained for 1 year.

APPENDIX G.

Biosafety Level-2 (BSL-2) Requirements

Biosafety Level-2 (BSL-2) Requirements

Biosafety Level 2 (BSL-2) is suitable for experiments involving agents of **moderate** potential hazard to personnel and the environment.

For example:

- Microorganisms of low biohazard potential, such as those in **Risk Group 2** or **BSL-2**.
- Recombinant DNA activity requiring BSL-2 physical containment, including animal studies that involve the construction of transgenic animals.
- Non-recombinant cell and/or tissue culture systems that require this level of containment.
- Oncogenic viral systems classified as low risk.
- Production activities with Risk Group 1 organisms.

The control of potential biohazards at the BSL-2 level is provided by use of standard microbiological practices with the addition of personnel protective equipment (lab coat and gloves).

The following are procedures are used with BSL-2 containment requirements. They are based on the recommendation of the *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) 5th Edition, 2007.

Standard Microbiological Practices

- **Access**

  Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.

- **Hand washing**

  Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.

- **Food**

  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 for this purpose only.

- **Eye protection**

  Personnel who use contact lenses should use eye protection in the lab.

- **Pipetting**

  Mouth pipetting is prohibited; mechanical pipetting devices are used.

- **Sharps**

  Policies for the safe handling of sharps are instituted.

- **Splashes & Aerosols**

  All procedures are performed carefully to minimize the creation of splashes or aerosols.

- **Decontamination**

  Work surfaces are decontaminated upon completion of work, or at the end of the day, and after any spill or splash of viable material with disinfectants that are effective against the agents of concern.

- **Insect/Rodent Control Program**

  An insect and rodent control program is in effect.

Special Practices

- **Access**

  Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections. The principal investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.

- **Policies & Procedures**

  The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.

- **Biohazard Signs**

  A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate information to be posted includes:
  - the agent(s) in use;
  - the biosafety level;
  - the required immunizations;
  - the investigator's name and telephone number;
  - any personal protective equipment that must be worn in the laboratory;
  - any procedures required for exiting the laboratory.

- **Immunizations**

  Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

- **Manual/SOPs**

  Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory director. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

- **Training**

  The Principal Investigator or Laboratory Director ensures that laboratory and support personnel receive appropriate training about the potential hazards associated with:
  - the work involved;
  - the necessary precautions to prevent exposures;
  - the exposure evaluation procedures.

- Personnel receive annual updates or additional training as necessary for procedural or policy changes.

- **Sharps**

  A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.

  Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

  Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

  Syringes that re-sheathe the needle, needleless systems, and other safety devices are used when appropriate.

  Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal according to any local, state, or federal regulations.

- **Potentially Infectious Material**

  Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.

- **Decontamination**

  Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant:

  - on a routine basis;
  - after work with infectious materials is finished;
  - and especially after overt spills, splashes, or other contamination by infectious materials.

  Prior to its removal from the facility, contaminated equipment must be decontaminated according to any government regulations and NSU IBC approved procedures before it is sent for repair or maintenance or packaged for transport in accordance with applicable regulations.

- **Spills & Accidents**

  Spills and accidents that result in overt exposures to infectious materials are immediately reported to the Principal Investigator and Laboratory Director. Medical evaluation, surveillance, and treatment are provided as appropriate through the NSU Medical Center or other medical facility, and written records are maintained accordingly.

- **Cleaning**

  Sinks in the BSL-2 area should be cleared routinely using appropriate disinfectant such as a chlorine-containing abrasive and flushed with a suitable chemical decontaminant.

  Water baths and all water reservoirs should be washed periodically with a suitable chemical decontaminant.

  Once a month, work spaces that do not get daily attention with germicide should be cleaned, as well as other lab areas where clutter accumulates (e.g., storage areas).

  The laboratory director will set up a routine schedule to perform surface cleaning with appropriate chemical disinfectant of large equipment (such as incubators) as part of laboratory good practices.

  Supplies should be rotated and outdated material thrown out. Unlabeled material should be eliminated.

  Clutter should be cleaned up.

- **Custodial Services**

  Only personnel with appropriate authorization may enter a BSL-2 facility while BSL-2 research activity is in progress. The laboratory director and/or faculty member supervising the research laboratory is responsible for arranging such custodial services with the NSU Operations and Maintenance Director.

- **Animals**

  Animals not involved in the work being performed are not permitted in the laboratory.

Safety Equipment (Primary Barriers)
Properly maintained certified biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are to be used when:

- Procedures that have the potential to create infectious aerosols or splashes are conducted. These may include:
  - centrifuging,
  - grinding,
  - blending,
  - vigorous shaking or mixing,
  - sonic disruption,
  - opening containers of infectious materials whose internal pressures may be different from ambient pressures,
  - inoculating animals intra-nasally,
  - harvesting infected tissues from animals or embryonate eggs.

- High concentrations or large volumes of infectious agents are used.
  Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.

Equipment

- **Face protection** (goggles, mask, face shield, or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.
- **Protective laboratory coats, gowns, smocks, or uniforms** designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.
- **Gloves** are worn when hands may contact potentially infectious materials, contaminated surfaces, or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated and removed when work with infectious materials is completed or when the integrity of the glove is compromised.
- Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.). They should not be worn outside the lab. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

Procedures for Receiving and Inspecting Samples
The PI will designate a responsible person for the purchase of all infectious materials to be used in the BSL-2 lab.

Infectious materials will be shipped to the laboratory in accordance with the appropriate government standards and, as appropriate for international shipping, standards of the International Air Transportation Association (IATA) for shipping of infectious biological materials.

Upon receipt of the package, it will be placed on a tray covered with absorbent material and opened only in the Biological Safety Cabinet to prevent any potential exposure to personnel in case the container leaked during transport.

Personnel assigned to open packages will wear lab smock, gloves, and eye protection.

If any containers are found to be damaged, leaking, or otherwise contaminated, they will be immediately isolated into a plastic bag along with all packaging materials. The spill will be disinfected and cleaned up. The Principal Investigator, lab director, or designee will be notified immediately. The incident will be reported to the NSU IBO and as necessary, to appropriate agencies.

If, after inspection, the samples are intact, they can be placed into labeled secondary containers (unbreakable plastic containers or metal tubes) and then transferred to a storage area.

Only staff who are authorized to do so can remove samples from storage. Removal and use of all such materials must be entered into the logbook.

Unused cultures can be returned to storage after the outer container has been properly disinfected.

Laboratory Facilities (Secondary Barriers)
In a BSL-2 lab, the following conditions are necessary:

- **Doors**

  Doors that can be locked and secured should be installed for facilities that house restricted areas.

- **Public**

  University authority should consider locating new laboratories away from public areas having regular personnel movement (e.g., in hallways, passage to classrooms or offices, etc.).

- **Sink**

  Each laboratory contains a sink for hand washing.

- **Cleaning**

  The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.

- **Bench Tops**

  Bench tops are impervious to water and resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.

- **Lab Furniture**

  Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.

- **Biological Safety Cabinets**

  Biological safety cabinets should be installed in a manner such that fluctuations of the room's air supply and exhaust air do not cause them to operate outside their parameters for containment. Locate BSCs away from doors, away from windows that can be opened, away from heavily traveled laboratory areas, and away from other potentially disruptive equipment so as to maintain the BSC's air flow parameters for containment.

- **Eyewash Station**

  An eyewash station is readily available.

- **Lighting**

  Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

- **Ventilation**

  There are no specific ventilation requirements. 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. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

APPENDIX H.

Requirements for Animal Biosafety Program

Requirements for Animal Biosafety Levels

Working with animals poses potential additional health and safety hazards that require extra precautions. The specific requirements will depend on the types of activities (e.g., surgery, feeding animals, use of anesthetic agents, etc.) and the specific species used.

Personnel should follow the following guidelines:
Follow the specific requirements established in the IACUC-approved protocol and the facility requirements. However, in general:

- **Wash hands** after handling an animal or anything that an animal has touched. The most common way of contracting an animal-transmitted infection is placing the infectious material directly into the mouth.
- **Never smoke, drink, or eat** in an animal area or before washing hands.
- **Wear protective clothing** as recommended/required by the facility for the species and operations:
    - ✓ Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing;
    - ✓ Protective clothing helps prevent potentially contaminated material from leaving an animal area; and
    - ✓ Do not wear the protective clothing outside of the animal area and do not take protective clothing home.
- **Use the personal protective equipment (PPE)** recommended/required for the species and operations.
    - ✓ Workers shall wear the appropriate PPE (e.g., gloves, face shields, masks, and respirators) when required and follow their supervisor's instructions scrupulously.
    - ✓ Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials, and when handling animals.
    - ✓ Gloves and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.
    - ✓ Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated.
    - ✓ Eye and face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.
- **Get the appropriate training** and contact a supervisor with any questions. Contact the Chair, NSU IACUC as needed.

Basic Safety for the Necropsy of Infected Animals

- Ensure that the necropsy of infected animals is carried out in biological safety cabinets or designated necropsy space by trained personnel.

- Wear a surgeon's wrap-around gowns over laboratory clothing.
- Use a surgeon's mask and eye protection.
- Use other PPE recommended by the facility for the infectious agents present.
- Wear gloves.
- Wet the fur of the animal with a suitable disinfectant.
- Pin down or otherwise fasten small animals to metal in a tray.
- Before and after necropsy, disinfect the necropsy table, inside the BSC, and other potentially contaminated surfaces with a suitable germicide.
- Upon completion of necropsy, place all potential biohazardous materials in suitable containers and then sterilize the materials.
- Segregate contaminated mixed waste and store for appropriate disposal.
- Place contaminated instruments in a bath that contains a suitable disinfectant.
- Follow the facility requirements for sterilization.
- Clean contaminated rubber gloves in disinfectant before removal from the hands.
- Wearing gloves is not a substitute for hand washing; wash hands after necropsy and carcass disposal.
- Follow the facility's guidelines for the disposal of animal carcasses.

Procedures for Working in an Animal Biosafety Level 2 (ABSL-2) Facility

Animal Biosafety Level 2 (ABSL-2) includes pathogenic agents of moderate hazard potential (CDC Biohazard Class 2) and chemical hazard agents of moderate hazard potential. Before starting any Animal Biosafety Level 2 (ABSL-2) work a PI must:

- Obtain IBC approval for biosafety and IACUC approval for the research protocol.
- Make appropriate animal housing arrangements, subject to approval by the NSU IACUC.

The following Standard Operating Procedures (SOP) have been developed to provide guidance to those individuals working in rooms in which animals involved in chemical and biological hazards determined to be ABSL-2 are housed.

Overview

Access to the room where the work with animals is to be conducted is restricted. Laboratory personnel must have training in aseptic micro-isolator techniques, when applicable, and use of biological safety cabinets, in addition to specific safety training in handling the pathogenic and/or chemical agent(s) with which they are working.

Research and Animal Facility personnel should receive appropriate immunizations or tests for any agents handled or potentially present in the room prior to initiating the ABSL-2 portion of their project.

Procedures must be conducted in a Class II BSC.

Personal Protective Equipment

- Minimum PPE:
  - ✓ Solid front gown
  - ✓ Hair cover
  - ✓ Shoe covers
  - ✓ Mask

- ✓ Double gloves
- In addition:
  - ✓ N95 may be required
  - ✓ Face shield or other specific eye or face protection may be required

Equipment and Supplies
- Biohazard stickers for cage cards
- MB-10 (chlorine dioxide) disinfectant or Virkon-S
- Biological safety cabinet: Class II

Responsibilities
It is the PI's responsibility to ensure that all necessary project-specific safety training is provided to research personnel and support staff prior to any project being initiated. It is also the PI's responsibility to provide documentation to the IBO and OR-NSU of such training.
The PI and individuals working in the animal research facility are responsible for ensuring they have received proper training and that they are adhering to this SOP, as well as to posted precautions and guidelines in the facilities.

Procedures

Entry
- Remove the lab coat worn in the ASC facility and hang it on the garment rack provided outside of designated ABSL-2 animal space.
- PPE **must** be worn while working in ABSL-2 animal housing and procedure space. PPE is provided just outside specific ABSL-2 rooms. Don designated PPE prior to entry into the ABSL-2 areas.
- Proceed into the designated ABSL-2 room using an access card or key.

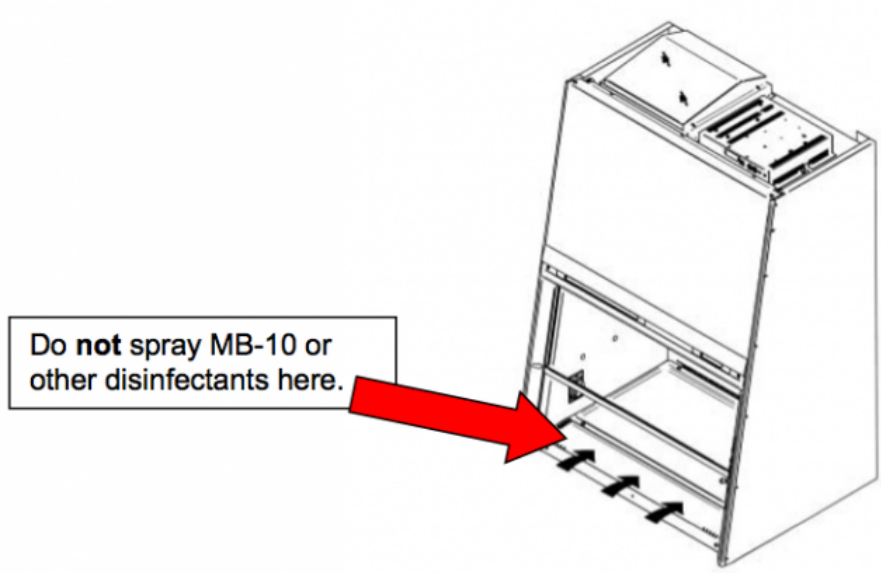
General Information
Investigators using the room will be assigned a cubicle and/or rack and shelf/shelves where their animals will be housed.
- A cubicle or rack may hold cages belonging to more than one investigator.
- Biohazard projects are not housed in cubicles that house ongoing chemical hazard projects.

ASC personnel will perform daily health checks of animals on studies involving infectious agents if the animals are housed on racks not held in cubicles.
- ASC personnel will perform daily health checks of animals on studies involving chemical agents by viewing the animals through the window of the isolation cubicle.
- The ASC will notify PIs of any animal health issues.
- All animal work will be conducted within the confines of the Class II BSC.
  - ✓ Always open animal cages in the Class II BSC using aseptic micro-isolator technique.
- Hypodermic needles and syringes are used only for parenteral injection or aspiration of fluids from laboratory animals and bottles with plastic/rubber diaphragms.
  - ✓ Only needle-locking syringes (i.e. luer locking) or disposable needle syringe units (i.e., the needle is integral to the syringe) are used for the injection or aspiration of infectious fluids.

- ✓ Needles should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use.
- ✓ The needle and syringe should be promptly placed in a puncture-resistant sharps container.

➢ Always spray or wipe down all interior surfaces of the Class II BSC with MB-10 or Virkon-S **before** and **after** working in the hood.

- ✓ Allow 10 minutes of contact time prior to wiping the surfaces with disposable towels.
- ✓ Do not spray the top grille of the Class II BSC (this is where the filter is located).
- ✓ Discard used towels in the waste container.



Immediately following infection of animals with pathogenic agents, place a biohazard sticker on their cage card(s) (ASC provides these specific stickers).

➢ Fill out the following information on the biohazard sticker for cage cards:

- ✓ PI name
- ✓ 24-hour contact phone number
- ✓ Date(s) infected
- ✓ Dose per animal
- ✓ Pathogenic agent
- ✓ Protocol number
- ✓ Husbandry by PI or ASC (circle one)

➢ Report all spills and accidents that result in overt exposure to infectious materials to a ASC Supervisor and the ASC office, (617) 358-8301 or LACF at (617) 353-5415

Removal of Dirty Cages and Bottles from Room

Biological agents

➢ Place a cage inside the BSC, remove the water bottle from the cage, and replace the lid.

- ✓ Put soiled cage, wire lid, and bedding in a semi-clear biohazard autoclave bag and place on a cart.
- ✓ Put water bottles in a separate, semi-clear biohazard autoclave bag on a cart.

- Closure ties are stored adjacent to the biohazard autoclave bags on the supply rack in the corridor outside rooms and in rooms.
- **Do not use** autoclave tape to close the bag.
- Thoroughly spray the exterior of all bags with MB-10 or Virkon-S after closing the bag with a nylon tie, beaded tie, or twist tie. Spray all surfaces and wheels of the cart(s).
- Cart(s) should be moved to the soiled side of the cage wash room.

Chemical agents

- Place a cage inside the BSC, remove the water bottle from the cage, and replace the lid.
    - ✓ Put soiled cage, wire lid, and bedding in a red biohazard bag and place on a cart.
    - ✓ Put water bottles in a separate red biohazard bag on a cart.
- Closure ties are stored adjacent to the biohazard autoclave bags on the supply rack in the corridor outside W-838/839 and in both rooms.
- **Do not use** autoclave tape to close the bag.
- Thoroughly spray the exterior of all bags with MB-10 after closing the bag with a nylon tie, beaded tie, or twist tie. Spray all surfaces and wheels of the cart(s).
- Cart(s) should be moved to the soiled side of the cage wash room on the 8th floor of W Building (W-8).

Disposal of carcasses

- Any dead animals must be removed from their cage (while in the BSC) and placed in a small, leak-proof red biohazard bag.
- Place a sticker with animal identification information on the outer bag, then thoroughly spray the bag with MB-10 or Virkon-S and place in the refrigerator. A cage card with a sticker showing the same animal identification information will be placed on the cage.
- The ASC will remove carcasses for incineration disposal three days after they are found in the refrigerator.

Exiting Procedures

- When work is complete and the BSC and all other work spaces have been decontaminated with MB-10 or Virkon-S, outer gloves should be removed and placed in the biohazard waste container.
- To exit the room, open the door and remove one shoe cover, stepping over the room threshold into the hallway with that foot.
- Remove the shoe cover from the other foot as it is brought into the hallway but before stepping into the hallway with the second foot
- Remove gown from the shoulders, turning it inside out.
- Discard disposable face protection, hair cover, mask, gown and gloves in the red biohazard trash receptacle in the hallway outside ABSL-2 room.
    - ✓ After leaving ABSL-2 room and discarding PPE, hands should be washed using the alcohol hand sprayer located on the wall immediately to the left of the doors to each ABSL-2 room.

APPENDIX I.

Principal Investigator Checkout Procedure

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Principal Investigator Checkout Procedure

PURPOSE

All Principal Investigators (PIs) conducting research in NSU facilities are required to complete this checkout procedure ***30 days prior*** to the completion of their association or affiliation with North South University. PIs must ensure and document in writing that all hazardous chemical, biological, and radioactive materials under their authorization/supervision are properly disposed, transferred to another laboratory, shipped, or removed to storage. Strict adherence to this policy will reduce the likelihood of accumulating orphaned chemicals, some of which may become dangerously unstable. Uncontrolled inventories of hazardous chemical, biological or radioactive materials eventually lead to storage problems, increased waste disposal costs, contamination and other potentially unsafe conditions. **The failure of any PI to complete or properly follow this checkout procedure will require that their departmental chairperson or his/her designee assume such responsibility. The NSU IBO and OR-NSU shall be informed in writing accordingly.**

Please note:

1. *OR-NSU must be given written notification of a PI's departure from the North South University by their department at least 30 days prior to his/her exit date.* The written notification is to be sent to: OR-NSU (Admin Building Rm 625) or electronically to bio.safety@northsouth.edu. Advance notice is required to allow adequate time for the scheduling of laboratory clean outs and compliance with governmental and/or university regulatory requirements. The attached PI Advance Notification form is provided to the PI for completion and submittal to OR-NSU as required.

2. The PI must include the following items in the written notification of departure:
   a. Forwarding mailing address
   b. NSU Department
   c. NSU Departmental chairperson's name
   d. Room numbers for all laboratories under that PI's supervision
   e. Date of departure
   f. Contact telephone numbers before and after departure
   g. Name of individual (and contact information) who will take responsibility of transferred chemicals, biological materials, and/or radioisotopes

3. Chemicals that will remain in the laboratory must have proper labels that include the chemical name, hazards, reactivity and date received or last utilized. Radioactive materials and radioactive samples must also have labels that include the radioisotope, activity, and date. Biological material remaining in the laboratory must be placed in leak proof or breakage resistant receptacles with the name and hazards associated with the microbial agent on the specimen container.

1. Chemicals will not be shipped through OR-NSU; outside vendors may be contacted to arrange legal shipments of such materials. However, the supervising department and/or NSU IBO will ensure, consistent with established procedures, that all chemical hazardous products are inspected prior to shipping to ensure that they are properly packaged for transport.

2. All biological materials that need to be shipped or relocated must be packed and transported following the International Air Transport Association (IATA) rules and regulations.

It is ultimately the responsibility of the department chairperson to make sure that all of the hazardous materials are shipped to a destination institution in accordance with standard national and international regulations.

3. All containment equipment such as biosafety cabinets, fume hoods, or centrifuges that were used with infectious agents at Biosafety Level 2 must be properly cleaned and decontaminated with an appropriate disinfectant for the agents used.

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PRINCIPAL INVESTIGATOR CHECKOUT CHECKLIST

The PI Checkout Checklist is provided to assist the PI with properly withdrawing from North South University.

1. Completed and submitted form for 30 days advance notification_____
   (Provide accurate and detailed information)

2. Chemicals & samples properly labeled & packaged_____
   (PI should consider donating unwanted new and reusable chemicals to fellow investigators with the help of the Department Chairperson and/or OR-NSU)

3. Biologicals materials properly labeled & packaged by the Department staff _____trained in procedures for shipping infectious agents and diagnostic materials

4. Laboratory cleanout completed_____

5. Equipment & laboratory ware properly decontaminated_____

6. Hazardous Chemicals inspected prior to shipping _____

7. Controlled Substance and Dangerous Drugs properly disposed/transferred_____

Please submit completed checklist to OR-NSU at Admin Building Rm 625 or electronically to bio.safety@northsouth.edu .

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PRINCIPAL INVESTIGATOR'S 30 DAYS ADVANCE NOTIFICATION PRIOR TO DEPARTURE FROM NSU

This is to officially notify the OR-NSU of my intent to leave North South University. This written notification is submitted to: OR-NSU (in person) or electronically to bio.safety@northsouth.edu, 30 days prior to my departure from NSU. The following information is provided as required in the Principal Investigator Checkout Procedure.

Name of Faculty/ PI: _____

Date of Departure: _____

Department: _____

Department Chair: _____

Room Numbers of all laboratories under PI supervision: _____

Contact Phone Numbers:
Before Departure: _____

After Departure: _____