WINSTON-SALEM STATE UNIVERSITY

INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) PROCEDURES FOR THE USE OF BIOHAZARDOUS MATERIALS, INFECTIOUS AGENTS AND RECOMBINANT DNA

School of Graduate Studies and Research
Office of Sponsored Programs
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INTRODUCTION AND PURPOSE

Winston-Salem State University recognizes the importance of conducting a broad spectrum of investigative research as well as classroom and laboratory educational activities that require the use of recombinant DNA technology, infectious agents, and biohazardous materials. Cognizant of the risks accompanying these activities, the University requires that these activities be subject to review and approval and continued oversite by the Institutional Biosafety Committee (IBC) to ensure that they are conducted in a safe and appropriate manner, and in accordance with the current editions of the National Institutes of Health (NIH) *Guidelines for Research Involving Recombinant DNA Molecules,* the Centers for Disease Control and Prevention (CDC) and National Institute of Health (NIH) publication entitled *Biosafety in Microbiological and Biomedical Labs* (BMBL), the CDC/United States Department of Agriculture (USDA) Select Agent Program, the Occupational Safety and Health Administration (OSHA) *Bloodborne Pathogen Standard* and the Occupational Safety and Health Administration (OSHA) *Occupational Exposure to Hazardous Chemicals in Laboratories Standard*. Adherence to these policies shall not exempt investigators employing recombinant DNA molecules or infectious agents in their research from compliance with other applicable laws, regulations or policies (e.g. research with human subjects, vertebrate animals, or radioactive materials). The appropriate paperwork must also be filed through the Institutional Review Board (IRB) and the Institutional Animal Care and Use Committee (IACUC).

The University is responsible for ensuring that research and teaching activities are carried out in a manner that protects students, University employees, and the community. The use of Recombinant DNA may or may not require the use of infectious biological agents. Therefore, the regulations and procedures are included for both. This policy applies to research that is: sponsored by the University; conducted by employees or agents of the University; or conducted using the University's property, facilities, students or non-public information.

LINES OF AUTHORITY AND CHARGE

The IBC was established in accordance with the National Institutes of Health (NIH) *Guidelines for Research Involving Recombinant DNA Molecules* under the authority of the Chancellor to ensure activities that require the use of recombinant DNA technology and infectious agents at the University are performed under optimum conditions, which, at a minimum, comply with all pertinent laws.

ABBREVIATIONS AND TERMS

Abbreviations:
ABSL - Animal Biological Safety Level

BSL - Biological Safety Level
CDC - Center for Disease Control
EHS - Environmental Health and Safety
HEPA - High-Efficiency Particulate Air
IBC - Institutional Biosafety Committee
IBSO - Institutional Biosafety Officer
LAR - Laboratory Animal Resources
MSDS - Material Safety Data Sheet
NIH - National Institutes of Health
NSF - National Science Foundation
NRC - National Research Council
ORDA - Office of Recombinant DNA Activities (NIH)
OSHA - Occupational Safety and Health Administration
PI - Principal Investigator
RAC - Recombinant DNA Advisory Committee (NIH)
rDNA - Recombinant DNA
OSPR - Office of Sponsored Programs and Research

Terms:
Animal Husbandry: A branch of agriculture concerned with the production and care of domestic animals.

Biological Toxin: A colloidal proteinaceous poisonous substance that is a specific product of the metabolic activities of a living organism and is usually very unstable, notably toxic when introduced into the tissue, and typically capable of inducing antibody formation.

Blood-Borne Pathogens: Pathogenic microorganisms that are present in human blood that can cause disease in humans. These pathogens include, but are not limited to HBV and HIV.

Class I Biosafety Cabinet: An enclosure with an inward airflow through the front opening. Provides protection for the worker and the laboratory environment but not to product being utilized in the cabinet.

Class II Biosafety Cabinet: An enclosure with an inward airflow through the front opening. Provides protection to the worker, the environment, and the product being utilized in the cabinet.

Containment: Used to describe safe methods for managing infectious agents in the laboratory environment where they are being handled and maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.

Host: Organism in which the rDNA replicates.

Infectious Agents: defined as those biological agents, both pathogenic and non-pathogenic, known to infect human as well as selected animal agents that may pose theoretical risks if inoculated into humans.

Negative Airflow: Directional airflow from areas exterior to a laboratory into the laboratory.

Primary Containment: methods to protect the internal laboratory environment.

Recombinant DNA: DNA prepared by breaking up and splicing together DNA from several different species of organisms.

Recombinant DNA Insert: That (those) strand(s) of foreign DNA being inserted into the host/vector.

Secondary Containment: methods to protect the environment external to the laboratory.

Sharps: Any object that can penetrate the skin, e.g., needle, scalpel, knife, etc.

Vector: Carrier used to introduce rDNA into the host system and that facilitates replication.

BIOHAZARDOUS MATERIALS

The Institutional Biosafety Committee (IBC) reviews the use of Biohazardous Material, which includes Recombinant DNA and Infectious Biological Agents.

Recombinant DNA

The NIH Guidelines define Recombinant DNA molecules as either: (i) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above.

Infectious Biological Agents

Infectious biological agents or biologically derived infectious materials present a risk or potential risk to the health of humans or animals, either directly through infection or indirectly through damage to the environment. Infectious agents have the ability to replicate and give rise to the potential for large populations in nature when small numbers are released from controlled situations.

Categories of Potentially Infectious Materials

1. Human, animal and plant pathogens (bacteria, parasites, fungi, viruses);
2. All human blood, blood products, tissues and certain body fluids;
3. Cultured cells and potentially infectious agents these cells may contain;
4. Clinical specimens; and
5. Infected animals and animal tissues.

INSTITUTIONAL BIOSAFETY COMMITTEE

Winston-Salem State University's Institutional Biosafety Committee is the committee responsible for ensuring that all research involving biohazardous material, which includes Recombinant DNA and infectious biological agents, is conducted in compliance with the *NIH Guidelines*. Research laboratories working with biohazardous agents, microorganisms and/or recombinant DNA technologies are special, often unique, work environments. The materials being used may pose special risks to persons working in or near the laboratory or to the environment should the material escape the containment procedures established for the laboratory. All research and educational activities that involve infectious agents, plant or animal pathogens, hazardous chemicals, recombinant DNA molecules, microorganisms, human etiologic agents, human tissues or body fluids, or gene therapy must be reviewed and approved by the Winston-Salem State University Institutional Biosafety Committee (IBC) prior to initiation.

IBC Committee Membership

The IBC members shall be selected so that they collectively have experience and expertise in recombinant DNA technology and infectious organisms and the capability to assess the safety of such activities and any potential risk to public health or the environment. At least two members shall not be affiliated with Winston-Salem State University (apart from membership on the IBC) and shall represent the interest of the community area with respect to the health and protection of the environment. When possible, there shall be at least one member from each department/ unit conducting recombinant DNA research.

Every effort will be made to schedule meetings to accommodate as many members as possible. Each member of the committee was chosen because of their expertise in their particular field. It is the members' professional responsibility to attend meetings and participate. Tentative meetings for the IBC will be held quarterly. Failure to attend at least 50% of these meetings will result in removal and replacement of the committee member.

Contacting the IBC

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Meetings

The IBC shall meet as needed, but at least once per year. A schedule of meetings shall be publicly posted when feasible. Meetings will be open to the public consistent with protection of privacy and proprietary interests. A quorum for conducting business shall consist of 50% of the current members plus one. At least one member not affiliated with the University (apart from serving the IBC) must also be present. The meetings will follow recognized parliamentary procedure. If the chairperson has a conflict of interest or is unavailable, a majority vote with a quorum from the IBC w/ the chair excusing him/her will be appropriate. Included in this vote shall be the designation of a specific person to sign the approval.

RESPONSIBILITIES

Chairperson, Institutional Biosafety Committee

1. Ensure that the Institutional Biosafety Committee is properly constituted and fulfills its requirements under the appropriate regulations, rules, etc.
2. Ensure that all members of the Institutional Biosafety Committee are adequately trained in appropriate containment practices, secondary containment procedures, and accidental spill containment procedures to fulfill their responsibilities as members of the Institutional Biosafety Committee.
3. Call and preside over meetings of the Institutional Biosafety Committee.
4. Review and insure compliance of all authorized researchers utilizing biohazardous materials.
5. Coordinate the review process of researchers seeking use of biohazardous material through research programs at the University.
6. Review all instances of noncompliance and recommend corrections to the University.
7. Notify the Principal Investigator of the results of the Institutional Biosafety Committee's review.

Institutional Biosafety Committee

1. Advise the Chancellor, Provost, Associate Provosts, Deans, and Department Chairs on matters related to biohazards and biosafety with their respective areas of responsibility.

2. Develop, recommend, and implement policies and procedures for biological risk assessment and biological risk reduction throughout the University.

3. Develop emergency plans for the containment and resolution of accidental spills and other related emergencies with an emphasis on risk reduction, personnel protection, and environmental protection.

4. Oversee all research and teaching activities involving biohazardous agents including review and approval prior to initiation, annual reviews and updates, reviews of laboratory safety equipment and procedures, and certification of compliance with all applicable rules and regulations governing the use of biohazardous materials and approve those research projects that are found to conform with the NIH Guidelines, OSHA and the CDC including **(a)** an independent assessment of the containment levels required by the NIH Guidelines for the proposed research; and **(b)** assessment, if applicable, of the facilities, procedures, practices, and training and expertise of personnel involved in the proposed use of infectious biological agents.

5. Ensure that all principal investigators are sufficiently trained in appropriate containment practices, secondary containment procedures, accidental spill containment, and their responsibilities as principal investigators.

6. Advise and provide technical expertise, whenever possible, to the Safety Officer on matters regarding biosafety.

7. Conduct investigation of serious violations or problems and to make recommendations to Chancellor for the resolution of continued non-compliance or serious infractions.

8. Conduct periodic inspections of laboratories to ensure compliance with established procedures.

Safety Officer

1. Investigate laboratory accidents and report problems, violations and injuries or illnesses associated with biohazardous research activities, to the Institutional Biosafety Committee.

2. Provide advice and assistance to the Institutional Biosafety Committee and Principal Investigators concerning containment procedures and practices, laboratory security, recommended laboratory containment equipment, rules, regulations, and other matters as may be necessary.

3. Provide oversight and assurance that laboratory safety containment equipment is functioning properly including field testing and certification, where appropriate, of all biosafety cabinets.

4. Serve as a member of the Institutional Biosafety Committee.

Health and Safety

1. Provide industrial hygiene and safety support for all laboratory operations.

2. Transport and dispose of all infectious waste in compliance with all applicable federal, state, and local ordinances.

3. Assist, as necessary, in the emergency response, cleanup, and decontamination of biological spills and accidents.

4. Provide Occupational Health training.

Office of Sponsored Programs (Compliance Officer)

1. Provide the necessary liaison between Principal Investigators, the Institutional Biosafety Committee, granting agencies, and regulatory agencies.

2. Serve as the administrator for the Institutional Biosafety Committee.

3. Provide all necessary documentation, forms, regulatory guidelines and regulations, etc. for Principal Investigators.

Principal Investigators (PI)

1. Ensure compliance with appropriate National Institute of Health guidelines and all conditions stated in the protocol approved by the Institutional Biosafety Committee.

2. Submit protocol applications for all activities or modifications of activities involving biohazardous materials and obtain approval by the Institutional Biosafety Committee prior to initiation of the activities or modifications.

3. Ensure that all laboratory staff, including students, are trained in the accepted procedures in laboratory practices, containment methods, disinfectant and disposal practices, and required actions in the event of an accidental spill.

4. Develop a Laboratory Safety Plan, including an emergency action plan for accidents and spills, (see addendum to this manual).

5. Ensure compliance with all shipping requirements for biological agents and toxins.

6. Ensure proper handling and disposal of all infectious wastes.

7. Request immunizations for laboratory personnel when working with biological agents for which there is an effective vaccine available.

8. Maintain all biosafety equipment in appropriate operating condition. Decontaminate laboratory equipment prior to maintenance or disposal.

9. Maintain records of microorganisms and toxins used in the laboratory and biosafety cabinets.

Laboratory Staff

1. Conduct no activities under the research protocol until the protocol is approved by the Institutional Biosafety Committee and appropriate training is completed.

2. Follow all procedures and containment methods established for activities conducted.

3. Properly utilize all laboratory protective equipment including proper clothing, personal protective equipment, and containment devices.

4. Report all accidents and spills to the Principal Investigator or the Institutional Biosafety Officer as soon as possible.

5. Report unsafe conditions to the Principal Investigator, the Institutional Biosafety Officer, or the Institutional Biosafety Committee.

PROTOCOL SUBMISSION AND REVIEW

The investigator must complete Winston-Salem State University Institutional Biosafety Committee (IBC) protocol application for the Use of Biohazardous Materials, Recombinant DNA and Infectious Agents and submitted to the IBC through *CAYUSE Hazard Safety*, the online system.

A. Protocol Application

The following application materials are required:

1. Application for the use of Biohazardous Materials, Infectious Agents and Recombinant DNA located in Appendix A.

2. Laboratory Safety Plan

A Laboratory Safety Plan must be prepared by the principal investigator and submitted to the OSPR before initiating the research project. The Laboratory Safety Plan will be

reviewed by the IBC for renewal with revisions for changes that have occurred in the laboratory during that year. Review and approval of a new Laboratory Safety Plan is required in order to continue research.

3. Training Certification.

APPLICATION PROCESSING

The Office of Sponsored Programs is responsible for coordinating the review process for applications to posses and use Recombinant DNA and infectious biological agents at Winston-Salem State University.

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Allow two weeks in advance of a proposal deadline to permit time for Committee review In the event that this is not possible, the proposal will be submitted to the sponsor to accommodate the deadline. However, the application must be completed and submitted to the IBC within 10 days of the proposal deadline. Approval to use Biohazardous Materials, Recombinant DNA and Infectious Biological Agents must be obtained prior to initiation of any research project. The Compliance Officer will prepare an official record file and forward the documents to the Chairperson of the Institutional Biosafety Committee for review.

COMMITTEE REVIEW/DECISION

Experiments at BL-1 Level of Containment

Exempt Studies

Certain studies are prohibited by the guidelines; other studies are exempt from review. However, such exemptions must be determined by the IBC after application is submitted by the principal investigator. The committee chairperson should be notified as soon as possible during the development of the proposal. Proposals which require IBC approval should be reviewed two weeks before final submission. It is not always possible for a proposal to receive IBC approval before submission. In these cases, a conditional approval shall be arranged with committee chairperson prior to submission.

All work involving recombinant DNA that is subject to the current *NIH Guidelines* must be reviewed and approved by the Institutional Biosafety Committee before such work can begin. If the application requires a BL-1 level of containment, the application may be exempt. However, such exemptions can only be determined by the IBC. The Guidelines require that the Committee be petitioned for proposed exemptions from the NIH Guidelines for those experiments, which are exempt from review and approval. The application and review process for such petitions is in accordance with the Recombinant DNA Procedure stated in this document.

The Chairperson or his/her designee (Committee Member) shall review the documents submitted and render a decision if the application is at the BL-1 level.

Experiments at Containment level BL-2

The Chairperson may select members from the Committee to serve on a subcommittee to conduct a site visit of the investigator's laboratory. The principal investigator may be asked to appear before the Committee to provide additional information. If the application requires a BL-2 level of containment, a Committee meeting will be held to review the documents submitted and to receive a report on the site visit and a certification of the BL level of containment.

No member of the Committee may be involved (except to provide information requested by the Committee) in the review or approval of a project in which he/she has been or expects to be engaged or has a direct financial interest.

It is not always possible for a proposal to receive IBC approval before submission. In these cases, a conditional approval shall be arranged with the committee chairperson prior to submission.

Notification

The Chairperson shall provide written notification of the Chairperson/Committee's decision to the principal investigator.

REVISED APPLICATIONS

1. **Revisions to Application for the use of Biohazardous Materials and Recombinant DNA**

   "No Changes to Proposal or Summary"

2. **Major Changes to Proposal**

Major changes in the proposal will require: (1). **Application for the use of Biohazardous Materials, Infectious Agents and Recombinant DNA.** These should be submitted to the CAYUSE Hazard Safety. Examples of such major changes are (a) a significant change in hosts or vectors; (b) a significant change in the physical location of the experiments; or (c) a change of the responsible investigator. The Compliance Officer will forward the materials to the Committee for review and approval.

3. Minor Changes to Proposal

- Submit an online report to the CAYUSE Hazard Safety.
- The Compliance Officer will consult with the Chairperson of the Committee and forward the material to the Committee if required.

CONTINUATION APPLICATIONS

A revised Application does not have to be submitted at the time of grant renewal if no significant changes have occurred. However, an IBC Continuing Review Form must be filled out and submitted to the Compliance Officer. A copy of the Form is attached as Appendix B.

RECORDS

The OSPR will maintain, for public viewing, minutes of IBC meetings, information about future meetings, a lay summary of each proposal submitted by principal investigators, and a list of reference material helpful to an understanding of the procedures involved in studies of Recombinant DNA molecules and infectious biological agents.

RISK ASSESSMENT AND SELECTION OF APPROPRIATE SAFEGUARDS

Risk Factors

Research involving biohazardous material is classified on the basis of perceived risk (RG1 - RG4) to humans into four biological and physical containment levels (BL1 - BL4). There are no laboratories at Winston-Salem State University certified to conduct BL-3 or BL-4 research. Risk operating levels are limited to RG1-RG2.

Risk Groups

1. Risk Group (RG1) This risk group contains agents that are not associated with disease in healthy adult humans.
2. Risk Group (RG2) This risk group contains agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.

Containment Levels

1. Biosafety Level (BL-1): The (BL-1) containment level is suitable for work involving agents of unknown or of a minimal potential hazard to laboratory personnel and the environment.
2. Biosafety Level (BL-2): This level of containment is suitable for work involving agents of a moderate potential hazard to personnel and the environment. The agents are associated with human disease, which is rarely serious, and for which preventive or therapeutic interventions are often reliable.

REGULATORY COMPLIANCE

1. Recombinant DNA activities - The NIH *Guidelines for Research Involving Recombinant DNA Molecules* governs all rDNA activities including those exempt by the guidelines.

2. Educational Activities and Non-rDNA activities involving microorganism and exempt rDNA microorganism – Activities involving these agents are not federally regulated but it is the position of the IBC that the procedures and containment levels outlined in CDC publication *Biosafety in Microbiological and Biomedical Laboratories* will govern such activities.

3. Biological Toxins - These agents are not governed by NIH or CDC regulations or guidelines. Although Material Safety Data Sheets (MSDS) are available for most of these agents, specific exposure levels, to our knowledge, have not been established. EHS will work with the PI to interpret the MSDS and to establish work and disposal procedures which will protect the users of the materials and the environment outside the laboratory.

4. Exposures to Blood-Borne Pathogens, Blood and Other Body Fluids - OSHA's standard on bloodborne pathogens will govern any activity involving human blood or other potentially infected body fluids. Compliance with this standard is administered by Health and Safety. Information on the university's blood-borne pathogen program can be found contacting the Safety Officer.

5. Chemicals - Chemical usage in educational and research laboratories are governed by OSHA Standard 1910.1450, *Occupational Exposures to Hazardous Chemicals in Laboratories*, and are administered by EHS.

6. Radioactive Materials and Radiation-Producing Devices - The radiation safety program is administered by a Radiation Safety Committee. WSSU does not currently have a Radiation Safety Committee. Please contact the Compliance Officer.

7. Disposal of Infectious Materials - Governed by the Environmental Protection Agency and administered by Health and Safety.

SAFETY AND TRAINING

Laboratory Safety Plan

All laboratory personnel must be informed of the hazards associated with the work and proper safety precautions. It is a continuing process that begins before a new employee starts laboratory work and requires regular supervision and emphasis. Each employee should receive a copy of the written Laboratory Safety Plan describing the safety precautions observed in his/her laboratory. Written information and reference material should be made available to laboratory personnel.

Biological Safety Workshops

Faculty and staff working in a laboratory involved with infectious biological agents are required to attend a Biological Safety Workshop every year held by the OSPR. Students working in laboratories where biological research with infectious agents is being conducted are required to attend a biological safety workshop every year.

These workshops will be provided by the university and may consist of topics related to biological research, laboratory safety, waste disposal methods, and state and federal regulations. Attendance at these workshops will be documented in the Laboratory Safety Plan. Failure to comply with this training requirement will result in suspension of the research project until compliance is achieved.

Biological Safety Information

The CDC/NIH publication Biological Safety in Microbiological and Biomedical Laboratories, HHS publication No. (CDC) 21-1112, 5th Edition, is an excellent resource for information pertaining to biological safety. This publication is available via the Internet at https://www.cdc.gov/labs/bmbl/. Click on http://www.cdc.gov/ and search "biological safety" to obtain additional information.

General Lab Rules

NO eating, drinking, smoking, handling contact lenses, or applying cosmetics in the laboratory at any time. Persons who wear contact lenses should also wear goggles or a face shield while working with infectious materials (respirators are available if desired).

Gloves must be worn when working with human primary cell cultures and when isolating pathogenic viruses from human body fluids.

Mouth pipetting is prohibited; mechanical pipetting devices are to be used at all times.

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

Wash hands

- after handling biohazardous materials,
- after removing gloves, and
- before leaving the laboratory.

Razor blades, scalpels, and hypodermic needles ("sharps") should be used only when absolutely needed and disposed in the proper containers.

Work surfaces are decontaminated at least once a day and after any spill of viable material.

All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving.

Plasticware should be substituted for glassware whenever possible.

Emergency Phone Numbers and Procedures

Emergency Phone Numbers

Fire and Medical Emergencies	750-2911
Police	750-2911
Principal Investigator's Home Phone	[*555-5555*]
Health Services	750-3300
Director of EHS, Eric D. Steelman	750-3466
University Compliance Officer, Dr. Islam Khan	750-2982

Emergency Procedures

In case of fire, pull the fire alarm and evacuate immediately. Appropriate judgment should be exercised in deciding whether to store or contain any hazardous materials prior to evacuation.

Any injury to a laboratory worker shall be reported immediately to the Principal Investigator, and timely and appropriate action shall be taken to evacuate such employee from the laboratory and to obtain appropriate medical treatment. Administer first aid outside of the lab if the injured person is ambulatory.

If an accident involves a biohazardous spill, move the injured person away from the spill. Inactivate the spill after attending to the injured person.
Do not attempt to move a non-ambulatory person unless it is absolutely necessary.

Remove from the injured person all protective clothing (i.e., lab coat) that may have been contaminated, and rip away the protective clothing only if necessary. Do **NOT** move the injured person to remove the protective clothing. Wash any contaminated skin with disinfectant such as Betadine or Envirocide.

Reporting Laboratory Accidents and Exposures

All laboratory accidents, which result in major spills or exposure of laboratory personnel, shall be reported immediately to the departmental chairperson, Dean, the Institutional Biosafety Committee Chairperson and the Safety Officer. A major reportable spill is a spill in which the principal investigator determines that there is any risk at all that the spill was not contained. Accidents, exposures, potential exposures, and clinical illness must be reported. Reportable accidents include any incident causing serious exposure to personnel or danger of environmental contamination. A written accident report should be submitted to the Safety Officer, within 48 hours of the accident. The Institutional Biosafety Committee is available to assist investigators in the selection of appropriate safeguards.

Proper Use of Biological Safety Cabinets ("Laminar Flow Hoods")

To assure sterility inside cabinet and establish proper air flow for containment, the blower should be turned on at least ten minutes before infectious materials are to be put in the biosafety cabinet.

The biosafety cabinet air flow ("Magnehelic") gauge should be checked (reading is equal to approximately 0.5 inches) to assure proper operation of the cabinet before placing any materials into it. Readings indicate relative pressure drop across the HEPA filter. Higher readings may, therefore, indicate filter clogging. Zero readings may indicate loss of filter integrity.

Wipe inner surfaces (especially the pan) with a solution of the disinfectant chosen as effective for the agent you are using and allow to dry. Always keep a bottle of disinfectant (e.g., 5% bleach, 70% ethanol, etc.) in the cabinet for decontaminating, or in case of a spill.

NEVER place anything over the front or rear grille of a biosafety cabinet.

Disrupting the air flow into the front grill allows contaminated air from inside the cabinet to blow into the lab or directly at the person sitting at the cabinet! It also allows non-sterile air from the room to blow into the biosafety cabinet over your experiments!

Materials should be placed in the cabinet so as not to block air flow into the rear grille. Leave a few inches for air to flow around things. Any disruption of the air flow in the cabinet decreases its effectiveness.

Remember: **"A biosafety cabinet is only as safe as the person using it."**

Before manipulating infectious materials, try to make sure that you have everything you need in the cabinet. The fewer times you pull your hands out of the cabinet, the less disruption of the air flow.

Work should be performed on the center of the work surface of the cabinet whenever possible. Work outward progressing from clean to dirty (contaminated). However, infectious agents

should not be placed directly adjacent to or directly on the intake grills.

After manipulating infectious agents, make sure all are in tightly closed containers before removing them. Wipe down the surface of all equipment used in manipulations (pipettors, etc.) with disinfectant before removing from the cabinet. All waste and disposable items should be left in the cabinet until properly decontaminated or contained.

After the cabinet has been emptied, wipe exposed surfaces including the front grille and splash area with disinfectant. Allow the blower to run for a minimum of ten minutes to purge any aerosols from inside the cabinet before shutting off the blower.

NOTE: Class IIA cabinets recirculate about 70% of the air inside themselves and exhaust the remainder to the lab. Any use of volatile solvents should be kept to a minimum or done elsewhere. Dangerously high levels of volatile fumes can accumulate inside the cabinet and pose a threat of fire or explosion.

Decontamination Procedures

Infectious agents are to remain in the Biosafety Cabinet until they are properly decontaminated. Infectious material should NEVER be in an open vessel OUTSIDE of the cabinet.

Any items used in conjunction with infectious material must be decontaminated by wiping with either 5% (v/v) diluted bleach or 70% ethanol.

Chemical Sterilization: Whenever possible, materials should be immersed in a solution of bleach (NaOCl) (household bleach diluted 1 part with 19 parts water, or 5% (v/v)) for minimum of fifteen minutes before any further handling. Disposables such as pipette tips, test tubes, petri plates, etc. should be immersed, filled, or rinsed with 5% (v/v) bleach and allowed to stand for fifteen minutes before being thrown away in a Contaminated Material Container. Immersion in 70% ethanol may be an acceptable means of decontamination for items that are incompatible with bleach.

Autoclaving: All solid, contaminated waste should be autoclaved in clear autoclavable bags. Following the appropriate sterilization cycle, the clear bag may be disposed of as regular trash.

Decontamination of liquid: This should be done by adding undiluted bleach, NaOCl, (sold as 6% w/v concentration) to a final concentration of AT LEAST 0.3% w/v NaOCl (or 5% dilution). Also rinse the vessel with bleach provided in the wash bottle in the cabinet. Mix well and allow to stand for fifteen minutes before being poured into the drain. Rinse with copious amounts of cold water. Liquid waste that is not compatible with NaOCl should be autoclaved for at least 30 minutes, using slow exhaust before disposal.

Volatile or organic solvents such as fixatives that are, by their nature, toxic to biological materials need not be chemically decontaminated or autoclaved.

Plasticware and other reusable items: These items are to be chemically decontaminated and

rinsed (whenever possible) before removal.

Note: Avoid autoclaving large quantities of bleach solution. Flush decontaminated liquids down the drain and rinse non-disposable items with water before removal. If it is impractical to rinse items, the NaOCl (bleach) should be neutralized by adding 1 mL of 5% sodium thiosulfate per mL of 5% hypochlorite ion.

Mixtures of Infectious Agents and Radiochemicals

The infectious agent should be neutralized first then the whole solution can be treated as radioactive waste. Care should be taken in choosing a chemical disinfectant. Some can cause more problems that they solve. **(e.g., Chlorine compounds such as bleach should NOT be used to disinfect anything containing ^{125}I because the chlorine will cause the volatilization of radioactive iodine!)**

Spills

Inside of a containment device: Please refer to Appendix G of the Biosafety Process Manual.

Leaking tubes in the centrifuge: If the rotor is sealed, and removable, place it in the biosafety cabinet before opening. Before attempting to deal with the leak, prepare a pan of disinfectant solution large enough to immerse the entire rotor in (iodine based solutions are recommended over Bleach because of corrosion). Don any protective gear needed to clean up spills and carefully remove the lid from the rotor. Retrieve unbroken tubes, wipe outside with disinfectant, and leave them in the cabinet, out of the way. The broken tube should be removed with a forceps or other instrument if possible and immersed in a beaker of disinfectant solution for a minimum of fifteen minutes. All instruments and rotor pieces involved in the incident should be chemically decontaminated before re-use.

After proper decontamination, instruments and rotor pieces may be washed with a mild detergent according to the manufacturer's instructions. As an added measure of caution, the inside of the centrifuge (chamber) may be wiped out with a non-corrosive disinfectant.

If a tube has broken in a centrifuge that does not use a containment type rotor, DO NOT open the centrifuge. Turn off the power and allow sufficient time for aerosols that have been created to settle (approximately 30 min.). Don protective garb and respiratory protection before opening the chamber. Decontaminate the inside of the chamber with a noncorrosive disinfectant (70% Ethanol or Wescodyne) by thoroughly soaking the interior. A spray bottle or lab squeeze bottle is sufficient. Large amounts of liquid generated during decontamination may be removed by a disposable pipette attached to suction device attached to a disinfectant trap. Any paper waste generated during clean-up should be bagged and autoclaved.

Note: It is recommended that a sealed rotor or bucket be used when centrifuging infectious materials. If none is available, placing a **smaller** tube inside a larger sealable tube can provide some protection against aerosol creation in the event of breakage.

Biological/Radioactive emergencies/spills: These types of spills should be handled similarly to spills of infectious agents. The Safety Officer (750-2868) should be notified and may want to assist in clean-up. Determine if anyone has been contaminated; remove contaminated clothing and wash contaminated skin with soap and water. The lab should be evacuated, especially if an aerosol was generated.

The infectious agent should be neutralized first, taking care when choosing a disinfecting agent to avoid chemical incompatibility. For example, hypochlorite (bleach) will volatize radioactive iodine. Refer to the section of this manual that covers clean-up of spills for more details.

All major spills must be reported to the Principal Investigator. A major spill is one in which: 1) hazardous materials contact skin, eyes, etc., 2) a break in the skin occurs, 3) the spill splashes over an area larger than one foot in diameter, 4) the extent of the spill is undetermined, or 5) the spill involves an aerosol.

Cleaning Procedures

Pans and splash guards (inside and out) should be cleaned after every use.

Important things to wipe daily: 1) door handles and 2) water faucets on non-foot pedal operated sinks.

Pipettors and other shared small equipment should be wiped with 70% ethanol after use, ***before*** removing them from the cabinet.

Specimen Transport

Human specimens and diagnostic samples are frequently transported from clinical collection sites to research facilities. A properly labeled (biosafety sticker with specified agent identity) leak-proof transport carrier is available exclusively for this purpose. Examples of acceptable containers include either a Playmate® cooler, or the Nalgene® Bio Transport Carrier.

Packaging and Shipment of Biological Materials

The importation or shipments of biological materials are governed by the Center for Disease Control. Information required of the PI for receipt or shipment is contained on pages 415-422 (Appendix C) of CDC's *Biosafety in Microbiological and Biomedical Laboratories*.

Use of Animals

The requirements for the use of animals with biohazardous agents are similar to, but not identical to, the requirements for the use of the same agent in laboratory situations. The PI, in conjunction with Institutional Animal Care and Use Committee (IACUC), is responsible for determining the

appropriate Animal Biosafety Level (ABSL) for the specific agent being utilized. NIH's *Guidelines for Research Involving Recombinant DNA Molecules* or CDC's *Biosafety in Microbiological and Biomedical Laboratories* should be consulted for the appropriate classification and requirements for the use of the proposed agent.

General Lab Rules

NO eating, drinking, smoking, handling contact lenses, or applying cosmetics in [*Room XXXX* or *Room YYYY*] at any time. Persons who wear contact lenses should also wear goggles or a face shield while working with infectious materials (respirators are available if desired).

Gloves must be worn when working with human primary cell cultures and when isolating pathogenic viruses from human body fluids.

Mouth pipetting is prohibited; mechanical pipetting devices are to be used at all times.

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

Wash hands

- after handling biohazardous materials,
- after removing gloves, and
- before leaving the laboratory.

Razor blades, scalpels, and hypodermic needles ("sharps") should be used only when absolutely needed in [*Room XXXX*].

Work surfaces are decontaminated at least once a day and after any spill of viable material [*specify the appropriate disinfectant*].

All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving.

Plasticware should be substituted for glassware whenever possible.

Emergency Phone Numbers and Procedures

Emergency Phone Numbers

Fire and Medical Emergencies	750-2911
Police	750-2911
Principal Investigator's Home Phone	[*555-5555*]
Health Services	750-3300
Safety Officer,	[*555-5555*]
Compliance Officer, Dr. Islam Khan	750-2982

Emergency Procedures

In case of fire, pull the fire alarm and evacuate immediately. Appropriate judgment should be exercised in deciding whether to store or contain any

hazardous materials prior to evacuation.

Any injury to a laboratory worker shall be reported immediately to [*the Principal Investigator*], and timely and appropriate action shall be taken to evacuate such employee from the laboratory and to obtain appropriate medical treatment. Administer first aid outside of the lab if the injured person is ambulatory.

If an accident involves a biohazardous spill, move the injured person away from the spill. Inactivate the spill after attending to the injured person.
Do not attempt to move a non-ambulatory person unless it is absolutely necessary.

Remove from the injured person all protective clothing (i.e., labcoat) that may have been contaminated, and rip away the protective clothing only if necessary. Do **NOT** move the injured person to remove the protective clothing. Wash any contaminated skin with disinfectant such as Betadine or Envirocide.

Proper Use of Biological Safety Cabinets ("Laminar Flow Hoods")

To assure sterility inside cabinet and establish proper air flow for containment, the blower should be turned on at least ten minutes before infectious materials are to be put in the biosafety cabinet.

The biosafety cabinet air flow ("Magnehelic") gauge should be checked (reading is equal to approximately 0.5 inches) to assure proper operation of the cabinet before placing any materials into it. Readings indicate relative pressure drop across the HEPA filter. Higher readings may, therefore, indicate filter clogging. Zero readings may indicate loss of filter integrity.

Wipe inner surfaces (especially the pan) with a solution of [*the disinfectant chosen as effective for the agent you are using*] and allow to dry. Always keep a bottle of disinfectant (e.g., 5% bleach, 70% ethanol, etc.) in the cabinet for decontaminating, or in case of a spill.

NEVER place anything over the front or rear grille of a biosafety cabinet.

Disrupting the air flow into the front grill allows contaminated air from inside the cabinet to blow into the lab or directly at the person sitting at the cabinet! It also allows non-sterile air from the room to blow into the biosafety cabinet over your experiments!

Materials should be placed in the cabinet so as not to block air flow into the rear grille. Leave a few inches for air to flow around things. Any disruption of the air flow in the cabinet decreases its effectiveness.

Remember: **"A biosafety cabinet is only as safe as the person using it."**

Before manipulating infectious materials, try to make sure that you have everything you need in the cabinet. The fewer times you pull your hands out of the cabinet, the less disruption of the air flow.

Work should be performed on the center of the work surface of the cabinet whenever possible. Work outward progressing from clean to dirty (contaminated). However, infectious agents should not be placed directly adjacent to or directly on the intake grills.

After manipulating infectious agents, make sure all are in tightly closed containers before removing them. Wipe down the surface of all equipment used in manipulations (pipettors, etc.) with disinfectant before removing from the cabinet. All waste and disposable items should be left in the cabinet until properly decontaminated or contained.

After the cabinet has been emptied, wipe exposed surfaces including the front grille and splash area with disinfectant. Allow the blower to run for a minimum of ten minutes to purge any aerosols from inside the cabinet before shutting off the blower.

NOTE: Class IIA cabinets recirculate about 70% of the air inside themselves and exhaust the remainder to the lab. Any use of volatile solvents should be kept to a minimum or done elsewhere. Dangerously high levels of volatile fumes can accumulate inside the cabinet and pose a threat of fire or explosion.

Decontamination Procedures

Infectious agents are to remain in the Biosafety Cabinet until they are properly decontaminated. Infectious material should NEVER be in an open vessel OUTSIDE of the cabinet.

Any items used in conjunction with infectious material must be decontaminated by wiping with either 5% (v/v) diluted bleach or 70% ethanol.

Chemical Sterilization: Whenever possible, materials should be immersed in a solution of bleach (NaOCl) (household bleach diluted 1 part with 19 parts water, or 5% (v/v)) for minimum of fifteen minutes before any further handling. Disposables such as pipette tips, test tubes, petri plates, etc. should be immersed, filled, or rinsed with 5% (v/v) bleach and allowed to stand for fifteen minutes before being thrown away in a Contaminated Material Container. Immersion in 70% ethanol may be an acceptable means of decontamination for items that are incompatible with bleach.

Autoclaving: All solid, contaminated waste should be autoclaved in clear

autoclavable bags. Following the appropriate sterilization cycle, the clear bag may be disposed of as regular trash.

Decontamination of liquid: This should be done by adding undiluted bleach, NaOCl, (sold as 6% w/v concentration) to a final concentration of AT LEAST 0.3% w/v NaOCl (or 5% dilution). Also rinse the vessel with bleach provided in the wash bottle in the cabinet. Mix well and allow to stand for fifteen minutes before being poured into the drain. Rinse with copious amounts of cold water. Liquid waste that is not compatible with NaOCl should be autoclaved for at least 30 minutes, using slow exhaust before disposal.

Volatile or organic solvents such as fixatives that are, by their nature, toxic to biological materials need not be chemically decontaminated or autoclaved.

Plasticware and other reusable items: These items are to be chemically decontaminated and rinsed (whenever possible) before removal from Room [*YYYY*].

Note: Avoid autoclaving large quantities of bleach solution. Flush decontaminated liquids down the drain and rinse non-disposable items with water before removal from Room [*YYYY*]. If it is impractical to rinse items, the NaOCl (bleach) should be neutralized by adding 1 mL of 5% sodium thiosulfate per mL of 5% hypochlorite ion.

Mixtures of Infectious Agents and Radiochemicals

The infectious agent should be neutralized first then the whole solution can be treated as radioactive waste. Care should be taken in choosing a chemical disinfectant. Some can cause more problems that they solve. **(e.g., Chlorine compounds such as bleach should NOT be used to disinfect anything containing ^{125}I because the chlorine will cause the volatilization of radioactive iodine!)**

Spills

Inside of a containment device: Please refer to Appendix G of the Biosafety Process Manual.

Leaking tubes in the centrifuge: If the rotor is sealed, and removable, place it in the biosafety cabinet before opening. Before attempting to deal with the leak, prepare a pan of disinfectant solution large enough to immerse the entire rotor in (iodine based solutions are recommended over Bleach because of corrosion). Don any protective gear needed to clean up spills and carefully remove the lid from the rotor. Retrieve unbroken tubes, wipe outside with disinfectant, and leave them in the cabinet, out of the way. The broken tube should be removed with a forceps or other instrument if possible and immersed in a beaker of

disinfectant solution for a minimum of fifteen minutes. All instruments and rotor pieces involved in the incident should be chemically decontaminated before re-use.

After proper decontamination, instruments and rotor pieces may be washed with a mild detergent according to the manufacturer's instructions. As an added measure of caution, the inside of the centrifuge (chamber) may be wiped out with a non-corrosive disinfectant.

If a tube has broken in a centrifuge that does not use a containment type rotor, DO NOT open the centrifuge. Turn off the power and allow sufficient time for aerosols that have been created to settle (approximately 30 min.). Don protective garb and respiratory protection before opening the chamber. Decontaminate the inside of the chamber with a noncorrosive disinfectant (70% Ethanol or Wescodyne) by thoroughly soaking the interior. A spray bottle or lab squeeze bottle is sufficient. Large amounts of liquid generated during decontamination may be removed by a disposable pipette attached to suction device attached to a disinfectant trap. Any paper waste generated during clean-up should be bagged and autoclaved.

Note: It is recommended that a sealed rotor or bucket be used when centrifuging infectious materials. If none is available, placing a **smaller** tube inside a larger sealable tube can provide some protection against aerosol creation in the event of breakage.

Spills of infectious material outside of a containment device: to Please refer to Appendices H and I of the Biosafety Process Manual.

Biological/Radioactive emergencies/spills: These types of spills should be handled similarly to spills of infectious agents. Determine if anyone has been contaminated; remove contaminated clothing and wash contaminated skin with soap and water. The lab should be evacuated, especially if an aerosol was generated. Report spill to Safety Director.

The infectious agent should be neutralized first, taking care when choosing a disinfecting agent to avoid chemical incompatibility. For example, hypochlorite (bleach) will volatize radioactive iodine. Refer to the section of this manual that covers clean-up of spills for more details.

All spills of any nature must be described in your laboratory notebook. The description must include: 1) the type of spill, 2) the time and date it happened, 3) the time and date it was cleaned up, and 4) the time and date you autoclaved the waste from the spill.

All major spills must be reported to [*the Principal Investigator*]. A major spill is one in which: 1) hazardous materials contact skin, eyes, etc., 2) a break in the

skin occurs, 3) the spill splashes over an area larger than one foot in diameter, 4) the extent of the spill is undetermined, or 5) the spill involves an aerosol.

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VALIDATION AND HISTORY FOR BIOSAFETY PLAN

Principal Investigator Certification

I hereby certify that I have reviewed the contents of this manual and that it reflects my current operating policy for the laboratories #[*XXXX*] and [*YYYY*] located in [*Location*] research building.

[*Principal Investigator's Name*]
[*Principal Investigator's Title*]

Signature _____ Annual Review Date _____

Date Created:

APPENDIX A

HIV Research Occupational Health Policy

This policy is designed to protect employees who conduct research with HIV products. Researchers who handle manipulate, or assay live HIV cultures are covered under this policy. Winston-Salem State University requires the following of all employees and students who conduct HIV research.

Pre-laboratory employment: This is a requirement to work in the HIV lab Minors (under 18) are not allowed in HIV research labs.

Prior to beginning work in an HIV Research lab, an employee, student shall be given the following:

1. If the HIV researcher also works with human blood or other potentially infected material, all other aspects of the Bloodborne Pathogen Program (1) under the OSHA Bloodborne Pathogen standards (2) shall be implemented for that employee. The employer shall be offered free Hepatitis B virus screening and immunization, annual training, and have a written exposure control plan in the workplace.

2. The principal investigator is also required to establish criteria for "demonstrated proficiency in standard microbiological practices" for employees/students prior to them being allowed to work with HIV. This may include prior experience, work with other BSL2 or BSL3 agents, or specific training provided by the PI. This training does not have specific guidelines because it really is dependent on the type of research they are doing and the PI has the most intimate knowledge of what skills his employees need to have to be safe and successful.

Optional:
A confidential HIV test can be obtained by the employee at the local county Health Department. This test shall include HIV pretest and post test counseling. Test results shall be given to the employee in person in a face-to-face meeting with an infectious disease expert.

Post-exposure prophylaxis:
An exposure to an HIV culture in a research laboratory is considered a high-risk exposure according to Public Health Service CDC Guidelines (3). The following shall be implemented:

1. If the exposure is to intact or broken skin, or in the event of a puncture wound, immediately wash the affected area with water for five minutes. Soap may be used, if immediately available. If the exposure is to the eyes, they shall be rinsed for five minutes in an eyewash station. Other exposed mucous membranes (nose and mouth) shall be rinsed for five minutes with water.

2. The employee shall proceed immediately to the Alexander H. Ray Student Health

Center, located in the Thompson Student Center, (336) 750-3301 open Monday through Friday 8:00 a.m. to 9:00 p.m., or contact Campus Police at (336) 750-2911. It is important for the employee to seek treatment within the first two hours of exposure.

3. The current Public Health Service (CDC) chemoprophylactic treatment/ post-exposure prophylaxis (PEP) will be recommended to an employee with a high-risk exposure, within the first two hours of exposure upon evaluation. The medical use of chemoprophylaxis is a decision that will be made by an HIV specialist in conjunction with the employee, based upon the most current CDC recommendations, the nature of the exposure event, and other medical factors.

4. Counseling concerning the risks and benefits of the chemoprophylactic treatment may be given to the employee at the time of the initial evaluation after the exposure event. PEP will be initiated after obtaining consent from the employee. Baseline labs will be done at that time and followed by three and six month intervals.

5. Post-exposure follow-up, including the offer of HIV testing and counseling, shall be given in accordance with the CDC recommendations and the OSHA Bloodborne Pathogen rules.

References

1. OSHA. 29 CFR Part 19 10.1030. Occupational Exposure to Bloodborne Pathogens, Final Rule, Dec. 6, 1991
2. CDC. Public Health Service Guidelines for the Management of Health-Care Worker Exposures to HIV and Recommendations for Post exposure Prophylaxis; MMWR 1998
3. Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendations for Post exposure Prophylaxis MMWR June 29, 2001 / Vol. 50 / No. RR--11