IIII. BIOHAZARDS AND POTENTIALLY INFECTIOUS MATERIALS

A. Definition -- Biological safety or “biosafety” is the application of knowledge, techniques and equipment to prevent personal, laboratory and environmental exposure to potentially infectious agents or biohazards. Biosafety defines the containment conditions under which infectious agents can be safely manipulated. The objective of containment is to confine biohazards and to reduce the potential exposure of the laboratory worker, persons outside of the laboratory, and the environment to potentially infectious agents.

B. Biological Agent Classification

1. Risk Assessment

It is the responsibility of the principal investigator or laboratory director to conduct a risk assessment to determine the proper work practices and containment requirements for work with biohazardous material. The risk assessment process should identify features of microorganisms as well as host and environmental factors that influence the potential for workers to have a biohazard exposure. This responsibility cannot be shifted to inexperienced or untrained personnel. The risk assessment of any research project must be approved by IBC.

The principal investigator or laboratory director should consult with a Biosafety Officer to ensure that the laboratory is in compliance with established guidelines and regulations. When performing a risk assessment, it is advisable to take a conservative approach if there is incomplete information available. Factors to consider when evaluating risk include the following:

- **Pathogenicity:** The more severe the potentially acquired disease, the higher the risk. Salmonella, a Risk Group 2 agent, can cause diarrhea, septicemia if ingested. Treatment is available. Viruses such as Ebola, Marburg, and Lassa fever cause diseases with high mortality rates. There are no vaccines or treatment available. These agents belong to Risk Group 4.

- **Route of transmission:** Agents that can be transmitted by the aerosol route have been known to cause the most laboratory-acquired infections. The greater the aerosol potential, the higher the risk of infection. Work with *Mycobacterium tuberculosis* is performed at Biosafety Level 3 because disease is acquired via the aerosol route.

- **Agent stability:** The greater the potential for an agent to survive in the environment, the higher the risk. Consider factors such as desiccation, exposure to sunlight or ultraviolet light, or exposure to chemical disinfections when looking at the stability of an agent.

- **Infectious dose:** Consider the amount of an infectious agent needed to cause infection in a normal person. An infectious dose can vary from one to hundreds of thousands of organisms or infectious units. An individual’s immune status can also influence the infectious dose.

- **Concentration:** Consider whether the organisms are in solid tissue, viscous blood, sputum, etc., the volume of the material and the laboratory work planned (amplification of the material, sonication, centrifugation, etc.). In most instances, the risk increases as the concentration of microorganisms increases.

- **Origin:** This may refer to the geographic location (domestic or foreign), host (infected or uninfected human or animal), or nature of the source (potential zoonotic or associated with a disease outbreak).
Availability of data from animal studies: If human data is not available, information on the pathogenicity, infectivity, and route of exposure from animal studies may be valuable. Use caution when translating infectivity data from one species to another.

Availability of an effective prophylaxis or therapeutic intervention: Effective vaccines, if available, should be offered to laboratory personnel in advance of their handling of infectious material. However, immunization does not replace engineering controls, proper practices and procedures and the use of personal protective equipment (PPE). The availability of post-exposure prophylaxis should also be considered.

Medical surveillance: Medical surveillance programs may include monitoring employee health status, participating in post-exposure management, employee counseling prior to offering vaccination, and annual physicals.

Experience and skill level of at-risk personnel: Laboratory workers must become proficient in specific tasks prior to working with microorganisms. Laboratory workers may have to work with non-infectious materials to ensure they have the appropriate skill level prior to working with biohazardous materials. Laboratory workers may have to go through additional training (e.g., HIV training, BSL-3 training, etc.) before they are allowed to work with materials or in a designated facility.

Refer to the following resources to assist in your risk assessment:
NIH Recombinant DNA Guidelines
WHO Biosafety Manual
Biosafety in Microbiological & Biomedical Laboratories, 4th ed. (CDC/NIH)

2. Biosafety Levels
Biosafety levels are established by the Centers for Disease Control and Prevention to control biological hazards by means of handling practices, safety equipment (primary barriers) and facility design and construction (secondary barriers). Ranked from Biosafety Level 1 through 4, each subsequent biosafety level adds higher degrees of control aimed at preventing worker exposure and release of pathogens into the community.

Vertebrate animal biosafety levels also exist. These were created to account for human activities as well as potential hazards from animals themselves (biting, scratching, generation of allergens, and release of zoonotic disease). For a full discussion of biosafety levels consult Biosafety in Microbiological & Biomedical Laboratories, 4th ed. (CDC/NIH)
The following table is an overview of biosafety levels.

<table>
<thead>
<tr>
<th>BSL</th>
<th>Agents</th>
<th>Practices</th>
<th>Safety Equipment (primary barriers)</th>
<th>Facilities (secondary barriers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not known to consistently cause disease in healthy adults</td>
<td>Standard microbiological practices</td>
<td>None required</td>
<td>Open bench top sink required</td>
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<tr>
<td>2</td>
<td>Associated with human disease. Hazard = percutaneous injury, ingestion, mucous membrane exposure</td>
<td>BSL-1 practice plus; • Limited access • Biohazard warning • “Sharps” precautions • Biosafety manual</td>
<td>Use of Class I or II BSCs or other physical containment device. Lab coats, gloves, face protection as needed</td>
<td>BLS-1 plus • Autoclave available</td>
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<tr>
<td>3</td>
<td>Indigenous or exotic with potential for aerosol transmission. Disease may have serious or lethal consequences</td>
<td>BSL-2 practice plus; • controlled access • decontamination of wastes • decontamination of lab clothing • baseline serum</td>
<td>Primary Barriers = Class I or II BSCs or other physical containment devices used for all open manipulations of agents. Protective lab clothing, gloves, respiratory protection as needed</td>
<td>BSL-2 plus; • Physical separation from access corridors • Self-closing, double-door access • Exhausted air not recirculated • Negative airflow into laboratory</td>
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<tr>
<td>4</td>
<td>Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections, or related agents with unknown risk of transmission</td>
<td>BSL-3 practices plus; • Clothing change before entry • Shower on exit • All material decontaminated on exit from facility</td>
<td>Primary barriers – all procedures conducted in Class III BSC or class I or II BSC in combination with full-body, air-supplied, positive pressure suit</td>
<td>BSL-3 plus; • Separate building or isolated zone • Dedicated supply &amp; exhaust, vacuum and decon systems • Other requirements outlined in the text (BMBL)</td>
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3. Risk Groups
Infectious agents may be classified into risk groups based on their relative hazard. The table below, which was excerpted from the NIH Recombinant DNA Guidelines, presents the basis for the classification of biohazardous agents by risk group.

<table>
<thead>
<tr>
<th>Risk Group 1 (RG1)</th>
<th>Agents that are not associated with disease in healthy adult humans</th>
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</thead>
<tbody>
<tr>
<td>Risk Group 2 (RG2)</td>
<td>Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available</td>
</tr>
<tr>
<td>Risk Group 3 (RG3)</td>
<td>Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk)</td>
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<tr>
<td>Risk Group 4 (RG4)</td>
<td>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)</td>
</tr>
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</table>

C. Categories of Biohazards or Potentially Infectious Materials
1. Human, animal and plant pathogens:
   - Bacteria, including those with drug resistance
   - Plasmids
   - Fungi
   - Viruses, including oncogenic viruses:
• Parasites
• Prions
2. All human blood, blood products, tissues and certain body fluids.
3. Cultured cells (all human or certain animal) and potentially infectious agents these cells may contain.
4. Allergens.
5. Toxins (bacterial, fungal, plant, etc.).
6. Certain recombinant products (rDNA).
7. Clinical specimens.
8. Infected animals and animal tissues.

D. Recombinant DNA (rDNA)

1. Generation of rDNA
Experiments involving the generation of rDNA may require registration and approval by the IBC. The National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules is the definitive reference for rDNA research in the United States. Experiments not covered by the guidelines may require review and approval by outside agencies before initiation or funding. These experiments are not generally associated with biomedical research but are more common in the agricultural and environmental sciences.

If the experimental protocol is not covered by the guidelines, contact the Environmental Health and Radiation Safety Department by phone (215-707-2520) or email, for determination of further review.

If you have any specific questions about an organism not covered by the guidelines, contact the Office of Biotechnology Activities (OBA), National Institutes of Health by phone (301) 496-9838, FAX (301) 496-9839 or email. Updates to the NIH Recombinant DNA Guidelines are published in the Federal Register and are available at the OBA website.

2. Human Gene Transfer
All protocols involving the generation of rDNA for human gene transfer must be approved locally by the IBC and the IRB prior to submission to outside agencies and the initiation of experimentation.

3. Human Recombinant Vaccine Trials
Recombinant vaccine trials must be reviewed and approved by the IBC and the Institutional Review Board (IRB) before research participants can be enrolled. For more details about IBC approval of human recombinant vaccine protocols, call 215-204-6938 or 215-707-3390.

4. Transgenic Animals
Investigators who create transgenic animals must complete an rDNA registration document and submit it to EHRS for IBC approval prior to initiation of experimentation. In addition, an Institutional Animal Care and Use Committee (IACUC) protocol review form must be approved by IACUC. Contact the IACUC & IBC Coordinator for more information.

5. Transgenic Plants
Experiments to genetically engineer plants by recombinant DNA methods may require registration with the IBC. The NIH rDNA guidelines provide specific plant biosafety containment recommendations for experiments involving the creation and/or use of genetically engineered plants.

E. Other Potentially Hazardous Biological Materials

1. Human Blood, Blood Products, Body Fluids and Tissues
In 1991, the Occupational Safety and Health Administration (OSHA) promulgated a standard to eliminate or minimize occupational exposure to Hepatitis B Virus (HBV), Human
Immunodeficiency Virus (HIV) and other bloodborne pathogens. This federal regulation, "Occupational Exposure to Bloodborne Pathogens," mandates a combination of engineering and work practice controls, training, Hepatitis B vaccination, and other provisions to help control the health risk to employees resulting from occupational exposure to human blood and other potentially infectious materials which may contain these or other specified agents.

Biosafety Level 2 practices and procedures must be followed when handling human blood, blood products, body fluids and tissues because of the infectious agents they may contain. Biosafety Level 2 practices and procedures, consistent with "Standard Precautions" (previously known as Universal Precautions), requires all specimens of human blood or other potentially infectious materials to be treated as if they are infectious.

Free Hepatitis B vaccinations are available to all occupationally at-risk University employees through Occupational Employee Health (OEH) at the Temple University Hospital. Mandatory safety training that provides information on protection from occupational exposure to infectious materials is offered by EHRS on a monthly basis university-wide. For more information on the availability of free Hepatitis B vaccine, phone OEH at 215-707-4455. Training dates are available at the EHRS website.

Investigators using human blood, blood products, body fluids or tissues must complete a site-specific Exposure Control Plan. The completed plan must be readily available in the laboratory for all workers and for inspection.

Laboratory personnel (faculty, staff and students) who work in HIV or HBV research laboratories must fulfill additional OSHA requirements as follows:

a. The employee must attend EHRS New Employee Orientation, which includes Bloodborne Pathogens training, and must annually update Bloodborne Pathogens training thereafter.

b. The employee must have prior experience in the handling of human pathogens or tissue cultures before working with HIV or HBV.

c. In the laboratory, the employee must demonstrate proficiency in standard microbiological practices and techniques and in the practices and operations specific to the laboratory to the satisfaction of the principal investigator/laboratory supervisor before being allowed to work with HIV or HBV.

a. An employee with no prior experience in handling human pathogens must be trained in the laboratory prior to handling infectious materials. Initial work activities shall not include handling of infectious agents. A progression of work activities will be assigned as techniques are learned and proficiency is developed. Participation in work activities involving infectious agents will be allowed only after proficiency has been demonstrated to the satisfaction of the principal investigator/laboratory supervisor.

2. **Use of Animals**

The use of animals in research requires compliance with the Animal Welfare Act and any state or local regulations covering the care or use of animals, and approval of all research with animals by IACUC. Facilities for laboratory animals used for studies of infectious or non-infectious disease should be physically separate from clinical laboratories and facilities that provide patient care.

Vertebrate animal biosafety level criteria must be adhered to where appropriate. All animal protocols involving the use of rDNA; infectious or transmissible agents; human blood, body fluids or tissues; toxins; carcinogenic, mutagenic, teratogenic chemicals; or physically hazardous chemicals (reactive, explosive, etc.) must be submitted to EHRS for review and approval prior to final approval by the Institutional Animal Care and Use Committee.
The PI must notify EHRS in writing prior to initiation of experimentation at Animal Biosafety Level 2 or Animal Biosafety Level 3. Investigators who are uncertain how to categorize agents should call EHRS (215-707-2520).

3. **Tissue Culture / Cell Lines**

When cell cultures are known to contain an etiologic agent or an oncogenic virus, the cell line should be classified as the same level as that recommended for the agent.

*Established human cell lines* which are certified to be free of contamination from human hepatitis viruses, human immunodeficiency viruses, and other recognized bloodborne pathogens, are not considered to be other potentially infected material (OPIM) and are not covered by OSHA's Bloodborne Pathogen Standard.

Established human or other animal cell lines which are not known to be free of human pathogens or which are likely to be infected/contaminated with human microbes or agents classed as bloodborne pathogens, especially hepatitis viruses and human immunodeficiency viruses are covered by the OSHA's Bloodborne Pathogen Standard. The final judgment for making the determination that human or other animal cell lines in culture are free of bloodborne pathogens must be made by a biosafety professional in accordance with the requirements of the OSHA’s Bloodborne Pathogen Standard. Documentation that such cell lines are not potentially infectious materials, by means of certification from the supplier, should be a matter of written record and on file with the employer for OSHA review.

*Primate cell lines* derived from lymphoid or tumor tissue, all cell lines exposed to or transformed by a primate oncogenic virus, all clinical material (e.g., samples of human tissues and fluids obtained after surgical resection or autopsy), all primate tissue, all cell lines new to the laboratory (until proven to be free of all adventitious agents) and all virus and mycoplasma-containing primate cell lines are classified as Risk Group 2 and should be handled at Biosafety Level 2.

Studies involving suspensions of HIV prepared from T cell lines must be handled using Biosafety Level 3 practices and procedures.

Primate cell lines are covered by the OSHA's Bloodborne Pathogen Standard.

4. **Use of Mycobacterium tuberculosis in Research**

Tuberculosis (TB) is an airborne infection caused by the bacterium *Mycobacterium tuberculosis*. Although TB primarily affects the lungs, other organs and tissues may be affected as well.

**Laboratory**

Investigators intending to work with *Mycobacterium tuberculosis* in the laboratory must submit an Assurance on Hazardous Procedures form to EHRS before beginning work. Requirements imposed by EHRS must be met. Biosafety Level 2 practices and procedures, containment equipment, and facilities are required for nonaerosol-producing manipulations of clinical specimens. Propagation and manipulation of *Mycobacterium tuberculosis* cultures must be performed in a Biosafety Level 3 facility using Biosafety Level 3 practices and procedures. An agent summary statement for *Mycobacterium tuberculosis* can be found at the CDC website.

F. **Etiologic Agents**

The Public Health Service, through the Centers for Disease Control and Prevention (CDC), regulates importation and interstate shipment of certain biological agents termed, “etiologic agents”. A list of etiologic agents (42CFR72.3) is provided by CDC. If importing into the United States, the CDC will require the opportunity to review the material. The CDC will also require notification for interstate shipments of SARs or of a Select Biological Agent.
G. Select Agents
The federal government, through the Department of Health and Human Services and the Department of Agriculture, regulates certain biological agents and toxins that are considered a threat to the public health, animal or plant health and animal or plant products. The current list of these Select Agents is available directly from the CDC website. Investigators must register with the EHRS Select Agent Program, IBC and the appropriate federal agency prior to possession, use or transfer of any Select Agent. Investigators should contact EHRS (215-707-2520) to register any research project involved in using a select agent.