

II. Biohazards and Potentially Infectious Materials

A. Definition

An agent of biological origin that has the capacity to produce deleterious effects on humans, i.e. microorganisms, toxins, and allergens derived from those organisms; and allergens and toxins derived from higher plants and animals.

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 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)

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."

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

Laboratories and animal facilities are classified according to their design features, construction and containment facilities. These designations, called biosafety levels and animal biosafety levels respectively, provide appropriate containment for the various risk group agents. Biosafety Levels and Animal Biosafety Levels are discussed in greater detail below.

C. Categories of biohazards or potentially infectious materials:

1. Human, animal and plant pathogens:

- Bacteria, including those with drug resistance (See Appendix 1 [A-K] and Appendix 2 [L-Z])
 - Plasmids
 - Fungi (See Appendix 3)
 - Viruses, including oncogenic viruses (See Appendix 4 [A-K] and Appendix 5 [L-Z])
 - **Parasites** (See Appendix 6)
 - **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.
 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 Office of Environmental Health and Radiation Safety by phone (215- 898-4453) or email*, for determination of further review.

If you have any specific questions about a particular host-vector system 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 Institutional Review Board (IRB) prior to submission to outside agencies and the initiation of experimentation. For more details about IBC approval of human gene transfer protocols, call 215-898-4453. For information about IRB submissions, call 215-898-2614.

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 215-898-4453.

4. Transgenic Animals

Investigators who create transgenic animals must complete a 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 EHRS prior to receiving IACUC approval.

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. Copies of the University's

rDNA registration document and a copy of current NIH guidelines are available at the EHRS website.

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 vaccination is available to all occupationally at-risk University employees through Occupational Medicine at the Hospital of the University of Pennsylvania. 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, email or phone EHRS at 215-898-4453. Training dates are available at the EHRS website.

Investigators using human blood, blood products, body fluids or tissues must complete an Exposure Control Plan. The completed plan must be readily available in the laboratory for all workers. In addition, investigators must consult with the Office of Regulatory Affairs (ORA) at 215-898-2614 to ensure that all regulatory requirements relating to the use of human materials or subjects in research are met.

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

A. The employee must attend EHRS *Introductory Laboratory and Biological Safety Training at Penn* and annual update 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.

D. 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.

E. The employee must view the video "Working Safely with HIV in the Laboratory". A copy of the video is available for loan from EHRS (email or phone EHRS at 215-898-4453).

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. 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. PIs whose protocols involve bringing animals into hospital spaces must receive written permission from HUP Infection Control (215-662-6995).

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 (IACUC). The PI must notify ULAR and EHRS in writing prior to initiation of experimentation at Animal Biosafety Level 2 or Animal Biosafety Level 3. IACUC "guidelines" are available from ORA. Investigators who are uncertain how to categorize agents should email or call EHRS (215-898-4453).

Because of the possibility of exposure to potentially infectious agents (i.e., Rabies virus, Cercopithecine herpesvirus 1, Coxiella burnetti), ULAR and the School of

Veterinary Medicine have developed policies to protect personnel and students who have animal contact. These policies should be consulted and followed when working with animals that may harbor these agents.

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 characterized 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 known to be or likely 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 judgement 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 or other qualified scientist with the background and experience to review such potential contamination and risk, in accordance with the requirements of the OSHA's Bloodborne Pathogen Standard. Documentation that such cell lines are not OPIM 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.

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. After decades of decline, the incidence of TB began increasing again in 1985. By 1992, the incidence had increased over 20 percent. Starting in 1992, however, the trend reversed, and the rate began to decline. The rate of reported tuberculosis has dropped 47 percent between 1992 and 2001. This has been attributed to improved TB control programs.

Outbreaks of tuberculosis, including drug resistant strains, have occurred in health-care environments. Several hundred employees have become infected after workplace exposure to tuberculosis, requiring medical treatment. A number of health-care workers have died.

The number of reported cases of tuberculosis in the Philadelphia area has dropped from 333 in 1993 to 147 in 2002. The statewide rate of tuberculosis is approximately 3.1 cases per 100,000 population. In contrast, Philadelphia County has a higher case rate of 9.7 per 100,000, and there are segments of the community with even higher rates.

The CDC published Guidelines for Preventing the Transmission of *Mycobacterium tuberculosis* in Health-Care Facilities, 1994 [MMWR 1994; 43 (No. RR-13)] and Guidelines for Environmental Infection Control in Health-Care Facilities [MMWR 2003; 52 (No. RR-10)]. The guidelines contain specific information on ventilation requirements, respiratory protection, medical surveillance and training for those personnel who are considered at-risk for exposure to tuberculosis.

Healthcare Environment

All persons working in healthcare settings where active TB is seen are strongly recommended by the Centers of Disease Control and Prevention (CDC) to have annual screening for TB. The University of Pennsylvania Medical Center admits the third largest number of TB cases in Pennsylvania. If you work in a clinical or hospital environment at the University of Pennsylvania (or have other contact with patients), it is strongly recommended that you be screened for TB every year. This screening is provided at no charge to you by Occupational Medicine. It consists of a brief questionnaire and, when appropriate, skin testing or chest x-ray.

Laboratory

Investigators intending to work with *Mycobacterium tuberculosis* in the laboratory must obtain written approval from EHRS before beginning work. 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](#).

Consult Penn's Tuberculosis Infection Control Plan for more information.

5. Use of Vaccinia Virus

Investigators must get written EHRS approval to use vaccinia virus in research prior to initiating work with the virus. All personnel who work with standard strains of vaccinia virus, recombinant virus or other similar Orthopoxviruses that infect humans must be given mandatory counseling and be offered vaccination by

Occupational Medicine at HUP. Consult the EHRS Protocol for the Approval to Use Vaccinia Virus in Research for more detailed information.

F. Clinical Laboratories

Clinical laboratories, especially those in health care facilities, receive clinical specimens with requests for a variety of diagnostic and clinical support services. Typically, the infectious nature of clinical material is unknown, and specimens are often submitted with a broad request for microbiological examination for multiple agents (e.g., sputa submitted for "routine," acid-fast, and fungal cultures). It is the responsibility of the laboratory director to establish standard procedures in the laboratory that realistically address the issue of the infective hazard of clinical specimens.

Except in extraordinary circumstances (e.g., suspected hemorrhagic fever), the initial processing of clinical specimens and serological identification of isolates can be done safely at Biosafety Level 2. This requires the use of Standard Precautions with all clinical specimens of blood or other potentially infectious material. Additionally, other recommendations specific for clinical laboratories may be obtained from the National Committee for Clinical Laboratory Standards.

Biosafety Level 2 recommendations and OSHA requirements focus on the prevention of percutaneous and mucous membrane exposures to clinical material. Primary barriers such as BSC's or plexiglass splash shields should be used when performing procedures that might cause splashing, spraying, or splattering of droplets. BSC's also should be used for the initial processing of clinical specimens when the nature of the test requested or other information suggests the likely presence of an agent readily transmissible by infectious aerosols (e.g., *M. tuberculosis*), or when the use of a BSC is indicated to protect the integrity of the specimen.

The segregation of clinical laboratory functions and limited or restricted access to such areas is the responsibility of the laboratory director. It is also the director's responsibility to establish standard, written procedures that address the potential hazards and the required precautions to be implemented.

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. Investigators must contact EHRS to register with the University's Select Agent Program and the appropriate federal agency prior to possession, use or transfer of any Select Agent.