

3.3. Introduction to Biohazardous Materials and Research

Laboratory research involving biological agents are subject to various federal and state regulations depending on the nature of the agents used and the experimental manipulations in which they will be employed. The following section of this Manual is intended to serve as a guide to the various federal and state agencies that govern biological research and their laws, regulations, and guidelines.

Principal Investigators are responsible for understanding the scope of their research program, identifying the regulations to which their work is subject, and complying with those regulations. IUEHS Biosafety for the respective campus is available to assist the Principal Investigator should guidance be needed in identifying and complying with those laws, regulations, and guidelines.

Principal Investigators should also note that many granting agencies require that grant recipients certify compliance with all relevant laws, regulations, and guidelines to which their research is subject. The scope of these regulations includes procedures and facilities involved in protecting laboratory workers, the public, and the environment from laboratory biological hazards.

3.3.1. Microorganisms

The National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) publish guidelines for work with infectious microorganisms. The publication, entitled [Biosafety in Microbiological and Biomedical Laboratories \(BMBL\)](#) recommends that work be done using one of four levels of containment: Biosafety Level 1 (BSL-1), BSL-2, BSL-3 and BSL-4 ([see section 3.4](#)). The *NIH Guidelines (Appendix E1-3)* classifies pathogenic agents into one of four risk groups according to specific criteria. It is required by Indiana University that all laboratories adhere to these NIH/CDC guidelines. Noteworthy, there are no BSL-4 laboratories on any of the IU campuses.

3.3.2. Microorganisms Capable of Causing Infection in Healthy Humans

Investigators must register any project involving a pathogenic agent with the IBC and receive its approval before work is begun. Following receipt of the completed IBC Protocol Submission Form, the laboratory will be inspected by IUEHS Biosafety to ensure that it meets the containment requirements listed in *BMBL* for the agent being studied. If the lab meets the requirements, the work will be reviewed and approved or disapproved by the IBC.

3.3.3. Genetically Engineered Organisms and/or Microorganisms

Work with all genetically engineered organisms must comply with the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*. These guidelines classify recombinant or synthetic nucleic acid molecules experiments into four levels of containment (BSL-1, BSL-2, BSL-3, and BSL-4) based on the hazard of the microorganism and the procedures and quantities being used. Additionally, the United States Department of Agriculture (USDA) requires permits for field testing of genetically engineered plants. It is required by Indiana University that all laboratories follow and ensure compliance with these guidelines.

3.3.4. Registration Document

Each PI is responsible for submitting protocols for all experiments involving biohazardous materials at BSL-2 or higher, biological toxins, and recombinant or synthetic nucleic acid molecules, including those exempt from *NIH Guidelines*. IUEHS Biosafety for your respective campus inspects all laboratories where BSL-2 or BSL-3 biocontainment is required, and all BSL-1 laboratories which are which require an IBC protocol prior to protocol approval.

3.3.5. Review and Approval of Experiments

The IBC, which oversees recombinant and synthetic nucleic acid molecule research at Indiana University, or the IUEHS Biosafety for your respective campus will review and approve the submitted protocol or amendment based on the submission status according to the NIH Guidelines, which are generally summarized below. More specific information about the categories and corresponding approval can be found with the Office of Research Compliance.

3.3.5.1. Experiments covered by the NIH Guidelines

Many experiments involving recombinant or synthetic nucleic acid molecules require registration and approval by the IBC before work may be initiated.

Experiments that require IBC approval before initiation include those that involve:

- Risk Group 2, 3, 4, or Restricted Agents as host-vector systems, cloning DNA from Risk Group 2, 3, 4, or Restricted Agents into nonpathogenic prokaryotic or lower eukaryotic host-vector systems, infectious virus, or defective virus in the presence of helper virus in tissue culture;
- Whole plants or animals; and
- More than 10 liters of culture.

Experiments that must be registered at the time of initiation include those that involve:

- The formation of recombinant or synthetic nucleic acid molecules containing no more than 2/3 of the genome of any eukaryotic virus propagated in tissue culture, recombinant or synthetic nucleic acid molecules-modified whole plants, and/or recombinant or synthetic nucleic acid molecules-modified organisms associated with whole plants, except those that fall under Section III-A, III-B, III-C, or III-D of the NIH Guidelines; and
- The generation of transgenic rodents that require BSL-1 containment.

3.3.5.2. Experiments exempt from the NIH Guidelines

Experiments exempt from the *NIH Guidelines*, although requiring registration with the IBC, may be initiated immediately. IUEHS Biosafety will review the registration and confirm that the work is classified correctly according to the *NIH Guidelines*. Exempt experiments are those that:

- Use synthetic nucleic acids that can neither replicate nor generate nucleic acids capable of replicating in any living cell; are not designed to integrate into DNA, and do not produce a toxin that is lethal for vertebrates at an LD50 of <100 ng/kg body weight;
- Use recombinant or synthetic DNA molecules that are not in organisms or viruses;
- Consist entirely of DNA segments from a single nonchromosomal or viral

DNA source, though one or more of the segments may be a synthetic equivalent;

- Consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well-established physiological means;
- Consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species);
- Consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent;
- Do not present a significant risk to health or the environment as determined by the NIH Director, with the advice of the Recombinant DNA Advisory Committee (RAC), and following appropriate notice and opportunity for public comment;
- Contain less than one-half of any eukaryotic viral genome propagated in cell culture;
- Use *E. coli* K12, *Saccharomyces cerevisiae*, or *Bacillus subtilis* host-vector systems, unless genes from Risk Group 3 or 4 pathogens are cloned into these hosts;
- Involve the purchase or transfer of transgenic rodents for experiments that require BSL-1 containment; and
- Work with biohazardous materials at BL2 that does not utilize recombinant or synthetic nucleic acid molecules.

3.3.6. Human Blood, Unfixed Tissue, and Cell Culture

Please refer to the [Indiana University Bloodborne Pathogens Exposure Control Plan](#) for detailed information on handling human material.

Work with human material is regulated by the Occupational Safety and Health Administration (OSHA) [Bloodborne Pathogens Standard, 29 CFR 910.1030](#). Human blood, unfixed tissue, cell culture, and certain other body fluids are considered potentially infectious for bloodborne pathogens such as hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). All human clinical material shall be presumed infectious and handled using BSL-2 work practices. This concept is called Universal Precautions. Principal Investigators are responsible for registering their use of human materials so training and immunization can be provided as required by OSHA.

3.3.7. Select Agents

Select Agents are microorganisms and toxins that have potential for criminal misuse to cause harm. The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 restricts their possession and use, and requires the University to collect and maintain information on the location and use on campus of any select agents or toxins. Please contact IUEHS Biosafety for your respective campus immediately if you currently possess or plan to acquire any of the agents listed in [Appendix A](#) and have not yet reported that fact. Failure to provide notice may result in civil and criminal liability for individual researchers and/or the University. If you have questions, you may contact IUEHS Biosafety for your respective campus, or visit the federal Select Agent website www.selectagents.gov which provides links to select agent program information.

3.3.8. Non-Human Primate (NHP) Unfixed Tissue and Primary Cell Culture

Non-human primates and their tissues pose special zoonotic risks as many of their diseases are often transmissible to humans and can be a serious health hazard. Although there are a number of NHP viruses that can cause disease in humans, monkeys of the genus *Macaca*, or their unfixed tissues, can carry the virus Cercopithecine herpesvirus 1 (other terms used: Herpes B-virus, Herpesvirus simiae, or simply B-virus). B-virus is frequently carried by Rhesus and *Cynomologus* macaques, as well as other macaques. It can cause fatal encephalitis in humans.

Prior to working with any NHP primary cell cultures or unfixed tissues, PIs must register their work, and lab personnel must be trained in the safety procedures required for handling and post-exposure procedures. Sharps use with these materials is to be eliminated or restricted.