NIH Guidelines Resource

Non-Exempt Studies

A. Experiments that involve Federal Approvals Prior to Initiation
    i. Section III-A: The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally if such acquisition could compromise the ability to control disease agents in humans, veterinary medicine, or agriculture.
        1. **This type of study requires approval by: The IBC, the NIH Recombinant DNA Advisory Committee (RAC), and the NIH Director BEFORE Initiation.**
        2. At the request of an Institutional Biosafety Committee, NIH will make a determination regarding whether a specific experiment involving the deliberate transfer of a drug resistance trait falls under this section of The Guidelines and therefore requires RAC review and NIH Director approval.
    ii. Section III-B-1: Experiments Involving the Cloning of Toxin Molecules with LD_{50} of Less than 100 Nanograms per Kilogram Body Weight. (Examples would include cloning botulinum toxins; tetanus toxin; diphtheria toxin, and Staphylococcus aureus alpha toxin.)
    iii. Section III-B-2: Experiments that have been Approved (under Section III-A) as Major Actions under the NIH Guidelines.
        1. **All studies in III-B require IBC and NIH approval BEFORE initiation.**
    iv. Section III-C: Experiments Involving the Deliberate Transfer of Recombinant or Synthetic Nucleic Acid Molecules, or DNA or RNA Derived from Recombinant or Synthetic Nucleic Acid Molecules, into One or More Human Research Participants
        1. **These studies require IBC, IRB, and RAC approval BEFORE participant enrollment. There are specific requirements for these types of studies. The RAC discussions may be public.**

B. Experiments with no Federal Approval Requirement, but Involve Local Approval Prior to Initiation

Prior to the initiation of an experiment that falls into Section D of The Guidelines, the Principal Investigator must submit a registration document to the IBC and get approval of the study.

    i. Section III-D-1: Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems.
        1. Section III-D-1-a: Experiments involving the introduction of recombinant or synthetic nucleic acid molecules into Risk Group 2 agents will usually be conducted at Biosafety Level (BL) 2

containment. Experiments with such agents will usually be conducted with whole animals at BL2 or BL2-N (Animals) containment.

(i) Examples include transforming *Listeria monocytogenes* with a vector containing GFP, introducing sequence into Herpes simplex type 2 virus to have it express a model antigen such as OVA, and introducing gene-reporter fusions to study gene activity in *Plasmodium falciparum*

2. Section III-D-1-b: for RG3 agents

ii. Section III-D-2: Experiments in Which DNA From Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems.

1. Section III-D-2-a: Experiments in which DNA from Risk Group 2 or Risk Group 3 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment.

(i) Many of these experiments are exempt. Studies that are not exempt, are ones that convey pathogenicity, or virulence to the non-pathogenic host via gene transfer

iii. Section III-D-3: Experiments Involving the Use of Infectious DNA or RNA Viruses or Defective DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems. (helper virus = packaging plasmid system)

****This section is used for the creation of viral vectors**.

1. Section III-D-3-a: Experiments involving the use of infectious or defective Risk Group 2 viruses in the presence of helper virus may be conducted at BL2.
2. Section III-D-3-b: for RG3 viruses
3. Section III-D-3-e: Experiments involving the use of infectious or defective viruses in the presence of helper virus which are not covered in Sections III-D-3-a through III-D-3-d may be conducted at BL1

(i) most baculoviral vector work applies here, unless gene insert(s) raise the risk of the study, and therefore the BL required

iv. Section III-D-4: Experiments Involving Whole Animals

This section applies to: Experiments involving whole animals in which the animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acid molecules, or nucleic acids derived therefrom, into the germ-line (transgenic animals). **(Please see further clarification of work with rodents in this section, and later).**

AND, experiments involving viable recombinant or synthetic nucleic acid molecule-modified microorganisms tested on whole animals. Other than viruses which are only vertically transmitted, **the experiments may not be**

conducted at BL1-N containment. A minimum containment of BL2 or BL2-N is required.

1. Section III-D-4-a: Recombinant or synthetic nucleic acid molecules, or DNA or RNA molecules derived therefrom, from any source **except for greater than two-thirds of eukaryotic viral genome** may be transferred to any non-human vertebrate or any invertebrate organism and propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study.
2. Section III-D-4-b: For experiments involving recombinant or synthetic nucleic acid molecules, or DNA or RNA derived therefrom, involving whole animals, including transgenic animals, and not covered by Section III-D-1, or Section III-D-4-a, the appropriate containment shall be determined by the IBC.
3. Section III-D-4-c: Exceptions under Section III-D-4
    (i) Section III-D-4-c-(1): Experiments involving the **generation** of transgenic rodents that require BL1 containment are described under Section III-E-3, Experiments Involving Transgenic Rodents.
    (ii) Section III-D-4-c-(2): The **purchase or transfer** of transgenic rodents is exempt from the NIH Guidelines under Section III-F, Exempt Experiments

v. Section III-D-5: Experiments Involving Whole Plants

These experiments require high containment. Biological Containment methods are specifically defined. For a description and discussion of biological containment methods, please see Appendix P-III of the Guidelines.

1. Section III-D-5-a: BL3-P (Plants) or BL2-P + biological containment is recommended for experiments involving most exotic infectious agents with recognized potential for serious detrimental impact on managed or natural ecosystems when recombinant or synthetic nucleic acid molecule techniques are associated with whole plants.
2. Section III-D-5-b: BL3-P or BL2-P + biological containment is recommended for experiments involving plants containing cloned genomes of readily transmissible exotic infectious agents with recognized potential for serious detrimental effects on managed or natural ecosystems in which there exists the possibility of reconstituting the complete and functional genome of the infectious agent by genomic complementation in planta.
3. Experiments in III-D-5-c require BL4-P containment, which is **not permitted** at NDSU
4. Section III-D-5-d: BL3-P containment is recommended for experiments involving sequences encoding potent vertebrate toxins

introduced into plants or associated organisms. Recombinant or synthetic nucleic acid molecules containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD_{50} of <100 nanograms per kilogram body weight fall under Section III-B-1, Experiments Involving the Cloning of Toxin Molecules with LD_{50} of Less than 100 Nanograms Per Kilogram Body Weight, and require NIH/OBA and Institutional Biosafety Committee approval before initiation.

5. Section III-D-5-e: BL3-P or BL2-P + biological containment is recommended for experiments with microbial pathogens of insects or small animals associated with plants if the recombinant or synthetic nucleic acid molecule-modified organism has a recognized potential for serious detrimental impact on managed or natural ecosystems.

vi. Section III-D-6: Experiments Involving More than 10 Liters of Culture.

In one container, or multiple containers. This type of experiment is subject to containment conditions as described in Appendix K of The Guidelines.

vii. Section III-D-7 a-d: Experiments Involving Influenza Viruses.

This section of the Guidelines specifically applies to human H2N2, HPAI, 1918 H1N1 strains of Influenza virus.

C. Experiments that require Local Approval Simultaneous with Initiation

The following protocols required notification **simultaneously with initiation** of the study. The IBC reviews and approves all of the following proposals, but IBC review and approval prior to initiation of the experiment is not required.

i. Section III-E-1: Experiments Involving the Formation of Recombinant or Synthetic Nucleic Acid Molecules Containing **No More than Two-Thirds** of the Genome of any Eukaryotic Virus.

1. Recombinant or synthetic nucleic acid molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family being considered identical) may be propagated and maintained **in cells in tissue culture** using BL1 containment.
2. For such experiments, it must be demonstrated that the cells lack helper virus/**no replication competent virus** for the specific Families of defective viruses being used. If this criteria is not met, the study is subject to Section III-D-3.
3. The DNA may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than two-thirds of a genome.

ii. Section III-E-2: Experiments Involving Whole Plants (requiring lower containment)

1. Section III-E-2-a: BL1-P is recommended for all experiments with recombinant or synthetic recombinant or synthetic nucleic acid molecule-containing plants and plant-associated microorganisms not covered in

Section III-E-2-b or other sections of the NIH Guidelines. Examples of such experiments are those involving recombinant or synthetic nucleic acid molecule-modified **plants that are not noxious weeds or that cannot interbreed with noxious weeds** in the immediate geographic area, and experiments involving whole plants and recombinant or synthetic nucleic acid molecule-modified **non-exotic microorganisms that have no recognized potential for rapid and widespread dissemination or for serious detrimental impact** on managed or natural ecosystems (e.g., Rhizobium spp. and Agrobacterium spp.).

2. Section III-E-2-b: BL2-P or BL1-P + biological containment is recommended for the following experiments:
   (i) Section III-E-2-b-(1). Plants modified by recombinant or synthetic nucleic acid molecules that are **noxious weeds** or can interbreed with noxious weeds in the immediate geographic area.
   (ii) Section III-E-2-b-(2). Plants in which the introduced DNA represents the complete genome of a non-exotic infectious agent.
   (iii) Section III-E-2-b-(3). Plants associated with recombinant or synthetic nucleic acid molecule-modified **non-exotic microorganisms that have a recognized potential for serious detrimental impact** on managed or natural ecosystems.
   (iv) Section III-E-2-b-(4). Plants associated with recombinant or synthetic nucleic acid molecule-modified **exotic microorganisms that have no recognized potential for serious detrimental impact** on managed or natural ecosystems.
   (v) Section III-E-2-b-(5). Experiments with recombinant or synthetic nucleic acid molecule-modified **arthropods** or small animals associated with plants, or with arthropods or small animals with recombinant or synthetic nucleic acid molecule-modified microorganisms associated with them if the recombinant or synthetic nucleic acid molecule-modified microorganisms have **no recognized potential for serious detrimental impact** on managed or natural ecosystems.

iii. Section III-E-3: Experiments Involving Transgenic Rodents.
This section covers experiments involving the **generation of rodents** in which the animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acid molecules, or nucleic acids derived therefrom, into the germ-line (transgenic rodents). Only experiments that require BL1 containment are covered under this section (experiments that require BL2, BL3, or BL4 containment are covered under Section III-D-4).

1. Section III-E-3-a. Experiments involving the breeding of certain BL1 transgenic rodents are exempt under Section III-F, Exempt Experiments