ENVIRONMENTAL HEALTH AND SAFETY
University of Nebraska-Lincoln

UNIVERSITY OF NEBRASKA - LINCOLN

BIOSAFETY GUIDELINES

Revised

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v. 2.2

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https://ehs.unl.edu/committees/ibc

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ADDITIONAL MATERIALS

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Previous Review Dates	
October 2014	v. 1.0
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June 2020	v. 2.1
January 2024	v. 2.2

1 Introduction

Hazards associated with pathogens and recombinant or synthetic nucleic acids may be encountered in many UNL research, clinical/diagnostic, and/or teaching activities. The University must comply with certain regulatory requirements, generally recognized consensus standards, and/or funding agency guidelines to remain in good standing and retain funding eligibility, particularly as it relates to eligibility for funding from the National Institutes of Health (NIH).

The UNL Biosafety Guidelines describe the methods, means, procedures, and policies that govern the conduct of work by UNL employees and/or within UNL facilities, that presents possible biohazard risks to employees, visitors, the public, or the environment. These Guidelines also delineate roles and responsibilities for Principal Investigators (PI), Laboratory Workers, the University Biosafety Officer (BSO), the Institutional Biosafety Committee (IBC), and certain Administrative Officials.

1.1 Scope

The UNL Biosafety Guidelines apply to clinical/diagnostic, research, and teaching activities involving any of the following items individually or in combination with each other:

- **Recombinant or Synthetic Nucleic Acid molecules** (*see definitions in Section 1.2*)

Exception: Experiments conducted in academic teaching lab courses for demonstrative purposes and not considered "research projects" are not subject to IBC Review.

- **Human, animal, and plant pathogens** (bacteria, virus, yeast, fungus, prions, & parasitic agents) including growth, manipulation and/or other handling and use *in vitro* or *in vivo*.
- **Toxins of biological origin***,* when the originating organism is grown for the purpose of obtaining toxin. Toxins obtained in pure form commercially are not covered, except for Select Agent toxins. Any amount of a Select Toxin requires submission of an IBC protocol.
- **Human blood and other potentially infectious materials**, as defined by the United States Occupational Safety and Health Administration (OSHA) and the **UNL Exposure Control Plan**.
- **Human and non-human primate cells and organ/tissue cultures**
- **Select Agents and Toxins**, as defined by the United States Departments of Agriculture or Health and Human Services, including **Dual Use Research of Concern (DURC)**, involving agents and research categories as defined in Appendix A of this document.
- **Genetically-modified animals or plants** including growth, breeding, manipulation or other use of the organism. Field trials of transgenic plants authorized by the United States Department of Agriculture - Animal and Plant Health Inspection Service (USDA-APHIS) are not subject to review by the IBC.

- **Field Collection or Sampling of Wild Animals**, when there is risk of exposure to zoonotic diseases. See section 4.7 for detailed parameters regarding these types of studies.

Activities not subject to IBC review

Clinical/diagnostic, research, and teaching activities that:

- **Are** properly conducted at biosafety containment level 1 (BSL-1); and
- **do not** involve human, animal, or plant pathogens capable of causing disease in healthy organisms; and/or
- involve only the *in vitro* use of nucleic acids (e.g., PCR, naked siRNA, sequencing) **and do not** involve the cloning and propagation of recombinant or synthetic nucleic acid molecules in cells or organisms; and
- the nucleic acid molecules are not able to produce infectious forms of a biological select agent or encode for the functional form of a select agent toxin. (See EHS SOP "Select Agents" for details.)

Important: Contact the BSO (ibc@unl.edu) if you are unsure if you are in need of an IBC protocol.

1.2 Definitions

The following terms are used throughout this document and their meaning is defined here.

Biologics – any compound or material of biological origin. This includes but is not limited to recombinant nucleic acid molecules, plasmids, microorganisms, viral vectors, toxins, etc.

Genetically-modified – artificial modification of the genetic code of an organism by any means other than regular breeding methods. This includes germline (transgenic) and somatic/transient modifications, gene knock-out/knock-in, gene silencing, gene editing, etc.

Pathogenic agents – any microbiological (bacteria, virus, yeast, fungus, prion, parasite) agent or biological toxin that is capable of causing disease in humans, animals or plants. Lab-adapted strains of microbes are not included under this definition; examples include K12-derived *E. coli* strains and *S. cerevisiae*.

Principal investigator (PI) – any person with primary authority over the work conducted, whether the work is research-, clinical/diagnostic- or teaching-related.

Recombinant or Synthetic Nucleic Acids (r/sNA) – (i) molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell, i.e., recombinant nucleic acids; (ii) nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids, or (iii) molecules that result from the replication of those described in (i) or (ii) above.

This definition encompasses recombinant or synthetically derived nucleic acids, including those that are chemically or otherwise modified analogs of nucleotides (e.g., morpholinos), or both.

Work activities – any activity involving conducting experiments on or with, testing, sampling, or analyzing any biological material or sample from a human, animal or plant that falls into one of the categories outlined in Section 4.

DURC - Dual Use Research of Concern (DURC) is life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, material, or national security.

1.3 Regulatory Authority

The UNL Biosafety Guidelines are based on several regulatory requirements, as well as nationally recognized consensus standards and guidelines. The following list is illustrative; not exhaustive. Other regulations, standards, or funding agency requirements may apply to specific work activities. PIs must identify and familiarize themselves with all applicable requirements pertaining to their particular work activities.

- NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (Herein referred to as "NIH Guidelines"), U.S. Department of Health and Human Services, National Institutes of Health. 59 FR 34496 (Final Rule) and subsequent amendments. https://osp.od.nih.gov/policies/biosafety-and-biosecurity-policy#tab2/
- *Biosafety in Microbiological and Biomedical Laboratories*, (latest edition). (Herein referred to as "BMBL or CDC Guidelines"), U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention. https://www.cdc.gov/labs/BMBL.html
- Possession, Use, and Transfer of Select Agents and Toxins, 42 CFR 73, U.S. Department of Health and Human Services, Public Health Service; 7 CFR 331 and 9 CFR 121, U. S. Department of Agriculture, Animal and Plant Health and Inspection Service. https://www.selectagents.gov/
- 49 CFR 171-178, Hazardous Materials Transportation regulations, incorporating by reference the International Air Transport Association (IATA), Dangerous Goods Regulations (DGR), *Latest edition*
- 29 CFR 1910.1030, Occupational Exposure to Bloodborne Pathogens, U.S. Department of Labor, Occupational Safety and Health Administration
- 42 CFR Subpart F, Importations (Particularly 71.54, Etiological Agents, Hosts, and Vectors)
- 9 CFR Parts 92, 94, 95, 96, 122, and 130, Importation of Etiologic Agents of Livestock, Poultry, and Other Animal Diseases
- 7 CFR Part 330, Importation and Domestic Transfer of Plant Pests

- 7 CFR Part 340, Introduction of Plants Genetically Engineered to Produce Industrial Compounds
- 15 CFR Parts 730 to 799, Export of Etiologic Agents of Humans, Animals, Plants, and Related Materials
- United States Government (USG) Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern. https://www.phe.gov/s3/dualuse/Pages/default.aspx

Biological materials such as reagents, cell lines, plasmids, and vectors are often subject to the terms of a Material Transfer Agreement (MTA), which governs the transfer of tangible research materials between two organizations, when the recipient intends to use it for his or her own research purposes. The MTA defines the rights of the provider and the recipient with respect to the materials and any derivatives. The UNL Offices of Sponsored Programs and Technology Development have primary authority related to MTAs; therefore, the specific procedures and policies related to MTAs are beyond the scope of these Guidelines. Nevertheless, PIs must understand MTA implications that may impact their work.

1.4 Violations

Non-conformance with *NIH Guidelines* and nationally recognized standards may endanger human, animal, or plant health. Violations of *NIH Guidelines* regardless of the specific funding source can jeopardize funding from the NIH and other granting agencies for **all of UNL**. Non-conformance may result in civil and/or criminal penalties.

Violations of the NIH Guidelines and adverse incidents, as summarized in the EHS SOP, ***Incident Reporting – National Institutes of Health (NIH) Guidance***, must be reported to the NIH. PIs are responsible to notify the BSO (or Director of EHS in the absence of the BSO). The BSO, IBC Chair, and Office of Research Responsibility (ORR) will cooperate to investigate the incident and file necessary verbal and written reports with the NIH. The ORR is responsible to communicate with the Senior Administrative Official. The Senior Administrative Official is responsible to determine and administer appropriate disciplinary actions, if any, and in accordance with existing UNL human resource and responsible conduct of research policies and procedures.

2 Roles and Responsibilities

2.1 Senior Administrative Oversight

The UNL Chancellor is the Senior Administrative Officer overseeing biosafety activities at the University of Nebraska-Lincoln. The UNL Chancellor has delegated this authority to the Vice Chancellor for Research & Economic Development (VCRED). The VCRED has further delegated oversight of biosafety to the Assistant Vice Chancellor for Research and Research Integrity Officer. (Figure 2-1)

2.1.1 *The Assistant Vice Chancellor for Research and Research Integrity Officer (AVCR) or delegate is responsible for:*

- Acting as the Administrative Advisor to the IBC in matters of administrative action and representing the interests of the University community.
- Requesting resources required to carry out the provisions of UNL Biosafety Guidelines. (*Resources are allocated through the Office of Research and the Department of Environmental Health and Safety.)*
- Appointing the IBC committee members and designating a committee chairperson. Ensuring that those selected are appropriately qualified and trained regarding laboratory safety and implementation of the *NIH Guidelines. (NIH Guidelines, Section IV-B-1)*
- Determining and administering disciplinary action for willful violation of *NIH Guidelines*, BMBL, UNL Biosafety Guidelines or other authoritative safety documents.
- Keeping the VCRED apprised of issues regarding operation of the UNL Biosafety Program and protocol termination/suspension for serious non-compliance/non-conformance to regulatory requirements and/or UNL policies. In cooperation with the IBC Chair and BSO, reporting incidents to NIH/OSP as specified in the NIH Guidelines.
- In cooperation with the Biosafety Officer, forwarding public comments on IBC actions to NIH/OSP, as applicable.

2.2 Department Head/Chair or Dean/Director

Department Heads/Chairs and/or Deans/Directors are responsible for:

- Indicating support of a PI's protocol prior to consideration by the IBC.
- Ensuring that adequate facilities are available and maintained to support the proposed protocol.
- Ensuring that biosafety requirements and safety policies and procedures are enforced at the departmental level.
- Ensuring that faculty using biological materials follow laboratory decommissioning procedures when they are leaving UNL or relocating their lab.

2.3 Institutional Biosafety Committee

The IBC is responsible for:

- Investigating potential violations of the *NIH Guidelines* in coordination with the BSO and the AVCR.
- Reviewing protocols and amendments, including independent assessment of the containment levels required by the *NIH Guidelines* for the proposed research; and consideration of information provided in the protocol or by the BSO regarding facilities, procedures, practices, and training and expertise of personnel involved in the protocol.

- Setting final containment levels for certain experiments as described in the *NIH Guidelines*, specifically Section III-D-2-a (Experiments in which DNA from RG 2-3 or *Restricted Ag*ents are Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems); Section III-D-4-b (Experiments Involving Whole Animals); and III-D-5 (Experiments Involving Whole Plants).
- Adopting standard emergency procedures covering accidental spills and personnel contamination resulting from activities subject to the *NIH Guidelines* and the UNL Biosafety Guidelines. This includes reviewing and approving alternate procedures proposed by individual PIs, as applicable.
- The IBC Chair is responsible for ensuring that IBC members are appropriately trained regarding laboratory safety and implementation of the *NIH Guidelines*. *(NIH Guidelines, Section IV-B-1-h)*
- The UNL IBC also functions as the Institutional Review Entity (IRE) for review of IBC protocols that fall under the DURC policy oversight
- If a research project is subject to the DURC policy, and such is confirmed by the IRE, then a risk mitigation plan will be developed and implemented. The risk mitigation plan will be collaboratively developed by the PI, BSO, and Research Compliance staff and presented to the IBC for review and approval.

2.4 Research Compliance Services, Export Controls

- Serve as subject matter experts who will advise the IBC and attend all meetings of the IBC when a protocol has the potential to trigger the USG DURC policy. Collaborate with the PI and BSO in developing a risk mitigation plan. Conduct a separate export control review if the protocol is determined to be subject to the USG DURC Policy. Inform the IBC and BSO of export control requirements that apply to a particular protocol.

2.5 Biosafety Officer (BSO)

The UNL Biosafety Officer reports to the Director of Environmental Health and Safety and is responsible for:

- Assisting PIs to complete the IBC protocol registration form in a manner that allows for thorough and complete committee review.
- Evaluating adequacy of facilities, equipment, procedures and techniques and reporting findings to the committee for their consideration in support of initial protocol review, amendment, and on-going evaluation/institutional oversight.
- Notifying PIs of the meeting date, time and location at which their protocol will be considered by the committee and notifying the PI of the results of the IBC review and basis for approval or denial of the proposed project. Following up with PIs concerning contingencies and other issues identified by the IBC following protocol review.
- Receiving and maintaining records of submitted faculty pathogen inventories.
- Investigating and reporting to the IBC and AVCR, significant problems related to accidents and illnesses, operations, non-compliance with NIH Guidelines or other authoritative source, or other adverse circumstances related to proposed or approved

protocols. In cooperation with the IBC Chair and AVCR, reporting incidents to NIH/OSP as specified in the NIH Guidelines.

- Providing technical services/advice and training to the IBC and PIs regarding NIH Guidelines, standard written safety, emergency, and security procedures and assisting them to train laboratory staff, as requested. Written standard procedures published by EHS that are substantially a restatement of recognized consensus, standards or regulatory requirements do not require IBC approval; other policies and procedures require review and approval by the IBC. Training of the IBC regarding their roles and responsibilities is conducted at least annually and individually upon initial assignment to the committee.
- Serving as a voting member of the IBC and providing administrative support to the IBC, such as establishing and distributing agendas and minutes; maintaining records and files of protocols, registration documents, etc.; responding to FOIA requests; and other similar duties.
- Serving as the contact person on the annual OSP roster of IBC members. Filing the IBC membership roster with NIH/OSP, including (i) a roster of IBC members clearly indicating the chair, contact person, BSO, plant expert, and animal expert; (ii) biographical sketches of all IBC members, including community members. This roster must be filed when changes occur in membership and at least annually, as applicable.
- In cooperation with the AVCR, forwarding public comments on IBC actions to NIH/OSP, as applicable.
- Serving as the Institutional Contact for Dual Use Research (ICDUR). Submitting DURC risk mitigation plans to the USG funding agency (or NIH in the case of qualifying work that is not USG funded) for approval. Conducting annual reviews of all active DURC risk mitigation plans.

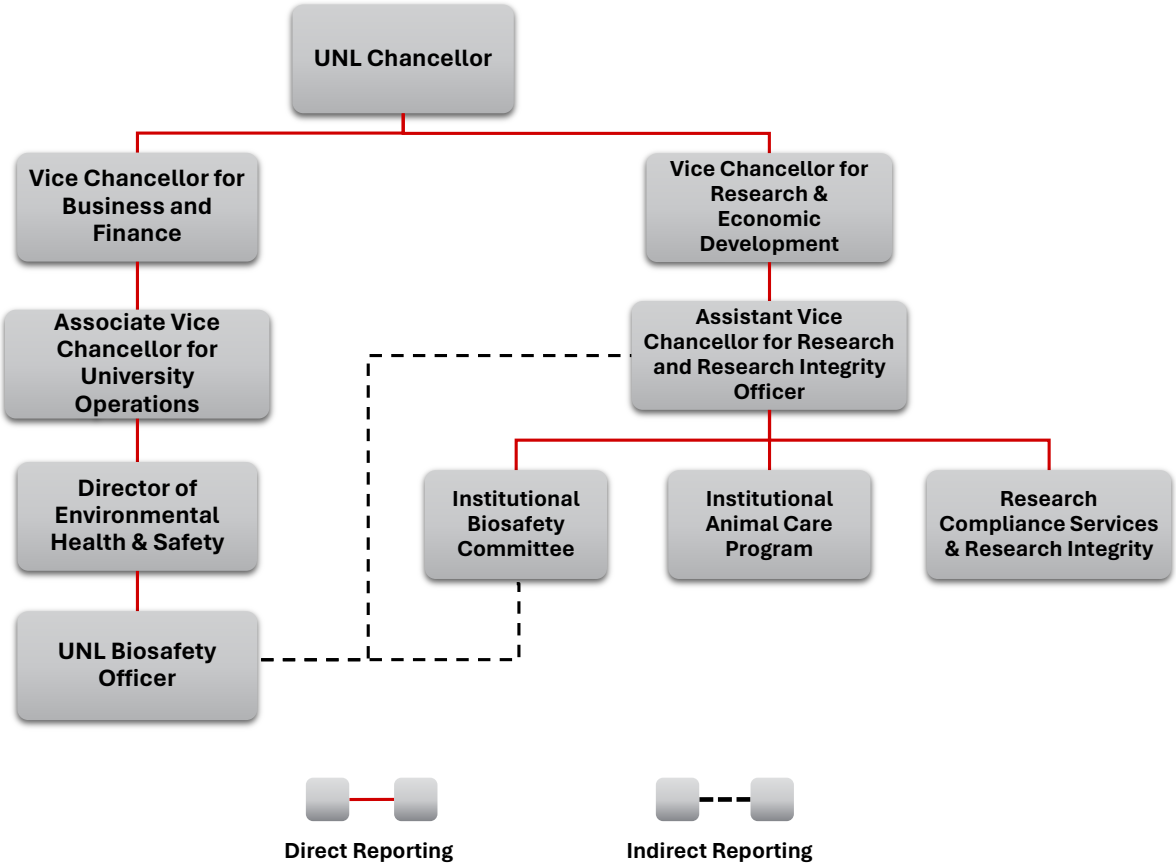


Figure 2-1 Organizational Chart for the UNL Biosafety Program

2.6 Principal Investigator (PI)

PIs are responsible for adhering to all responsibilities and expectations articulated by the NIH in Section IV-B-7; and the following:

- Adhering to and training all personnel in all applicable rules, regulations and standard practices, including but not limited to NIH and CDC (BMBL) guidelines.
- Maintaining IBC protocol forms by submitting **Amendment, Minor Modification, *Annual Update Forms*** as needed and ***Termination Forms*** for protocols that are no longer active. (See subsection 5.5.4 Protocol Terminations for details)
- Adhering fully to UNL policies and procedures for work activities subject to the UNL Biosafety Guidelines.
- Adhering fully to other UNL policies related to responsible conduct of research (http://research.unl.edu\orr\rcr.shtml).
- Restricting activities to those that are approved by the IBC and abstaining from initiating or modifying research/experiments, as applicable under these Guidelines.
- Making available to all laboratory personnel a copy of the biosafety manual; training and supervising laboratory workers in good microbial techniques and other practices and procedures related to safety, security, personal protective equipment (use, limitations, and maintenance), accidents, and emergency preparation/response. See the EHS SOP, ***Biosafety Training***, for further training guidance/requirements. NIH encourages PIs to retain training records.
- Routinely supervising the performance of laboratory workers to assure a safe workplace and correct work errors and conditions that are a risk to the worker or the environment.
- Maintaining a current and accurate inventory of all pathogenic agents in his/her possession at UNL and submitting inventory documentation as described in the EHS SOP, ***Pathogen Inventories*** to the UNL Biosafety Officer at least annually.
- Immediately reporting violations of the *NIH Guidelines*, injuries and illnesses attributable to occurrences in the laboratory, personnel contamination, spills, and loss of containment to the Biosafety Officer or Chair of the IBC. See EHS SOP, ***Biohazard Incident Reporting*** for more information.
- Complying with applicable shipping regulations, permit requirements, and Material Transfer Agreements.
- Ensuring that facilities and equipment are maintained to support the required biosafety containment level and enforcing laboratory access limitations to maintain adequate security.
- Informing laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).

2.7 Laboratory Workers

Laboratory workers are responsible for:

- Completing relevant training as required by the IBC and provided by EHS and the PI.

- Restricting activities/experiments subject to the UNL Biosafety Guidelines to those that are authorized under an approved IBC protocol.
- Being familiar with hazards posed by all agents used in the laboratory regardless of whether he/she directly works with them.
- Keeping a current and accurate inventory of the pathogenic agents used and notifying the PI of any inventory changes.
- Knowing and adhering to all emergency procedures established by the PI.
- Reporting all occupational accidents, illnesses, and injuries to the PI and in accordance with UNL policy, as described in the EHS SOP, ***On-the-Job and Student Injuries***.
- Following all laboratory practices established by the PI.

3 Institutional Biosafety Committee

3.1 Membership

As mandated by the *NIH Guidelines*, at a minimum the IBC is composed of no fewer than five (5) members selected for their collective expertise in recombinant and/or synthetic nucleic acids. A list of current members is maintained on the IBC webpage at: http://ehs.unl.edu/committees/ibc

- Two (2) members of the IBC are not affiliated with UNL (do not have faculty appointments).
- At least one (1) member has expertise in plant, plant pathogen, or pest containment procedures.
- At least one (1) member has expertise in animal containment procedures.
- One (1) member represents laboratory technical staff.
- One (1) member is the Biosafety Officer.
- At the present time, UNL is not engaged in human gene therapy studies or clinical trials; therefore, such an expert is not included on the committee.

The AVCR appoints the committee chair and members for a term of three (3) years. The term of the Biosafety Officer is consistent with the term of employment. The members listed above are vested with voting rights. However, they must abstain from voting if they are engaged or have a vested interest in a project proposal that is before the committee for consideration.

A quorum consists of at least 50% of the total membership of the committee and a vote of approval requires at least a simple majority of the members in attendance. The Chair or designee must be present. The Biosafety Officer or designee must be present for approval of BSL-3 protocols.

3.2 Authority

3.2.1 *Suspension/Termination of Existing protocols*

The committee has authority to withdraw or suspend protocol approval in response to violations of the NIH Guidelines or UNL biosafety policies and procedures, including but not limited to:

- Failure to maintain an existing approved protocol or;
- Failure to complete required training or;
- Failure to adhere to safety and containment design and principles.

In general, the PI is expected to implement corrective actions in a timely manner (upon notice of a deficiency). The AVCR may also administer additional consequences, up to and including suspension of access to research funds. Refer to the supplemental IBC policy on remediation of non-compliance with UNL Biosafety Guidelines for additional detail. This policy is available on the IBC website (http://ehs.unl.edu/violations-unl-biosafety-guidelines).

3.2.2 *Reinstatement of Suspended Protocols*

A suspended protocol can be reinstated when the following occurs:

1) The violation has been addressed/corrected to the satisfaction of the IBC and AVCR and;
2) The PI has submitted an explanation, in writing, to the IBC of his/her reasons for non-compliance with the UNL Biosafety Guidelines and actions taken to prevent reoccurrence.

The IBC will discuss reinstatement at the next meeting following completion of the items above and a decision will be made about reinstating full approval of the protocol. The PI will receive a letter notifying him/her as to the IBC's decision.

3.2.3 *Unapproved work activities*

If it is discovered that a PI is conducting work activities for which he/she is not approved, the IBC or BSO on behalf of the committee will notify the PI and require immediate submittal of a biosafety protocol or protocol amendment and suspension of work activities if the activities are subject to review by the committee prior to initiation. The AVCR may also administer additional consequences, up to and including suspension of access to research funds. Refer to the supplemental IBC policy on remediation of non-compliance with UNL Biosafety Guidelines for additional detail. This policy is available on the UNL IBC website (http://ehs.unl.edu/violations-unl-biosafety-guidelines).

3.3 Meetings

IBC meetings are scheduled once per month during the academic year from September through May and as needed during the summer months. Meetings are conducted in a manner that facilitates discussion, public attendance, and PI participation. Committee

business is always conducted in a live meeting. The schedule and meeting location is available on the EHS website at http://ehs.unl.edu/committees/ibc and is posted on the IBC webpage listed above as well as on the University calendar accessed at http://events.unl.edu. PIs are notified of the meeting date at which their protocol will be discussed and are invited to attend at their discretion.

Minutes of the IBC Meetings will contain sufficient detail to serve as a record of the major points of discussion and the committee's rationale for specific decisions and contain the following information:

- Date and time of meeting
- Approval status of prior meeting minutes
- Individuals in attendance
- Whether and why meeting was closed
- All major motions, including rationale
- Major points of order and discussion
- Whether motions were approved
- Time of meeting adjournment

Minutes of the committee are maintained by the BSO and distributed at the next meeting for review and approval by the IBC. Minutes are available to the public upon request in accordance with **NEB. REV. STAT. §§ 84-712 to 84-712.09 (1999, CUM. SUPP. 2006, Supp. 2007)**, but are not posted electronically on a publicly accessible webpage. Minutes of the committee meetings or subject protocols may be redacted after consultation with NU legal counsel. Redaction of personal information (such as home phone numbers, addresses, etc.) does not require review by NU legal counsel. Redaction is usually reserved for information that is necessary to protect trade secret information; confidential commercial information; information that, if disclosed, could directly compromise institutional or national security; and similar information.

In accordance with the NIH Guidelines Section IV-B-2-a-(7), all public comments made on IBC actions and the IBC's response will be forwarded to NIH/OSP. The Biosafety Officer, in cooperation with the Office of Research Responsibility, is responsible for this reporting.

4 Specific Requirements for IBC Protocols

More than one of the following subsections may apply to a given protocol; in which case, the most stringent reporting and initiation requirements apply.

4.1 Recombinant or Synthetic Nucleic Acid Molecules

PIs are required to submit a completed IBC protocol registry form when conducting research experiments involving recombinant or synthetic nucleic acids or materials containing recombinant or synthetic nucleic acids, regardless of whether the experiment is given exempt

status under the *NIH Guidelines*. The review, approval and initiation requirements for work that falls under the *NIH Guidelines* are listed below.

4.1.1 *NIH Guidelines, Section III-F (NIH Exempt Experiments)*

Those protocols involving experiments described in Section III-F of the *NIH Guidelines* (Exempt Experiments) and which are appropriately conducted at biosafety level 1 (BSL-1) containment are reviewed only by the BSO and Chair of the IBC. The purpose of this review is to verify exemption status and to generate documentation for the PI that may be required by funding agencies relative to NIH compliance. Experiments described in a protocol of this nature **may** be initiated simultaneously with submittal of the completed protocol registry form.

Work with the following recombinant or synthetic nucleic acid molecules is exempt from the *NIH Guidelines, but still requires review and approval by the UNL IBC*:

III-F-1: Those synthetic nucleic acids that: (1) can neither replicate nor generate nucleic acids that can replicate in any living cell (e.g., oligonucleotides or other synthetic nucleic acids that do not contain an origin of replication or contain elements known to interact with either DNA or RNA polymerase), and (2) are not designed to integrate into DNA, and (3) do not produce a toxin that is lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight. If a synthetic nucleic acid is deliberately transferred into one or more human research participants and meets the criteria of Section III-C, it is not exempt under this Section.

III-F-2: Those that are not in organisms, cells, or viruses and that have not been modified or manipulated (e.g., encapsulated into synthetic or natural vehicles) to render them capable of penetrating cellular membranes.

III-F-3: Those that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature.

III-F-4: Those that consist entirely of nucleic acids from a **prokaryotic** host (indigenous plasmids or viruses included) when propagated in that host (or a closely related strain of the same species), or when transferred to another host by well-established physiological means.

III-F-5: Those that consist entirely of nucleic acids from a **eukaryotic** host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated in that host (or a closely related strain of the same species).

III-F-6: Those that 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. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice of the RAC after appropriate notice and opportunity for public. See Appendices A-I through A-VI of the *NIH Guidelines* for a list.

III-F-7: Those genomic DNA molecules that have acquired a transposable element provided the transposable element does not contain any recombinant and/or synthetic DNA.

III-F-8: Those that do not present a significant risk to health or the environment..., as listed in Appendix C of the *Guidelines*:

- **Appendix C-I**
  - The recombinant or synthetic nucleic acid molecules are propagated and maintained in cells in tissue culture and contain less than 50% of any eukaryotic viral genome (all viruses from a single family being considered identical). *There are other exceptions to this rule (**Appendix C-I-A**). Check with the BSO.*
- **Appendix C-II**
  - Experiments using an *E. coli* K-12 host-vector system except those listed in Appendix C-II-A, provided that: (i) the host does not contain conjugation proficient plasmids or generalized transducing phages; or (ii) lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids shall be used as vectors. (**Note: strain BL21 is not a K-12 strain.**) There are some restrictions on the vectors used (***Appendix C-II-A***). BSL-1 containment is suggested.
- **Appendix C-III**
  - Experiments with *Saccharomyces* host-vector systems. There are some restrictions (***Appendix C-III, C-III-A***). BSL-1 containment is suggested.
- **Appendix C-IV**
  - Experiments involving *Kluyveromyces lactis* host-vector systems. There are some restrictions (***Appendix C-IV, C-IV-A***). BSL-1 containment is suggested.
- **Appendix C-V**
  - Experiments with *Bacillus subtilis* or *B. licheniformis* host-vector systems and in which reversion to spore formation is $< 10^{-7}$. There are some other restrictions (***Appendix C-V, C-V-A***). BSL-1 containment is suggested.
- **Appendix C-VI**
  - Experiments with r/sNA derived entirely from extrachromosomal elements of gram positive organisms listed in ***Appendix C-VI*** and propagated in those same organisms. There are some restrictions (***Appendix C-VI-A***).
- **Appendix C-VII**
  - The domestic purchase or transfer of transgenic rodents (e.g., not constructed at UNL) for experiments that require BSL-1 containment are exempt from the *NIH recombinant DNA Guidelines*. (***Appendix C-VII***)
- **Appendix C-VIII**
  - The breeding of two different transgenic rodents or the breeding of a transgenic rodent and a non-transgenic rodent with the intent of creating a new strain of transgenic rodent that can be housed at BSL-1 containment will be exempt from the NIH Guidelines if:
    - (1) Both parental rodents can be housed under BSL-1 containment; and
    - (2) neither parental transgenic rodent contains the following genetic modifications: (i) incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or (ii) incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); and
    - (3) the transgenic rodent that results from this breeding is not expected to contain more than one-half of an exogenous viral genome from a single family of viruses. (***Appendix C-VIII***)

4.1.2 *NIH Guidelines, Section III-E*

Those protocols involving experiments described in Section III-E of the *NIH Guidelines* are reviewed by the full IBC. Experiments described in a protocol that fall under this section and which are appropriately conducted at BSL-1 **may be initiated simultaneously** with submittal of the completed protocol registration form. This section also includes experiments that do not fall into any other section of the NIH Guidelines, for example:

- Experiments involving the introduction of Risk Group 1 (RG1) 1 nucleic acid molecules into RG1 organisms such as E. coli BL21
- *N*on-viral RG1 or Risk Group 2 (RG2) r/sNA used in tissue culture systems, or
- *Use of Baculovirus vectors*

Section III-E also includes several subsections and they are described in brief below:

- ***Experiments involving the formation of r/sNA molecules that contain no more than two-thirds of the genome of any eukaryotic virus [III-E-1]***
- ***Experiments involving whole plants, Section III-E-2***
  - Experiments with r/sNA modified whole plants and plant-associated microorganisms safely conducted at BL1-P containment. Example: *Agrobacterium spp.* and *Rhizobium spp.* [III-E-2-a]
  - BL2-P or BL1-P + biological containment is recommended for the following experiments [III-E-2-b]
  - Plants modified by recombinant or synthetic nucleic acid molecules that are noxious weeds or can interbreed with noxious weeds in the immediate geographic area. [III-E-2-b-(1)]
  - Plants in which the introduced DNA represents the complete genome of a non-exotic infectious agent. [III-E-2-b-(2)]
  - Plants associated with r/sNA-modified non-exotic microorganisms that have a recognized potential for serious detrimental impact on managed or natural ecosystems. [III-E-2-b-(3)]
  - Plants associated with r/sNA-modified exotic microorganisms that have no recognized potential for serious detrimental impact on managed or natural ecosystems. [III-E-2-b-(4)]
  - Experiments with r/sNA-modified arthropods or small animals associated with plants or with arthropods or small animals with r/sNA-modified microorganisms associated with them if the r/sNA-modified microorganisms have no recognized potential for serious detrimental impact on managed or natural ecosystems. [III-E-2-b-(5)]
- ***Experiments involving transgenic rodents, Section III-E-3***
  This section covers creation of genetically-modified rodents (including knock-out animals) that can be safely housed at BSL-1 containment. Animals that require BSL-2 or higher containment are covered under Section III-D-4.

[1] See Appendix B of the NIH Guidelines for a list of organisms divided into Risk Groups

4.1.3 *NIH Guidelines, Section III-D*

Those protocols involving experiments described in Section III-D of the *NIH Guidelines* are reviewed by the full IBC. Experiments described in a protocol of this nature **cannot be initiated without IBC approval**. The subsections of III-D are listed below and include experiments involving:

- ***Human or animal pathogens (Risk group 2 or greater) as host-vector systems (III-D-1)***
- ***DNA from human or animal pathogens (Risk group 2 or greater) cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems (III-D-2)***

  
  Note: Work with Cas9 genes from S. pyogenes in these organisms falls into this section unless the organisms fall into Section III-F.

- ***Using infectious viruses (including replication defective) or helper virus systems (III-D-3)***
- ***Certain whole animal experiments (III-D-4)***
  This section covers administration of recombinant nucleic acid-containing materials to animals as well as creation of genetically-modified rodents (including knock-out animals) that require being housed at BSL-2, BSL-3 or BSL-4 containment.
- ***Certain whole plant experiments (III-D-5)***
- ***Large scale culture preparations (> 10 liters) (III-D-6)***
- ***Experiments with Influenza viruses (III-D-7)***

4.1.4 *NIH Sections III- A and B (Major Actions)*

Those protocols involving experiments described in Section III- A, or -B of the *NIH Guidelines* are reviewed by the full IBC. Experiments described in a protocol of this nature **cannot** be initiated without approval by the IBC **and** NIH. Experiments that fall into Sections III-A and III-B may also trigger the USG Policy for Dual Use Research of Concern (DURC), please refer to Appendix A for more information on DURC experiments.

- ***III-A-1-a (The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally (see Section V-B), if such acquisition could compromise the ability to control disease agents in humans, veterinary medicine, or agriculture.***
- ***III-B-1 (Experiments Involving the Cloning of Toxin Molecules with LD50 of Less than 100 Nanograms per Kilogram Body Weight) Deliberate formation of recombinant or synthetic nucleic acid molecules containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and Shigella dysenteriae neurotoxin). Specific approval has been given for the cloning in Escherichia coli K-12 of DNA containing genes coding for the biosynthesis of toxic***

molecules which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight.

- Experiments that involve cloning genes coding for toxin molecules toxic for vertebrates that have an LD50 of > 100 nanograms per kilogram bodyweight and < 100 micrograms per kilogram body weight require **IBC approval and registration with NIH OSP prior to initiation** per Appendix F of the *NIH Guidelines*.

4.2 Human, Animal, and Plant Pathogens

As defined in Section 1.2, pathogenic agents are those that are infectious to humans, animals and/or plants and include bacteria, viruses, yeast, fungi, prions, and parasites. Work of **any kind** with a pathogenic agent requires review and approval by the IBC. Additionally, clinical, diagnostic, research, or teaching activities normally and appropriately conducted at BSL-2 or higher containment; and any work requiring relevant USDA, CDC or other federal permits, will be subject to review and approval by the IBC. This category of work may be initiated **only** after submission of a completed IBC protocol registration form and approval by the IBC. Use of recombinant nucleic acid molecules and technology in conjunction with pathogenic agents may result in additional review and approval requirements specified in the *NIH Guidelines*.

4.3 Toxins of Biological Origin

Work with toxins of biological origin is also subject to review and approval by the IBC when the toxin used is expressed in or produced by biological organisms and isolated for use in the lab. Toxins obtained in pure form from commercial sources are not covered by these guidelines, except those that are on the Select Toxin List (see Section 4.6 for more information about work with select toxins). Work with unregulated quantities of Select Toxins (see EHS SOP, **Select Agents and Toxins**) requires registration with the IBC and use of a log to track inventory and use of the toxin.

4.4 Human Blood and Other Potentially Infectious Materials (OPIM)

Work activities with materials potentially containing Bloodborne pathogens (BBP), as defined below, requires compliance with OSHA's Bloodborne Pathogens Standard as described in UNL's ***Bloodborne Pathogen/ Exposure Control Plan (BBP/ECP)***. This includes, but is not limited to, initial and annual refresher Bloodborne Pathogen training and recommended vaccinations.

Clinical, diagnostic, research, or teaching activities involving human blood or OPIM are subject to review and approval by the IBC. Work may be initiated only after submission of a completed IBC protocol registration form and approval by the IBC. These materials include:

- Human blood, blood components, and products made from human blood;
- The following human body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, any body fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids;

- Any unfixed tissue or organ (other than intact skin) from a human (living or dead);
- HIV-containing cells or tissue cultures, organ cultures, and HIV- or HBV-containing culture medium or other solutions;
- Blood, organs, or other tissues from experimental animals infected with pathogens present in blood that can cause disease in humans.

4.4.1 *HIV, HBV and other Bloodborne Pathogens Research Labs*

Research and teaching laboratory activities involving the culture, production, concentration, experimentation, and manipulation of Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), or other bloodborne pathogens may be initiated only after submission of a completed IBC protocol registry form and approval from the IBC.

4.5 Human and Non-Human Primate Cells and Organ/Tissue Cultures

Work with all human or non-human primate cells and organ/tissue cultures including those that are potentially infectious or contaminated with bloodborne pathogens, well-established cell lines, human embryonic stem cells and pluripotent cells and their derivatives are covered by the UNL ***BBP/ECP*** and subject to review and approval by the IBC. Work may be initiated only after submission of a completed IBC protocol registry form and approval by the IBC.

Work activities with established human cell lines that are characterized to be free of contamination from HBV, HIV, and other recognized bloodborne pathogens **are not** subject to BBP requirements, but review and approval by the IBC **is** still required. Documentation of the tests verifying the cells to be pathogen free is required and a copy should be attached to the submitted IBC protocol.
See Appendix C of the UNL BBP/ECP for further details related to human cell lines covered by the BBP/ECP.

4.6 Select Biological Agents and Toxins

Some biological agents and toxins, referred to as Select Agents and Toxins by the U.S. Departments of Health and Human Services (HHS) or Agriculture (USDA), have potential to pose severe threat to public, animal, or plant health or to animal or plant products.

Possession* **of select agents (including select toxins over a certain amount) requires issuance of a certificate of registration by the appropriate federal agency (USDA/APHIS or HHS/CDC).**

Note: The only exception to this requirement relates to received diagnostic specimens suspected to contain select agent organisms or toxins. When a select agent is positively identified, notification must be sent to CDC/APHIS by the identifying lab and the samples must be destroyed or transferred to a registered facility. See EHS SOP,* ***Select Agents and Toxins – Clinical and/or Diagnostic Laboratory Activities**.*

Work with or possession of select agents and toxins can be initiated only after consultation with the BSO and Institutional Responsible Official, submission of a completed IBC protocol registry form, approval from the IBC, and completion of the registration process with the appropriate federal agency (HHS/CDC or APHIS). Possessing Select Toxins in less than

certain permissible amounts does not require registration with a federal agency, but does require registration with the IBC and consultation with the BSO. See the EHS SOP, ***Select Agents and Toxins*** for more details about work with select agents.

4.7 Genetically-Modified Animals and Plants

The *NIH Guidelines* do not permit experiments involving deliberate release of genetically-modified organisms into the environment unless, as provided in Section I-A-1 of the *NIH Guidelines*, another federal agency has jurisdiction over the experiment and approves the proposed release.

4.7.1 *Animals*

All live vertebrate animal research at UNL is subject to review by the Institutional Animal Care and Use Committee (IACUC) regardless of IBC review requirements. Following is a summary of general requirements pertaining to genetically-modified animals. Refer to the EHS SOP, ***Recombinant and/or Synthetic Nucleic Acid Molecule Experiments Requiring IBC Review*** for a full discussion and to determine if your project must be approved by the IBC prior to initiation. For the purposes of these guidelines, activities involving genetically-modified animals are divided into two categories:

- **Rodents**
  The purchase or transfer of genetically-modified rodents is exempt from the *NIH Guidelines*, but experiments involving creation or administration of r/sNA to rodents may not be exempt (See sections III-E-3 and III-D-4 of the *NIH Guidelines*). Those activities exempt from the *NIH Guidelines* require submission of an IBC protocol registry form with review by the BSO and Chair of the IBC. Rodent activities that are not exempt from the NIH Guidelines must be reviewed and approved by the full IBC prior to initiation.
- **Other Vertebrates and Invertebrates**
  Except as described above, nearly all activities involving genetically-modified animals must be reviewed and approved by the full IBC prior to initiating work. This includes all species within the kingdom Animalia.

4.7.2 *Plants*

Review and approval of genetically-modified plant experiments (including growing of transgenic plants; administration of any pathogenic microorganism, arthropod or nematode to genetically-modified plants, etc.) conducted in laboratory, growth chamber or greenhouse settings is required in compliance with the Sections III-D-5, III-E and III-E-2 of *NIH Guidelines* as described in Subsection 4.1 above.

PIs planning genetically-modified plant field trials pursuant to a valid and current APHIS permit must submit their application to the UNL Biotechnology Quality Management System (BQMS) committee. The BQMS committee is responsible for review of these applications. The IBC is notified of the actions of the BQMS committee on a regular basis.

4.8 Field Collection or Sampling of Wild Animals

The following activities involving the field collection or sampling of wild animals requires submission of an IBC protocol registry form and approval by the IBC prior to initiation of work activities due to the risk of zoonotic diseases. Persons conducting such research should be familiar with potential implications associated with the Select Agent regulations, as summarized in the ***EHS Guidance: Reporting Requirements for Select Agents and Toxins Identified in Field-collected Samples*** (https://ehs.unl.edu/EHS%20Guidance_SAReportingReqforFieldSamples.pdf).

- Trapping and handling of wild animals for surveillance of zoonotic agents (infectious to humans and animals) designated at Risk Group 2 or higher.
- Trapping and handling of wild animals that may transmit significant or life threatening zoonotic diseases (e.g. rabies, Hantavirus Pulmonary Syndrome) as determined by risk assessment of the target species and proposed procedures.
- Laboratory processing of diagnostic samples collected from the above studies.

5 Protocol Development, Approval, and Maintenance

An IBC Biosafety protocol will be required for each PI working with materials described in Section 2; it should cover **all** research activities requiring registration with the IBC. If you are working with multiple model systems or microorganisms but the basic techniques and procedures are the same, the IBC encourages you to enter all of the information in one protocol form with a generalized title. The individual projects can be described separately in the "Research Description" section of the protocol form. A separate protocol will be required for PI's directing/managing teaching labs and/or core/diagnostic facilities that exist outside of the PI's research activities; and for experiments or work activities that are not closely related in objectives, techniques, or procedures.

The IBC approval process begins with the PI completing all required documentation and supporting material necessary for thorough review by the IBC and Biosafety Officer (BSO). Documentation and supporting materials consist of the following items:

- A completed NuRamp IBC New Protocol form, and;
- A lab-specific biosafety manual (See EHS SOP, ***Preparing a Laboratory Biosafety Manual***).

In addition, IBC approval will require:

- Completion of required training (See Section 5.2.2) by all personnel listed in the protocol.
- Satisfactory completion of a Pre-approval lab safety survey by the EHS Biosafety Staff.

Protocols approved by the IBC are valid indefinitely contingent on the PI following the requirements for protocol maintenance described in Subsection 5.5.

5.1 Protocol Development and Submission

Protocols are registered electronically using the NuRamp research administration system found at https://nuramp.nebraska.edu/login. Log-in credentials default to the PI's *My.UNL*

username and password. Further details and instruction about IBC protocol development and the information needed to complete the IBC New Protocol Form can be found on the IBC webpage (https://ehs.unl.edu/committees/ibc). The information requested in the protocol form is necessary and required to support consideration of the following:

- Dual use considerations (as further described in Appendix A)
- Risk assessment, leading to proper selection of appropriate containment level, safety equipment, and laboratory practices and procedures.
- Training and expertise of the PI and laboratory workers

5.2 Pre-approval Requirements

In addition to preparing and submitting the New Protocol Form, there are several additional requirements to be completed prior to IBC review or they will be contingencies of approval following IBC review.

5.2.1 *Preparing a Laboratory Biosafety Manual*

A biosafety manual is an important piece of documentation for every laboratory. The manual is not submitted for IBC review; however, it is evaluated by the BSO as part of the administrative review process described below. EHS provides a number of standard Safe Operating Procedures (SOPs) related to accidents/injuries, spills, emergency preparedness/response that the PI may opt to include in their manual. If the PI opts to develop individual procedures in lieu of these standard procedures provided on the EHS web page (http://ehs.unl.edu/sop/), then those lab-specific procedures must be submitted with the protocol for review and approval by the IBC. The EHS SOP, ***Preparing a Laboratory Biosafety Manual*** provides guidance on the content required for a biosafety manual. The ***Biosafety Manual Table of Contents Form*** described in the SOP should be completed and submitted with the protocol form through NuRamp. The form can be downloaded from http://ehs.unl.edu/forms/

5.2.2 *Training*

Training in the principles and practices of general biosafety is essential to maintaining a safe work environment and it is the responsibility of each PI to ensure that his/her lab personnel are properly trained. All employees of UNL are required to take the following EHS courses[2]:

- *Core – Injury and Illness Prevention Plan*
- *Core – Emergency Preparedness Training*
- *Chemical Safety Training* (if assigned tasks with potential for exposure to hazardous chemicals)

Biosafety training is required of all PIs and laboratory personnel working on IBC approved research protocols. This training must be completed **prior** to working on experiments/protocols that require IBC approval. Additionally, laboratory workers must receive annual refresher training on biosafety. Biosafety training requirements are further detailed in the EHS SOP, ***Biosafety Training***, but below is a list of training material that applies to most IBC protocols.

- **Biosafety Research Compliance** (required for all UNL employees working with biological materials subject to UNL's Biosafety Guidelines. Training covers the oversight of biological research at UNL, risk assessment, protocol development, and the NIH Guidelines)
    - ***Biosafety 100: Research Compliance*** - web-based - (UNL EHS)[2]
- **Biosafety Procedures and Practices** (as applicable to approved protocol)
    - All biosafety levels– web-based – ***Biosafety 101*** (UNL EHS)[2]
    - BSL-2/ABSL-2– web-based – ***Biosafety 101*** and ***Biosafety 201*** (UNL EHS)[2]
    - BSL-3 – ***Biosafety 101*** and ***Biosafety 201*** (UNL EHS)[2] AND additional training provided by BSL-3 facility director or alternative as prescribed by the BSO and/or IBC.
- **Bloodborne Pathogens Training -** PIs and their staff working with bloodborne pathogens or other potentially infectious materials (OPIM) including human cell lines are required to take training annually in addition to other required training.
    - ***Bloodborne Pathogens for Laboratory Workers*** – web-based - (UNL EHS)[2]
- Additional training may be necessary as applicable to the research approved. Examples include autoclave operation training, radiation safety training, export control awareness, animal handling, etc.

IMPORTANT: Completion of training is a condition of continued IBC protocol approval and failure to comply may result in suspension or termination of an IBC protocol. See Section 3.2.1 for details about violation of the UNL Biosafety Guidelines.

5.3 Protocol Administrative Review

The protocol review process is depicted in Appendix B to these Guidelines. A unique Protocol ID number is assigned to each protocol when a New Protocol Form is started. All future correspondence related to an approved protocol must reference this protocol number. The initial review process involves thorough evaluation by EHS Biosafety Staff. Revisions to the protocol may be requested from the PI during this process. Prompt response to the revisions request by the PI will facilitate timely submission of the protocol to the IBC for review.

A protocol is released to the IBC or IBC Chair for review only after it has been accepted as substantially complete by the BSO, the PI has electronically signed the form, and the department head/chair has indicated his/her support of the protocol by electronic signature.

[2] Web-based training can be accessed from the EHS website at http://ehs.unl.edu

After the BSO determines the protocol form to be substantially complete, it is sent back to the PI for his/her electronic signature. When "signing" the document, the PI must agree to the following:

To the best of my knowledge, the information in this document is a true and accurate description of the research that will be conducted in my lab. I understand my responsibilities under the NIH Guidelines and other national standards or regulations as summarized below:

- *I must adhere to all sections of the **UNL Biosafety Guidelines** relevant to my project(s) and ensure that all personnel involved in this project are aware of their responsibilities in the conduct of this research.*
- *I must adhere to the **NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules**, the current edition of the CDC/NIH **Biosafety in Microbiological and Biomedical Laboratories** manual, the **Select Agent Rules** (42 CFR Part 73, 7 CFR Part 331, 9 CFR Part 121) and other authoritative and/or regulatory sources as appropriate.*
- *I must amend my protocol and seek IBC approval prior to implementing major changes to my approved protocol; further, I must complete the annual update form in a timely fashion and provide notification of minor changes that do not require submission of a formal amendment to my protocol.*
- *I am responsible for the safe conduct of the experiments to be conducted and must ensure that all associated personnel complete required training relative to this work, as described in the **UNL Biosafety Guidelines** and the **NIH Publication, "Investigator Responsibilities"** (reprinted in the UNL Biosafety Guidelines).*

After the PI has signed off on the protocol form, notification is sent to the Department Head/Chair/Director (DH/C/D) that the protocol is ready for review. **NOTE**: A PI conducting research and currently serving as a DH/C/D cannot sign off on their own protocol and a Dean/Director/Vice Chancellor will need to be indicated as the DH/C/D on the protocol form. The DH/C/D will review and sign off on the protocol unless they deem revisions are necessary, if so, it will be sent back to the PI for revisions. On the routing page of the form, the DH/C/D must agree to the following statements before signing off on the protocol:

I fully support the research project(s) described in this protocol form. To that end I acknowledge the following:

- *I will ensure that the PI adheres to all sections of the **UNL Biosafety Guidelines** relevant to their project(s) and all biosafety requirements and safety policies and procedures are enforced at the departmental level*
- *I will ensure that adequate facilities are available to the PI for the safe conduct of the proposed experiments.*

- *I will ensure that all associated personnel complete required training relative to this work, as described in the **UNL Biosafety Guidelines** and the **NIH Publication, "Investigator Responsibilities"** (reprinted in the UNL Biosafety Guidelines).*

Once the DH/C/D signs off on the protocol it is released for viewing by the IBC committee and placed on the agenda for discussion at the next meeting The BSO will supplement the information in the protocol with a report to the IBC on the facilities, laboratory biosafety manual, personnel training and other relevant information.

5.3.1 *Laboratory Safety Surveys*

For all new IBC protocols, EHS biosafety staff will conduct a pre-approval safety survey of the facilities and review lab-specific procedures and the biosafety manual to verify compliance with safety guidelines. Findings will be communicated to the IBC at the time of protocol consideration and/or prior to issuance of the final approval notification.

For protocol Amendment Forms, results from the previous annual safety survey are reported to the IBC when protocol amendment forms are reviewed.

5.4 IBC Review

The IBC meets monthly, when there is pending committee business. Committee meeting schedules are published on the EHS web page. Protocols must be submitted for pre-review at least three (3) weeks before the upcoming meeting in order to be eligible for review at the meeting. IBC meetings are open to the public. If the PI requests a closed meeting, or redaction of information from the publicly available protocol or meeting minutes, the BSO will arrange for review of the request by NU legal counsel prior to consideration by the committee. NU legal counsel will determine the appropriate level of public restriction or redaction.

Protocols requiring review by the full committee **must be complete no less than 10 days prior to the next scheduled meeting** (this includes all requested revisions and signatures by the PI and DH/C/D). In some cases, a longer period may be necessary to allow for scheduling and completion of a pre-approval safety survey by the EHS biosafety staff. The entire protocol review and approval process takes 6-8 weeks on average from initial submission to final committee approval with the minimum processing time being 3-4 weeks. PIs are encouraged to communicate with the BSO early in their planning stages to avoid delays in approval.

The meeting agenda and minutes from the previous meeting are sent to the IBC one (1) week prior to the scheduled meeting date. Protocols will not be included on an upcoming meeting agenda unless the PI and DH/C/D have signed off on the new protocol or amendment form. Following discussion and review, possible actions by the IBC are:

(1) a vote to approve as submitted;

(2) a vote to approve the protocol contingent upon specific conditions set forth by the committee;

(3) a vote to table the protocol for discussion at the next meeting pending revision or submission of additional information by the PI to address specific concerns of the committee;

(4) denial of approval.

Following committee action, the BSO will notify the PI of the Committee's decision by e-mail letter. A formal approval letter will be issued only after the PI has satisfied all contingencies imposed by the IBC. Timing of initiation of work activities described in the protocol was previously discussed in Section 4 of these Guidelines.

5.4.1 *Review of Protocols Exempt from the NIH Guidelines*

New protocols determined to be "Exempt" from the NIH Guidelines (Experiments in Section III-F) and conducted at BSL-1 are reviewed and approved solely by the BSO and IBC Chair. This determination is made during the administrative review process.

5.4.2 *Contingencies of Approval*

Sometimes protocols are approved in principal with minor stipulations or contingencies that need to be addressed before a final approval letter is sent to the PI. Following the IBC meeting, the BSO will send an email letter to the PI explaining the outcome of the IBC review and detail what issues need to be addressed. At this point, the protocol form or amendment form is marked as "Revisions Requested" and opened for editing by the PI. This allows the PI to make any requested edits to the form or to upload any missing attachments. Some examples of contingencies that do not require editing of the form are:

- Training needed for personnel
- Missing components of a biosafety manual, or
- Outstanding issues from the last laboratory safety survey.

When these items are addressed, their completion can be conveyed to the committee by submitting comments through the submit revisions button in NuRamp.

5.4.3 *Tabled Protocols*

When a protocol is tabled until the next IBC meeting, a letter outlining the issues to be resolved is sent to the lead PI listed on the protocol. At this point the protocol form or amendment form is marked as "Revisions Requested" and opened for editing by the PI. This allows the PI to make any requested edits to the form or to upload any missing attachments. Protocols are not tabled unless substantial questions are raised during review by the committee or essential information is missing from the protocol to assess the risk and safety of the proposed procedures.

It is the PI's responsibility to address the issues in a timely manner to ensure re-review can be completed at the next scheduled IBC meeting. The PI will also be invited to attend the IBC meeting at which his/her protocol will be re-reviewed by the committee.

5.5 Protocol Maintenance

5.5.1 *Annual Updates*

Annually on the anniversary of the approval of a New Protocol Form, the PI will be required to submit an ***Annual Update Form (AUF)***. The PI will be notified by email to login to the NuRamp system and submit the form. Reminders will be sent at the following intervals: 30 days before the anniversary date, one week before the anniversary date, and on the anniversary date. If an Annual Update or Amendment Form is not submitted by the renewal date, EHS biosafety staff will contact the PI and offer assistance with completing the form.

The purpose of the AUF is to inform the IBC of the continuing status of an approved research protocol and to allow the PI to update certain information. The information that can be edited on this form includes:

- Changes to Personnel (except the PI; this change requires a full Amendment)
- Changes to Facilities (Facility inspection by the BSO is required.)
- Changes to Funding
- Changes to Disinfectants
- Changes to Decontamination/Disposal procedures
- Updates to Pathogen Inventory

There are also questions aimed at helping a PI determine if an amendment form is needed by asking about changes to research projects that would be considered **Major modifications**.

Submission of an Annual Update Form is a condition of continued protocol approval and failure to submit annual update forms or amendments in a timely manner may result in the IBC taking action to suspend or withdraw approval of the protocol until the requested information/documents are received. See Section 3.2.1 for details about protocol suspension.

Annual Update Forms are reviewed by EHS Biosafety staff. The staff and willapprove the forms and notify the PI of any necessary follow up actions. The IBC is given a summary of approved Annual Updates at each IBC meeting.

5.5.2 *Protocol Amendments*

Major changes to a protocol after initial approval must be reviewed and approved by the IBC through submission of an ***Amendment Form***. Amendments can be submitted at any time after a protocol is approved. They also supersede the requirement for an ***Annual Update Form*** if submitted within 60 days of the Annual Update due date.

What constitutes "Major Changes" and require an Amendment to the Protocol?

Major/Significant changes to your protocol includes adding or changing any of the following:

- Principal Investigator
- Genes and genomic fragments studied or used in your recombinant nucleic acid work
- Host/Vector systems and genome editing technologies used
- Infectious agents or biological toxins
- Work with substances from humans or certain vertebrate animals not previously described in the protocol (e.g. non-human primates)
- Genetically-modified animal or plant work not previously described in the protocol
- Collection of or sampling of wild animals with a zoonotic disease risk not previously described in the protocol
- Administration of biologics and/or pathogens to animals not previously described in the protocol
- Administration of biologics to plants not previously described in the protocol
- Anything else that may have an impact on the biosafety level of the work being performed

Work described above that requires submission of an ***Amendment Form*** must **not** be initiated prior to submission of the form to the IBC. Some work may commence after the amendment form undergoes administrative review by the BSO; other work will require IBC approval prior to initiation. Review of protocol amendments will generally be the same as for New Protocol Forms. As such, approval of an Amendment Form may change the renewal date for Annual Updates.

5.5.3 *Administrative Approval of Amendment Forms*

In some cases, the changes described in an amendment form may not require review by the full IBC. In such cases such, the BSO and the IBC Chair will review and approve the amendment form on behalf of the IBC. The IBC will be informed of these approvals at the next convened meeting of the committee. The following conditions must be met in order for a protocol to be eligible for administrative review and approval:

- Changes do not affect the risk assessment or containment level
- Changes do not affect the applicability of already approved sections of the NIH guidelines and do not fall under a section that requires committee review prior to initiation.
- Changes are consistent with approved projects and research objectives

5.5.4 *Minor Modification Form*

Changes to a protocol that are considered “Minor” in nature can be requested through submittal of a ***Minor Modification Form*** through NuRamp. This form can be submitted at any time after initial approval. Below is a list of the categories of changes that are

considered “Minor” by the IBC. The Minor Modification Forms are reviewed and can be approved by the BSO without full committee review:

- Changes to personnel (except the PI; this change requires a full Amendment)
- Changes to facilities (Facility inspection by EHS Biosafety staff is required.)
- Changes to funding
- Changes to Disinfectants
- Changes to decontamination/disposal procedures

If the proposed changes do not fall into the categories above, then an **Amendment Form** must be submitted. Additionally, the **Minor Modification Form** does not replace the requirement for submission of the **Annual Update Form**.

Minor Modification Forms are reviewed by the EHS biosafety staff and they will approve the forms and notify the PI of any necessary follow up actions. The IBC is given a summary of approved Minor Modifications at each IBC meeting.

5.5.5 *Protocol Terminations*

When approved IBC research projects are no longer active due to faculty retirement, leaving the university, or for other reasons, the IBC protocol must be terminated by submitting a Termination Form. The form asks about the final disposition of the materials described in the protocol (e.g., materials are destroyed, transferred to another PI at UNL or externally, taken with the PI to another university, etc.)Submission of this form must be completed immediately upon project completion/termination and prior to faculty leaving UNL*.

> ***Note**: Exceptions to this can be made if lab staff are not leaving with the faculty member and will remain at UNL to finish a project or experiments. If this should occur, the protocol can remain active, but the faculty member must remain in contact with the IBC or designate another faculty member to serve as a representative and responsible person for the protocol. Contact the EHS Biosafety staff for help with this process (ibc@unl.edu or 402.472.4925).*

If a faculty member is leaving UNL and shutting down his/her lab, other requirements for lab decommissioning apply and are detailed in the EHS SOP, ***Laboratory Decommissioning***.

5.6 Post-approval Monitoring

5.6.1 *Laboratory Safety Surveys*

EHS is tasked by the IBC to conduct regular inspections of all IBC approved facilities to ensure continued observance of safety procedures, adequacy of facilities and equipment, adherence to the approved protocol, and compliance with the *NIH Guidelines*.

5.6.2 *Post-Approval Monitoring Visits*

EHS Biosafety staff will conduct post-approval monitoring (PAM) visits with the lead PI on an IBC protocol at an appropriate interval. The frequency of visits will be based on several factors including but not limited to:

- IBC compliance history
- Infectious agents in use
- Human genes manipulated as well as methods used (CRISPR, etc.);
- Viral vectors used;
- Regulatory requirements (APHIS permit, etc.);
- Recent injuries, exposure incidents, or near-misses.

EHS may conduct frequent PAM visits with PIs of new protocols as means of providing lab start up assistance to the PI related to safety procedures and practices and to offer assistance with lab personnel training as requested by the PI.

5.6.3 *Pathogenic Agent Inventory*

Pursuant to best practices in biosafety stewardship and in order to avoid stockpiles of unknown or abandoned pathogenic agents at UNL, it is institutional policy for all faculty and staff to maintain an inventory of all pathogenic agents in their possession at UNL. The inventory must be kept current and accurate at all times. An updated copy of the inventory must be submitted to EHS biosafety staff whenever new agents are added/removed or at least annually. For PIs with active IBC protocols, the inventory is submitted as part of the IBC protocol form and will be verified annually via the Annual Update Form submitted through NuRamp. The inventory must at a minimum contain the following information:

- Genus and species of microbiological agents; or name and species of origin for biological toxins
- Strain information: list all strains of the agents possessed. (*If known, include the genotype of the microbe indicating all antibiotic resistance genes and any mutations that may increase virulence, host range or pathogenicity*)
- Location of agent
- Status of agent (e.g. long-term storage, active use, etc.)

The procedure and other details about submitting and maintaining these inventories can be found in the EHS SOP, ***Pathogen Inventories***.

5.6.4 *Biosafety Refresher Training*

Annual refresher training is required for all workers on active IBC protocols. This training does not need to be comprehensive, but should at least cover a basic biosafety topic or focused special topic that pertains to the research in the lab. Several options exist to meet this required training.

A. Complete the ***Biosafety Refresher Training*** module online at ehs.unl.edu
    - *This online module covers common safety issues observed in labs as well as special topics. EHS will update the annual refresher training each fall, therefore the content will be refreshed each year.*

B. Request EHS staff members to conduct instructor-led training at a location of your choice in a biosafety topic that is particularly relevant to your laboratory.

C. Hold a meeting with laboratory workers:

- Review relevant lab-specific or EHS procedures; and/or
- Discuss a relevant near miss incident or laboratory acquired infection and lessons learned (this could be an incident that occurred anywhere);
- Watch a safety video as a lab. A curated YouTube playlist of biosafety-related videos is available at this address: https://go.unl.edu/zp39.

If option A or B is chosen, EHS will keep records of the training and provide those records to the PI upon request. If option C is chosen, the training must be documented by recording the date of training, the attendees and the content of the training. A sample refresher-training log is available in the EHS SOP, ***Biosafety Training****.*

Additional training may be necessary as applicable to the research approved. Examples include autoclave operation training, radiation safety training, export control awareness, animal handling, etc.

5.6.5 *Medical Surveillance*

The BSO in consultation with the PI and a medical professional is responsible for recommending medical surveillance requirements specific to a protocol for consideration by the IBC. The medical surveillance regime generally includes the following components, as appropriate:

- Medical history and counseling including previous exposure(s) and the need for preventative immunization or pre-exposure prophylaxis including the related risks of vaccination
- Level of immunity for an employee and whether the employee may be immunocompromised
- Other tests and procedures as the medical professional may deem necessary (e.g., serum banking, post-exposure management, etc.)

The Biosafety Officer will assist the PI in arranging for immunizations and medical counseling for all indicated employees when deemed necessary by the IBC.

The process for obtaining Hepatitis B vaccinations for employees working on projects falling under the OSHA Bloodborne Pathogens standard are discussed in the ***UNL Exposure Control Plan*** found on the EHS website (http://ehs.unl.edu/documents)

Appendix A: Dual Use Research of Concern (DURC)

The United States Government (USG) issued a policy on September 24, 2014 that requires institutional oversight of DURC research. [a] This policy became effective on September 24, 2015.

Dual Use Research of Concern (DURC) is defined as life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security.

The USG has limited the scope of this Policy to a well-defined subset of life sciences research that involves 15 agents and toxins and seven categories of experiments.

- **Agents and Toxins**
  1. Avian influenza virus (highly pathogenic)
  2. *Bacillus anthracis*
  3. Botulinum neurotoxin
  4. *Burkholderia mallei*
  5. *Burkholderia pseudomallei*
  6. Ebola virus
  7. Foot-and-mouth disease virus
  8. *Francisella tularensis*
  9. Marburg virus
  10. Reconstructed 1918 Influenza virus
  11. Rinderpest virus
  12. Toxin-producing strains of *Clostridium botulinum*
  13. Variola major virus
  14. Variola minor virus
  15. *Yersinia pestis*

- **Categories of Experiments**
  1. Enhances the harmful consequences[1] of the agent[2] or toxin[3]
  2. Disrupts immunity[4] or the effectiveness of an immunization[5] against the agent or toxin without clinical and/or agricultural justification
  3. Confers to the agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions[6] against that agent or toxin or facilitates their ability to evade detection methodologies
  4. Increases the stability[7], transmissibility[8], or the ability to disseminate the agent or toxin
  5. Alters the host range[10] or tropism[11] of the agent or toxin
  6. Enhances the susceptibility of a host population[12] to the agent or toxin
  7. Generates or reconstitutes an eradicated[13] or extinct[14] agent or toxin listed above

Research Assessment

Work with the above mentioned agents/toxins under the circumstances mentioned above requires additional review by the UNL IBC. Contact the UNL Biosafety for further information or if you are planning any work that could be considered to fall under DURC.

[a] Information from the "United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern" http://www.phe.gov/s3/dualuse

The USG DURC Oversight policy requires that the PI conducting possible DURC research assess whether the research produces, aims to produce, or is reasonably anticipated to produce one or more of the listed experimental effects. This is accomplished by checking the appropriate boxes in Section I of page one of the IBC protocol form. Additional documentation is required should the IBC determine that the work does meet the scope of the USG *Policy for Institutional DURC Oversight*. This documentation includes:

- An assessment of the benefits of the research that considers the risks of conducting the work
- A draft risk mitigation plan for the research developed in conjunction with the PI and the USG funding agency for the research. Annual review of all active risk mitigation plans is required.

The UNL DURC policy requires the following records to be maintained for the term of the research grant or contract plus three years after its completion, but no less than eight years:

- Institutional DURC reviews
- Completed risk mitigation plans
- Training records

Training Resource

- Video - https://www.youtube.com/watch?v=0yS1ur24j40 (NIH/OSP)

Footnotes

[1]**Harmful Consequences:** The ability of a biological agent or toxin to critically alter normal biological functions, inflict damage on public health resources, material, and public safety. This would include augmenting properties such as virulence, infectivity, stability, transmissibility, or the ability of the biological agent or toxin to be disseminated.

[2]**Biological agent:** As is consistent with 18 U.S.C. § 178, any microorganism (including, but not limited to, bacteria, viruses, fungi, or protozoa), or infectious substance, or any naturally occurring, bioengineered or synthesized component of any such organism or infectious substance, capable of causing (A) death, disease, or other biological malfunction in a human, an animal, a plant, or other living organism; (B) deterioration of food, water, equipment, supplies, or material of any kind; or (C) deleterious alteration of the environment.

[3]**Toxin:** As is consistent with 18 U.S.C. § 178, any toxic material or product of plants, animals, microorganisms (including, but not limited to, bacteria, viruses, fungi, or protozoa), or infectious substances, or a recombinant or synthesized molecule, whatever their origin and method of production, and includes (A) any poisonous substance or biological product that may be engineered as a result of biotechnology produced by a living organism; or (B) any poisonous isomer or biological product, homolog, or derivative of such a substance.

[4]**Immunity:** Encompasses all aspects of host immunity (e.g., active, adaptive, adoptive, passive, innate, and immune modulators).

[5]**Immunization:** Refers to the active or passive induction of immunity through inoculation (e.g., natural inoculation or vaccination) with an immunizing agent or with antibodies; this includes antitoxins and toxoids.

[6]**Clinically and/or agriculturally useful prophylactic or therapeutic interventions;** Includes first or second line prevention and treatment measures or alternative therapeutics used with special populations (e.g., pregnant women and pediatric patients), in the form of vaccines, antibiotics, antivirals, antiparasitics, antibodies, herbicides, fungicides, algaecides, insecticides, etc.

[7]**Stability:** The ability of a biological agent to remain viable when exposed to various environmental factors, including temperature, relative humidity, atmospheric pollution, and sunlight. Stability also includes persistence in a host.

[8]**Transmissibility:** The ease with which an agent spreads from host to host or from vector to host (e.g., via arthropod vectors).

[9]**Disseminate:** The process by which infectious diseases or toxins are dispersed. The same routes of entry pertinent to natural spread of diseases are also relevant when their etiologic agents are delivered intentionally (e.g., inhalation of biological agent disseminated as an aerosol, or ingestion of a biological agent disseminated through a water supply.

[10]**Host range:** The number of different species or populations that can become infected by a biological agent, causing disease in the host or allowing it to become a carrier.

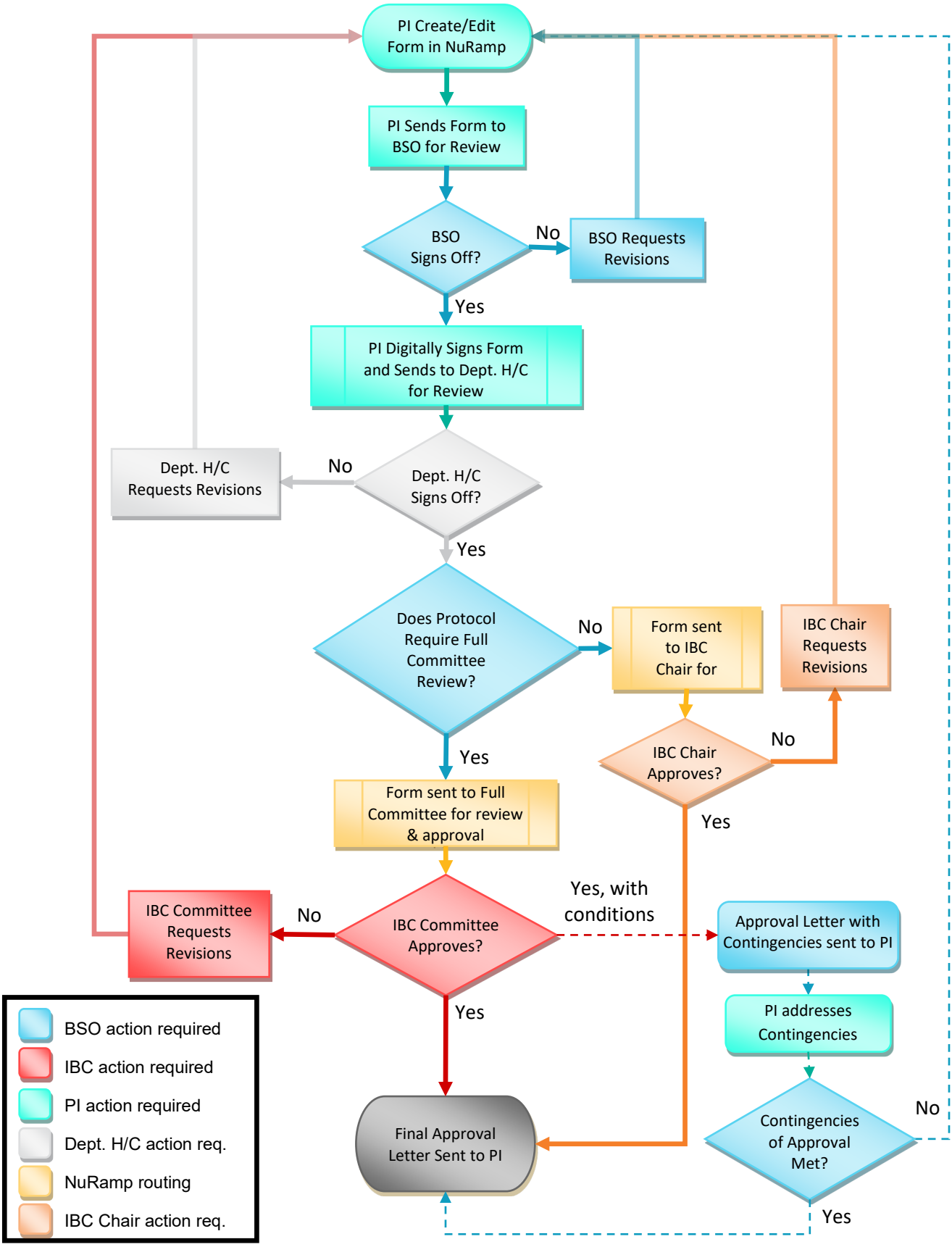
[11]**Tropism:** The specificity of a biological agent or toxin for a particular host tissue or cell.

[12]**Host population:** A collective of organisms that constitutes a specific group or occur in a specified habitat. In the context of the criteria, the use of this phrase implies that the misapplication of the knowledge, products, or technologies derived from the research has the potential to broadly impact a population of host organisms.

[13]**Eradicated agent:** A biological agent that has been exterminated through surveillance and containment resulting in the permanent reduction to zero in the worldwide incidence in the transmission of the agent and the infection/disease it causes; intervention measures are no longer needed. Eradicated agents are thought to no longer exist in circulation in plants, animals, and the environment. J Note: Reconstituted eradicated agents of concern are those for which there are no known or widely available prophylactic or therapeutic interventions, those that could evade diagnostics, or those for which there is no known immunity.

[14]**Extinct agent:** These agents are thought to no longer exist in nature or in the laboratory.

IBC Protocol Approval Process



Appendix C: Applicability of the NIH Guidelines to RNA interference Experiments[1]

What is RNA interference (RNAi)?

RNA interference (RNAi) is a method of suppressing gene expression in cells by introducing a double stranded RNA molecule that is complementary to a portion of your target gene. This double stranded RNA gets taken up by a complex designed to fight RNA based viruses. Using the introduced RNA as a targeting template, the complex binds to any RNA that is complementary to that template and degrades it, in this case the mRNA of your target gene. In this way, expression of a target gene is reduced or eliminated.

What are the most commonly used methods of RNAi?

Depending on the organism worked with, there are different methods of RNAi that can be used. Focusing on mammalian cell culture and mammalian animal models, the primary methods include: short interfering RNA (siRNA), short hairpin RNA (shRNA), microRNA (miRNA). In organisms, like *C. elegans* and *Drosophila,* long double stranded RNA dsRNA may be used also.

siRNA: When using siRNA the researcher will select a 20-22 nucleotide sequence that is unique to the target gene. A RNA oligonucleotide of this sequence and its complement are synthesized, mixed and allowed to anneal to form an RNA duplex. This duplex is then transfected into cells or introduced into animals. The duplex is then taken up by the RNAi machinery as outlined above. The resulting suppression of the target gene's mRNA is transient (3-7 days) as the amount of siRNA within the cells is reduced by degradation and cell division.

shRNA: To achieve a longer lasting gene suppression, the shRNA method was developed. In this method, a DNA cassette is made that contains a 19-29 nucleotide target sequence, a loop domain, and then a 19-22 nucleotides sequence complementary to the target sequence. When transcribed, the RNA twists into the short hairpin structure which brings the complementary target RNA sequences together to form an RNA duplex. This duplex is then processed and taken up by RNAi machinery as above. These shRNA DNA cassettes are placed into plasmid vectors and either directly administered to cells or animals or used to make viral vectors which then introduce the construct by infection. Depending on the method of introduction, the resulting shRNA gene suppression can be transient or persistent.

miRNA: miRNA are naturally occurring genes that code for an RNA that adopts a short hairpin structure. This hairpin is then processed by the RNAi machinery into a mature miRNA of 21 nucleotides which suppresses gene expression as described above. Many miRNAs are complementary to more than one gene's mRNA and thus can suppress the expression of

[1] This document originally prepared by Vanderbilt Environmental Health & Safety office, Vanderbilt University, https://www.vumc.org/safety/bio/recombinant-dna-molecule-use

several gene products at once. As with shRNA, the miRNA gene is placed into plasmid vectors and either directly administered to cells or animals or used to make viral vectors which then introduce the construct by infection. Depending on the method of introduction, the resulting shRNA gene suppression can be transient or persistent.

dsRNA: Many organisms, most notably *C. elegans* and *Drosophila*, have the capacity to take up dsRNA segments of 200-400 nucleotides in length and then process them into 21 nucleotide pieces which are taken up by the RNAi complex as above. These dsRNA are generally expressed from a plasmid though there are kits for *in vitro* generation of dsRNA. Whole *C. elegans* and *Drosophila* cell culture can passively absorb dsRNA. Targeted microinjection of dsRNA is also used. Gene suppression is transient but long lived. Some RNAi mediated suppression in *C. elegans* persists into first generation offspring.

How is RNAi regulated under the NIH Guidelines for Research Involving Recombinant DNA Molecules?

In section I-B of the NIH Guidelines, recombinant DNA molecules are defined 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." It further goes on to say that, "If the synthetic DNA segment is not expressed in vivo as a biologically active polynucleotide or polypeptide product, it is exempt from the NIH Guidelines."*

siRNA duplexes are synthesized and not replicated within recipient cells. Thus, it is not considered recombinant DNA and is exempt from the NIH Guidelines. In contrast, plasmids containing shRNA, miRNA and dsRNA cassettes are replicated within E. coli during their creation and amplification protocols. Thus, they are r/sNA and regulated by the NIH Guidelines. A risk assessment that evaluates the nature of the insert, the intended recipient, and the transmission method used would be needed to determine the exact regulations that apply to any given experiment.