Committee on Microbiological Safety Policy Manual

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Introduction

I. Purpose

Harvard University's Committee on Microbiological Safety (COMS) serves as the Institutional Biosafety Committee (IBC) for Harvard as well as the Harvard-affiliated medical and research institutions listed in Appendix A ("Affiliated Institutions").

II. Scope

COMS is responsible for reviewing all research projects involving specific materials, COMS Regulated Materials ("CRM"), as defined in the Policy Introduction (II) Scope at Harvard and the Affiliated Institutions. Projects using these materials at institutions under COMS purview cannot commence without COMS approval.

COMS Regulated material(s) or CRM are defined as:

- recombinant or synthetic nucleic acids as defined in the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)
- human or nonhuman primate blood, cells, tissues, fluids and secretions
- biological toxins subject to the National Select Agent Registry Program or
- bacteria, virus, fungi, yeast, parasites and prions

III. Function

So that the biological aspects of the research are conducted in a safe manner using established biosafety standards, principles and practices, COMS shall establish, maintain and update policies and procedures on the proper use of CRM. COMS also shall establish minimum standards and best practices for the oversight and administration of research with CRM that may pose safety, health, or environmental risks, including, for example, requirements for education and training and for laboratory safety policies. All COMS materials must comply with applicable biosafety standards and applicable federal, state and local laws and

regulations and also shall take into consideration relevant worker safety, public health, agricultural and environmental protection, and ethical standards.

IV. Roles and Responsibilities

Harvard University

Harvard is responsible for running COMS and thus has the authority to establish policies and procedures that COMS shall follow in its initial and continuing review and approval of applications, proposals, and activities. For research conducted at or sponsored by Harvard, Harvard also is responsible for compliance with all training and other safety requirements imposed by COMS or by federal, state or local legislation or regulation. Specifically, Harvard shall:

A) Establish and implement policies for the safe conduct of research involving CRM
B) Establish and maintain COMS in compliance with the NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules (NIH Guidelines).
C) Appoint a Biological Safety Officer (BSO) to serve as a member of COMS.
D) Appoint at least one individual with expertise in plant, plant pathogen, or plant pest containment principles to serve either as a regular member of COMS or as an ad hoc member of COMS. This person must be present any time COMS considers a research proposal governed by the NIH Guidelines Appendix P, Physical and Biological Containment for Recombinant DNA Research Involving Plants. Appoint at least one individual with expertise in animal containment principles to serve as a member of COMS.
E) Choose as members of COMS only people with appropriate expertise. Provide appropriate training for the COMS Chair and all COMS members, including without limitation the Biological Safety Officer and other containment experts.
F) For research conducted at or sponsored by Harvard, provide appropriate training on laboratory safety and implementation of the NIH Guidelines to all Principal Investigators and laboratory staff.
G) For research conducted at or sponsored by Harvard, establish mechanisms for having all research involving recombinant or synthetic nucleic acid molecules or other hazardous biological materials reviewed by COMS.
H) For research conducted at or sponsored by Harvard, establish mechanisms for compliance with the NIH Guidelines by all Principal Investigators (PIs) including, for example, the requirements that:

1) All aspects of NIH Guidelines Appendix M must be appropriately addressed by any PI who is conducting human gene therapy trials;
2) No research participant may be enrolled in a human gene transfer experiment until the Recombinant DNA Advisory Committee (RAC) review process has been completed, COMS approval has been obtained, Institutional Review Board approval has been obtained, and any other applicable regulatory authorization has been obtained.

I) For research conducted at or sponsored by Harvard, determine the necessity for health surveillance of personnel involved in connection with individual recombinant or synthetic nucleic acid molecules projects; and if appropriate, establish and maintain a health surveillance program for such projects. Such a program shall be required if personnel are:
   1) Engaged in large-scale research or production activities involving viable organisms containing recombinant or synthetic nucleic acid molecules that require Biosafety Level Three (BL3) containment.
   2) Engaged in animal research involving viable recombinant or synthetic nucleic acid molecules-containing microorganisms that require BL3 or greater containment.

J) For research conducted at or sponsored by Harvard, work with COMS to report any significant problems, violations, or any significant research-related accidents or illnesses to applicable governmental agencies, following the COMS Incident Reporting and COMS Clinical Trial Standard Operating Procedures (SOP).

K) For research conducted at or sponsored by Harvard, provide COMS-approved training as outlined in the COMS Training SOP

L) COMS holds an annual public meeting, in accordance with the Boston Public Health Commission Laboratory Regulation. The COMS encourages members of the public to attend this public meeting to learn more about the review process. To request attendance of a meeting, contact COMS@hms.harvard.edu

Affiliated Institutions

The Affiliated Institutions recognize and agree that Harvard has the authority to establish policies and procedures that COMS shall follow in its initial and continuing review and approval of applications, proposals, and activities. For research conducted at or sponsored by an Affiliated Institution that Affiliated Institution is responsible for making sure that its laboratories and personnel are in compliance with all training and other safety requirements imposed by COMS or by federal, state or local legislation or regulation. Specifically, each Affiliated Institution shall:

A) Establish and implement policies for the safe conduct of research involving CRM.

B) Appoint a Biological Safety Officer (BSO) with appropriate expertise to serve as the primary liaison between the Affiliated Institution and COMS. Provide appropriate training for the Biological Safety Officer.

C) For research conducted at or sponsored by the Affiliated Institution, provide appropriate training on laboratory safety and implementation of the NIH Guidelines to all Principal Investigators and laboratory staff.

D) For research conducted at or sponsored by the Affiliated Institution, establish mechanisms for having all research involving CRM reviewed by COMS.

E) For research conducted at or sponsored by the Affiliated Institution, establish mechanisms for compliance with the NIH Guidelines by all Principal Investigators (PIs) including, for example, the requirements that:

    1) All aspects of NIH Guidelines Appendix M must be appropriately addressed by any PI who is conducting human gene therapy trials;
    2) No research participant may be enrolled in a human gene transfer experiment until the RAC review process has been completed, COMS approval has been obtained, Institutional Review Board approval has been obtained, and any other applicable regulatory authorization has been obtained.

F) For research conducted at or sponsored by the Affiliated Institution, determine the necessity for health surveillance of personnel involved in connection with individual CRM projects; and if appropriate, establish and maintain a health surveillance program for such projects. Such a program shall be required if personnel are:

    1) Engaged in large-scale research or production activities involving CRM that requires BL3 containment.
    2) Engaged in animal research involving CRM require BL3 or greater containment.

G) For research conducted at or sponsored by the Affiliated Institution, follow the COMS Incident Reporting and COMS Clinical Trial Standard Operating Procedures (SOP).

H) For research conducted at or sponsored by the Affiliated Institution, provide training as outlined in the COMS Training SOP

Committee on Microbiological Safety (COMS)

As the IBC responsible for the review and approval of all research involving CRM to be conducted at or sponsored by Harvard or any of the Affiliated Institutions, COMS shall:

A) Maintain membership of no fewer than five members selected for their collective experience and expertise in recombinant or synthetic nucleic acid molecules technology and for their ability to assess the safety of recombinant or

synthetic nucleic acid molecules research and identify any potential risk to public health or the environment. COMS membership shall include:

1) At least two members not affiliated with Harvard or any of the Affiliated Institutions (apart from their membership on COMS) and who represent the interest of the surrounding community with respect to health and protection of the environment.
2) At least one individual with expertise in plant, plant pathogen, or plant pest containment principles when experiments utilizing Appendix P, Physical and Biological Containment for Recombinant DNA Research Involving Plants, require prior approval by the COMS.
3) At least one member representing Harvard's laboratory technical staff
4) The Institutional BSO when an Affiliated Institution conducts recombinant or synthetic nucleic acid molecules research at BL3, BL4, or Large Scale (greater than 10 liters of biological culture),
5) On a rotating basis, Institutional BSOs from Affiliated Institutions that do not conduct recombinant or synthetic nucleic acid molecules research at BL3, BL4, or Large Scale (greater than 10 liters of biological culture),
6) Members with expertise and training in recombinant or synthetic nucleic acid molecules research involving human research participants
7) Members with expertise in recombinant or synthetic nucleic acid molecules technology, biological safety, and physical containment
8) Ad hoc members knowledgeable in institutional commitments and policies, applicable law, standards of professional conduct and practice, community, and the environment

B) Review CRM research conducted at or sponsored by Harvard or the Affiliated Institutions in accordance with the requirements set forth in the NIH Guidelines, approving only those research projects that are found to conform with the NIH Guidelines (as outlined in the COMS Protocol Review and COMS Protocol Maintenance SOPs). Such review and approval may involve setting containment levels as specified in NIH Guidelines Sections III-D-4-b, Experiments Involving Whole Animals, and NIH Guidelines Section III-D-5, Experiments Involving Whole Plants.
C) Periodically review recombinant or synthetic nucleic acid molecules research conducted at or sponsored by Harvard or the Affiliated Institutions listed above to check compliance with the NIH Guidelines.
D) Ensure adequacy of emergency plans and procedures for addressing accidental spills and personnel contamination resulting from or related to rCRM.
E) Follow the COMS Incident Reporting and COMS Clinical Trial Standard Operating Procedures (SOP).

Harvard's COMS Office of Biological Safety, located at Harvard Medical School, bears administrative responsibility for COMS, and shall:

A) On behalf of all COMS-supported institutions, file an annual report with NIH/OSP that includes: (i) a roster of all COMS members clearly indicating the Chair, contact person, BSOs, plant expert (if applicable), animal expert, experts in human gene therapy; and (ii) biographical sketches of all new COMS members.
B) Make available to the public all COMS meeting minutes and any documents submitted to or received from federal funding agencies that these agencies are required to make available to the public.
C) File an annual report with the Boston Public Health Commission (BPHC) which includes: (i) a list of recombinant or synthetic nucleic acid molecules studies approved by COMS; (ii) a roster of all COMS members, and (iii) minutes of COMS meetings.
D) File an annual report with the Cambridge Biosafety Committee (CBC) which includes: (i) a list of recombinant or synthetic nucleic acid molecules studies approved by COMS in the City of Cambridge; (ii) a roster of all COMS members,
E) Notify Principal Investigators of the results of COMS review of their research proposals through each institutional biosafety officer
F) Establish mechanisms for communication between COMS and the relevant Institutional Review Boards (IRB) and Institutional Animal Care and Use Committees (IACUC).
G) Ensure biological safety laboratory inspections in accordance with COMS Laboratory Inspection SOP
H) Ensure reporting of laboratory incidents per COMS Laboratory Incident Reporting SOP and COMS Clinical Trial SOP
I) Maintain a secure electronic database for COMS protocol documentation
J) Appropriately archive COMS records, including:
    1) Records of research projects reviewed by COMS,
    2) COMS minutes,
    3) Other documents related to COMS activities.
K) Monitor national, state and local regulatory trends and communicate any changes to the Biosafety Officers and responsible institutional representatives.
L) Develop CRM training materials

Institutional Biological Safety Officers (BSOs)

Harvard and the Affiliated Institutions each are responsible for their own compliance with all training and other safety requirements imposed by COMS or by federal, state or local legislation or regulation. To this end, the Institutional Biological Safety Officers (BSOs) shall, for their own institutions:

A) Assess the risk of proposed research applications and make recommendations to COMS as to appropriate containment, procedures, and personal protective equipment.
B) Oversee laboratory and vivarium inspections in accordance with the COMS Laboratory Inspection SOP
C) Immediately report to COMS and their institution any significant problems, violations, or any significant research-related accidents or illnesses as defined in SOP
D) Develop emergency plans and procedures for handling accidental spills and personnel contamination and investigating laboratory accidents resulting from or related to CRM
E) Provide advice and guidance on laboratory security
F) Provide technical advice and guidance to PIs and COMS on research safety procedures
G) Monitor:
    1) Institutional Animal Care and Use Committee (IACUC) applications,
    2) Institutional Review Board (IRB) applications
    3) Institutional Material Transfer Agreements (MTAs)
    4) Grants & Contracts Office

Principal Investigators

Principal Investigators bear ultimate responsibility for conducting their research in compliance with all training and other safety requirements imposed by COMS or by federal, state or local legislation or regulation.

A) In general, a PI shall:
    1) Obtain COMS approval before purchase, receipt, storage, initiation or modification of any and all research involving CRM.
    2) Immediately report any significant problems, violations, or any significant research-related accidents or illnesses to his or her Institutional BSO, and work with COMS to report applicable governmental agencies, following the COMS Incident Reporting and COMS Clinical Trial Standard Operating Procedures (SOP).
    3) Make sure that he or she has received adequate training on good microbiological techniques

4) Adhere to institutional emergency plans and procedures for handling accidental spills and personnel contamination resulting from or related to recombinant or synthetic nucleic acid molecules or infectious agent research.
5) Comply with all applicable shipping requirements for CRM

B) Before initiating research involving CRM, the PI shall:
1) Make an initial determination of whether the project involves recombinant or synthetic nucleic acid molecules or Dual Use Research of Concern.
2) Make a determination as to the required levels of physical and biological containment in accordance with the NIH Guidelines;
3) Select appropriate microbiological practices and laboratory techniques to be used for the research;
4) Working with the institutional BSO as appropriate, instruct and train laboratory staff in: (i) protocol (ii) practices and techniques required to ensure safety, and (iii) the emergency plans and procedures for handling accidental spills and personnel contamination resulting from or related to CRM research;
5) Educate laboratory staff about the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection);
6) Working with the institutional BSO, address all aspects of NIH Guidelines Appendix M prior to submission of a human gene transfer experiment to NIH OSP (See COMS Clinical Trial SOP).

C) During the conduct of research involving CRM, the PI shall:
1) Supervise the safety performance of the laboratory staff to ensure that the required safety practices and techniques are employed;
2) Investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures as outlined in the COMS Laboratory Incident Reporting SOP
3) Immediately report to the institutional BSO any work errors and conditions that may result in the release of CRM and work the institutional BSO to correct any such errors or conditions.
4) Ensure the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity and genotypic and phenotypic characteristics) for CRM.

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COMS Protocol Review

I. Purpose

COMS reviews and approves research at Harvard University and its affiliates that involves COMS Regulated Materials ("CRM") as defined in the Policy Introduction (II) Scope.

II. Applicability

Any COMS application that is approved by the committee must follow the guidance of this policy and the recommendations in the NIH Guidelines Section III: Experiments Covered by NIH Guidelines.

III. Implementation Procedures

A. Review Procedure for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

Research involving recombinant or synthetic nucleic acid molecules is covered under one of six sections (Sections III-A through III-F) of the National Institutes of Health (NIH) Guidelines for the Use of Recombinant DNA Molecules (NIH Guidelines). The review process differs depending on which section the research falls under. The Principal Investigator (PI) is responsible for submitting the completed and signed COMS protocol document to their institutional biosafety officer (BSO). The protocol document requires the PI to make an initial determination of which section of the NIH Guidelines (if any) their research falls under. The BSO then conducts a risk assessment and verifies the PI's initial determination of the NIH Guideline section is correct. If the BSO risk assessment results in the protocol requiring further review due to diversity of laboratory activities, common laboratory areas, or other concerns, further committee review may be needed. Further information may be provided in the BSO risk assessment.

1) NIH Section III-A
    a. *Experiments Covered*
        i. Experiments that involve 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 use

of the drug to control disease agents in humans, veterinary medicine, or agriculture, will be reviewed by RAC."

ii. Consideration should be given as to whether the drug resistance trait to be used in the experiment would render that microorganism resistant to the primary drug available to and/or indicated for certain populations, for example children or pregnant women.

iii. At the request of COMS, NIH/OSP will make a determination regarding whether a specific experiment involving the deliberate transfer of a drug resistance trait falls under Section III-A-1-a and therefore requires RAC review and NIH Director approval. An Institutional Biosafety Committee may also consult with NIH/OSP regarding experiments that do not meet the requirements of Section III-A-1-a but nonetheless raise important public health issues. NIH/OSP will consult, as needed, with one or more experts, which may include the RAC.

b. Risk Assessment

i. The institutional BSO will conduct a risk assessment of the proposed research. See the Risk Assessment SOP. The results of the risk assessment will include:

- *Recommended biosafety level*
- *Containment*: The containment, procedures and, if necessary, additional stipulations for the protocol. If available, agent-specific guidance, e.g. risk group or biosafety level, from the NIH Guidelines, CDC/NIH BMBL, or Public Health Agency of Canada Pathogen Safety Data Sheets.

c. Committee Review

i. Protocols that fall un*der Section III-A*, will be assigned at least one committee member reviewer and discussed at an upcoming COMS meeting.

ii. Protocol initiation requires review by the NIH Recombinant DNA Advisory Committee (RAC) and approval by COMS and the NIH Director.

2) NIH Section III-B

*a. Experiments Covered (***Require NIH/** OSP **and Institutional Biosafety Committee Approval Before Initiation)**

Section III-B-1: Experiments that involve the "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)."

b. Risk Assessment

i. The institutional BSO will conduct a risk assessment of the proposed research. See the Risk Assessment SOP. The results of the risk assessment will include:

- *Recommended biosafety level*
- *Containment*: The containment, procedures and, if necessary, additional stipulations for the protocol. If available, agent-specific guidance, e.g. risk group or biosafety level, from the NIH Guidelines, CDC/NIH BMBL, or Public Health Agency of Canada Pathogen Safety Data Sheets.

Committee Review

Applications that fa*ll under Section* III-B will be assigned at least one committee member reviewer and discussed at an upcoming COMS meeting.

Protocol initiation requires approval by the NIH Office of Science Policy (OSP) and COMS.

3) NIH Section III-C (i.e. human gene therapy clinical trials)

a. Experiments Covered

Clinical trials that involve the deliberate transfer into human research participants of either:

1. Recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acid molecules, or

2. Synthetic nucleic acid molecules or DNA, or RNA derived from synthetic nucleic acid molecules that meet any one of the following criteria:

a. Contain more than 100 nucleotides; or

b. Possess biological properties that enable integration into the genome (e.g., *cis* elements involved in integration); or

c. Have the potential to replicate in a cell; or

d. Can be translated or transcribed.

b. Risk Assessment

i. The BSO is responsible for providing COMS will all relevant trial information and providing an overview of the trial at the COMS meeting.

c. Committee Review

i. Applications that falls under Section III-C will be assigned at least one committee member reviewer and discussed at an upcoming COMS meeting.

a. ii. Protocol initiation requires approval by COMS, the local Institutional Review Board (IRB), and review by the NIH RAC (Note: if the protocol is exempt from NIH Guidelines Appendix M requirements, RAC review is not required, but COMS and IRB approval is still necessary) All material relating to clinical studies must be submitted to the COMS Office of Biological Safety 6 weeks prior to the next COMS meeting. Local institutional deadlines vary. PIs/ Clinical Coordinators should consult with their institutional biosafety officer for their local institutional deadline. All human gene therapy studies require two scientific appointed reviewers. Non-recombinant or Synthetic nucleic acid COMS Regulated Materials ("CRM"), as defined in the Policy Introduction (II) Scope, require a minimum of one scientific appointed reviewer. The COMS Chair may request an additional reviewer.

b. At the COMS meeting the application is reviewed by full committee and a vote is recorded.

4) NIH Section III-D

a. Experiments Covered:

i. Experiments that involve the introduction of recombinant or synthetic nucleic acid molecules into Risk Group 2 agents (or higher)

ii. Experiments in which DNA from Risk Group 2 or Risk Group 3 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes

iii. 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

iv. Experiments involving whole animals in which the animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acids, or DNA or RNA molecules derived there from, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals

v. Experiments to genetically engineer plants by recombinant DNA methods where BL3-P containment is recommended.

vi. Experiments Involving More than 10 Liters of Culture

vii. Experiments with some strains of influenza viruses generated by recombinant methods

b. Risk Assessment

i. The institutional BSO will conduct a risk assessment of the proposed research. See the Risk Assessment SOP. The results of the risk assessment will include:

- *Recommended biosafety level*
- *Containment*: The containment, procedures and, if necessary, additional stipulations for the protocol. If available, agent-specific guidance, e.g. risk group or biosafety level, from the NIH Guidelines, CDC/NIH BMBL, or Public Health Agency of Canada Pathogen Safety Data Sheets.
- If necessary, a recommendation for further committee member review

c. Committee Review

i. If Institutional BSO, Director of COMS, or the COMS Chair recommends further committee member review, at least one committee member reviewer will be assigned and the protocol will be reviewed at an upcoming COMS meeting.

ii. If further committee member review is not recommended, the protocol will be discussed at an upcoming COMS meeting

iii. After discussion at a COMS meeting, the BSO will send an approval letter signed by the COMS Chair to the PI indicating that work may commence under the biosafety level and stipulations indicated in the letter.

5) NIH Section III-E

a. Experiments Covered

i. Experiments involving the formation of recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus

ii. Experiments involving recombinant DNA-modified whole plants, and/or experiments involving recombinant DNA-modified organisms associated with whole plants where BL2-P or lower containment is recommended

iii. Experiments involving the generation of rodents in which the animals' genomes have been altered by stable introduction of recombinant DNA, or DNA derived there from, into the germ-line (transgenic rodents).

b. Risk Assessment

i. The institutional BSO will conduct a risk assessment of the proposed research. See the Risk Assessment SOP. The results of the risk assessment will include:

- *Recommended biosafety level*
- *Containment*: The containment, procedures and, if necessary, additional stipulations for the protocol. If available, agent-specific guidance, e.g. risk group or biosafety level, from the NIH Guidelines, CDC/NIH BMBL, or Public Health Agency of Canada Pathogen Safety Data Sheets.
- If necessary, a recommendation for further committee member review

c. Committee Review

i. If Institutional BSO, Director of COMS, or the COMS Chair recommends further committee member review, at least one committee member reviewer will be assigned and the protocol will be discussed at an upcoming COMS meeting.

ii. If further committee member review is not recommended, the BSO and the Director of COMS will verify that III-E is the appropriate section of the Guidelines. The BSO will email the PI that the work under section III-E may commence under the biosafety level and stipulations indicated by the BSO. The protocol will be reviewed at an upcoming COMS meeting.

iii. After discussion at a COMS meeting, the BSO will send an approval letter signed by the COMS Chair to the PI indicating that work may continue under the biosafety level and stipulations indicated in the letter.

6) NIH Section III-F

a. *Experiments Covered*

i. Experiments involving the use of recombinant or synthetic nucleic acid molecules that are exempt from the NIH Guidelines. However, other federal and state standards of biosafety may still apply to such research (for example, the Centers for Disease Control and Prevention (CDC)/NIH publication Biosafety in Microbiological and Biomedical Laboratories). The following Sections III F 1 through 8 outline the exemptions under the NIH Guidelines.

a) Section 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.

b) 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.

c) Section 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.

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

e) Section 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 only in that host (or a closely related strain of the same species).

f) Section 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 comment (see Section IV-C-1-b-(1)-(c), *Major Actions).*

g) Section 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.

h) Section III-F-8. Those that do not present a significant risk to health or the environment (see Section IV-C-1-b-(1)-(c), *Major Actions*), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment.

b. *Risk Assessment*

i. The institutional BSO will conduct a risk assessment of the proposed research. (See Risk Assessment policy). The results of the risk assessment will include:
   - *Recommended biosafety level*
   - *Containment*: The containment, procedures and, if necessary, additional stipulations for the protocol. If available, agent-specific guidance, e.g. risk group or biosafety level, from the NIH Guidelines, CDC/NIH BMBL, or Public Health Agency of Canada Pathogen Safety Data Sheets.
   - If necessary, a recommendation for further committee member review

c. Committee Review

i. If Institutional BSO, Director of COMS t, or the COMS Chair recommends further committee member review, at least one committee member reviewer will be assigned and the protocol will be discussed at an upcoming COMS meeting. Following the COMS meeting, the COMS Office will send an approval letter that is signed by the COMS chair electronically to the PI.

ii. If further committee member review is not recommended, the BSO and the Director of COMS will verify that III-F is the appropriate section of the Guidelines. .

iii. After review at a COMS meeting, the BSO will send an approval letter signed by the COMS Chair to the PI indicating that work may continue under the biosafety level and stipulations indicated in the letter.

B. Review Procedure for Research Not Involving Recombinant or Synthetic Nucleic Acid Molecules

Research not involving recombinant or synthetic nucleic acid molecules is covered under the definition of CRM (see Policy Introduction (II) Scope). . The review process is dependent on the risk assessment conducted by the institutional BSO and input from the Director of COMS and the COMS Chair.

1) COMS Regulated Materials that are not regulated by OSP

a. *Experiments Covered*

i. Experiments involving the use of non-recombinant CRM, regardless of their pathogenicity to humans.

b. *Risk Assessment*

i. The institutional BSO will conduct a risk assessment of the proposed research. See the Risk Assessment SOP. The results of the risk assessment will include:

- *Recommended biosafety level*
- *Containment*: The containment, procedures and, if necessary, additional stipulations for the protocol. If available, agent-specific guidance, e.g. risk group or biosafety level, from the NIH Guidelines, CDC/NIH BMBL, or Public Health Agency of Canada Pathogen Safety Data Sheets.
- Agent-specific, procedure-relevant COMS precedent.
- If necessary, a recommendation for further committee member review

c. Committee Review

i. If the application involves the use of Select Agents (as defined by the HHS and USDA) and/or the containment recommendation is biosafety level 3, then the application will be assigned at least one committee member reviewer and presented at an upcoming COMS meeting.

ii. If the application does not involve the use of Select Agents or BL3 containment:

1. The Institutional BSO, Director of COMS, or the COMS Chair may recommend further committee member review, at which time at least one committee member reviewer will be assigned and the protocol will be discussed at an upcoming COMS meeting.
2. If further committee member review is not recommended, the BSO and the Director of COMS will verify that the protocol does not involve recombinant or synthetic nucleic acid molecules. The BSO will email the PI that the work may commence immediately under the biosafety level and stipulations indicated by the BSO. The protocol will be discussed at an upcoming COMS meeting.
3. After discussion at a COMS meeting, the BSO will send an approval letter signed by the COMS Chair to the PI indicating that work may continue under the biosafety level and stipulations indicated in the letter.

2) Human and non-human primate blood, unfixed tissues or cell lines

a. *Experiments Covered*

i. Experiments involving the use of human and non-human primate blood, unfixed tissues or cell lines.

b. *Risk Assessment*

i. The institutional BSO will conduct a risk assessment of the proposed research. See the Risk Assessment SOP. The results of the risk assessment will include:

- *Recommended biosafety level*
- *Containment*: The containment, procedures and, if necessary, additional stipulations for the protocol. If available, agent-specific guidance, e.g. risk group or biosafety level, from the NIH Guidelines, CDC/NIH BMBL, or Public Health Agency of Canada Pathogen Safety Data Sheets.
- If necessary, a recommendation for further committee member review

c. Committee Review

i. The Institutional BSO, Director of COMS, or the COMS Chair may recommend further committee member review, the protocol will be discussed at an upcoming COMS meeting.

ii. If further committee member review is not recommended, the BSO and the Director of COMS will verify that the protocol does not contain recombinant or synthetic nucleic acid molecules. The BSO will send an approval letter signed by the COMS Chair to the PI indicating that work may commence under the biosafety level and stipulations indicated in the letter.

3) Biological toxins subject to the National Select Agent Program

a. *Experiments Covered*

i. Experiments involving the use of biological toxins subject to the National Select Agent Registry Program.

b. *Risk Assessment*

i. The institutional BSO will conduct a risk assessment of the proposed research. See the Risk Assessment SOP. The results of the risk assessment will include:

- *Recommended biosafety level*
- *Containment*: The containment, procedures and, if necessary, additional stipulations for the protocol. If available, agent-specific guidance, e.g. risk group or biosafety

level, from the NIH Guidelines, CDC/NIH BMBL, or Public Health Agency of Canada Pathogen Safety Data Sheets.

- If necessary, a recommendation for further committee member review

c. Committee Review

i. The Institutional BSO, Director of COMS, or the COMS Chair may recommend further committee member review, the protocol will be discussed at an upcoming COMS meeting.

ii. If further committee member review is not recommended, the BSO and the Director of COMS will verify that the protocol does not contain recombinant or synthetic nucleic acid molecules.

iii. For non-recombinant or synthetic nucleic acid molecules applications that don't require further review, the COMS application submitted serves as a record and work can commence prior to the next meeting. For recombinant or synthetic nucleic acid molecules applications or those that require reviewer, work may not commence until after COMS vote has occurred.

V. Policy Authority

The Committee on Microbiological Safety shall enforce this policy.

a. Related Policies

COMS Risk Assessment Policy

COMS Clinical Trial Policy

VI. References

A. NIH Guidelines

B. CDC/NIH BMBL 5th edition (see Table 2)

C. CDC/USDA Select Agent Regulations

Date Approved: 11/18/11

Last Revision Date: 11/18/16

Risk Assessment Policy

I. Purpose

The purpose of this policy is to define risk assessment and what is required for each application submitted to COMS involving COMS Regulated Materials ("CRM") as defined in the Policy Introduction (II) Scope.

II. Applicability

Any COMS application that will be reviewed by the committee must follow the guidance of this policy and the recommendations in the NIH Guidelines Section IIA *Risk Assessment.*

III. Definitions

A. Biological Agent:

Potentially infectious materials or recombinant agents that are classified as Risk Group 1-3 of the NIH Guidelines.

B. Risk assessment:

Risk assessment is a process used to identify the hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person's exposure to an agent, the likelihood that such exposure will cause a LAI, and the probable consequences of such an infection. The information identified by risk assessment will provide a guide for the selection of appropriate biosafety levels and microbiological practices, safety equipment, and facility safeguards that can prevent LAIs (BMBL 5th Ed., Section II, p.21).

IV. Implementation procedures

A. General Information

Institutional Biosafety Officers (BSO) must perform a qualitative biological risk assessment (RA) of all proposed research protocols involving CRM. All projects are subject to COMS review and approval. The information to be considered in the RA is elaborated below in section B. Additionally, based on the conclusions drawn in the RA, the BSO may recommend that the proposed protocol receive additional review by a COMS committee member. This process is outlined in the COMS Protocol Review SOP.

B. Procedure:

The following is a list of hazards associated with CRM, categories of risk, and contents of the risk assessment. These should all be considered in the RA, as applicable:

1. *Factors influencing hazard of the agent (as applicable)*
    - Pathogenicity
    - Host range
    - Infectious dose (may differ based on route of transmission)
    - Agent stability
    - Concentration of agent
    - Animal study data
    - Effective treatment/prevention (e.g., availability of antibiotics or vaccine)
    - Origin of agent (e.g., academic laboratory, commercial source)
    - Strain validation
    - Predominate route(s) (Note: route(s) of exposure in laboratories may differ than routes of transmission observed in nature)
    - Delivery of genetic material to cell independently (e.g., viral vector) cells

2 Experimental category (as applicable):

- Transferring antibiotic resistance (Section III A)
- Creation of a toxin molecule less than 100ng/kg (Section III B)
- Experiments involving the deliberate transfer of recombinant or synthetic nucleic acid molecules or derived, into one or more human research participants. (Section III C)
- Using risk group agents as host-vector systems (Section III D1)
- Experiments involving recombinant or synthetic nucleic acid molecules from risk group agents and transferring into nonpathogenic prokaryotes or lower eukaryotes (Section III D2)
- Experiments involving use of infectious recombinant or synthetic nucleic acid molecules or RNA viruses or Defective DNA and RNA viruses in the Presences of Helper virus in Tissue Culture Systems (Section III D 3)

- Experiments involving whole animals (Section III D 4)
- Experiments involving whole plants (Section III D 5)
- Experiments involving more than 10liters of culture (Section III D6)
- Experiments involving influenza virus (Section III D 7)
- Experiments involving formation of recombinant or synthetic nucleic acid molecules containing no more than 2/3 of the genome of any eukaryotic virus (Section III E)

3. *Contents of the Risk Assessment (as applicable):*
    - Risk Group
    - Biosafety Level
    - Safety features to reduce risk of replication-competent generating viruses (e.g., genes separated into different plasmids, deletion of 3' LTR (i.e., self-inactivating)
    - Expression of Tat (Tat is a transcriptional activator responsible for high replication rates)
    - Viral DNA integration into the host genome
    - Gene insert (e.g., oncogenic, toxin, altering of cell cycle)
    - Tropism (ability to infect human cells)
    - Human and Old World Non-Human Primate cell and/or tissue use
    - Review of sharps in use and safe sharp alternatives
    - Generation of aerosols (e.g., centrifugation, cell-sorting)
    - Unique procedures/equipment
    - Large volume work [greater than 10L or high concentration of biological organism (greater than 10^6 agent)
    - Animal involvement:
        - Type of animal
        - Potential for animal activity to generate aerosols
        - Infectious agent shedding
        - Handling (e.g., bites, scratches, allergens)
        - Use of a permissive host, engraftment of permissive cells

4. *Hazards associated with materials containing unknown infectious agents (e.g., clinical samples, cell culture) (as applicable)*
    - Source of material
    - Suspected or potential infectious agent(s)
    - Availability of medical or epidemiologic data (e.g. morbidity or mortality rates)
    - Potential route of transmission

5. *Laboratory/ Containment:*
    - Space and facilities available
    - Training and experience of staff
    - qualifications of those utilizing the agent,
    - Laboratory design consistent with BMBL biological containment levels and risk group levels for the organism(s) in use
    - Laboratory procedures consistent with BMBL work practices for the recommended biological containment level
    - Ability to contain unique equipment or procedures

V. Policy Authority

The Committee on Microbiological Safety shall enforce this policy.

VI. Related Policies

A. COMS Protocol Review Policy

VII. References

A. NIH Guidelines

B. NIH Risk Group Classifications (see Appendix B)

C. CDC/NIH BMBL 5th edition (see Appendix C)

Date Approved: 11/18/11

Last Revision Date: 11/18/16

Incident Reporting Policy

I. Purpose

This policy outlines the incident reporting requirements for Principal Investigators, Biosafety Officers, and institutions whose COMS Regulated Materials ("CRM"), as defined in the Policy Introduction (II) Scope, is covered by the Committee on Microbiological Safety.

II. Applicability

Any COMS application that is approved by the committee must follow the guidance of this policy and the recommendations in the NIH Guidelines.

III. Definitions

A. CRM incident:

Any incident involving a CRM. These incidents must be reported to COMS. Local health departments (Boston Public Health Commission and/or Cambridge Biosafety Committee) may also require the reporting of a CRM. Please refer to Appendix D: Regulatory Agency Reporting Procedure for further procedures on reporting to health departments including lists of reportable CRM.

B. Recombinant DNA Incident:

Section IV-B-2-b-(7) of the *NIH Guidelines* states that IBCs should report "...any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses" to NIH OSP within 30 days. Appendix G of the *NIH Guidelines* specifies certain types of accidents that must be reported on a more expedited basis. According to NIH Guidelines Appendix G-II-B-2-k, spills or accidents in BL2 laboratories resulting in an overt exposure must be immediately reported to NIH OBA (as well as the IBC). According to NIH Guidelines Appendix G-II-C-2-q and Appendix G-II-D-2-k, spills or accidents occurring in high containment (BL3 or BL4) laboratories resulting in an overt or potential exposure must be immediately

reported to NIH OSP (as well as the IBC, and BSO). Local health departments under COMS (City of Boston and Cambridge) also require reporting recombinant DNA incidents (See Tables 1 and 2 below).

C. Potential Exposure:

A possible personal contact with a Biosafety Level Three (BL3) recombinant or synthetic CRM. According to the NIH guidelines, this contact would be reportable to NIH Office of Science Policy (NIH OSP). Examples of potential exposures to a BL3 agent are any accidents, equipment failure, or splash to intact skin.

D. Overt Exposure:

A definitive contact with a Biosafety Level Two or Biosafety Level Three recombinant or synthetic CRM. According to the NIH guidelines, this contact would be reportable to NIH Office of Science Policy (NIH OSP). Examples of overt exposures are needle sticks and splashes of recombinant or synthetic nucleic acid molecules agent on personnel.

IV. Implementation procedures

A. Responsibilities

1) Principal Investigator:

As stated in the Memorandum of Understanding and Agreement, signed by the Principal Investigator (PI) of COMS-approved research, PIs are required to report potential or overt exposures to CRM to their Institutional Biological Safety Officer (BSO). Additionally, the NIH Guidelines state that reporting of accidents or illnesses to the NIH is the responsibility of the PI, the BSO and the Institutional Biosafety Committee (IBC). This policy mandates the reporting through the BSO. The following excerpts from the COMS application memorandum highlight the PI requirements under this policy:

"By signing this document I agree to immediately notify COMS if a member of the laboratory staff develops symptoms of illness related to an agent involved in this study and if there is accidental release of a biohazardous agent into the environment.

And in a separate paragraph, "*By signing this document I accept full responsibility for laboratory biosafety training, for the maintenance of a safe workplace and for immediate reporting of accidental exposures to biohazardous agents."*

2) Biosafety Officer:

As stated above, the biosafety officer is responsible for reporting any incident involving rCRM to COMS and to the appropriate government agencies, as listed under Appendix E. In some cases, an entity may designate an institutional responsible official to complete said reporting. The BSO is also responsible for presenting any incident and corrective action plans that have preceded each COMS meeting.

3) COMS:

The committee is responsible for reviewing and discussing incidents at each committee meeting and ensuring that each institution has complied with all applicable regulations for incident reporting. The committee also requires that each Principal Investigator comply with all applicable regulations for incident reporting.

B. Reporting Considerations

1) Procedure

a. Personnel involved in any personal potential or overt exposure must be provided all appropriate medical evaluation and surveillance.
b. The BSO or duly designated representative will notify the Director of COMS and/or the COMS Chair the initial details of the incident. The BSO, or duly designated institutional official, will then notify all appropriate regulatory agencies as specified in Appendices D and E. Notification of the agencies should take place in accordance with reporting requirements as specified in Appendix D.
c. BSO should investigate the incident to identify route cause, training needs, and corrective action measures.
d. A verbal summary of the incident shall be provided by the BSO at the next scheduled COMS meeting and will be recorded in the meeting minutes.
e. For incidents involving laboratory acquired infections, breach of containment or overt exposures, and/or violations of the COMS approval, (or lack thereof), PIs must prepare a written response detailing the laboratory event and corrective actions taken to mitigate the event. The letter should be submitted to COMS one week prior to the next scheduled COMS meeting so that it can be discussed during the meeting. COMS will document its review in the meeting minutes.

2) Reporting of Significant reporting events:(See Appendix D)

a. Spills and accidents which result in overt exposures to any organisms containing rCRM must be immediately reported.

b. Illnesses and/or symptoms potentially related to rCRM in use in the BL3 laboratory must be immediately reported.
c. Breach of BL3 containment which results in potential or overt exposures to organisms containing rCRM released into the environment must be immediately reported.
d. Breach of containment resulting from failure of mechanical systems (e.g. HVAC, loss of power) and laboratory equipment (Biosafety cabinet, centrifuge, ventilated animal cages) must be immediately reported.

3) Reporting of Incidents at the COMS meeting:

BSO should provide verbal report, which shall include, but not limited to, the following:

a. The nature of the incident (e.g. personnel exposure, spill, loss of containment, loss of transgenic animal, failure to obtain IBC approval, failure to follow approved containment conditions, other)
b. The COMS approval number
c. Federal, state or local agencies to which incident is being reported
d. A description of the incident, including the following information:
    i. The recombinant agent or material involved. (if applicable)
    ii. The incident/violation location (e.g. laboratory biosafety level, vivarium, non-laboratory space).
    iii. The person(s) involved in the incident/violation, including others present at the incident location. [position title only] (e.g., graduate student, post doc, animal care worker, and facility maintenance worker).
    iv. Actions taken immediately following the incident/violation to limit any health or environmental consequences of the event, as well as the [position titles] of the individual(s) who took those actions.
    v. The training received by the individual(s) involved and the date(s) the training was conducted.
    vi. The institutional or laboratory standard operating procedures (SOPs) for the research and a determination of whether there was any deviation from these SOPS at the time of the incident/violation.
    vii. Any deviation from the COMS-approved containment level or other COMS approval conditions at the time of the incident/violation.
    viii. The personal protective equipment in use at the time of the incident/violation.
    ix. The occupational health requirements for laboratory personnel involved in the research.
    x. Any medical treatment/surveillance provided after the incident.
    xi. Any injury or illness associated with the incident.

xii. Any equipment failures that occurred.

xiii. Any other relevant information identified during the review/investigation of the event

xiv. Measures taken by the Institution to mitigate identified problems (e.g., review by COMS, root cause analysis)

4) Multi-institutional research:

There may be circumstances where Principal Investigators are collaborating with other institutions that are not covered by COMS. The Principal Investigator must report to their BSO any incident that occurs under a COMS protocol. PIs should be aware that they may have additional reporting obligations to other institutions should their work be registered at other Institutional Biosafety Committees (IBCs).

VI. Policy Authority

The Committee on Microbiological Safety shall enforce this policy.

a. Related Policies

VII. References

a. NIH Guidelines

b. CDC/NIH BMBL 5th edition (see Table 2)

Date Approved: 3/11/11

Last Revision Date: 11/18/16

Policy on Minutes of IBC Meeting

I. Purpose

To describe the policy for completing the minutes of convened meetings of the Harvard University Institutional Biosafety Committee, commonly referred to as COMS (Committee on Microbiological Safety).

II. Applicability

The *NIH Guidelines for Research Involving Recombinant DNA Molecules* (NIH Guidelines) require that IBC minutes and documents be made available to the public on request. (Section IV-B-2-a-7) The NIH Office of Biotechnology Activities has issued two documents pertaining to minutes (Q&A 5/14/04; Guidance Memo 2/23/07). Where there are discrepancies in *Robert's Rules of Order Newly Revised* and Guidance from NIH, NIH Guidance is followed. In order to ensure consistency, the following is adopted.

III. Definitions

A. Principal Investigator:

The Principal Investigator (PI), or also known as a Project Director or Program Director, is one or more individuals designated by the institution to direct the project or program supported by the NIH grant. Having more than one PI does not diminish the responsibility of the individual PI. On behalf of the institution, the PI(s) is responsible for full compliance with the *NIH Guidelines* in the conduct of recombinant DNA research.

B. COMS Office of Biological Safety:

The Office records and ensures timely review of public comments and reports comments and COMS response to the NIH OSP and institutions supported by COMS.

IV. Implementation procedures

Information in the minutes must document that COMS has fulfilled its obligations for review and oversight of projects as noted in section IV-B-2-b of the *NIH Guidelines*. The Committee's rationale for particular decisions is clear.

Detail will exceed the standard set in *Robert's Rules*. Minutes will document the date and place of the meeting, whether minutes of the prior meeting were approved, whether and why the meeting was open or closed, all major motions, whether the motions were approved and the time of adjournment. Structure of the minutes will reflect the agenda.

Specifically;

- Attendance will include voting members, ex-officio members, ad-hoc reviewers, consultants, Principal Investigators and guests.
- Members who recuse themselves from discussion or voting on a review due to a conflict of interest.
- Members who leave the meeting for any reason. Quorum must be maintained.
- Members who attended the meeting under discussion may offer modifications to minutes before the IBC.
- Section of the NIH Guidelines pertinent to the research involved in recombinant DNA applications.
- Technical information related to the proposed project:
    - Host(s) and vector(s) to be used
    - Agent characteristics such as virulence and pathogenicity.
    - Function of the inserted DNA sequence
    - Types of manipulations
    - Containment conditions to be implemented

Distribution of Minutes

- The COMS Office of Biological Safety staff distribute draft minutes as part of the COMS agenda for the meeting at which minutes are scheduled to be approved.
- Those present at the convened meeting may submit corrections to the Office of Biological Safety office prior to the meeting as well as during the meeting. The Office staff may correct administrative errors as appropriate.
- Distribution of approved minutes is through Office of Biological Safety.
    - Minutes are submitted to regulatory agencies by this office and distributed to each institution.
    - Other requests for minutes are also processed by this office. Note that the *NIH Guidelines* require meeting minutes be available to the public on request.

COMS Provision of Public Comments to the NIH Office of Biotechnology Activities

In accordance with the NIH Guidelines, COMS shall allow for public review of its actions through the provision of meeting minutes to those that have requested such documentation. COMS, in consultation with the appropriate institutional Biosafety Officer and Office of General Counsel, shall review and respond to all written public comments received in response to public review of meeting minutes in a manner that is consistent with any redaction policy noted in these policies and procedures. Public comments and COMS' response shall be forwarded in writing to the NIH Office of Biotechnology Activities by the Director of COMS in a timely manner at the below address:

National Institutes of Health

6705 Rockledge Drive, Suite 750, MSC 7985

Bethesda, MD 20892-7985 (20817 for non-USPS mail)

NIH Guidelines require COMS to provide copies of its minutes to any member of the public, *respond to public comments received, and report such comments and COMS' response to the NIH.* In addition the guidelines encourage COMS to have its meetings open to the public.

V. Policy Authority:

The Office of Biological Safety of the Harvard Medical School is responsible for supporting the Committee on Microbiological Safety. This includes preparation of IBC meeting minutes as well as the associated record keeping.

VI. Related Policies

- POLICY ON REDACTING MINUTES OF IBC MEETING

VII. References

Date Approved: 3/11/11

Last Revision Date: 11/18/16

Policy on Redacting Minutes of IBC Meeting

I. Purpose

To describe the policy for redacting the minutes of convened meetings of the Harvard University Institutional Biosafety Committee, commonly referred to as COMS (Committee on Microbiological Safety).

II. Applicability

The *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (NIH Guidelines) require that IBC minutes and documents be made available to the public on request. (Section IV-B-2-a-7) The previous Section (IV-B-a-2-6) acknowledges that the protection of privacy and proprietary interests is sufficient to redact portions of minutes. The NIH Office of Biotechnology Activities has issued two documents pertaining to minutes (Q&A 5/14/04; Guidance Memo 2/23/07). In order to ensure redaction is performed consistently, the following procedure is adopted.

III. Definitions

A. Principal Investigator:

The Principal Investigator (PI), or also known as a Project Director or Program Director, is one or more individuals designated by the institution to direct the project or program supported by the NIH grant. Having more than one PI does not diminish the responsibility of the individual PI. On behalf of the institution, the PI(s) is responsible for full compliance with the *NIH Guidelines* in the conduct of recombinant DNA research.

B. Office of Biological Safety:

Records and ensures timely review of public comments. Reports comments and COMS response to the NIH OSP and affiliated institutions.

IV. Implementation Procedures

A. Information not redacted:

- Statement that members recuse themselves from discussion/voting due to a conflict of interest.

- Basic information related to risk assessment and containment levels required by the *NIH Guidelines* for proposed research

B. Information not released to the Public:

- Home telephone numbers and home addresses of COMS members
- Information that is likely to compromise institutional or national security.
- Whether and/or where Select Agent work is ongoing.
- Trade secret or other confidential information
- Principal investigator names
- Laboratory locations
- COMS members names
- Guests names
- Proprietary information is received from sponsors of clinical gene transfer studies
- Proprietary information is received from investigators with patents pending.
- Proprietary information related to personnel matters.

COMS Provision of Public Comments to the NIH Office of Biotechnology Activities

In accordance with the NIH Guidelines, COMS shall allow for public review of its actions through the provision of meeting minutes to those that have requested such documentation. COMS, in consultation with the appropriate institutional Biosafety Officer and Office of General Counsel, shall review and respond to all written public comments received in response to public review of meeting minutes in a manner that is consistent with any redaction policy noted in these policies and procedures. Public comments and COMS' response shall be forwarded in writing to the NIH Office of Biotechnology Activities by the Director of COMS in a timely manner at the below address:

National Institutes of Health

6705 Rockledge Drive, Suite 750, MSC 7985

Bethesda, MD 20892-7985 (20817 for non-USPS mail)

V. NIH guidelines require COMS to provide copies of its minutes to any member of the public, *respond to public comments received, and report such comments and COMS' response to the NIH.* In addition the guidelines encourage COMS to have its meetings open to the public.

VI. Policy Authority

The Office of Biological Safety of the Harvard Medical School is responsible for supporting the Committee on Microbiological Safety. This includes preparation of IBC meeting minutes as well as the associated record keeping.

VII. Related Policies

a. POLICY ON MINUTES OF IBC MEETING

VII. References

Date Approved: 5/26/06

Revised: 2/26/15

Policy for Validation and Use of Attenuated Organisms

I. Purpose

To validate the identity of attenuated organisms derived from virulent organisms that originally required BSL3 containment when the attenuation results in a reduction of required biosafety containment for possession or use.

III. Applicability

This policy applies to registrations of attenuated pathogens sought to be used at BSL-2 when the wild type organism required BSL-3 containment. COMS and the institutional biosafety officer will review the biological inactivation procedures. This policy does not apply to pathogens irreversibly inactivated (e.g., chemically) in a BSL3 laboratory. Principal Investigators must ensure they are in compliance with the federal select agent program requirements for excepted organisms.

IV. Definitions

A. Master Stock
A "master" stock is a culture of a particular organism that has been validated and serves as the reference strain. It is created by selecting 1 colony forming unit (CFU) or plaque forming unit (PFU) and expanding in a small volume. It is used to produce the seed stock.

B. Seed Stock:
A "seed" stock is a vial(s) of stock culture that is prepared from the "master" stock. Each master and seed stock vial is only be used once. A new vial of seed stock is used to prepare a "working" stock for a single routine experiment.

V. Implementation of Procedures

A. Registration of the attenuated RG3 organism

1. As is the case with all proposed studies at institutions under COMS purview, work with any organism cannot be initiated without prior approval by COMS.
2. The investigator must submit a registration describing the proposed work and the validation procedures that will be or have been used.
3. Documentation of the results of validation testing must be submitted to COMS for review and permanent archiving.
4. COMS will review the validation results by appointing two reviewers with the necessary expertise.
5. COMS will present the results at a convened COMS meeting and the committee will vote on whether to accept the results.
6. The investigator will be informed of the COMS decision.

7. Unless COMS grants an exception, until attenuation is validated and COMS approves the results, the attenuated RG3 organisms must be stored in BSL3, and all experiments with attenuated RG3 organisms must take place at BSL3. COMS will consider applications for lesser containment (exceptions) on a case-by-case basis. The source and/or documentation of the strain may be considered in this context.

B. Validation

1. Harvard University has no BSL4 laboratories and so attenuated risk group 4 (RG4) agents cannot be validated at Harvard.
2. If the organism cannot be validated as attenuated, it must be used at the containment level of the wild-type organism (BSL3).
3. Validation of the original organism sample and subsequent master and seed lots prepared must be performed in a laboratory equipped for and experienced with strain validation. This laboratory may be at a Harvard-COMS institution or may be at an off-campus validation laboratory. Refer to **Appendix K** for validation methods.
4. After the initial validation of the original organism, the attenuated RG3 organism must be grown and aliquoted in quantities sufficient for use until the project is completed. Consequently, vials of a master stock that can be used to prepare batches of seed stock are necessary. Each vial of seed stock is to be used for one set of experiments and then discarded without prolonged propagation.
5. Attenuated RG3 organisms must be stored in a secured, limited access facility.
6. A detailed, legible log of each vial used (master and seed lots) must be kept by the investigator.

C. **Maintenance**

1. Vials of master stock and seed stock derived from the master stock must be decontaminated and discarded after a single use.
2. Hence, numerous aliquots must be generated by the receiving laboratory.
    a. Seed Stock must be derived only from the master stock.
    b. Revalidation is also acceptable if Seed Stock is not available

D. Transfer to another Laboratory

1. Attenuated RG3 organisms or their derivatives may not be transferred to another laboratory without COMS approval.

VI. Policy Authority

The Committee on Microbiological Safety shall enforce this policy.

VII. Related Policies

VIII. References

Centers for Disease Control and Prevention/Division of Select Agents and Toxins

Select Agent Program Exclusion List

http://www.selectagents.gov/SelectAgentsandToxinsExclusions.html

Date Approved: 11/30/12

BL2+ Policy

I. Purpose

This policy was created to define proper BL2+ work practices for research with COMS Regulated Materials ("CRM"), as defined in the Policy Introduction (II) Scope, registered with the Harvard Medical School Committee on Microbiological Safety (COMS).

II. Applicability

All laboratories having a COMS-approved registration requiring BL2+ practices.

III. Definitions

BL2+:
Application of specific BL3 work practices to enhance the biosafety of BL2 work practices.

IV. Implementation Procedures

A. General Information

Contact your institutional biosafety officer should you have any questions about BL2+ procedures. Laboratory space and specific work practices for BL2+ work must be reviewed by the institutional biosafety officer prior to beginning research.

B. Practices

Personal Protection Equipment (PPE):

- A disposable solid front gown that is impervious to fluids is required. This gown may be reused unless it becomes contaminated or its integrity is compromised, in which case it must be replaced. Double gloves are required. Skin should not be exposed during work with infectious materials. Options for protection of the skin include extended cuff gloves and gowns with closed cuffs.
- Eye protection is required if manipulations of infectious materials are performed outside of a biosafety cabinet. Eye and face protection (goggles, mask, face shield or other splash guard) is used for anticipated splashes or sprays of infectious or other hazardous materials.
- Respiratory protection, such as a positive air purified respirator (PAPR) or fitted N95 respirator, may be required for laboratory activities that may generate aerosols. This requirement must be evaluated by the institutional biosafety officer.
- All PPE must be dedicated to the BL2+ laboratory and must be autoclaved following use.

Work Practices:

- A sign must be placed on the outside of the laboratory door and designated biosafety cabinet when BL2+ work practices are taking place inside the biosafety cabinet. This sign can be removed when work inside the cabinet is completed and the cabinet has been decontaminated.
- All work involving the manipulation of infectious materials must be conducted in the biosafety cabinet or other physical containment device (e.g. centrifuge rotor covers). Any transfer of materials to or from incubators, freezers, centrifuges should be transported in a primary leak-proof container that is in a secondary container. If manipulation of infectious materials outside of the biosafety cabinet is unavoidable, the use of eye protection and respiratory protection may be required, particularly when a splash hazard is present, as described above.
- All centrifugation must be conducted using rotors with o-rings and/or centrifuge safety covers.
- Efforts should be made to reduce aerosols.
- Depending on the agent in use and experiment being conducted, an absorbable pad may be appropriate.
- The work area must be disinfected before and after each experiment has concluded with a disinfectant and contact time appropriate for the agent(s) in use. This includes laboratory equipment such as centrifuges and biosafety cabinets. Research equipment and materials, including pipetting devices, left inside the biosafety cabinet to be re-used must be thoroughly decontaminated before and after use.
- Any cultures that are to be removed from the BL2+ laboratory for further research or liquid waste disposal must be decontaminated on the outside of each container and placed in a secondary container. The outside of the secondary container must be decontaminated prior to removal from the biosafety cabinet using procedures approved by your safety officer.

Waste Procedures: Inside the Biosafety Cabinet

- A solid waste collection container must be placed inside the cabinet to collect solid experimental materials. Petri dishes should be sealed closed prior to disposal. No infectious agents or materials may be left unattended inside the biosafety cabinet.
- Liquid waste must be inactivated prior to being removed from the biosafety cabinet using a disinfectant appropriate for the work. Aspiration tubing must be flushed with disinfectant following completion of work.
- Solid waste (including paper wrappers and outer gloves) and all other materials generated inside the biosafety cabinet in a BL2+ laboratory are considered contaminated and must be disposed in an autoclavable biohazard waste bag within the biosafety cabinet prior to leaving the laboratory. All sharps waste must be disposed of in a sharps container.

Waste Procedures: Autoclave

- An autoclave must be available but does not need to be located within the BL2+ laboratory. Solid waste materials generated in the biosafety cabinet or that have come in contact with infectious materials must be moved to the autoclave in secondary containment.

- All laboratory supplies and paper used inside the biosafety cabinet must be disposed in an autoclavable bag and these materials must be autoclaved as discussed above.
- Autoclaves used for waste decontamination must be validated quarterly. Contact your BSO to implement a validation program.

Waste Procedures: Outside the Biosafety Cabinet

- Laboratory materials outside of the biosafety cabinet (media, gels, PCR etc.) do not need to be placed in an autoclavable container if they have not come into contact with BL2+ agents. A rigid waste container with a lid must be provided to collect all the waste inside the BL2+ laboratory.
- Shipping cartons that have not come into contact with infectious materials and have not been placed inside a biosafety cabinet can be disposed of as regular trash.

V. BL2+ Training:

- PIs are responsible for ensuring their staff have proper training in BL2+ practices and must provide agent-specific training.

VI. Policy Authority

The Office of Biological Safety of the Harvard Medical School is responsible for supporting the Committee on Microbiological Safety. This includes preparation and revising of the COMS Policy Manual for committee review and approval. The institutional biosafety officer enforces and interprets this policy in collaboration with the Committee on Microbiological Safety (COMS).

VII. Related Policies

- Liquid Waste Policy
- Sharps Policy
- Solid Waste Policy

VIII. References

- BMBL 5th Edition

Date Approved: 4/29/11

Last Revision Date: 11/18/16

COMS Policy on Clinical Trial Studies

I. Purpose:

Investigators must obtain approval from COMS before administering CRM to human subjects.

II. Applicability:

All investigators that conduct work or are employed by a COMS-affiliated institution must have approval from COMS for any clinical trial involving human gene transfer, human xenotransplant or CRM.

III. Definitions:

A) Human Gene Transfer Studies: Research involving the deliberate transfer of recombinant or synthetic nucleic acids into human subjects. NOTE: All human gene transfer studies must be submitted for evaluation to COMS. COMS will make a recommendation whether the study should be reviewed by the NIH Office of Science Policy, Recombinant Advisory Committee (RAC).

B) Human Xenotransplants and Xenografts: Research and investigational therapeutic approaches involving the transfer of organs, tissue, or cells of animal origin into human subjects. Ex vivo use of animal tissue or cells for treating human subjects in a manner that may result in microorganisms being passed to human subjects.

C) COMS Regulated Materials ("CRM") as defined in the Policy Introduction (II) Scope in Human Subjects. Investigational treatment of human subjects with biological agents, whether they are potentially pathogenic or not must be reviewed by COMS if they involve an Investigational New Drug (IND).

D) Microorganisms: An organism that is too small to be seen clearly with the naked eye. Some of these organisms may cause disease.

IV. Implementation Procedures

A) Clinical Trial documents for submittal to Committee:
   i. Clinical Protocol
   ii. Investigator's Brochure
   iii. Informed Consent Form
   iv. COMS Clinical trial application form

v. *NIH Guidelines for Research Involving Recombinant DNA Molecules* (September 2009), Appendix M for gene transfer studies, "Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Molecules into the Genome of One or More Human Subjects."
vi. NIH/OSP RAC letter of review and comments

NIH regulations require Institutional Biosafety Committees await action by the NIH Recombinant DNA Advisory Committee (RAC) before approving and human study involving DNA transfer. The RAC can simply pass the protocol to the FDA or it can decide to evaluate the proposal at its next quarterly meeting. This procedure can delay study approval by as much as six months. However, if an investigator sends a Gene Transfer protocol to COMS at the same time as it is sent to the RAC, local approval can come immediately after the RAC acts.

B) Biosafety information:
The Institutional Biosafety Officer will submit the above study documents with a risk assessment containing: a) study summary, b) biosafety issues involved with the gene transfer product, c) similar studies approved by COMS d) description of study adverse event reporting procedure (NIH, FDA, IRB, and COMS) and the presence of a Data Safety Monitoring Board (DSMB).

C) Review process:
1) All material relating to clinical studies must be submitted to the COMS Office of Biological Safety 6 weeks prior to the next COMS meeting. Local institutional deadlines vary. PIs/ Clinical Coordinators should consult with their institutional biosafety officer for their local institutional deadline.

2) All human gene therapy studies require two scientific appointed reviewers.

3) Non-recombinant or Synthetic nucleic acid COMS Regulated Materials ("CRM"), as defined in the Policy Introduction (II) Scope, require a minimum of one scientific appointed reviewer. The COMS Chair may request an additional reviewer.

4) At the COMS meeting the application is reviewed by full committee and a vote is recorded.

D) Approved Clinical Trials:
Human trials involving gene transfer or xenotransplantation are approved for one year. No research participant shall be enrolled at a clinical trial site until the following documentation is provided below as follows:

1) Safety Reporting

Reporting to COMS Serious Adverse Events (SAE)
Principal Investigators must submit a written report on any serious adverse events that is both unexpected and associated with the use of gene transfer product. Investigators should also report events where there is a reasonable possibility that the product may have caused the event. Reporting is required for any finding from tests in laboratory animals that suggests a significant risk for human research participants including reports of mutagenicity; teratogenicity, or carcinogenicity.

This report labeled "safety report" must be submitted to NIH OSP as soon as possible, but not later than after the sponsor's initial receipt of the information7 days for serious adverse events that result in death or considered life-threatening. Serious adverse events that do not result in death or considered life-threatening should be reported as soon as possible, but no later than 15 days after the sponsor's initial receipt of the information. It should be noted that the event must be reported concurrent to the FDA.

Principal Investigators may delegate to another party, such as the corporate sponsor, the reporting functions set forth in NIH Guidelines, Appendix M, with written notification to the NIH OSP. A copy of this written notification to NIH must be provided to COMS. The Principal Investigator is still responsible for notifying COMS of any serious adverse events through the institutional Biosafety Officer as described above. SAEs that do not require reporting are those that are considered un-related to the study drug or fall out of reporting requirements with the institutional review board(s) that are overseeing the study.

Reporting to other Committees and Regulatory Agencies
Principal Investigators should adhere to any other serious adverse event reporting requirements in accordance with federal regulations, state laws, and local institutional policies and procedures, as applicable.

Specific Institutional Review Board may have additional requirements for adverse event reporting. Dana Farber /Harvard Cancer Institute (DF/HCC) studies (including multi-center trials) must report SAEs as soon as possible, but no later than 10 working days from notification of event on the DFCI IRB SAE Reporting form.

2) Annual Renewals
Clinical studies are approved for one year only. A renewal is necessary to proceed and may be submitted using the online application system. Renewals involve submittal of renewal report form of the year's activities and results. Renewals are required during the follow-up phase. The PI can adjust the renewal timing to correspond with annual reports to other Committees and Agencies (i.e. IRB and FDA).

A current Data Safety Monitoring Board (DSMB) report can be substituted for a renewal report form. Renewal for the subsequent year will be required one year after the date of the DSMB report.

3) Clinical Holds
Investigators must immediately notify COMS of an FDA required hold. In general the COMS approved clinical study will automatically go on COMS hold as well. A release of the FDA hold does not automatically constitute a release by COMS. Rather, the circumstances necessitating the original hold and the extenuating information

resulting in its release will be provided to the COMS Chair through the institutional Biosafety Officer. The Chair will determine whether the issues require committee discussion or if a release of the hold can go forward.

4) Amendments to approved clinical protocols

Clinical protocol amendments must be submitted electronically. The institutional Biosafety Officer then evaluates the changes and conducts a risk assessment. The Biosafety Officer generates a memorandum to the Committee Chair outlining the changes and recommending administrative (document updates, personnel changes) approval or full committee (scientific changes or PI change) review. Should the clinical trial site PI be changed, the new PI's CV must be submitted as part of the amendment.

5) Protocol Closures

Clinical trials that are being closed require notification from the PI to COMS. A clinical trial is considered completed by COMS under the following circumstances:

- Only data analysis is being conducted
- Study follow up is only to confirm long-term survival
- Patients are no longer receiving study drug or follow up and
- There is no further study enrollment of new patients
- Research samples from the patients are no longer being analyzed by laboratories

V. Policy Authority

The Committee on Microbiological Safety shall enforce this policy.

VI. Related Policies to Clinical Trials

A. Principal Investigator Responsibilities:

The Principal Investigator for a clinical trial is solely responsibility for its conduct. It is COMS policy that all materials, documents and other formal communications relating to a proposed human gene transfer or xenotransplantation study come from the Principal Investigator, not the sponsor. It is the responsibility of the Principal Investigator to be fully informed about issues that pertain to the safe conductance of his/her study. Hence, all written responses to Committee queries must be submitted on the Principal Investigator's letter head and must be signed and dated by the Investigator. Signature stamps and signatures by others in the Investigator's name are not acceptable. All communications between a study sponsor and COMS must go through the Principal Investigator. The sponsor may not communicate directly with COMS. Investigators must provide annual updates and reports to the COMS concerning the progress of clinical trials. Investigators are required to train clinical staff about the risks associated with the study, about safe procedures and the proper use safety equipment

B. Multiple Clinical Sites:

Many clinical studies involve multiple centers. When two (or more) centers fall under the COMS umbrella an application from a Principal Investigator at each institution is expected. However, identical protocols from different institutions can be considered together and approval for one will be approval for all. Each PI should submit a COMS application and they will be assigned a related protocol number (e.g. 11-100a, 11-100b…) and will be reviewed as a group.

C. Referrals to Human Gene Transfer and Xenotransplantation Trials at External Institution:
For human gene transfer and human xenotransplantation studies in which investigators associated with Harvard affiliated institutions recruit and follow participants but do not administer the test article will be fully reviewed by COMS. This means the Harvard institution must submit: a copy of the remote site IBC and IRB approvals, a completed COMS application form covering the entire study, a completed NIH recombinant DNA Guideline Appendix M (if required by the NIH), a completed FDA protocol, an FDA investigator s brochure, informed consent forms for both sites, NIH bio sketches of investigators at the non-Harvard institution, and a description of the facilities involved. COMS will defer or reject the application, if deficient. In a mirror situation, one in which the drug or tissue is administered in a Harvard Institution but recruitment and follow-up are done elsewhere, COMS will not require NIH bio sketches of investigators at the non-Harvard institution or a description of the facilities involved.

D. Tissue Processing Laboratories for Human Trials
It is COMS policy that processing of eukaryotic cells or tissues modified with recombinant DNA and destined for human recipients must be carried out in a laboratory accredited, or, in special cases, is actively seeking accreditation, by an independent, outside, clinical organization appropriate to the manipulations.

E. Laboratory Studies Closely associated with Clinical Studies
Research laboratory studies in support of a clinical study carried out in a hospital setting on materials taken from a clinical study can be registered with COMS or, if the Biosafety Officer deems it appropriate, responsibility can be placed with the hospital's infection control unit. In the latter case the Infection Control Unit will take full responsibility for technician safety and training.

F. Cooperative Arrangement with Dana-Farber Cancer Institute
The Dana-Farber Cancer Institute (DFCI) is not covered by COMS. DFCI has its own Biosafety Committee - the Biohazard Control Committee (BCC). On occasion COMS and the BCC are asked to approve the same gene transfer protocol. Principal Investigators will have submitted applications to the IBC serving their institution that include an identical IRB protocol, Investigator's Brochure and Appendix M plus an institution specific application form.

VII. References

NIH Guidelines Appendix M

Date Approved: 9/28/01

Last Revision Date: 4/29/11

Guidelines for Microbiologic Safety in Clinical Trials Involving Xenotransplantation

I. Purpose

The goals of the Xenotransplantation Advisory Committee (XTAC) include the protection of subjects in clinical trials of xenotransplantation (XT), protection of the community at large, and the facilitation of such studies whenever possible. These goals are not contradictory. However, adherence to optimal safety practices will always take precedence when these goals come into conflict.

II. Applicability

All laboratories with or seeking COMS approval for work involving xenotransplantation.

III. Definitions

A) Xenotransplantation: any study in which human tissues (including blood) come into contact (in vivo or ex vivo) with non-human fluids, cells, tissues, or organs. This includes cells or tissues intended for human uses that contact nonhuman cells in vitro (e.g., stem cells cultured with murine feeder cells).
B) Porcine endogenous retrovirus (PERV-A, B, and C): a family of C type retroviruses with some infectivity for human cell lines. No active infection of humans exposed to porcine tissues has been identified to date.
C) Porcine cytomegalovirus (PCMV): a herpes virus without known infectivity for human cells
D) Porcine gamma herpesvirus: (PGHV) an agent associated with post-transplant lymphoma in immunosuppressed swine.
E) Porcine circovirus: of unknown infectivity

IV. Implementation Procedures

A) General Concerns:

A central concern for any human study of XT is the possible introduction of novel infectious agents into the subjects and, subsequently, into their sexual and social contacts. This possibility has been reviewed extensively in the literature. For example, a number of potential pathogens have been described in swine including, but not limited to:

1. Porcine endogenous retrovirus (PERV-A, B, and C): a family of C type retroviruses with some infectivity for human cell lines. No active infection of humans exposed to porcine tissues has been identified to date.
2. Porcine cytomegalovirus (PCMV): a herpes virus without known infectivity for human cells
3. Porcine gamma herpesvirus: (PGHV) an agent associated with post-transplant lymphoma in immunosuppressed swine.

4. Porcine circovirus: of unknown infectivity

Many common pathogens of humans including mycobacteria, common bacteria (e.g., *S. suis* and S*almonella spp*.), parasites (*Toxoplasma gondii*), fungi (*Aspergillus spp*.) The risk of infection due to each of these organisms is unknown and immeasurable for XT procedures. Thus, the FDA has developed guidelines and restrictions for the performance of such trials including sample archiving from donor animals and recipients, testing for a variety of infectious agents, and lifelong- surveillance of recipients of xenogenic tissues (http://www.cdc.gov/mmwr/PDF/rr/rr5015.pdf). It is the responsibility of each investigator to become familiar with relevant regulations and background materials and to assure that each protocol will adhere to these guidelines.

A) Specific Concerns:

1) The sponsor must ensure that appropriate counseling is provided to subjects and their close contacts (family and or sexual partners) to minimize the potential risk of transmission of infectious agents to social and sexual contacts (see pages 5, 17 and 18 of guidance document). Subjects must be required to agree to barrier protection during sexual contacts and to report unexplained illnesses after XT. Subjects must also educate close contacts and relatives regarding potential risks. Pregnancy and unprotected sexual contacts are central concerns regarding the possible transmission of pathogens to a fetus (potentially via germ line transmission), to sexual contacts, and to society.

2) Informational materials regarding potential hazards should be developed for staff and participants.

3) Corporate sponsors are required to test donor animals and tissues for infectious agents (see pages 6-8, 16, 19-29 of guidance document) and to maintain archived blood and tissue samples. They must also report adverse events in clinical trials, and insure that appropriate and up-to-date microbiologic assays are in place for known and potential human pathogens. The sponsor of each study must maintain these records for 50 years. Surveillance samples are required from subjects, source animals, and health care workers (see pages 16, 27, 29, 33 of guidance document).

4) Clinical centers performing XT trials should have the capability to culture and identify potential pathogens on site or through collaborators.

5) Most clinical trials to date have tested blood cells or serum samples to ascertain the presence of potential infection during XT trials. Given that pathogens, including most viruses, have preferred tropism for specific tissues (e.g., brain, lymphocytes, liver); it is likely that such testing is not adequate to detect sub-clinical

infection. Thus, it is reasonable to test multiple tissues during the course of each study (e.g., biopsies, blood samples, autopsy samples) using the most sensitive assays available. The development of new assays will necessitate the re-testing of stored samples. The absence of appropriate assays will necessitate the utilization of resources to develop such assays. Thus, for example, if a study involves xenotransplantation of porcine tissues into the brain, it is reasonable to test any brain tissue samples for PERV DNA and RNA. Other clinical compartments available for testing (i.e., blood) can be used for serial testing of cells and sera for PERV DNA and RNA. The risk for infection may be increased in some trials by the need for immune suppression to prevent graft rejection. COMS considers the investigator responsible for all aspects of each XT trial. These responsibilities include, but are not limited to:

a) Data regarding microbiologic risks are to be provided by corporate sponsors to the investigator. The investigator will provide such information to both COMS and the relevant IRB as part of, or as an amendment to, each XT proposal.
b) The FDA requires that each XT trial includes appropriate infectious disease and epidemiological support to assure appropriate protection of subjects and their contacts throughout the trial and to assist in the evaluation of infectious syndromes if such occur.
c) Annual reports of XT trials must be provided to COMS for review as a condition of trial continuation. As studies progress, it is reasonable to ask investigators to obtain and provide data obtained from earlier clinical trial subjects and from other participating centers. Corporate sponsors and/or investigators must assure that the maximum possible effort (up-to-date assay systems) has been made to identify any infection due to known or unknown infectious agents.
d) The potential benefit to the patient and/or the scientific merit of the proposed trial must outweigh the perceived risks to the subject associated with XT procedures.
e) Administrative review or approval of XT trials will not be available.
f) Significant adverse events will be reported to the IRB and to COMS even if not considered related to the exposure to xenogenic tissues. SAE's from other centers performing clinical trial must also be reported to COMS in a timely fashion. Any adverse event which may have implications for microbiologic safety must also be reported to COMS. SAEs that do not require reporting are those that are considered un-related to the study drug or fall out of reporting requirements of the institutional review board(s) that are overseeing the study.
g) Life-long monitoring of all subjects is required. Assurance of such monitoring is the responsibility of the investigator and trial sponsors. Subjects unable to comply with this or other aspects of the trial should not be included as trial subjects.
h) Investigators should consider that review of complex XT trials is a time consuming process. The timely submission of materials will expedite the review process.
i) Protocol Closures: Clinical trials that are being closed require notification from the PI to COMS. All filings shall be distributed through the Biosafety officer at each respective institution. A clinical trial is considered completed by COMS under the following circumstances:
j) Only data analysis is being conducted
k) Study follow up is only to confirm long-term survival
l) Patients are no longer receiving study drug or follow up and
m) There is no further study enrollment of new patients
n) Research samples from the patients are no longer being analyzed by laboratories

V. Policy Authority

The Committee on Microbiological Safety shall enforce this policy.

VI. Related Policies

VII. References

NIH Guidelines Appendix M

Date Approved: 4/29/11

Last Revision Date: 12/16/16

COMS Training Policy

I. Purpose

The purpose of this policy is to provide the requirements for training to all personnel involved with a COMS registered protocol.

II. Applicability and Minimum Guidelines

A. All personnel (PI, staff, students) handling CRM must meet all institution-mandated training requirements.
B. All PIs registered with COMS must complete NIH Guidelines training prior to initiation of recombinant or synthetic nucleic acid molecules work and not less frequently than once every three years thereafter.
C. Initial awareness-level training on the NIH Guidelines must be provided to PIs that do not work with recombinant or synthetic nucleic acid molecules.
D. All personnel handling CRM must be provided registration and agent-specific biosafety training appropriate to the job activity. This training may be performed by the PI or by a knowledgeable designee. The training must be provided prior to initiation of work, when novel biohazards are added to the work, and as per institutional requirements, but not less frequently than once every three years.

III. Definitions

IV. Record Keeping Requirement:

Each institution or their designee maintains documentation of training for laboratories under COMS purview.

V. Implementation procedures

A. Monitoring Compliance and Sanctions for Non-compliance:
1) Each institution shall verify at the time of laboratory inspections
2) At the time of COMS registration renewal the PI or his designee will verify the PI and their laboratory members have taken the required biosafety training.
3) Untrained individuals will be prohibited from working on the registered biological research.
4) COMS registration will be suspended if training non-compliance is not corrected.

B. COMS Responsibilities:
1) Establishes minimum guidelines for required training at participating institutions
2) Ensures appropriate training for the Committee Chair and members
3) Provides materials/resources for institutions to meet training requirement
4) Notifies Institutional BSOs about new regulations related to training, changes in COMS processes, training modifications
5) Provides training to IACUC/IRB/Grants management personnel on COMS procedures

C. Institutional Responsibilities:
   1) Delivers and documents, through methods determined by the institution, annual biosafety training.
   2) Distributes or makes available training resources/references such as Biosafety Manual, Lab Inspection Checklist, autoclave validation process, spill kits, etc.
   3) Provides resources to laboratories to enable them to perform laboratory and registration-specific biosafety training upon request.

VI. Policy Authority

The Committee on Microbiological Safety shall enforce this policy.

VII. Related Policies

VIII. References

Date Approved: 4/29/11

Last Revision Date: 4/29/11

COMS Inspection Policy

I. Purpose

The purpose of this policy is to standardize the information provided by all BSOs to the Office of Biological Safety and COMS committee members on the laboratory inspection data.

II. Applicability

All laboratories that have a registered COMS application must have a current laboratory inspection date and be in compliance with findings that resulted from their laboratory inspection.

III. Definitions

IV. Implementation procedures

A. Oversight

Section IV-B-2-b-(1) of the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) states that the Institutional Biosafety Committee (known here as the Committee on Microbiological Safety (COMS) is responsible for the assessment of the facilities, procedures, practices, and training and expertise of personnel involved in recombinant (DNA) research. In order to fulfill these COMS requires:

1) Institutional Biosafety Officers (BSOs) submit on an annual basis a Standard Operating Procedure (SOP) including, but not limited to, inspection checklists, methods to address noncompliance,
2) BSOs submit on a monthly basis, completed inspection dates
3) BSOs should submit any significant or chronic biological safety issues that were identified.

B. Review:

Documents and inspection results submitted by BSOs to fulfill the requirements above will be reviewed by the Director of COMS. If necessary, the Director of COMS may raise specific documents or inspection results to the attention of COMS Chair and/or COMS.

C. Frequency

The NIH Guidelines require that BSOs (or alternate personnel trained by the BSOs) conduct periodic laboratory inspections to ensure that laboratory standards are rigorously followed. COMS requires that inspections of all laboratories

with active COMS registrations be conducted once per calendar year for Biosafety Level 2 (BL2) and Biosafety Level 3 (BL3). COMS requires Biosafety Level 1 (BL1) laboratories be inspected at least once per every other calendar year.

D. Reporting

Significant and/or chronic problems, biosafety violations, violations of the NIH Guidelines, or research-related accidents identified during laboratory inspections or otherwise must be reported to COMS as soon as the BSO is aware. The COMS Incident Reporting Policy should also be reviewed to determine if reporting to governmental agencies is required.

V. Policy Authority

The Committee on Microbiological Safety shall enforce this policy.

VI. Related Policies

COMS Incident Reporting Policy

VII. References

NIH Guidelines

Date Approved: 3/27/09

Last Revision Date: 11/18/16

Policy on Suitable Methods of Liquid Decontamination and Disposal

I. Purpose

To satisfy a Massachusetts regulation on the proper disposal of biologically contaminated liquid waste.

II. Applicability

In July 2008 the Massachusetts Department of Public Health required certain changes in the disposal of biological waste. One aspect of the new regulations was the requirement that the local Institutional Biosafety Committee approve the method of liquid effluent disinfection. This policy applies to all institutions that generate biological waste and use the Committee on Microbiological Safety (COMS) as their IBC of record.

III. Definitions

Medical or Biological Waste:
Waste that because of its characteristics may pose a potential hazard to human health or the environment when improperly treated, stored, transported, disposed of, or otherwise managed.

CRM as identified in Section XX and defined as medical or biological waste, and have been adapted from the requirements of 105 CMR 480.000:

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IV. Implementation Procedures

A. General Information

1) An EPA-approved disinfectant must be used, with demonstrated efficacy for the CRM in use, according to the manufacturer's label.
2) . The autoclave should be tested using biological and chemical indicators used periodically and approved by the Biosafety Officer.
3) An on-site treatment log is not required for liquid waste for drain disposal given that the COMS approves the liquid waste policy and an EPA-approved disinfectant with demonstrated efficacy for the CRM is used. Alternative methods for disinfection of liquid waste should be approved by COMS prior to instituting the method in the laboratory.

B. Procedure

Two Options are provided for Disinfection of Liquid Waste As Follows:

Option 1: Bleach disinfection

1) Effectiveness and EPA Approval:

Bleach, a sodium hypochlorite solution (NaOCl), is a broad-spectrum disinfectant that is an effective disinfectant for enveloped viruses (e.g. HIV, HBV, HSV), vegetative bacteria (e.g. Pseudomonas, Staphylococcus, and Salmonella), fungi (e.g. Candida), mycobacterium (e.g. M. tuberculosis and M. bovis), and non-enveloped viruses (e.g. Adenovirus and Parvovirus). E.g. Austin A1 mercury-free bleach and Clorox bleach EPA registration numbers are 1672-20004 and 5813-50, respectively.

2) Recommended Personal Protective Equipment:
    a. Lab coat
    b. Latex or nitrile gloves
    c. Safety glasses

3) Concentration:

The appropriate concentration of sodium hypochlorite for disinfecting liquid BL1 and BL2 waste, e.g. supernatants from cell culture, is 5000 ppm, approximately 0.5%. Household bleach is 5.2 - 6.1 % sodium hypochlorite; therefore a 1:10 (v/v) dilution of bleach to liquid biological waste is appropriate.

4) Procedure:
    All liquid waste should be collected in a final concentration of 10% bleach with a contact time of at least 20 minutes prior to disposal.

    After 20 minutes of contact, disinfected liquid waste is disposed of per institutional policy.

5) Stability and Storage:

Bleach should be stored according to manufacturer instructions, to maintain stability, typically between 50 and 70°F. According to Clorox, undiluted household bleach has a shelf life of six months to one year from the date of manufacture, after which bleach degrades at a rate of 20% each year until totally degraded to salt and water. Some manufacturer-prepared 1:10 bleach solutions, e.g. Bleach-Rite, contain a stabilizer that increases the shelf life to approximately 18 months.

6) Documentation:

An on-site treatment log and validation is not required for chemical disinfection of BL1 and BL2 liquid waste for drain disposal.

Option 2: Autoclave

1) Effectiveness:

Autoclaving is an effective means of sterilizing BL1 and BL2 liquid waste. Sterilization refers to the complete killing of all living organisms, including spores. The methods which rely on heat must be evaluated for each

load or cycle by using a recording thermometer, thermocouple, parametric monitoring device, thermal indicator strip or by an equivalent method approved in writing by MA DPH. The method must be qualitatively validated quarterly using a method of 1 x 10^4 minimum challenge population of a bacterial organism that is most resistant to any aspect of the treatment technology guidelines established by MA DPH (MA DPH 105 CMR 480.150).

2) Recommended Personal Protective Equipment:
   - Lab coat
   - Latex or nitrile gloves
   - Heat resistant gloves
   - Safety glasses

3) Procedure:
   - Collect BL1 and BL2 liquid waste in autoclavable, leak proof containers that are never more than ¾ full.
   - Place containers in an autoclavable tray in the autoclave. LOOSEN each container top and place indicator tape on each top.
   - Adequate cycle time varies depending on load, type of autoclave, and secondary containment. Every autoclave facility needs to determine optimal conditions (Time, Temperature, and Pressure) for their autoclave for waste. Typical cycle times for sterilizing liquid waste range from 45 to 90 minutes at 250°F (121°C) and 15 psi.
   - If allowed, pour sterilized liquid waste down the sink and flush the drain with water, or follow institutional policy.

4) Documentation: An on-site treatment log and validation must record the autoclave cycles as well as quarterly validation of the waste. Records must be retained for 3 years.

5) Laboratory/Containment

V. Policy Authority

The Office of Biological Safety of the Harvard Medical School is responsible for supporting the Committee on Microbiological Safety. This includes preparation and revising of the COMS Policy Manual for committee review and approval. The Committee on Microbiological Safety (COMS) authorizes this policy.

VI. Related Policies

Suitable Methods of Solid Biowaste Decontamination and Disposal
Suitable Methods for Use of Sharps

VII. References

105 CMR 480.000 Minimum Requirements for the Management of Medical or Biological Waste (State Sanitary Code Chapter VIII), effective July 11, 2008.

Date Approved: 3/30/12

Last Revision Date: 3/30/12

Policy on Use of Sharps

I. Purpose

To establish a policy on the safe use of sharps.

II. Applicability

This policy applies to all institutions that use sharps and use the Committee on Microbiological Safety (COMS) as their IBC of record.

III. Definitions

A) Sharps:

B) Discarded medical and research items that may cause puncture or cuts, including, but not limited to, all needles, syringes, lancets, pen needles, glass Pasteur pipettes, broken

C) glassware, glass slides, cover slips, scalpels and razor blades, suture needles, dental wires, and disposable razors used in connection with a medical or research procedure.

IV. Implementation Procedures for Safe Sharps Use

A) Procedure

1) Eliminate the use of devices sharp enough to puncture your skin (including glass) whenever possible
2) Use a sharp with an engineered safety feature when such a device is available and feasible for your procedure.
3) Get trained in proper techniques before using sharp devices in conjunction with biohazardous materials.
4) Use scalpels in the appropriate and safe manner.
5) Do not leave sharp devices out in the environment any longer than necessary.
6) Eliminate recapping needles.
7) Do not put excessive force on a sharps device
8) Use an appropriate sharps container for disposal of sharps waste.
9) Do not fill sharps containers more than 2/3
10) Wear appropriate PPE in accordance with institutional policy
11) Storage: Sharps must be safely stored in the laboratory when not in use. Sharps that are used must be stored for Biowaste Sharps disposal in a rigid sharps container. If a sharp must be re-used, it should be placed in a rigid container with a lid.

B) Policy Authority

The Office of Biological Safety of the Harvard Medical School is responsible for supporting the Committee on Microbiological Safety. This includes preparation and revising of the COMS Policy Manual for committee review and approval. The Committee on Microbiological Safety (COMS) authorizes this policy.

B) Related Policies

1) Suitable Methods of Liquid Biowaste Decontamination and Disposal
2) Suitable Methods for Solid Biowaste Decontamination and Disposal

C) References

105 CMR 480.000 Minimum Requirements for the Management of Medical or Biological Waste (State Sanitary Code Chapter VIII), effective July 11, 2008.

Date Approved: 9/21/2012

Revised: 12/16/2016

Suitable Methods of Solid Biological Waste Decontamination and Disposal

I. Purpose

To satisfy the 105 CMR 480.000 Minimum Requirements for the Management of Medical or Biological Waste regulation on the proper disposal of biologically contaminated solid waste.

II. Applicability

In July 2008 the Massachusetts Department of Public Health required certain changes in the disposal of biological waste. This policy applies to all institutions that generate biological waste and use the Committee on Microbiological Safety (COMS) as their IBC of record. This procedure applies for final treatment and disposal of solid biological waste.

III. Definitions

Medical or Biological Waste:
Waste that because of its characteristics may cause, or significantly contribute to, an increase in mortality or an increase in serious irreversible or incapacitating reversible illness; or pose a substantial present potential hazard to human health or the environment when improperly treated, stored, transported, disposed of, or otherwise managed.

Solid biological waste:

Identified and defined as medical or biological waste, and have been adapted from the requirements of 105 CMR 480.000 if they are contaminated with COMS Regulated Materials ("CRM") as defined in the Policy Introduction (II) Scope:

Pathological Waste:
The following types of pathological waste are identified and defined as pathological waste, and have been adapted from the requirements of 105 CMR 480.000:

- Human anatomical parts
- organs
- tissues and body fluids removed and discarded during surgery
- autopsy, or other medical or diagnostic procedures
- specimens of body fluids and their containers; and
- discarded material saturated with body fluids other than urine.

Contaminated Animal Waste:
The following types of animal waste are identified and defined as contaminated animal waste, and have been adapted from the requirements of 105 CMR 480.000:

- Contaminated carcasses
- body parts
- body fluids
- blood or bedding from animals known to be exposed to a CRM.

IV. Implementation Procedures

A. General Information

Contact your institutional biosafety officer should you have any questions about solid biological waste disposal.

B. Procedure

Two options are provided for disinfection of solid biological waste.

Option 1: Off-Site Biological Waste Disinfection

1. Policy
Institutional policies will dictate the selection of the solid biowaste collection company in accordance with all applicable City, State, and Federal Regulations. Institutional policies must dictate the selection of a solid biowaste collection company in accordance with all applicable City, State, and Federal regulations.

2. Recommended Personal Protective Equipment
The following should be worn while handling or moving solid biological waste collection containers.

- Lab coat
- Disposable safety gloves
- Safety glasses

3. Procedure:

- The solid biological waste collection containers should be placed in locations that are easily accessible to all laboratory users of biological materials.

- Solid biological waste should be added to the waste collection container.

- When the experiment is completed, all solid biological waste containers should have a cover placed over the working container.

- Institutional policies will dictate the personnel and department responsible for the collection, temporary storage of, and replacement of solid waste containers.

4. Storage:

An on-site storage facility shall be provided for all biological waste until it is picked up for off-site disposal. The facility must be separate from all other storage and laboratory areas and must be used only for the storage of biological waste.

5. Documentation:
An on-site treatment log and validation is required for all solid biological waste at the waste collection designated area.

Option 2: Autoclave

1. Policy:
Autoclaving is an effective means of sterilizing BL1 and BL2 solid waste. Sterilization refers to the complete killing of all living organisms, including spores.

2. Recommended Personal Protective Equipment:

- Lab coat
- Disposable safety gloves
- Heat resistant gloves
- Safety glasses

3. Procedure:

- Collect BL1 and BL2 solid waste in autoclavable, leak proof containers. Do not fill containers more than ¾ full.
- Place containers in an autoclavable tray in the autoclave. Fold over but do not seal each biohazard waste container and place indicator tape on each top.
- Adequate cycle time varies depending on load, type of autoclave, and secondary containment. Every autoclave facility needs to validate their autoclave for waste sterilization to determine the appropriate cycle parameters. Typical cycle times for sterilizing solid waste range from 45 to 90 minutes at 250°F (121°C) and 15 psi.
- The methods which rely on heat must be evaluated for each load or cycle by using a recording thermometer, thermocouple, parametric monitoring device, thermal indicator strip or by an equivalent method approved in writing by MA DPH. The method must be qualitatively validated quarterly using a method of 1×10^4 minimum challenge population of a bacterial organism that is most resistant to any aspect of the treatment technology guidelines established by MA DPH (MA DPH 105 CMR 480.150).Please see Appendix A for further instructions.
- Institutional policies will dictate the personnel and department responsible for the collection, temporary storage of, and replacement of solid waste containers.
- Documentation of autoclaving must be completed using a site-specific log provided by 105 CMR 480.000. Please see “Resources” for site specific log from the 105 CMR 480.000. Should an institution choose to use their own log it must be approved by the MA DPH.

4. Storage:
An on-site storage facility shall be provided for all biological waste until it is picked up for off-site disposal. The facility must be separate from all other storage and laboratory areas and must be used only for the storage of biological waste.

5. Documentation:
An on-site treatment log and validation is required for all solid biological waste. Records must be retained for 3 years.

V. Policy Authority

The Office of Biological Safety of the Harvard Medical School is responsible for supporting the Committee on Microbiological Safety. This includes preparation and revising of the COMS Policy Manual for committee review and approval. The Committee on Microbiological Safety (COMS) authorizes this policy.

VI. Related Policies

Suitable Methods of Liquid Biowaste Decontamination and Disposal
Suitable Methods for Use of Sharps

VII. Resources

Log:
105R 480.000 on-site treatment log

Supplies:
Supplies and information about validation of the biological waste treated in an autoclave using *Geobacillus stearothermophilus* are available. COMS does not endorse particular products or suppliers and other suppliers may be used in addition to those suggested (Appendix A).

Equipment:
Autoclave vendor information can be found in Appendix B.

VIII. References

105 CMR 480.000 Minimum Requirements for the Management of Medical or Biological Waste (State Sanitary Code Chapter VIII), effective July 11, 2008.

Date Approved: 12/17/2010

Last Revision Date: 12/17/2010

COMS Policy on Recommended Containment Levels for Adenoviral Vectors in Laboratory Rats, Laboratory Mice and Laboratory Rabbits

I. Purpose: Provide containment requirements for use of adenoviral vectors in Laboratory

Rats, Laboratory Mice and Laboratory Rabbits.

II: Applicability:

All COMS projects involving the use of adenoviral vectors in Laboratory

Rats, Laboratory Mice and Laboratory Rabbits must comply with the requirements of this policy.

III. Definitions:

A) BL2-N(72hr):
Animals are housed in BL2-N containment for the first 72 hours following inoculation with viral vector according to the guidelines of the specific institution. Animal care during this time period is handled by either the laboratory personnel (best practice) or animal care workers, depending on the institution. Waste materials such as bedding, feces and urine should be disposed as of biohazardous waste. After a minimum of 72 hours, animals must be placed in a clean cage before animals can be housed at BL1-N for the remainder of experiment. Please consult with your Biosafety Officer, IACUC and/or Animal Facility Manager on approved procedures at your Institutions animal facility.

IV. Implementation procedures

A. Inoculation
Inoculations of adenoviral vectors into animals are to be performed within a biological safety cabinet under biosafety level 2 (BL2) conditions.
Safer, engineered needles or needle less systems should be used, when possible. Inoculations should be conducted by trained personnel only.
The site of inoculation should be thoroughly cleansed to prevent contamination of bedding materials.

B. Housing
1. The level of housing containment for animals inoculated with viral vectors that can infect human cells is dependent on the characteristics of the viral vector, the animal host, inoculation method, and the transgene.

For most experiments where common, well described replication incompetent adenoviral vectors are inoculated into rodents, the required housing containment is dependent on the expressed transgene (see table below).

2. A list of common, well described adenoviral vector systems is being developed and will be added to this policy when it becomes available. Vectors not on this list may be approved with at a higher containment level.

Transgene Type	Housing
Reporter genes (e.g., green fluorescent protein, LacZ)	BL1-N
Genes with biological activity	BL2-N for first 72 hours post inoculation followed by BL1-N housing (denoted as BL2-N(72hr))
Oncogene or toxin gene (or transgenes with high oncogenic or toxic potential)	BL2-N housing for the life of the animal

C. BL2-N(72hr):
   1. Definition - Animals are housed in BL2-N containment for the first 72 hours following inoculation with viral vector. Laboratory personnel are responsible for animal care during this time period. Waste materials such as bedding, feces and urine should be disposed as of biohazardous waste. After 72 hours, lab personnel will place animals in a clean cage and animals can be housed at BL1-N for remainder of experiment. Please consult with your IACUC and/or Animal Facility Manager on approved procedures at your Institution's animal facility.
   2. Rationale – Studies suggest that the potential for shedding of replication competent virus (RCV) is low but not unfeasible.[i,ii] Therefore, a reduction in containment to BL1-N after 72 hours, reduces the risk of exposure to shed virus and allows for a sensible safety factor.

D. Exceptions:
   1. Animals engrafted or injected with human cells or animal hosts that are permissive for adenovirus replication (e.g., cotton rat, hamster), may be approved at a higher containment level.
   2. Depending on the specific project attributes, COMS may require BL2-N housing for the life of the animal regardless of the expressed transgene.
   3. This policy is subject to change as new information on viral shedding becomes available.
   4. This policy is specific to lab rats, lab mice, not other rodent species. Other animal species may be examined on a case by case basis by COMS.

V. Policy Authority:

The Office of Biological Safety (OBS) of the Harvard Medical School is responsible for supporting the Committee on Microbiological Safety. This includes preparation and revising of the COMS Policy Manual for committee review. The Committee on Microbiological Safety (COMS) authorizes this policy.

VI. Related Policies

VII. References

[i] Oualikene W, Gonin P, Eloit, M. (1994) Short and long term dissemination of deletion mutants of adenovirus in permissive (cotton rat) and nonpermissive (mouse) species. Journal of General Virology 75 pp 2765-2768.

[ii] Ying B, Toth K, Spencer JF, Meyer J, Tollefson AE, Patra D, Dhar D, Shashkova EV, Kuppuswamy M, Doronin K, et al: INGN 007, an oncolytic adenovirus vector, replicates in Syrian hamsters but not mice: comparison of bio distribution studies. Cancer gene therapy 2009, 16:625-637.

Date Approved: 10/29/2010

Last Revision Date: 12/17/2010

Policy on Recommended Containment Levels for use of Retroviral Vectors in Laboratory Rats, Laboratory Mice and Laboratory Rabbits

I. Purpose:

Provide containment requirements for use of retroviral vectors in Laboratory

Rats, Laboratory Mice and Laboratory Rabbits.

II: Applicability:

All COMS projects involving the use of retroviral vectors in Laboratory

Rats, Laboratory Mice and Laboratory Rabbits must comply with the requirements of this policy.

III. Definitions:

A) BL2-N(72hr):
Animals are housed in BL2-N containment for the first 72 hours following inoculation with viral vector according to the guidelines of the specific institution. Animal care during this time period is handled by either the laboratory personnel (best practice) or animal care workers, depending on the institution. Waste materials such as bedding, feces and urine should be disposed as of biohazardous waste. After a minimum of 72 hours, animals must be placed in a clean cage before animals can be housed at BL1-N for the remainder of experiment. Please consult with your Biosafety Officer, IACUC and/or Animal Facility Manager on approved procedures at your Institutions animal facility.

IV. Implementation procedures

A. Inoculation

1. Inoculations of retroviral vectors that can infect human cells (e.g., vectors pseudotyped with VSV-G Env protein) into animals are to be performed within a biological safety cabinet under biosafety level 2 (BL2) conditions.
2. Inoculations with ecotropic viral vectors (i.e., vectors that cannot infect human cells) can be performed under biosafety level 1 (BL1) conditions and inoculated animals housed in animal biosafety level 1 (BL1-N) conditions.
3. Safer, engineered needles or needle less systems should be used, when possible. Inoculations should be conducted by trained personnel only.
4. The site of inoculation should be thoroughly cleansed to prevent contamination of bedding materials.

B. Housing

1. The level of housing containment for animals inoculated with viral vectors that can infect human cells is dependent on the characteristics of the viral vector, the animal host, inoculation method, and the transgene.
2. For most experiments where common, replication incompetent second or third generation retroviral vectors (and packaging systems) are inoculated into animals, the required housing containment is dependent on the expressed transgene (see table below).
3. A partial list of common, well described viral vector systems are located in Appendix F and common packaging systems in Appendix G. Vectors not on this list may be approved with at a higher containment level.

Transgene Type	Housing
Reporter genes (e.g., green fluorescent protein)	BL1-N
Genes with biological activity	BL2-N for first 72 hours post inoculation followed by BL1-N housing (denoted as BL2-N(72hr))
Oncogene or toxin gene (or transgenes with high oncogenic or toxic potential)	BL2-N housing for the life of the animal

C. BL2-N(72hr):

1. Rationale – Studies suggest that the potential for shedding of replication competent virus (RCV) is low but not unfeasible in non-permissive hosts (even if RCV were present in the original vector inoculum)[i]. Therefore, based on guidance from the NIH[ii], a reduction in containment to BL1-N after 72 hours reduces the risk of exposure to shed virus and allows for a sensible safety factor.

D. Exceptions:

1. In light of their potential to support replication of infectious HIV-1, animals engrafted or injected with human cells or animal hosts that are permissive for retrovirus replication (e.g., SCID mice with humanized immune systems), may be approved at a higher containment level.
2. Depending on the specific project attributes, COMS may require BL2-N housing for the life of the animal regardless of the expressed transgene.
3. This policy is subject to change as new information on viral shedding becomes available.
4. This policy is specific to lab rats, lab mice, not other rodent species. Other animal species may be examined on a case by case basis by COMS.

V. Policy Authority:

The Office of Biological Safety (OBS) of the Harvard Medical School is responsible for supporting the Committee on Microbiological Safety. This includes preparation and revising of the COMS Policy Manual for committee review. The Committee on Microbiological Safety (COMS) authorizes this policy.

VI. Related Policies

VII. Reference

[i] Karlen S and Zufferey R. (2007). Declassification of rodents exposed to third generation HIV-based vectors into class 1 animals. Applied Biosafety 12(2) pp. 93-99.

[ii] National Institutes of Health Recombinant DNA Advisory Committee (RAC). *Biosafety Considerations for Research with Lentiviral Vectors*.

COMS Lentiviral Vector Policy

Adopted by COMS: 3/29/13

I. Purpose

To provide guidance for investigators and reviewers for biosafety requirements and best practices for laboratory work with retroviral vectors at institutions affiliated with the Harvard Committee on Microbiological Safety (COMS).

II. Applicability

This policy applies to all new and current projects including work with retroviral vectors at institutions affiliated with COMS. Use of retroviral vectors in laboratory animals is addressed in a separate COMS policy (Policy on Recommended Containment Levels for use of Retroviral Vectors in Laboratory Rats, Laboratory Mice and Laboratory Rabbits).

III. Definitions

A. Retrovirus. Retroviruses are RNA viruses that use reverse transcriptase to produce DNA from an RNA template.
B. Lentivirus. Lentiviruses are a subset of retroviruses. They are characterized by slowly progressive infections, complex genomes and the ability to infect non-replicating cells. Lentiviruses include human immunodeficiency viruses (HIV-1 and HIV-2), simian immunodeficiency virus (SIV) and feline immunodeficiency virus.
C. Retroviral vector. Retroviral vectors are composed of recombinant or synthetic transgene sequences derived from retroviruses, viral packaging and regulatory elements.
D. Tropism. Tropism is the ability of a virus to replicate in specific cells (e.g., from a host species) or tissue. An ecotropic virus has a host range limited to the original host. An amphotropic virus can infect cells of multiple hosts.

IV. Implementation Procedures

A. General Information

Biosafety recommendations for use of retroviral vectors will be made on a case-by-case basis by COMS in consultation with institutional biosafety officers. The general guidelines provided here do not limit the ability of COMS to require biosafety practices for specific projects.

B. Assessment of Biosafety of Retroviral Vectors

There are several factors that affect the biosafety of retroviral vectors and these should be considered in determining what practices will be followed in using these agents.

a. **Tropism**. Use of retroviral vectors that cannot infect human cells is generally safer than use of those that can infect human cells. Some ecotropic wild-type retroviruses, such as feline leukemia virus, cannot naturally infect human cells, while others, such as HIV-1 and HIV-2, can naturally infect human cells. Pseudotyping alters the host-range of retroviral vectors. The most common modification affecting host range is use of the vesicular stomatitis virus (VSV) G envelope protein, which allows the retroviral vector to infect a wide range of animal cells, including human cells.

b. **Potential for Generation of Replication Competent Virus (RCL).** Reducing the potential for generation of RCL increases the safety of retroviral vectors. Modifications to lentiviral vectors have been made which reduce the chance that RCV will be generated. Specific modifications that reduce the chance that RCL will be generated include the following.
   i. limited homology between vector and helper sequences
   ii. separation of vector and packaging functions on multiple plasmids (e.g. 4 or more plasmids in third and greater generation lentiviral vectors)
   iii. elimination of accessory genes (e.g. *tat*) from packaging plasmid
   iv. use of self-inactivating vectors.

c. **Quantity of Vector Used.** Use of high-titer and/or large scale preparations can increase the hazards of using retroviral vectors

d. **Nature of Inserted Genes.** Use of potentially hazardous genetic inserts (e.g. oncogenes) increases the risk associated with use of retroviral vectors. A list of high-risk gene activities can be found in Section D, entitled "High Risk Gene Activity Examples."

e. **Generations**. Lentiviral vectors are separated into several "generations" based on some of these characteristics. The characteristics that define each generation of lentiviral vector are:

   i. First generation: A LV packaging system that includes all HIV-1 genes except *env*.
   ii. Second generation: A LV packaging system that lacks *env* and all auxiliary HIV-1 genes, i.e. *vpr*, *vif*, *vpu* and *nef*. Examples: pCMV-dR8.91, pCMV-dR8.74, psPAX2
   iii. Third generation: A LV packaging system that includes only *gag*, *pol*, *rev* and a chimeric 5' LTR from HIV-1. A cDNA encoding *rev* is provided on a separate plasmid. A third generation packaging system offers maximal biosafety and involves the transfection of four different plasmids into the producer cells. Example: pMDL g/p RRE + pRSV-Rev.

C. Approach to Laboratory Biosafety with Retroviral Vectors

The determination of appropriate biosafety practices for use of retroviral vectors will require careful consideration of the factors above by the investigator, the biosafety officer and the COMS. Some general guidelines are given in Appendix A, however specific projects might raise issues in addition to those below and so COMS is not limited to specific practices by this guideline. These guidelines are applicable to laboratory scale lentiviral preparations; 100ml or less of infectious packaged virus are to be handled for preparations involving high risk gene inserts in 2nd or 3rd generation lentiviral preparations.

D. High Risk Gene Activity Examples (this list is not exhaustive, and other gene activity may be high risk based on risk assessment)

- **Oncogene/Proto-Oncogene**: A gene that promotes autonomous growth in cancer cells. Oncogenes are generally mutated forms of normal cellular genes (proto-oncogenes). Insertional mutagenesis of an oncogene could lead to uncontrolled growth.
- **Tumor Suppressor**: A gene that normally limits cell proliferation. When a tumor suppressor gene is mutated (altered), it may fail to limit proliferation, thereby promoting oncogenesis. Knocking out a tumor suppressor gene may lead to tumor development.
- **Cell Cycle Maintenance and DNA Damage Disruption**: Maintenance of genomic integrity is essential to avoid cellular transformation, neoplasia, or cell death. DNA synthesis, mitosis, and cytokinesis are important cellular

processes required for cell division Several DNA damage checkpoints exist, and if the genes that control this internal system are damaged, cells that are mutated may continue to divide leading to cancer.

- **Apoptosis Prevention**: Apoptosis is a natural, genetically controlled series of steps that occurs when a cell is old, unhealthy, or severely damaged. In some cases, however, genetic mutations derail apoptosis. If this occurs, it can promote proliferation and oncogenesis.
- **Transcription Factor**: A protein that binds DNA and regulates whether genes are transcribed or not. Transcription factors bind to regulatory regions in the genome and help control gene expression. Mutated forms of transcription factors may contribute to tumor development downstream.
- **Epithelial to Mesenchymal Transition** (EMT): cellular program of development of cells characterized by loss of cell adhesion, and increased cell mobility. EMT may be essential for numerous developmental processes including mesoderm formation and neural tube formation. Initiation of metastasis involves invasion, which has many phenotypic similarities to EMT. Mutated forms of genes involved in the EMT pathway could be downstream contributors of cancer.
- **Angiogenesis**-The formation of new blood vessels, especially blood vessels that supply oxygen and nutrients to cancerous tissue. Mutated forms of genes involved in angiogenesis may contribute downstream to cancer growth and metastasis.
- **Cell-Cell Adhesion**- determines the polarity of cells and participates in the maintenance of the cell societies called tissues. Cell-cell adhesiveness is generally reduced in human cancers. Reduced intercellular adhesiveness allows cancer cells to disobey the social order, resulting in destruction of histological structure, which is the morphological hallmark of malignant tumors. Mutated forms of genes involved in cell to cell adhesion are downstream contributors to cancer
- **Holotoxins**- are complete protein toxins, including all functional domains of the toxin that are required for its effect on an intact cell. The use of gene inserts for holotoxins is not addressed in this policy. Biosafety practices for vectors containing an insert with the gene(s) for a holotoxin will be considered if such a proposal is submitted for review.

V. Policy Authority

The Office of Biological Safety of the Harvard Medical School is responsible for supporting the COMS. This includes preparation and revising of the COMS Policy Manual for committee review and approval. COMS authorizes this policy.

VI. Related Policies

A. COMS Policy on Recommended Containment Levels for Adenoviral Vectors in Laboratory Rats, Laboratory Mice and Laboratory Rabbits
B. COMS Policy on Recommended Containment Levels for Retroviral Vectors in Laboratory Rats, Laboratory Mice and Laboratory Rabbits
C. Sharps Policy

VII. References

A. Biosafety Considerations for Research with Lentiviral Vectors, Recombinant DNA Advisory Committee (RAC) Guidance Document. Accessed from the Office of Biotechnology Activities website on 11/7/16.)

B. Lentiweb http://lentiweb.com/lvdesign.php
C. Lentiviral Vector Guidelines on the web:
    - http://www.safety.rochester.edu/ibc/HIVSIVguidelines.html
    - http://www.safety.duke.edu/biosafety/VectorPolicy.htm
    - http://ehs.uky.edu/docs/pdf/bio_viral_vectors_0001.pdf
    - http://oba.od.nih.gov/oba/rac/Guidance/LentiVirus_Containment/pdf/Lenti_Containment_Guidance.pdf

Appendix A: List of Harvard-affiliated medical and research institutions for which COMS serves as the Institutional Biosafety Committee and the Biosafety Officer for that institution.

Institution	**Biosafety Officer**
Beth Israel Deaconess Medical Center, Boston, MA	Rob Griffin, Nanette Moss
Harvard Faculty of Arts & Sciences, Cambridge, MA	Sid Paula
Harvard Medical School (HMS), Boston, MA	Marissa Cardwell
Harvard T.H. Chan School of Public Health, Boston, MA	Marissa Cardwell
Harvard Dental School, Boston, MA	Marissa Cardwell
Joslin Diabetes Center, Boston, MA	Michael Melisi
Massachusetts Eye and Ear Infirmary, Boston, MA	Tony Gemmellaro, Mark Liffers
WYSS Institute, Boston, MA	Bob Rasmussen

Appendix B: Risk Group Classifications WHO and NIH Guidelines

RG	WHO	NIH Guidelines
RG1	A microorganism that is unlikely to cause human disease or animal disease	Agents not associated with disease in healthy adult humans. Includes a list of animal viral etiologic agents in common use.
RG2	A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventative measures are available and the risk of spread of infection is limited.	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.
RG3	A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).
RG4	A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available

Appendix C: BMBL, 5th Edition, Biological Safety Levels

BL1	Suitable for work involving well-characterized agents not known to consistently cause disease in immuno-competent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building.
BL2	Suitable for work involving agents that pose moderate hazards to personnel and the environment. 1) laboratory personnel have specific training in handling pathogenic agents 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.
BL3	Suitable for work with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure. Manipulation of infectious materials must be conducted within BSCs, other physical containment devices, or by personnel wearing appropriate personal protective equipment. Physical separation from access corridors, self-closing/double-door access, exhaust air not recirculated, negative airflow, controlled access
BL4	Suitable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, aerosol transmission, or related agent with unknown risk of transmission.

Appendix D: Regulatory Agency Reporting Procedure

Regulatory Agency	**Jurisdiction**	**Reporting Requirements / Procedure**	**Timing to Report**
NIH OSP	All institutions receiving NIH funding for recombinant or synthetic nucleic acid molecule research	Telephone or Email Correspondence to OSP *In some cases, it may be appropriate to contact the NIH/OSP by telephone or email to determine if NIH/OSP considers the incident to be reportable.*	Within 30 days. Note: certain types of incidents require <u>immediate</u> reporting. Consult regulation
Boston Public Health Commission	City of Boston only 1)rDNA BL3 and BL4 Labs 2) Reportable Infectious Agents and Toxins (see regulation for specific list of materials) 3) Clinical labs 4) Animal Bites	1) Telephone or Email Correspondence 2) For laboratories with BPHC BL3 Permits: a. Employees exhibiting symptoms or may have been exposed to agents in use in the BPHC permitted BL3 laboratory b. Employees absent for two or more consecutive working days, where Institutional Occupational Health personnel have reasonable suspicion that the illness may be related to an exposure to agent in use in the BPHC permitted BL3 laboratory c. Failures, malfunctions, or renovations of major	1) Within thirty (30) days an institution shall report any significant problems with or violations of the Guidelines and any significant rDNA related accidents or illnesses to the Executive Director and the Boston RDNA Advisory Committee. Any such problems, accidents, or illnesses which have a potential impact on the public health and safety shall be reported immediately. 2) Immediate reporting

		mechanical or security systems of the BPHC permitted BL3 laboratory 3) Clinical Laboratory Reporting Form To Report: Complete form and fax 4) Animal Bite Reporting Form To report: Complete reporting form and	3) Report immediately by phone suspect or confirmed cases 4) Fax Completed Form to BPHC-CDC
Cambridge Public Health Department	1)All institutions receiving NIH funding for rDNA: 2) BL3 Labs	1) Telephone or Email Correspondence to Director of Environmental Health 2) Telephone or Email Correspondence to Director of Environmental Health	
MA DPH	State of MA Biological Waste (see regulation)		
CDC/APHIS Select Agent Program	Select Agents (see regulation for complete list)	Select Agent Responsible Official must report	RO must contact APHIS or CDC immediately upon discovery of a theft, loss, or a release (occupational exposure or release of an agent or toxin outside of the primary barriers of the biocontainment area) of a Select Agent and Toxin not authorized under a federal act.

Appendix E: Regulatory Agency Contact List for Reporting of incidents

Agency Contact	Regulation	Website/Forms
Completed reports may be sent to OSP via email at NIHGuidelines@od.nih.gov Human Gene Transfer (HGT) Adverse Events (AEs) should still be reported to the NIH Office of Science Policy (OSP). HGT AEs should be emailed to HGTprotocols@mail.nih.gov	The NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules (NIH Guidelines)	Incident Reporting Template Human Gene Transfer Adverse Events Reporting Template
Boston Public Health Commission, Communicable Disease Control Division 1010 Massachusetts Avenue, Boston, MA 02118 Phone: 617-534-5611 Fax 617-534-5905	1) BPHC Recombinant DNA Technology: Use Regulations 2) BPHC Disease Surveillance and Reporting Regulation 3) BPHC Biological Laboratory Regulation	BPHC Reporting Form Surveillance Regulation Lab Regulation Guidelines FAQs
Sam Lipson. Director of Environmental Health, Cambridge Public Health Department 119 Windsor Street, Ground Level Cambridge, MA 02139 Phone 617-665-3838 Fax 617-665-3888 slipson@challiance.org	Recombinant DNA Ordinance Cambridge Biosafety Regulation	No Specific Reporting Template. Regulations: http://www.cambridgepublichealth.org/services/regulatory-activities/biosafety/
APHIS Select Agent Program	42 CFR 73.0 Select	Form 3 Report of Theft

4700 River Road Unit 2, Mailstop 22, Cubicle 1A07 Riverdale, MD 20737 Fax: 301-734-3652 Email: Agricultural.Select.Agent.Program@aphis.usda.gov	Agent Rule	Loss or Release Regulations
CDC Select Agent Program 1600 Clifton Road NE, Mailstop A-46, Atlanta, GA 30333 Fax 404-718-2096 Email: lrsat@cdc.gov	42 CFR 73.0 Select Agent Rule	Form 3 Report of Theft Loss or Release Regulations
Massachusetts Department of Public Health Office of General Counsel 250 Washington Street, 2nd Floor Boston MA 02108 Phone: 617-624-5213	105 CMR Department of Public Health Minimum Requirements for the Management of Medical or Biological Waste (State Sanitary Code Chapter VIII)	No Specific Reporting Template. Regulations

Appendix F: Top 10 Most Commonly Used Retroviral Vectors in Animals

Vector	Source
pLenti (various versions)	Invitrogen
pRRL	Salk Institute
MSCV	Many
pLKO	Open BioSystems, AddGene, Others
pBabe	Many
MLV	Many
pSICO	AddGene, others
Lenti-Lox	Many
pSMPUW	Cell Biolabs
MoMuLV	Many
pHAGE	R. Mulligan
pMMP	R. Mulligan

Appendix G: Common Packaging System

1. Invitrogen ViraPower - http://tools.invitrogen.com/content/sfs/manuals/virapower_lentiviral_system_man.pdf
2. Phoenix Ampho Packaging System (Obigen) - http://www.orbigen.com/objects/catalog/product/extras/1461_RVK-10001.pdf
3. Trans-Lentiviral™ Packaging System (Open Biosystems) - http://www.openbiosystems.com/Viral%20Packaging/TransLentiviral%20Packaging%20Syst/
4. pPack Packaging Systems (System Biosciences) - http://www.systembio.com/index.php?id=lentiviral-technology_delivery-systems_ppack
5. Lenti-X Packaging Systems (Clontech) - http://www.clontech.com/products/detail.asp?product_id=171915&tabno=2
6. Packaging Systems from HGTI
7. pCMV-R8.74 and pMD2G from Didier Trono - http://tronolab.epfl.ch/page71945.html
8. psPAX2 – Originally from Trono (deposited at AddGene), second gen packaging vector - http://www.lablife.org/p?a=vdb_view&id=g2%2e7Qg9zRofxbsyPKiTgbD%2eFpe1fTQ%2d

Appendix H: Suggested Vendors for Biological Waste Validation Supplies

COMS does not endorse particular products or suppliers.
Other suppliers may be used in addition to those suggested in this appendix.

I. Mesa Laboratories
II. VWR

Appendix I: Suggested Vendors for Autoclaves

COMS does not endorse particular vendors for equipment.
Other vendors may be used in addition to those suggested in this appendix.

Ranger Engineering
P.O. Box 3111
Framingham, MA 01705
(508) 877-3166

Appendix J: Laboratory Biosafety Guidelines for Retroviral Vectors

The following practices are recommended for all work with retroviral vectors.

1. Sharps, including glass Pasteur pipettes and needles should be eliminated from laboratory procedures involving the use of infectious packaged virus. Sharps are only allowed for work with infectious packaged virus *in vivo*. Strict adherence to sharps precautions must be followed if there are no alternatives to the use of sharps for *in vivo* work.
2. Use of sharps should be minimized for any manipulation of virally transduced materials, i.e., cell lines or fluids, and tissues from animals inoculated with the viral vector. In instances where there are no alternatives to sharps for work with virally transduced materials, strict adherence to sharps precautions must be followed. Biosafety cabinets must be used and annually certified.
3. Agent specific training is required.
4. Aerosol-proof rotors or centrifuge buckets with safety caps should be used when centrifuging.

Lentiviral Vector	**Transgene**	**BSL Level**	**Requirements for work outside the biosafety cabinet**	**Volume Restriction <100mL**	**Waste**
2nd generation or lower	Low Risk	BL-2 with additional practices	Open bench work should be discussed with your BSO. Safety glasses and additional face protection may be required for additional manipulations.	No	Liquid disinfection must be completed with approved disinfectant before drain discharge Chemical disinfection may be used for most liquid and solid waste applications. Solid waste may be chemically inactivated followed by disposal in designated Biowaste containers.

2nd generation or lower	High risk (see list) (includes known oncogene or tumor suppressor where genes are knock-down or over-expressed)	BL-2 with additional practices	Open bench not permitted. Disposable double nitrile (or similar) gloves and solid-front laboratory coat must be worn for any work. Safety glasses and additional face protection may be required for additional manipulations.	Yes	Chemical disinfection may be used for most liquid and solid waste applications. Solid waste may be chemically inactivated followed by disposal in designated Biowaste containers. In some cases, waste treatment using autoclave may be required followed by disposal in designated Biowaste containers.
3rd generation	Low risk	BL-2 with additional practices	Open bench work should be discussed with your BSO. Disposable nitrile (or similar) gloves and laboratory coat must be worn for any work outside biosafety cabinet. Safety glasses and additional face protection may be required for additional manipulations.	No	Liquid disinfection must be completed with approved disinfectant before drain discharge. Solid waste may be chemically inactivated followed by disposal in designated Biowaste containers.

3rd generation	High risk (see list) (includes known oncogene or tumor suppressor where genes are knock-down or over-expressed)	BL-2 with additional practices	Open bench work not permitted. Disposable double nitrile (or similar) gloves and solid-front laboratory coat must be worn for any work. Safety glasses and additional face protection may be required for additional manipulations.	Yes	Chemical disinfection may be used for most liquid and solid waste applications. In some cases, waste treatment using autoclave may be required followed by disposal in designated Biowaste containers.

Appendix K: Suggested Laboratory Methods for Attenuated Organism Validation

1. It is best practice to isolate a single clone of the organism and expanding prior to performing the validation, for example by limited dilution cloning. This master stock is then used to reproduce a seed stock. The master stock is only used to prepare a batch of seed stock; hence, the number of vials of master stock prepared must be small.
2. A “seed” stock is prepared from the “master” stock. A master and seed stock vial is to be used as “single” use only; a vial of seed stock is for preparing a “working” stock for a single routine experiment. Ideally, the number of vials of seed stock prepared should be sufficient for the duration of the research funding.

3. Validation must include direct testing for the presence or absence of the virulence factors lost in attenuation by phenotypic or genetic means (or both). This is preferred to methods that demonstrate a genetic match between the attenuated organism and a control strain without testing for the virulence factors themselves (e.g. pulse field gel electrophoresis).

4. Controls for the validation must include properties of the wild-type strain (positive control) and an appropriate negative control.

5. A formal Validation Report must be submitted to COMS for review and approval prior to going forward with a COMS-approved research registration.