Tuskegee University's Biosafety Submission Instructions

Investigators using biohazardous agents should submit an application to Biosafety Committee (BC) for review. The review can be initiated by forwarding the application forms to the Office Grantsmanship and Compliance, located in Chappie James Center, Room 103.

Following a preliminary review by the Office of Grantsmanship and Compliance, the new application is submitted to the Biosafety Review Committee for full review.

Use of Biohazardous material in Research and Instruction

PURPOSE:

To ensure safe handling, storage, and disposal of potentially biohazardous materials, as defined below, used in research or instructional projects at Tuskegee University. Enforcement of this policy by Tuskegee University is meant to provide a safe working atmosphere and a well-controlled research environment. The Biosafety Committee (BC) review of proposals and projects provides the necessary compliance in accordance with federal regulations on use or Recombinant DNA.

POLICY:

All research and instructional activities involving biohazardous materials, as defined below, shall be reviewed and approved by the Biosafety Committee (BC) prior to the use of any such reagent. Projects submitted for sponsorship by external agencies should be submitted for BC review prior to the acceptance of funding. The Office of Grantsmanship and Compliance (OGC) located in the Chappie James Center coordinated the (BC) full committee review and the approval process.

APPLICABILITY:

The policy applies to all research and instructional activities, sponsored or un-sponsored, conducted under the auspices of Tuskegee University. University projects involving the use of biohazardous materials at other institutions should receive a Biosafety Committee approval from the cooperating institution. Copies of the BC approval from the cooperation institution(s) should be forwarded to the OGC.

DEFINITIONS:

Biohazardous Material

The categories below represent the areas of primary concern with respect to Biosafety. Projects involving material(s) included by any of those categories should be submitted for BC approval.

1. Infectious agents requiring handling conditions above Biosafety Level-1. (Biosafety Level determinations are based on the recommendations outlined by the CDC-NIH publication Biosafety in Microbiological and Biomedical Laboratories.)
2. Toxins, Microbiological, or Chemical to be used in animal or human studies, or used in the laboratories of teaching, testing, or research. (Refer to attached references) Biosafety Level determinations are based on the Recommendations outlined by the CDC-NIH Biosafety in Microbiological and Biomedical Laboratories.
3. Tissue, Blood, Fluids used in conjunction with animals, humans, or microorganisms.
4. Radiation
5. Recombinant DNA (unless exempted by national Institutes of Health Guidelines.) Including genetically altered animals requiring Biosafety Level 2 containment.
6. Whenever a contractual agreement or grant proposal requires Biosafety Committee approval for the safe handling of a biological or chemical product.

The BC also serves as an advisory committee for University projects that involve possible biohazards that do not appear to fall into one of these six areas. When it is unclear as to whether a material constitutes a potential biohazard, the BC or the Office of Environmental Health and Safety should be consulted. Questions should be directed to the OGC at 727-8985 or to the Office of Environmental Health safety Officer.

Materials and Methods:
A detailed flow chart for an experimental procedure that involves the use of biohazardous material or that produces biohazardous waste material should be included. The principle investigator (PI) should review the document before submission to the BC and consider whether the use of biohazardous material is described with appropriate detail.

REQUEST FOR APPLICATION REVIEWS:
The Biosafety Committee's review and approval may be obtained by forwarding two copies of an application to the OGC. To avoid delays in review, the investigator should clearly identify projects that involve more than one biohazardous material. Applications should be submitted 14 days prior to the BC meeting date for review.

APPROVAL PROCEDURE:
Following a preliminary review by the Office of Grantsmanship and Compliance, the new application is scheduled for consideration at the monthly meeting of the BC.

Application for renewal or ongoing project(s) is submitted to the Office of Grantsmanship and Compliance for a preliminary review also. The renewal application is then submitted to the Biosafety Committee for full review. Also, the Office of Environmental Health and Safety (EH&S) will review the EH&S records to ensure that the respective laboratory areas have the necessary safety equipment and/or have implemented the appropriate safety procedures. If the involved laboratory areas have not been previously reviewed by the EH&S officer he/she will contact the principal investigator to arrange a safety inspection for renewal project. When contacted by EH&S, the PI should discuss the written Safety Protocol(s) for the laboratory. A written Safety Protocol for the containment, handling, and disposal of biohazardous material is required for all active projects, especially where biohazardous material comes into contact with animals. Animal studies will not be approved by the Institutional Animal Care and Use Committee that use materials considered biohazards. Approval must be obtained from the BC before approval is granted for the Institutional Animal Care and Use Committee.

Following review of the renewal project by the BC, including a safety inspection by EH&S, the OGC provides the principal investigator with written notification of the approval status of the project. It is the responsibility of the PI to ensure that approval letters are properly directed to any funding agency or sponsor.

OGC#_____

TUSKEGEE UNIVERSITY BIOSAFETY COMMITTEE

APPLICATION FOR THE USE OF BIOHAZARDOUS MATERIALS

Tuskegee University's Biosafety Committee must review your proposal or project, according to Institutional Policy. Please complete pages 1-8 and 9-13 if required.

Date: _____

Project Title: _____

Principal Investigator: _____

E-mail Address: _____ Phone Number: _____

College: _____Department_____

Advisor (if applicable):_____

Co-Investigator:_____Address:_____ Phone Number: _____

Co-Investigator:_____Address:_____Phone Number: _____

Funding Source: _____

Project Duration: Start Date: _____ End Date: _____

Check one that applies:

Proposed Project ☐
- a) Research ☐
- b) Teaching* ☐
- c) Demonstration ☐
- d) Resubmission ☐

Continuous/ Active Project ☐
- a) Research ☐
- b) Teaching* ☐
- c) Demonstrations ☐
- d) Other ☐

*__Please provide course name & number:__ _____

Please Complete the Information Below for the use of Biohazard Material(s):

A. Infectious Agents:

(Check All That Apply)	**Yes**	**No**	**If Yes, (Circle Biosafety Level) *Refer to Biosafety Level Charts**
Viral	☐	☐	1 2 3 4
Bacterial	☐	☐	1 2 3 4
Fungal	☐	☐	1 2 3 4
Other(s)_____	☐	___	1 2 3 4

B. Toxins:
(Check All That Apply)

Carcinogen	___	____	1 2 3 4
Tetrogen	____	____	1 2 3 4
Mutagen	____	____	1 2 3 4
Microbiological	____	____	1 2 3 4
Chemical	____	____	1 2 3 4
Other(s) _____	____	____	1 2 3 4

C. Tissues, Blood, Fluids, Plants [Including Cellular Culture(s) or Line(s)]:

1. Animal	____	____	**If Yes, Submit an Application to Animal Care and Use Committee and complete Attachment B and Attachment C**
2. Human	____	____	**If Yes, Submit an Application to Human Participant Committee**
3. Radiation:	____	____	**If Yes, Submit an Application to Radiation Safety Committee**
4. Recombinant DNA:	____	____	**If Yes, Complete Attachment D.**
5. Transgenic animals:	____	____	**If Yes, Complete Attachment E.**

A. Please describe the methodology of the project. (F**ill out Attachment A** including additional sheets if necessary)

B. Please explain your plan for the biohazard material(s)

1. Please identify the source:
   a. Purchased

   b. Copy of MSDS Sheets

   c. Explain the method biohazard material will be transported to campus.

      1. before laboratory experiment(s)
      2. after laboratory experiment(s)

   e. Route of administration (examples: injection or ingestion), if applicable.

   f. Please explain the waste disposal containment after the experiment.

C. Please provide a flow chart of experiment(s) with focus on the containment of biohazardous material(s). (**Fill out Attachment B** including additional sheets if necessary)

D. Please provide a map/outline of the laboratory that will be utilized for experiments with a focus on laboratory safety items. Please remember to include the safety items used for the containment of the biohazard material(s) purchased or produced by experiment. **(Fill out attachment C** including additional sheets if necessary)

E. Please describe training(s) and protective clothing provided for staff using biohazardous agents and laboratory equipment. In addition attach a copy of Standard Office Procedures SOP's for laboratory.

F. Please fill out **Attachment D, Registration Document for Recombinant DNA and/or Infectious Agent, on page 8**

- Submit pages 9-12 **only if required** as instructed on page 8.
- Submit Attachment E, Registration of Transgenic animals on page 13 only if required.

Investigator Signature: _____ Date: _____
Advisor Signature: _____ Date: _____
(if applicable)

PLEASE RETURN COMPLETED FORMS TO OGC
Chappie James Center Room 103

PRINCIPAL INVESTIGATOR'S SIGNATURE

- I attest that the information contained in the attached application is accurate and complete. I agree to comply with the requirements pertaining to shipment and transfer of infectious agents and/or recombinant DNA. I am familiar with and agree to abide by the provisions of the current NIH/CDC Guidelines and other specific granting agency instructions pertaining to the proposed project.

- I further attest that all research personnel are familiar with and understand the potential biohazards, proposed precautions, and appropriate emergency procedures, and that the practices and techniques required to ensure safety will be followed. I agree to accept responsibility for training of all laboratory workers involved in the project.

- I hereby adopt the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (4th Edition) as the principal Biosafety manual for my laboratory, or, I will provide a supplemental Biosafety Laboratory manual in addition to, or in place of, the CDC/NIH manual as I deemed necessary or when specifically requested by the Bio-Safety Committee. I understand that a supplemental Biosafety manual must be approved by the Bio-Safety Committee before research can commence.

- Written reports will be submitted to the Environmental Health and Safety through the Bio-Safety Committee concerning:

  1. Any accident that results in inoculation, ingestion, and inhalation of infectious agents or recombinant DNA or any incident causing serious exposure of personnel or danger of environmental contamination:

  2. Any problems pertaining to operation and implementation of containment safety procedures or equipment or facility failure or security: and,

  3. Any new information bearing on the Guidelines such as technical information relating to hazards and safety procedures or innovations.

- I will not carry out the work described in the attached application until it has been filed with and accepted by Bio-Safety Committee or, when necessary, until it has been approved by the Bio-Safety, other appropriate oversight committees and all sponsoring agency requirements have been met.

_____ _____

Investigator (Signature) Date

_____ _____

Advisor Signature (If Applicable) Date

ATTACHMENT A
OGC# _____

METHODOLOGY OF THE PROGECT AND EXPERIMENTS.
Please be sure to explain where biohazardous materials will be used.

Investigator Signature: _____ Date: _____

Advisor Signature: _____ Date: _____
(If applicable)

ATTACHMENT B
OGC #_____

FLOW CHART OF EXPERIMENT (S)

Investigator Signature: _____ Date: _____

Advisor Signature: _____ Date: _____
(If applicable)

ATTACHMENT C
OGC#_____

MAP/OUTLINE OF LABORATORY

Investigator Signature: _____ Date: _____

Advisor Signature: _____ Date: _____
(if applicable)

Attachment D

Registration Document for Recombinant DNA and/or Infectious Agents

Tuskegee University
BIOSAFETY COMMITTEE

TO DETERMINE WHO SHOULD COMPLETE THIS APPLICATION, ANSWER QUESTIONS BELOW.

1. Does this application involve the use of Recombinant DNA? Yes No

If **'Yes'** check the section(s) which apply. **Consult Appendix A to determine the appropriate NIH section(s) of the proposed experiment(s).**

SECTIONS*:* **III A III B III C III D.....**must receive approval from BC before initiation of experiments
III E.....…… must notify BC simultaneously upon commencement of research
III F*.....……. exempt from NIH guidelines for recombinant DNA

***If your research falls in section III F and there are NO infectious agents involved you do not need to complete the rest of attachment D (pages 9-12).**

2. Does this application involve the use of Infectious Agents? Yes No

If **'Yes'** check the risk group(s) which apply **Consult Appendix E to determine the risk group of the agent(s*).***

Risk Group 3..... must receive approval from BC before initiation of experiments
Risk Group 2..... must receive approval from BC before initiation of experiments
Risk Group 1**.....exempt from Tuskegee University guidelines

****If your research involves only Risk Group 1 and NO Recombinant DNA then you do not need to complete the rest of attachment D (pages 9-12).**

3. Does this application involve the use, importation or construction of transgenic animals? Yes*** No
****If yes complete Attachment E (page 13).**

SECTION I - RECOMBINANT DNA

Make duplicates of this section and complete it for each host vector system used.

1. Biological sources of DNA. List Genus/Species or common name of the source organism of the insert DNA.

2. Nature of the inserted DNA sequences. List gene names, biological markers, sequences, promoters, etc., and describe the function/activity of the DNA or its product.

3. Which of the following host-vector systems will be used for this research? Check box(es) and provide further details requested.

Vector

Adenovirus Name strain and describe wild type deletions: _____
_____
Is this strain replicative defective? Yes No

Retrovirus Vector backbone:

Murine Name strain_____

Lentivirus: Name HIV genes present or attach map _____

Name envelope packaging system(s) _____

Other _____

Does the packaging cell line generate amphotrophic virus? Yes No
Does the packaging cell line generate ecotrophic virus? Yes No

Adeno-associated virus

Vaccinia virus

Bacterial plasmids Name plasmids:_____

Baculovirus

Agrobacterium spp. Name species: _____

Other _____

Host

Include bacterial host used to propagate vector plasmid which will be used to generate recombinant virus.

E. coli K12: Name derivative or strain: _____

Other bacteria: Give genus/species/strain: _____

Laboratory animals: Name species: _____

Tissue culture: (Check all that apply.) Cell designation: _____

Human Established cell line Primary cell culture Transformed cell line

Non human primate Plant Other

Other host_____

4. Will you attempt to express a foreign gene? No Yes

What protein(s) will be produced? _____

5. What containment conditions will be implemented as specified in the *NIH Guidelines* Consult Appendix A?

Physical Containment: Check all that apply. Consult Appendix A.

General: ___BSL 1 ___BSL2 ____BSL3 ____Not Applicable
Animals: ___ BSL1-N ___BSL2-N ____BSL3-N ____Not Applicable
GLSP BSL1-Large Scale BSL2-Large Scale BSL3-Large Scale Not Applicable
Other _____

Biological Containment --prokaryotic host. See Appendix B.

____EK1 _____EK2 ____Not Applicable

6.

Yes No Will the recombinant DNA molecule contains greater than 2/3 of the genome of any eukaryotic virus? Section III-E-1.

Yes No Will the research involves greater than 10 liters of culture at any one time? Section III-C-6

Yes No Will there be deliberate formation of rDNA containing genes for the biosynthesis of **toxic** molecules **lethal** for vertebrates at an LD50 of less than 100 ng/kg body weight? Section III-B-1

Yes No Will there be deliberate transfer of drug resistance trait to microorganisms not known to acquire the trait naturally if such acquisition could compromise the use of the drug to control disease agents? Section III-A-1-a

Yes No Will there be transfer of rDNA into human or animal pathogens in Risk Groups 2 or 3? Section III-C-1

SECTION II - INFECTIOUS AGENTS

Complete this section for each agent to be studied. Duplicate pages as necessary.

1. Agent Strain Risk Group Biosafety Level

a. _____ __
b._____
c. _____ _____
d. _____ _____
e. _____ _____
f. _____
g. _____ ____

2. Source

Is the source of the infectious agent your lab or another lab or a commercial company? Write the name of the lab or person or company below.

a. _____
b. _____
c. _____
d. _____
e. _____
f. _____
g. _____

3. Are experiments being done to alter the tropism of the agent? __No ___Yes If yes, please explain alteration.

SECTION III – LOCATION OF LABORATORY AND STOCK CULTURES; OCCUPATIONAL HEALTH

This Section Must Be Completed

I. a) Building and room where experiments will be conducted: _____

b) Where will stock cultures be stored? _____

II. Occupational Health:

a) Hazardous to Humans No____ Yes_____, If Yes, answer b and c

b) If applicable, is a vaccine available? No___ Yes_____ If so, provide names of persons vaccinated, date vaccinated and name of institution where they were vaccinated.

c) Describe medical surveillance practices for laboratory research personnel and for animal handlers.

ATTACHMENT E

Tuskegee University
BIOSAFETY COMMITTEE
REGISTRATION OF TRANSGENIC ANIMALS

Date _____

Principal Investigator _____ Degree _____

Circle one:
Faculty Staff Student Visiting Scholar Resident Post-doctoral Fellow

Department _____ Address _____

Office Phone _____ Lab Phone _____ Fax _____

E-mail address _____

1. Provide a non-technical summary of transgenic animals that will be made, used or imported as they relate to the overall objectives of your research project.

2. List genes that will be inserted or knocked out by the stable Introduction of recombinant DNA Into the germ-line and provide a description of the function of these genes, If possible.

3. Will these modifications to the genome cause the animal to shed Infectious agents, toxins or create other hazards for the animal handlers and research staff? Please Circle: **Yes or No**

4. If yes, explain the hazard the animal will present to staff handling the animals. Provide safety precautions required to be observed in housing and handling these animals.

APPENDIX A

SUMMARY OF EXPERIMENTS COVERED BY THE "NIH GUIDELINES"

The NIH Guidelines can be found at http://www4.od.nih.gov/oba/guidelines.html. Appendix B of this application describes Risk Groups (RG).

Please check the appropriate box (es) if the category accurately describes your experiment. If your experiment does not fall into any of these categories please contact the Bio-Safety Committee, Chairman. **COMPLETE AND RETURN THIS APPENDIX WITH YOUR APPLICATION.**

Section III-A Experiments that require Institutional Biosafety Committee (IBC) approval, Recombinant DNA Advisory Committee (RAC) review, and NIH Director approval before initiation of experiments.

III-A-1-a. 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.

Section III-B Experiments that require NIH/OBA and IBC approval before initiation.

III-B-1 Deliberate formation of rDNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD_{50} of less than 100 nanograms per kg body weight (e.g., microbial toxins such as tetanus toxin).

Section III-C Experiments that require IBC and Institutional Review Board (IRB) approvals, and NIH/OBA registration before initiation.

III-C-1 Experiments involving the deliberate transfer of (1) recombinant DNA or (2) DNA or RNA derived from recombinant DNA into one or more human subjects.

Section III-D Experiments that require IBC approval before initiation of experiments.

III-D-1-a Introduction of recombinant DNA into Risk Group 2 (RG-2) agents is usually conducted at BL2 containment. Experiments with such agents will usually be conducted with whole animals at BL2 or BL2-N containment. (See Appendix B for information on Risk Groups.)

III-D-1-b Introduction of recombinant DNA into Risk Group 3 (RG-3) agents is usually conducted at BL3 containment. Experiments with such agents will usually be conducted with whole animals at BL3 or BL3-N containment. (See Appendix B for information on Risk Groups.)

III-D-2-a Experiments in which DNA from RG- 2 or RG- 3 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes may be conducted at BL2 containment. Experiments in which DNA from RG-4 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment after demonstration that only a totally and irreversibly defective fraction of the agent's genome is present in a given recombinant. The IBC may approve the specific lowering of containment for particular experiments to BL1. Many experiments in this category are exempt from the "NIH Guidelines". (See Appendix B for information on Risk Groups.)

III-D-3-a Experiments involving the use of infectious or defective RG-2 viruses in the presence of helper virus may be conducted at BL2 containment. (See Appendix B for information on Risk Groups.)

III-D-3-b Experiments involving the use of infectious or defective RG-3 viruses in the presence of helper virus may be conducted at BL3 containment. (See Appendix B for information on Risk Groups.)

III-D-3-d Experiments involving the use of infectious or defective restricted poxviruses in the presence of helper virus shall be determined on a case-by-case basis following NIH/OBA review. A USDA permit is required for work with plant or animal pathogens.

III-D-3-e Experiments involving the use of infectious or defective viruses in the presence of helper virus which are not covered in Sections III-D-3-a through III-D-3-d may be conducted at BL1.

III-D-4-a Recombinant DNA, or DNA or RNA molecules derived there from, from any source except for greater than two-thirds of eukaryotic viral genome may be transferred to any non-human vertebrate or any invertebrate organism and propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study. Animals that contain sequences from viral vectors, which do not lead to transmissible infection either directly or indirectly as a result of complementation or recombination in animals, may be propagated under conditions 16 of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study. Experiments involving the introduction of other sequences from eukaryotic viral genomes into animals are covered under Section III-4-b. It is important that the investigator demonstrate that the fraction of the viral genome being utilized does not lead to productive infection.

III-D-4-b Experiments involving recombinant DNA, or DNA or RNA derived therefrom, involving whole animals, including transgenic animals, and not covered by Sections III-D-1 or III-D-4-a, may be conducted at the appropriate containment determined by the IBC.

III-D-4-c-1 Experiments involving the generation of transgenic rodents that require BL1 containment are described under Section III-E-3.

III-D-4-c-2 The purchase or transfer of transgenic rodents is exempt from the "NIH Guidelines" under Section III-F and Appendix C-VI.

III-D-5 Experiments to genetically engineer plants by rDNA methods, to use plants for other experimental purposes, to propagate such plants, or to use plants together with microorganisms or insects containing rDNA, may be conducted at various recommended containment conditions. See the "NIH Guidelines", Section III-D-5.

III-D-6 Experiments involving more than 10 liters of culture. The appropriate containment will be decided by the IBC. Where appropriate Appendix K of the "NIH Guidelines" will be used to determine containment conditions.

Section III-E Experiments that require IBC notice simultaneously with initiation.

III-E Experiments not included in Sections III-A, III-B, III-C, III-D, III-F and their subsections are considered in this section. All such experiments may be conducted at BL 1.

III-E-1 Experiments involving the formation of rDNA molecules containing no more than 2/3 of the genome of any eukaryotic virus (All viruses from a single Family being considered identical.)may be propagated and maintained in cells in tissue culture using BL1 containment. It must be shown that the cells lack helper virus for the specific Families of defective viruses used. The DNA may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than tow-thirds of a genome.

III-E-2 Experiments involving rDNA-modified whole plants, and/or experiments involving rDNA-modified organisms associated with plants, except those that fall under Section III-A, III-B, III-C, III-D, III-F. See Section III-E-2 for recommendation of containment levels.

Section III-F Experiments that are exempt from *NIH Guidelines.* Registration with the IBC is not necessary except for transgenic rodents.

III-F-1 Recombinant DNA molecules that are not in organisms or viruses.

III-F-2 Recombinant DNA molecules that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.

III-F-3 Recombinant DNA molecules that consist entirely of DNA from a procaryotic 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.

III-F-4 Recombinant DNA molecules that consist entirely of DNA from a eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or closely related strain of the same species).

III-F-5 Recombinant DNA molecules 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. See Appendix A-I through A-V of the “NIH Guidelines”.

III-F-6 Recombinant DNA experiments that do not present a significant risk to health or the environment as determined by the NIH Director, RAC and following appropriate notice and opportunity for public comment. See Appendix C of the NIH Guidelines.

APPENDIX B

SUMMARY OF BIOLOGICAL CONTAINMENT COVERED BY THE NIH GUIDELINES

The "NIH Guidelines" can be found at http://www4.od.nih.gov/oba/guidelines.html. The subject of Biological Containment is located in Appendix I of the "NIH Guidelines".

COMPLETE AND RETURN THIS APPENDIX WITH YOUR APPLICATION

In consideration of biological containment, the vector (plasmid, organelle, or virus) for the recombinant DNA and the host (bacterial, plant, or animal cell) in which the vector is propagated in the laboratory will be considered together. Any combination of vector and host which is to provide biological containment shall be chosen or constructed so that the following types of "escape" are minimized: (i) survival of the vector in its host outside the laboratory, and (ii) transmission of the vector from the propagation host to other non-laboratory hosts. The following levels of biological containment (host-vector systems) for prokaryotes are established: EK1 and EK2. Specific criteria will depend on the organisms to be used.

Host-Vector Systems requiring EK1

The host is always Escherichia coli K-12 or a derivative thereof, and the vectors include non-conjugative plasmids (e.g., pSC101, Co1E1, or derivatives thereof and variants of bacteriophage, such as lambda). The Escherichia coli K-12 hosts shall not contain conjugation-proficient plasmids, whether autonomous or integrated, or generalized transducing phages. Hosts and vectors which are comparable in containment to Escherichia coli K-12 with a non-conjugative plasmid or bacteriophage vector.

Host-Vector Systems requiring EK2

Host-Vector 2 Systems provide a high level of biological containment as demonstrated by data from suitable tests performed in the laboratory. Escape of the recombinant DNA either via survival of the organisms or via transmission of recombinant DNA to other organisms should be <1/10 under specified conditions. Specific Host-Vector 2 systems are:

Escherichia coli K-12 Host-Vector 2 systems (EK2) in which the vector is a plasmid, no more than 1/10 host cells shall perpetuate a cloned DNA fragment under the specified non-permissive laboratory conditions designed to represent the natural environment, either by survival of the original host or as a consequence of transmission of the cloned DNA fragment.

Escherichia coli K-12 Host-Vector 2 systems (EK2) in which the vector is a phage, no more than 1/10 phage particles shall perpetuate a cloned DNA fragment under the specified non-permissive laboratory conditions designed to represent the natural environment, either as a prophage (in the inserted or plasmid form) in the laboratory host used for phage propagation, or survival in natural environments and transferring a cloned DNA fragment to other hosts (or their resident prophages).

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they relate to the overall objectives of your research project.

2. List genes that will be inserted or knocked out by the stable introduction of recombinant DNA into the germ-line and provide a description of the function of these genes, if possible.

3. Will these modifications to the genome cause the animal to shed infectious agents, toxins or create other hazards for the animal handlers and research staff? Please Circle: **Yes or No**

4. If yes, explain the hazard the animal will present to staff handling the animals. Provide safety precautions required to be observed in
housing and handling these animals.

APPENDIX C
CLASSIFICATION OF HUMAN ETIOLOGIC AGENTS ON THE BASIS OF HAZARD

This appendix is taken directly from the document “NIH Guidelines for Research Involving Recombinant DNA Molecules”. ttp://www4.od.nih.gov/oba/guidelines.html

RISK GROUPS

Risk assessment is ultimately a subjective process. The investigator must make an initial risk assessment based on the Risk Group of an agent. Agents are classified into four Risk Groups (RGs) according to their relative pathogenicity for healthy adult humans.

In deciding on the appropriate containment for an experiment, the initial assessment from the following Risk Group classification should be followed by a thorough consideration of the agent itself and how it is manipulated. Factors to be considered in determining the level of containment include agent factors such as: virulence, pathogenicity, infectious dose, environmental stability, route of spread, communicability, operations, quantity, availability of vaccine or treatment, and gene product effects such as toxicity, physiological activity, and allergenicity. Any strain that is known to be more hazardous that the parent (wild type) strain should be considered for handling at a higher containment level. Certain attenuated strains or strains that have been demonstrated to have irreversibly lost known virulence factors may qualify for a reduction of the containment level compared to the Risk Group assigned to the parent strain.

A final assessment of risk based on these considerations is then used to set the appropriate containment conditions for the experiment. The containment level required may be equivalent to the Risk Group classification of the agent or it may be raised or lowered as a result of the above considerations.

The Biosafety Committee must approve the risk assessment and the biosafety containment level.

Careful consideration should be given to the types of manipulation planned for some higher Risk Group agents. For example, the RG2 dengue viruses may be cultured under the Biosafety Level (BL) 2 containment, however, when such agents are used for animal inoculation or transmission studies, a higher containment level is recommended. Similarly, RG3 agents such as Venezuelan equine encephalomyelitis and yellow fever viruses should be handled at a higher containment level for animal inoculation and transmission experiments.

Individuals working with human immunodeficiency virus (HIV), hepatitis B virus (HBV) or other bloodborne pathogens should consult Occupational Exposure to Bloodborne Pathogens; Final Rule (Cal/OSHA Regulation). BL2 containment is recommended for activities involving all blood-contaminated clinical specimens, body fluids, and tissues from all humans, or from HIV- or HBVinfected
or inoculated laboratory animals. Activities such as the production of research-laboratory scale quantities of HIV or other bloodborne pathogens, manipulating concentrated virus preparations, or conducting procedures that may produce droplets or aerosols, are performed in a BL2 facility using the additional practices and containment equipment recommended for BL3. Activities involving industrial scale volumes or preparations of concentrated HIV are conducted in a BL3 facility, or BL3 Large Scale if appropriate, using BL3 practices and containment equipment.

Exotic plant pathogens and animal pathogens of domestic livestock and poultry are restricted and may require special laboratory design, operation and containment features.

Basis for the Classification of Biohazardous Agents by Risk Group

Risk Group 1 (RG1) Agents that are not associated with disease in healthy adult humans

Risk Group 2 (RG2) Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available

Risk Group 3 (RG3) 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)

Risk Group 4 (RG4) Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community Risk)

This appendix includes those biological agents known to infect humans as well as selected animal agents that may pose theoretical risks if innoculated into humans. Included are lists of representative genera and species known to be pathogenic; mutated, recombined, and non-pathogenic species and strains are not considered. Non-infectious life cycle stages of parasites are excluded.

This appendix reflects the current state of knowledge and should be considered a resource document. Included are the more commonly encountered agents and is not meant to be all inclusive. Information on agent risk assessment may be found in the *Agent Summary Statements* of the CDC/NIH publication, *Biosafety in Microbiological and Biomedical Laboratories*. Further guidance on agents not listed in Appendix B may be obtained through: Centers for Disease Control and Prevention, Biosafety
Branch, Atlanta, Georgia 30333, Phone: (404) 639-3883, Fax: (404) 639-2294; National Institutes of Health, Division of Safety, Bethesda, Maryland 20892, Phone: (301) 496-1357; National Animal Disease Center, U.S. Department of Agriculture, Ames, Iowa 50010, Phone: (515) 862-8258.

Risk Group 1 (RG1) Agents
RG1 agents are not associated with disease in healthy adult humans. Examples of RG1 agents include asporogenic *Bacillus subtilis* or *Bacillus licheniformis* (see Appendix C-IV-A of the NIH Guidelines, *Bacillus subtilis* or *Bacillus licheniformis* Host-Vector Systems, Exceptions), *Escherichia coli*-K12 (see Appendix C-II-A of the NIH Guidelines, *Escherichia coli* K-12 Host-Vector Systems, Exceptions), and adeno-associated virus types 1 through 4. Those agents not listed in Risk Groups (RGs) 2, 3 and 4 are not automatically or implicitly classified in RG1; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.

Risk Group 2 (RG2) Agents
RG2 agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are
often available.
Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia
--*Acinetobacter baumannii* (formerly *Acinetobacter calcoaceticus*)
--*Actinobacillus*
--*Actinomyces pyogenes* (formerly *Corynebacterium pyogenes)*
--*Aeromonas hydrophila*

--*Amycolata autotrophica*
--*Archanobacterium haemolyticum* (formerly *Corynebacterium haemolyticum*)
--*Arizona hinshawii* - all serotypes
--*Bacillus anthracis*
--*Bartonella henselae, B. quintana, B. vinsonii*
--*Bordetella* including *B. pertussis*
--*Borrelia recurrentis, B. burgdorferi*
--*Burkholderia* (formerly *Pseudomonas species* except those listed in Appendix B-III-A (RG3))
--*Campylobacter coli, C. fetus, C. jejuni*
--*Chlamydia psittaci, C. trachomatis, C. pneumoniae*
--*Clostridium botulinum, Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani*
--*Corynebacterium diphtheriae, C. pseudotuberculosis, C. renale*
--*Dermatophilus congolensis*
--*Edwardsiella tarda*
--Erysipelothrix rhusiopathiae
--*Escherichia coli* - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including *E. coli*
O157:H7
--*Haemophilus ducreyi, H. influenzae*
--*Helicobacter pylori*
--*Klebsiella* - all species except *K. oxytoca* (RG1)
--*Legionella* including *L. pneumophila*
--*Leptospira interrogans* - all serotypes
--*Listeria*
--*Moraxella*
--*Mycobacterium* (except those listed in Appendix B-III-A (RG3)) including *M. avium* complex, *M. asiaticum, M. bovis* BCG
vaccine strain, *M. chelonei, M. fortuitum, M. kansasii, M. leprae, M. malmoense, M. marinum, M. paratuberculosis, M.*
scrofulaceum, M. simiae, M. szulgai, M. ulcerans, M. xenopi
--*Mycoplasma*, except *M. mycoides* and *M. agalactiae* which are restricted animal pathogens
--*Neisseria gonorrhoea, N. meningitidis*
--*Nocardia asteroides, N. brasiliensis, N. otitidiscaviarum, N. transvalensis*
--*Rhodococcus equi*
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--*Salmonella* including *S. arizonae, S. cholerasuis, S. enteritidis, S. gallinarum-pullorum, S. meleagridis, S. paratyphi*, A, B, C, *S.*
typhi, S. typhimurium
--*Shigella* including *S. boydii, S. dysenteriae*, type 1, *S. flexneri, S. sonnei*
--*Sphaerophorus necrophorus*
--*Staphylococcus aureus*
--*Streptobacillus moniliformis*
--*Streptococcus* including *S. pneumoniae, S. pyogenes*
--*Treponema pallidum, T. carateum*
--*Vibrio cholerae, V. parahemolyticus, V. vulnificus*
--*Yersinia enterocolitica*

Risk Group 2 (RG2) - Fungal Agents

--*Blastomyces dermatitidis*
--*Cladosporium bantianum, C. (Xylohypha) trichoides*
--*Cryptococcus neoformans*
--*Dactylaria galopava* (*Ochroconis gallopavum*)
--*Epidermophyton*
--*Exophiala* (*Wangiella*) *dermatitidis*
--*Fonsecaea pedrosoi*
--*Microsporum*
--*Paracoccidioides braziliensis*
--*Penicillium marneffei*
--*Sporothrix schenckii*
--*Trichophyton*

Risk Group 2 (RG2) - Parasitic Agents
--*Ancylostoma* human hookworms including *A. duodenale, A. ceylanicum*
--*Ascaris* including *Ascaris lumbricoides suum*
--*Babesia* including *B. divergens, B. microti*
--*Brugia* filaria worms including *B. malayi, B. timori*
--*Coccidia*
--*Cryptosporidium* including *C. parvum*
--*Cysticercus cellulosae* (hydatid cyst, larva of *T. solium*)
--*Echinococcus* including *E. granulosis, E. multilocularis, E. vogeli*
--*Entamoeba histolytica*
--*Enterobius*
--*Fasciola* including *F. gigantica, F. hepatica*
--*Giardia* including *G. lamblia*
--*Heterophyes*
--*Hymenolepis* including *H. diminuta, H. nana*
--*Isospora*
--*Leishmania* including *L. braziliensis, L. donovani, L. ethiopia, L. major, L. mexicana, L. peruvania, L. tropica*
--*Loa loa* filaria worms
--*Microsporidium*
--*Naegleria fowleri*
--*Necator* human hookworms including *N. americanus*
--*Onchoerca* filaria worms including, *O. volvulus*
--*Plasmodium* including simian species, *P. cynomologi, P. falciparum, P. malariae, P. ovale, P. vivax*
--*Sarcocystis* including *S. sui hominis*
--*Schistosoma* including *S. haematobium, S. intercalatum, S. japonicum, S. mansoni, S. mekongi*
--*Strongyloides* including *S. stercoralis*
--*Taenia solium*
--*Toxocara* including *T. canis*
--*Toxoplasma* including *T. gondii*
--*Trichinella spiralis*
--*Trypanosoma* including *T. brucei brucei, T. brucei gambiense, T. brucei rhodesiense, T. cruzi*
--*Wuchereria bancrofti* filaria worms

Risk Group 2 (RG2) - Viruses
Adenoviruses, human - all types

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Alphaviruses (Togaviruses) - Group A Arboviruses
--Eastern equine encephalomyelitis virus
--Venezuelan equine encephalomyelitis vaccine strain TC-83
--Western equine encephalomyelitis virus
Arenaviruses
--Lymphocytic choriomeningitis virus (non-neurotropic strains)
--Tacaribe virus complex
--Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)
Bunyaviruses
--Bunyamwera virus
--Rift Valley fever virus vaccine strain MP-12
--Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)
Calciviruses
Coronaviruses
Flaviviruses (Togaviruses) - Group B Arboviruses
--Dengue virus serotypes 1, 2, 3, and 4
--Yellow fever virus vaccine strain 17D
--Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV)
Hepatitis A, B, C, D, and E viruses
Herpesviruses - except Herpesvirus simiae (Monkey B virus) (see Appendix B-IV-D, Risk Group 4 (RG4) - Viral Agents)
--Cytomegalovirus
--Epstein Barr virus
--Herpes simplex types 1 and 2
--Herpes zoster
--Human herpesvirus types 6 and 7
Orthomyxoviruses
--Influenza viruses types A, B, and C
--Other tick-borne orthomyxoviruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I
through IV)
Papovaviruses
--All human papilloma viruses
Paramyxoviruses
--Newcastle disease virus
--Measles virus
--Mumps virus
--Parainfluenza viruses types 1, 2, 3, and 4
--Respiratory syncytial virus
Parvoviruses
--Human parvovirus (B19)
Picornaviruses
--Coxsackie viruses types A and B
--Echoviruses - all types

--Polioviruses - all types, wild and attenuated
--Rhinoviruses - all types
Poxviruses -all types except Monkeypox virus (see Appendix B-III-D, Risk Group 3 (RG3) - Viruses and Prions) and restricted
poxviruses including Alastrim, Smallpox, and Whitepox (see Section V-L, Footnotes and References of Sections I through IV of
the NIH Guidelines)
Reoviruses - all types including Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)
Rhabdoviruses
--Rabies virus - all strains
--Vesicular stomatitis virus - laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow
Togaviruses (see Alphaviruses and Flaviviruses)
--Rubivirus (rubella)

Risk Group 3 (RG3) Agents
RG3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.

Risk Group 3 (RG3) - Bacterial Agents Including Rickettsia
--*Bartonella*
--*Brucella* including *B. abortus, B. canis, B. suis*
--*Burkholderia* (*Pseudomonas*) *mallei, B. pseudomallei*
--*Coxiella burnetii*
--*Francisella tularensis*
--*Mycobacterium bovis* (except BCG strain, see Appendix B-II-A, Risk Group 2 (RG2) - Bacterial Agents Including *Chlamydia*),
M. tuberculosis
--*Pasteurella multocida* type B -"buffalo" and other virulent strains
--*Rickettsia akari, R. australis, R. canada, R. conorii, R. prowazekii, R. rickettsii, R, siberica, R. tsutsugamushi, R. typhi* (*R.*
mooseri)
--*Yersinia pestis*

Risk Group 3 (RG3) - Fungal Agents
--*Coccidioides immitis* (sporulating cultures; contaminated soil)
--*Histoplasma capsulatum, H. capsulatum* var.. *duboisii*

Risk Group 3 (RG3) - Parasitic Agents
None

Risk Group 3 (RG3) - Viruses and Prions
Alphaviruses (Togaviruses) - Group A Arboviruses
--Semliki Forest virus
--St. Louis encephalitis virus
--Venezuelan equine encephalomyelitis virus (except the vaccine strain TC-83, see Appendix B-II-D (RG2))
--Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV of the NIH

Guidelines)
Arenaviruses
--Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)
Bunyaviruses
--Hantaviruses including Hantaan virus
--Rift Valley fever virus
Flaviviruses (Togaviruses) - Group B Arboviruses
--Japanese encephalitis virus
--Yellow fever virus
--Other viruses as listed in the reference source (see Section V-C, Footnotes and References of Sections I through IV of the NIH
Guidelines)
Poxviruses
--Monkeypox virus
Prions
--Transmissible spongioform encephalopathies (TME) agents (Creutzfeldt-Jacob disease and kuru agents)(see Section V-C,
Footnotes and References of Sections I through IV of the NIH Guidelines, for containment instruction)
Retroviruses
--Human immunodeficiency virus (HIV) types 1 and 2
--Human T cell lymphotropic virus (HTLV) types 1 and 2
--Simian immunodeficiency virus (SIV)
Rhabdoviruses
--Vesicular stomatitis virus

Risk Group 4 (RG4) Agents. **These agents are not allowed at Tuskegee University.**
RG4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.

Risk Group 4 (RG4) - Bacterial Agents
None

Risk Group 4 (RG4) - Fungal Agents
None

Risk Group 4 (RG4) - Parasitic Agents
None

Risk Group 4 (RG4) - Viral Agents
Arenaviruses (Togaviruses) - Group A Arboviruses
--Guanarito virus
--Lassa virus
--Junin virus
--Machupo virus
Bunyaviruses (Nairovirus)
--Crimean-Congo hemorrhagic fever virus
Filoviruses
--Ebola virus

--Marburg virus
Flaviruses (Togaviruses) - Group B Arboviruses
--Tick-borne encephalitis virus complex including Absetterov, Central European encephalitis, Hanzalova, Hypr, Kumlinge,
Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses
Herpesviruses (alpha)
--Herpesvirus simiae (Herpes B or Monkey B virus)
Hemorrhagic fever agents and viruses as yet undefined

Animal Viral Etiologic Agents in Common Use
The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents is
associated with disease in healthy adult humans; they are commonly used in laboratory experimental work.
A containment level appropriate for Risk Group (RG1) human agents is recommended for their use. For agents that are
infectious to human cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for
Risk Group (RG2) human agents is recommended.
Baculoviruses
Herpesviruses
--Herpesvirus ateles
--Herpesvirus saimiri
--Marek's disease virus
--Murine cytomegalovirus
Papovaviruses
--Bovine papilloma virus
--Polyoma virus
--Shope papilloma virus
--Simian virus 40 (SV40)
Retroviruses
--Avian leukosis virus
--Avian sarcoma virus
--Bovine leukemia virus
--Feline leukemia virus
--Feline sarcoma virus
--Gibbon leukemia virus
--Mason-Pfizer monkey virus
--Mouse mammary tumor virus
--Murine leukemia virus
--Murine sarcoma virus
--Rat leukemia virus

Murine Retroviral Vectors
Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered, under BL1 containment.

Appendix D

***Biosafety in Biomedical and Microbiological Laboratories**
BMBL Table 1
Summary of Recommended Biosafety Levels for Infectious Agents

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy adults	Standard Microbiological Practices	None required	Open bench top sink required
2	Associated with human disease, hazard = percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers = Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPEs: laboratory coats; gloves; face protection as needed	BSL-1 plus: Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of lab clothing before laundering		

	• Baseline serum	
	Primary barriers = Class I or II BCSs or other physical containment devices used for all open manipulations of agents; PPEs: protective lab clothing; gloves; respiratory protection as needed	
	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhausted air not recirculated • Negative airflow into laboratory	
4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission	
	BSL-3 practices plus: • Clothing change before entering • Shower on exit • All material decontaminated on exit from facility	
	Primary barriers = All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	
	BSL-3 plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decon systems • Other requirements outlined in the text	

*Biosafety in Microbiological and Biomedical Laboratories, 4th ed., CDC/NIH, May 1999: (http://bmbl.od.nih.gov)

***Vertebrate Animal Biosafety Level Criteria**

Summary of Recommended Biosafety Levels for Activities in Which Experimentally or Naturally Infected

Vertebrate Animals Are Used

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy human adults.	Standard animal care and management practices, including appropriate medical surveillance programs	As required for normal care of each species.	Standard animal facility No recirculation of exhaust air Directional air flow recommended Handwashing sink recommended
2	Associated with human disease. Hazard: percutaneous exposure, ingestion, mucous membrane exposure.	ABSL-1 practices plus: Limited access Biohazard warning signs Sharps precautions Biosafety manual Decontamination of all infectious wastes and of animal cages prior to washing	ABSL-1 equipment plus primary barriers: containment equipment appropriate for animal species; PPES: laboratory coats, gloves, face and respiratory protection as needed.	ABSL-1 facility plus: Autoclave available Handwashing sink available in the animal room. Mechanical cage washer used

3
Indigenous or exotic agents with potential for aerosol transmission; disease may have serious health effects.
ABSL-2 practices plus:
Controlled access
Decontamination of clothing before laundering
Cages decontaminated before bedding removed
Disinfectant foot bath as needed
ABSL-2 equipment plus:
Containment equipment for housing animals and cage dumping activities
Class I or II BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols.
PPEs: appropriate respiratory protection
ABSL-2 facility plus:
Physical separation from access corridors
Self-closing, double-door access
Sealed penetrations
Sealed windows
Autoclave available in facility
4
Dangerous/exotic agents that pose high risk of life threatening disease; aerosol transmission, or related agents with unknown risk of transmission.
ABSL-3 practices plus:
Entrance through change room where personal clothing is removed and laboratory clothing is put on; shower on exiting
All wastes are decontaminated before removal from the facility
ABSL-3 equipment plus:
Maximum containment equipment (i.e., Class III BSC or partial containment

equipment in combination with
full body, air-supplied positivepressure
personnel suit) used
for all procedures and activities
ABSL-3 facility plus:
Separate building or
isolated zone, Dedicated
supply and exhaust,
vacuum and
decontamination systems
Other requirements
outlined in the text

*Biosafety in Microbiological and Biomedical Laboratories, 4th ed., CDC/NIH, May 1999: (http://bmbl.od.nih.gov)