

INSTITUTIONAL BIOSAFETY COMMITTEE
GUIDELINES FOR THE REVIEW OF
RESEARCH ACTIVITIES INVOLVING RECOMBINANT DNA

Purpose

When research activities conducted under the University's auspices involve the use of recombinant DNA, it is the legal and ethical responsibility of the University, and of the institutional Biosafety Committee acting on its behalf, to ensure that these activities comply with the "NIH Guidelines for Research Involving Recombinant DNA Molecules" (*NIH Guidelines*) published in the Federal Register on July 5, 1994 (vol. 59), and amendments) - www4.od.nih.gov/oba/rac/guidelines/guidelines.html.

These guidelines define recombinant DNA as "(i) molecules that are constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above."

The Institutional Biosafety Committee

In compliance with the federal regulations, the University has established an Institutional Biosafety Committee (IBC), administered through the Research Compliance Office, to ensure fulfillment of the University's legal and ethical responsibilities relative to recombinant DNA research. It is the responsibility of the IBC to review proposed recombinant DNA research activities to be conducted under the University's auspices, and to assign the appropriate biosafety level. All research involving recombinant DNA conducted under the University's auspices is subject to the jurisdiction of the IBC, regardless of the source of funding (internal, external) of such research. No project involving recombinant DNA should proceed without the explicit written approval or knowledge of the Committee. This approval is obtained by completing and submitting for review a written protocol describing the research objectives and methods, including: (1) the source(s) of the recombinant DNA; (2) the nature of the inserted DNA sequences; (3) the hosts and vectors to be used; (4) whether a deliberate attempt will be made to obtain expression of a foreign gene in the cloning vehicle, and if so, what protein; and (5) the containment condition (biosafety level) required by the NIH guidelines.

Types of Action on Proposed Projects

In reviewing proposals, the IBC may take any one of the following actions:

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1. Declare the research exempt from the federal guidelines.
 Certain classes of experiments are exempt from the NIH guidelines. However, because it is the responsibility of the IBC to ensure University compliance with the guidelines, exempt status must be approved by the IBC. Investigators requesting exemption need to submit a written protocol for review, and should state in their application the basis for the requested exemption (see below for categories of research which are exempt).
2. Approve the research procedures at the containment level indicated.
3. Approve the research procedures subject to modifications.
4. Disapprove the research procedures.
5. Defer action on the proposal pending receipt of additional information or further clarification of specific terms as may be identified.

Any changes to previously approved projects, including new sources of recombinant DNA or use of new hosts or vectors, must be brought to the attention of the IBC, which will then consider whether the original project approval and assessment of containment level requires modification.

If a proposal receives final disapproval by the IBC and the principal investigator wishes a further hearing on the matter, an appeal may be made to the Vice Provost for Research. In this case, an ad hoc appeals committee will be convened, consisting of the Chair of the IBC and two or more persons who have served on the IBC as well as any special consultants that may be required.

Classification of Risk / Containment Requirements

The NIH guidelines designate various categories of recombinant DNA research activities and their appropriate containment levels. The classification of microorganisms on into Risk Group 1, Risk Group 2, Risk Group 3, or Risk Group 4, agents, is based on the association with or cause of human disease as follows:

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 <i>often</i> available
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions <i>may</i> 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 <i>not usually</i> available (high individual risk and high community risk)

The *NIH Guidelines* stipulate the required containment procedures for research activities involving recombinant DNA. Four "Biosafety Levels (BLs)" have been defined, with BL1 the least stringent and BL4 the most stringent. These levels consist of combinations of laboratory practices and techniques;

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safety equipment and laboratory facilities appropriate for the operations performed and are based on the potential hazards imposed by the agents used and for the laboratory function and activity. These containment mechanisms can be divided into two categories: (i) a set of standard practices that are generally used in microbiological laboratories and (ii) special procedures, equipment, and laboratory installations that provide physical barriers that are applied in varying degrees according to the estimated biohazard.

Generally, RG1 agents require BL1 containment, RG2 agents require BL2 containment, etc. However, each determination of the appropriate containment should be based on an initial risk assessment and a thorough consideration of the agent itself and how it is to be manipulated. Specific BL1 and BL2 containment requirements can be found later in this document. For BL3 and BL4 requirements please contact the Chair of the IBC.

The *NIH Guidelines* also define containment levels for research involving plants (BL1-P through BL4-P) and for animals of a size or having growth requirements that preclude the use of conventional primary containment systems used for small laboratory animals (BL1-N through BL4-N). The specifics regarding these containment levels can be found in the *NIH Guidelines* Section II-B, Appendix P (plants) and Appendix Q (animals).

In addition to physical containment there is a third containment mechanism – the application of highly specific biological barriers. Natural barriers exist that limit either (i) the infectivity of a vector or vehicle (plasmid or virus) for specific hosts, or (ii) its dissemination and survival in the environment.

Since the physical and biological means of containment are complimentary, different levels of containment can be established that apply various combinations of physical and biological barriers along with a constant use of standard practices. More information on containment can be found in the *NIH Guidelines* Appendix G and I.

Categories of Recombinant DNA Research Activities

In most cases, only IBC approval or awareness of proposed research is required. However, in some cases approval from NIH and/or other federal agencies may also be required.

Experiments Requiring IBC and Federal Review and Approval Before Initiation (59 FR, Sections III-A, III-B, III-C).

Experiments in this category include:

1. Deliberate formation of recombinant DNA containing genes for the biosynthesis of toxic molecules lethal for vertebrates at an LD₅₀ of less than 100 nanograms per kilogram body weight.
2. Experiments considered as “Major Actions” under the *NIH Guidelines*.

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3. Deliberate transfer of a drug-resistant trait to microorganisms that are not known to acquire it naturally, if such acquisition could compromise the use of the drug to control disease agents in human or veterinary medicine or agriculture.
4. Deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into human subjects. This research will also require Human Subjects Review Board review and approval before it can be initiated.

Experiments Requiring IBC Review and Approval Before Initiation (59 FR, Section III-D)

These experiments include:

1. Experiments using Risk Group 2, Risk Group 3, Risk Group 4 or restricted agents as host-vector systems.
 - a. The containment level for these experiments is based on the class of the agent used, with Risk Group 2 agents usually requiring BL2; Risk Group 3 usually requiring BL3; and Risk Group 4 usually requiring BL4. Use of restricted agents requires special NIH review and approval on a case-by-case basis.
2. Experiments in which DNA from Risk Group 2, Risk Group 3, Risk Group 4, or restricted agents is cloned in nonpathogenic prokaryotic or lower eukaryotic host-vector systems.
 - a. Many experiments in this category will be exempt from the guidelines (see below). For those that are not, transfer of DNA from Risk Group 2 or Risk Group 3 agents into nonpathogenic prokaryotes or lower eukaryotes require BL2 containment; transfer from Risk Group 4 agents into nonpathogenic prokaryotes or lower eukaryotes requires BL4 containment unless it can be demonstrated that only a totally and irreversibly defective fraction of the agent's genome is present in a given recombinant, in which case only BL2 containment is required. Use of restricted agents requires special NIH review and approval on a case-by-case basis.
3. Experiments involving the use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus in tissue culture systems.
 - a. Use of Risk Group 2 viruses may be conducted at BL2 containment; Risk Group 3 at BL3; and Risk Group 4 at BL4. Use of infectious or defective restricted poxviruses requires special NIH review and approval on a case-by-case basis. All others require BL1 containment.
 - b. Note: A USDA permit is required for work with plant or animal pathogens.
 - c. Caution: Special care should be used in the evaluation of containment levels for experiments which are likely to either enhance the pathogenicity or extend the host range of viral vectors under conditions that permit a productive infection. In such cases serious consideration should be given to increasing physical containment by at least one level.
4. Experiments involving whole animals or plants.

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- a. Recombinant DNA, or DNA or RNA molecules derived from recombinant DNA, from any source except for greater than two-thirds of a eukaryotic viral genome may be transferred to any non-human vertebrate or any invertebrate organism and propagated under conditions of physical containment equivalent to BL1 or BL1-N and appropriate to the organism under study. The investigator must demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. A USDA permit is required for work with plant or animal pathogens. For all other experiments involving whole plants and animals, the IBC will determine appropriate containment.
5. Experiments involving more than 10 liters of culture.
 - a. Appropriate containment will be determined by the IBC. Where appropriate, Appendix K from the *NIH Guidelines* should be used.

Experiments That Require IBC Notice Simultaneous with Initiation (59 FR, Section III-E)

Experiments not included in Sections III-A, III-B, III-C, III-D and III-F are considered here and may be conducted at BL1 containment. For experiments in this category, a proposal shall be submitted to the IBC at the time the experiment is initiated. The IBC reviews and approves all such proposals, but IBC review and approval prior to initiation of the experiment is not required.

1. Experiments involving the formation of recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus.
 - a. Recombinant DNA molecules may be propagated and maintained in cells in tissue culture using BL1 containment but it must be demonstrated that the cells lack helper virus for the specific families of defective virus being used. The DNA may contain fragments of the genome of viruses from more than one family but each fragment must be less than two-thirds of a genome.
2. Experiments involving whole plants
 - a. Except those that fall under III-A, III-B, III-D or III-F. Knowledge of the organisms and judgment based on accepted scientific practices should be used in all cases in selecting the appropriate containment level.
3. Experiments involving transgenic rodents
 - a. This involves the generation of rodents in which the animal's genome has been altered by the stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line. Only experiments that require BL1 containment are covered here. Experiments requiring BL2-BL4 require IBC review and approval *before* initiation.

Experiments That Are Exempt (59, Section III-F)

Certain classes of experiments are exempt from the procedures of the NIH guidelines concerning containment, record keeping, etc. These include experiments in which the recombinant DNA molecules:

1. Are not in organisms or viruses (Section III-F-1).

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2. Consist entirely of DNA segments from a single non-chromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent (Section III-F-2).
3. Consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species) or when transferred to another host by well-established physiological means (Section III-F-3).
4. Consist entirely of DNA 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) (Section III-F-4).
5. 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. Although these experiments are exempt, it is recommended that they be performed at the appropriate Biosafety level for the host or recombinant organism (Section III-F-5). Following is a list of natural exchangers. The list will be updated periodically by NIH.

Sublist A

1. Genus *Escherichia*
2. Genus *Shigella*
3. Genus *Salmonella* (including *Arizona*)
4. Genus *Enterobacter*
5. Genus *Citrobacter* – including *Levinea*
6. Genus *Klebsiella* – including *oxytoca*
7. Genus *Erwinia*
8. *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas fluorescens* and *Pseudomonas mendocina*
9. *Serratia marcescens*
10. *Yersinia enterocolitica*

Sublist B

1. *Bacillus subtilis*
2. *Bacillus licheniformis*
3. *Bacillus pumilus*
4. *Bacillus globigii*
5. *Bacillus niger*
6. *Bacillus nato*
7. *Bacillus amyloliquefaciens*
8. *Bacillus aterrimus*

Sublist C

1. *Streptomyces aureofaciens*

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2. *Streptomyces rimosus*
3. *Streptomyces coelicolor*

Sublist D

1. *Streptomyces aureofaciens*
2. *Streptomyces rimosus*
3. *Streptomyces coelicolor*

Sublist E

1. One-way transfer of *Streptococcus mutans* or *Streptococcus lactis* DNA into *Streptococcus sanguis*

Sublist F

1. *Streptococcus sanguis*
 2. *Streptococcus pneumoniae*
 3. *Streptococcus faecalis*
 4. *Streptococcus pyogenes*
 5. *Streptococcus mutans*
6. Recombinant DNA molecules containing less than one-half of any eukaryotic genome (all viruses from a single Family being considered identical) that are propagated and maintained in cells in tissue culture (Appendix C-I) except:
 - (i) Experiments requiring IBC and federal review and approval before initiation
 - (ii) Experiments involving DNA from Risk Groups 3, 4, or restricted organisms or cells known to be infected with these agents,
 - (iii) Experiments involving the deliberate introduction of genes coding for the biosynthesis of molecules toxic for vertebrates,
 - (iv) Whole plants regenerated from plant cells and tissue cultures that do not remain axenic cultures
 7. Experiments involving Escherichia coli K-12 host-vector systems provided that: (i) the E. coli host shall not contain conjugation-proficient plasmids or generalized transducing phages and (ii) lambda or lambdoid or Ff bacteriophages or nonconjugative plasmids shall be used as vectors (Appendix C-II), except:
 - (i) Experiments requiring IBC and federal review and approval before initiation
 - (ii) Experiments involving DNA from Risk Group 3, 4 or restricted organisms or cells known to be infected with these agents,
 - (iii) Large scale experiments (e.g., more than 10 liters of culture) and
 - (iv) Experiments involving the cloning of toxin molecule genes coding for the biosynthesis of molecules toxic for vertebrates.
 8. Experiments involving *Saccharomyces cerevisiae* or *Saccharomyces uvarum* host-vector systems (Appendix C-III) except:

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- (i) Experiments requiring IBC and federal review and approval before initiation
 - (ii) Experiments involving DNA from Risk Group 3, 4 or restricted organisms or cells known to be infected with these agents,
 - (iii) Large scale experiments (e.g., more than 10 liters of culture) and
 - (iv) Experiments involving the cloning of toxin molecule genes coding for the biosynthesis of molecules toxic for vertebrates.
9. Experiments involving asporogenic *Bacillus subtilis* strains which do not revert to sporeformers with a frequency greater than 10^{-7} (Appendix C-IV) except:
- (i) Experiments requiring IBC and federal review and approval before initiation
 - (ii) Experiments involving DNA from Risk Group 3, 4 or restricted organisms or cells known to be infected with these agents,
 - (iii) Large scale experiments (e.g., more than 10 liters of culture) and
 - (iv) Experiments involving the cloning of toxin molecule genes coding for the biosynthesis of molecules toxic for vertebrates.
10. Recombinant DNA molecules derived entirely from extrachromosomal elements of gram positive organisms (listed below), propagated and maintained in the organisms listed below (Appendix C-V) except:
- (i) Experiments requiring IBC and federal review and approval before initiation
 - (ii) Experiments involving DNA from Risk Group 3, 4 or restricted organisms or cells known to be infected with these agents,
 - (iii) Large scale experiments (e.g., more than 10 liters of culture) and
 - (iv) Experiments involving the cloning of toxin molecule genes coding for the biosynthesis of molecules toxic for vertebrates.

Bacillus amyloliquefaciens
Bacillus amylosacchariticus
Bacillus anthracis
Bacillus subtilis
Bacillus aterrimus
Bacillus brevis
Bacillus cereus
Bacillus globigii
Bacillus licheniformis
Bacillus megaterium
Bacillus pumilus
Bacillus thuringiensis
Bacillus natto
Bacillus niger
Bacillus pumilus
Bacillus sphaericus
Bacillus subtilis
Bacillus thurigiensis
Clostridium acetobutylicum

Lactobacillus casei
Listeria grayi
Listeria monocytogenes
Listeria murrayi
Pediococcus acidilactici
Pediococcus damnosus
Pediococcus pentosaceus
Staphylococcus aureus
Staphylococcus carnosus
Staphylococcus epidermidis
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus avium
Streptococcus cremoris
Streptococcus dorans
Streptococcus equisimilis
Streptococcus faecalis
Streptococcus fereus
Streptococcus ferns

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Streptococcus lactis
Streptococcus mitior
Streptococcus mutans
Streptococcus pneumoniae
Streptococcus pyogenes

Streptococcus salivarius
Streptococcus sanguis
Streptococcus sobrinus
Streptococcus thermophilus

11. Investigators with recombinant DNA activities exempt from the NIH guidelines should indicate the basis for the exemption status on the protocol form (i.e., refer to the appropriate category of exempt projects from the above list). All determinations of exempt status will be made by the IBC.

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Classification of Microorganisms on the Basis of Hazard

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*; adeno- associated virus (AAV) types 1 through 4; and recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus. A strain of *Escherichia coli* is an RG1 agent if it (1) does not possess a complete lipopolysaccharide (i.e., lacks the O antigen); and (2) does not carry any active virulence factor (e.g., toxins) or colonization factors and does not carry any genes encoding these factors.

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 (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*

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- *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. chelonae*, *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 gonorrhoeae*, *N. meningitidis*
- *Nocardia asteroides*, *N. brasiliensis*, *N. otitidiscaviarum*, *N. transvalensis*
- *Rhodococcus equi*
- *Salmonella* including *S. arizonae*, *S. choleraesuis*, *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*
- *Microsporium*
- *Paracoccidioides braziliensis*
- *Penicillium marneffeii*
- *Sporothrix schenckii*
- *Trichophyton*

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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. granulosus*, *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. peruviana*, *L. tropica*
- *Loa loa* filaria worms
- *Microsporidium*
- *Naegleria fowleri*
- *Necator* human hookworms including *N. americanus*
- *Onchocerca* 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
- Alphaviruses (Togaviruses) - Group A Arboviruses
 - Eastern equine encephalomyelitis virus
 - Venezuelan equine encephalomyelitis vaccine strain TC-83
 - Western equine encephalomyelitis virus

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- **Arenaviruses**
 - Lymphocytic choriomeningitis virus (non-neurotropic strains)
 - Tacaribe virus complex
 - Other viruses as listed in the reference source
- **Bunyaviruses**
 - Bunyamwera virus
 - Rift Valley fever virus vaccine strain MP-12
 - Other viruses as listed in the reference source
- **Caliciviruses**
- **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
- **Hepatitis A, B, C, D, and E viruses**
- **Herpesviruses - except Herpesvirus simiae (Monkey B virus) (see 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
- **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 Risk Group 3 (RG3) - Viruses and Prions) and restricted poxviruses including Alastrim, Smallpox, and Whitepox**
- **Reoviruses - all types including Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)**
- **Rhabdoviruses**

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- 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 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 (RG2))
 - Other viruses as listed in the reference source
- Arenaviruses
 - Flexal
 - Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)

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- 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
- Poxviruses
 - Monkeypox virus
- Prions
 - Transmissible spongiform encephalopathies (TME) agents (Creutzfeldt-Jacob disease and kuru agents)
- 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

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
 - Guaranito virus
 - Lassa virus
 - Junin virus
 - Machupo virus
 - Sabia

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- 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)
- Paramyxoviruses
 - Equine morbillivirus
- 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 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 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

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- 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.

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Containment Requirements for Biosafety Levels 1 and 2

The physical containment requirements for BL1 and BL2 follow. Investigators requiring information about BL3 or BL4 should check with the Chair of the Biosafety Committee.

BIOSAFETY LEVEL 1 (BL1)

Standard Microbiological Practices

- Access to the laboratory is limited or restricted at the discretion of the Principal Investigator when experiments are in progress.
- Work surfaces are decontaminated once a day and after any spill of viable material.
- All contaminated liquid or solid wastes are decontaminated before disposal.
- Mechanical pipetting devices are used; mouth pipetting is prohibited.
- Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.
- Persons wash their hands: (i) after they handle materials involving organisms containing recombinant DNA molecules and animals, and (ii) before exiting the laboratory.
- All procedures are performed carefully to minimize the creation of aerosols.
- In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, changing room) and protective clothing (e.g., uniforms, laboratory coats) shall be provided that are appropriate for the risk of exposure to viable organisms containing recombinant DNA molecules.

Special Practices

- Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.
- An insect and rodent control program is in effect.

Containment Equipment

- Special containment equipment is generally not required for manipulations of agents assigned to BL1.

Laboratory Facilities

- The laboratory is designed so that it can be easily cleaned.
- Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
- Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.
- Each laboratory contains a sink for hand washing.
- If the laboratory has windows that open, they are fitted with fly screens.

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BIOSAFETY LEVEL 2 (BL2)

Standard Microbiological Practices

- Access to the laboratory is limited or restricted by the Principal Investigator when work with organisms containing recombinant DNA molecules is in progress.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All contaminated liquid or solid wastes are decontaminated before disposal.
- Mechanical pipetting devices are used; mouth pipetting is prohibited.
- Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.
- Persons wash their hands: (i) after handling materials involving organisms containing recombinant DNA molecules and animals, and (ii) when exiting the laboratory.
- All procedures are performed carefully to minimize the creation of aerosols.
- Experiments of lesser biohazard potential can be conducted concurrently in carefully demarcated areas of the same laboratory.

Special Practices

- Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.
- The Principal Investigator limits access to the laboratory. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.
- The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g., immunization) may enter the laboratory or animal rooms.
- When the organisms containing recombinant DNA molecules in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign incorporating the universal biosafety symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.
- An insect and rodent control program is in effect.
- Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before exiting the laboratory for non-laboratory areas (e.g., cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.
- Animals not involved in the work being performed are not permitted in the laboratory.
- Special care is taken to avoid skin contamination with organisms containing recombinant DNA molecules; gloves should be worn when handling experimental animals and when skin contact with the agent is unavoidable.
- All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

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- Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably autoclaved, before discard or reuse.
- Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Institutional Biosafety Committee and NIH/OBA. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
- When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.
- A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

Containment Equipment

- Biological safety cabinets (Class I or II) or other appropriate personal protective or physical containment devices are used whenever:
 - Procedures with a high potential for creating aerosols are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, intranasal inoculation of animals, and harvesting infected tissues from animals or eggs.
 - High concentrations or large volumes of organisms containing recombinant DNA molecules are used. Such materials may be centrifuged in the open laboratory if sealed beads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

Laboratory Facilities

- The laboratory is designed so that it can be easily cleaned.
- Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
- Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.
- Each laboratory contains a sink for hand washing.
- If the laboratory has windows that open, they are fitted with fly screens.
- An autoclave for decontaminating laboratory wastes is available.