

Office of Environment, Health & Safety

BIO SAFETY MANUAL



Berkeley
UNIVERSITY OF CALIFORNIA

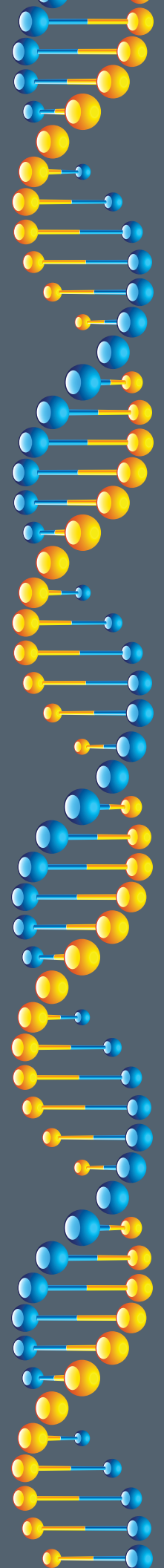


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Section I.

Introduction

UC Berkeley is committed to maintaining a healthy and safe workplace for all laboratory workers, students and visitors. This manual is an overview of the administrative steps necessary to obtain and maintain approval for the use of biological materials in laboratories, as well as a reference for good work practices and safe handling of other potentially infectious materials (OPIM).

A variety of procedures are conducted on campus which use many different agents. This manual addresses the biological hazards frequently encountered in laboratories. Biological hazards include infectious or toxic microorganisms (including viral vectors), potentially infectious human substances, and research animals and their tissues, in cases from which transmission of infectious agents or toxins is reasonably anticipated. Additionally, the use of recombinant or synthetic nucleic acids and genetically modified organisms, including plants and animals, may pose additional risks to laboratory personnel and the environment.

All campus researchers working with, or contemplating working with, such agents are therefore required to submit a Biological Use Authorization (BUA) application to be reviewed and approved by UC Berkeley's Institutional Biosafety Committee, known as the Committee for Laboratory and Environmental Biosafety (CLEB), prior to obtaining, storing, or initiating any experiment involving infectious agents or genetically modified organisms.

The information in this manual is meant to act as a guide for safe working procedures and a reference for current best practices in biological research. It does not, however, replace laboratory specific training and risk communication. If any additional information not covered in this manual is required, please contact the Office of Environment, Health & Safety (EH&S) biosafety program staff at bsa@berkeley.edu.

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Section II. **Institutional Review of Biological Research**

Introduction to CLEB

The Committee for Laboratory and Environmental Biosafety (CLEB) serves as UC Berkeley's Institutional Biosafety Committee (IBC). CLEB is comprised of subject matter experts, an animal health expert, and an occupational physician, as well as community members. The committee meets during the first week of every month to review Biological Use Authorizations.

The National Institutes of Health (NIH) mandates the establishment of an Institutional Biosafety Committee for all institutions that receive any support for recombinant or synthetic nucleic acid molecule research from NIH. At Berkeley, CLEB is also charged with reviewing projects involving infectious agents and human source material.

CLEB meetings are open to the public. Anyone interested in attending, however, must request an invitation prior to the meeting. The proceedings of the meeting are confidential, and the content of the discussion should not be deliberated outside of the meeting. Thus, any attendees must sign a confidentiality agreement.

If you have any questions regarding CLEB, contact the EH&S biosafety program staff at bs@berkeley.edu

Prerequisites to Initiation of Work

Prior to conducting work with biological material, research groups ("laboratories") must have an approved application for a biological use authorization (BUA), which has been submitted by the principal investigator (PI). BUAs are required for work with recombinant DNA molecules, genetically modified organisms or plants, infectious agents, human clinical samples, cell lines or other potentially infectious materials.

Prior to approval of the BUA application, there are a few prerequisites that the

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laboratory and members of the laboratory must complete.

1. The PI must submit a BUA application for review
2. All personnel must complete the required trainings
3. An initial inspection of the laboratory facilities must be conducted by a member of the EH&S biosafety program.

The following sections will detail the processes necessary to obtain final approval of the BUA application, which allows for initiation of work.

Completion and Submission of the BUA Application

The UC Berkeley biological use authorization (BUA) application is reviewed by CLEB.

Experiments Requiring a BUA

A BUA application must be submitted for the following experiments:

All recombinant or synthetic nucleic acid molecule experiments. This includes transgenic plants and transgenic animals in which the germ line of the animal has been changed. This is true regardless of whether the experiment is considered exempt by the National Institutes of Health (NIH) in the *Guidelines for the Use of Synthetic and Recombinant DNA Molecules*, Section III-F. CLEB determines whether an experiment is exempt. The investigator may not make this determination.

Use of infectious agents categorized as NIH Risk Group 2 or higher. To identify the risk group of any particular agent, please follow the link below:

absa.org/riskgroups/

Select agents and toxins, including tetrodotoxin and conotoxin. CLEB must review the protocol for research with these agents, even in cases where the toxin amount is below the minimum threshold under the *Code of Federal Regulations: Select Agent Standard*.

selectagents.gov/SelectAgentsandToxinsList.html

Where to Obtain the BUA Application Form

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UC Berkeley currently uses an MS Word format BUA application, which can be found on the biosafety page of the [EH&S website](#). Additionally, a copy of the form can be requested from the biosafety program at bs@berkeley.edu.

How to Complete the BUA Application

The BUA application must be filed one month before the CLEB meeting at which it will be reviewed. Please see the biosafety page of the [EH&S website](#) for submission deadlines.

When completing the BUA application, be as thorough as possible and complete all applicable sections. In the “Scope of Work” narrative, describe the purpose of the experiment, the procedures to be conducted, the agents used, and incident response protocols in lay terms. This information is essential to the deliberations of the committee, and will allow reviewers to fully understand the experiments that are proposed and the potential risks associated with the research. Additionally, if any work will be conducted that includes the use of recombinant nucleic acid molecules, the template in Attachment II-A of the BUA application must be completed. CLEB will not review the application if this information is missing. The template includes the following information:

Host (e.g., HEK293):

Vector (e.g. Lentivirus):

Source of Gene (e.g., jellyfish):

Type of Gene (e.g., marker):

Gene Expressed (e.g., yes (gfp)):

If Gene is expressed, protein produced (e.g., Green Florescent Protein):

Recommended Level of Containment (e.g., Biosafety Level 2)

Section of the Guidelines: (e.g., Section III-D-1)

This information is required by the NIH for all experiments that include recombinant DNA. The committee uses this information to evaluate the risks posed by any recombinant experiments.

Submission of the BUA Application

Once complete, please submit the BUA application to the EH&S biosafety

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program staff by sending it to bsso@berkeley.edu.

If the research involves biological use and animals, you will need to obtain CLEB approval prior to submitting a request for approval to the Animal Care and Use Committee (ACUC). Plan to submit your BUA application prior to the deadline for the CLEB meeting that precedes the ACUC meeting where your Master Animal Use Protocol (MAUP) will be reviewed.

Training Requirements

EHS 101: UC Laboratory Safety Fundamentals - This course is required for all researchers working in laboratories on the UC Berkeley campus.

EHS 201: Biosafety in Laboratories - This in-person training course is required for all researchers conducting work covered by a BUA.

EHS 204: Biosafety in Laboratories Refresher - All researchers conducting work covered by a BUA must complete this course every three subsequent years following completion of EHS 201.

EHS 202: Bloodborne Pathogens - This online course is required in addition to the training listed above whenever a researcher is working with human cell lines, non-human primate cell lines, human primary tissue, blood, or any other potentially infectious material. This training must be completed annually.

EHS 203: PI Responsibilities - This course is required for all principal investigators conducting or overseeing research covered by a BUA.

See the [EH&S Website](#) for more information on biosafety training. The courses listed above may be accessed through the UC Learning Center. Please see the [EH&S training page](#) for more information.

In addition to the courses offered by EH&S, personnel in each laboratory must take a laboratory-specific training. The PI of each laboratory should complete the template provided by EH&S for this training and present the information to the laboratory. The training must be documented each time it is presented and administered to new people as they join the laboratory. The laboratory-specific PowerPoint presentation should also be updated and submitted to EH&S when research objectives and procedures change. Contact bsso@berkeley.edu or visit the biosafety page on the [EH&S website](#) to obtain the template for laboratory-

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specific training.

Inspection of the Laboratory Facilities

EH&S biosafety staff conduct initial inspections of all laboratory spaces to be included on the biological use authorization. These inspections cover administrative controls, elements of general laboratory safety, and specific requirements related to the containment level of the experiments. The inspection is meant to be consultative rather than punitive. Reasonable safety measures will be implemented based on the specific experiments and agents used in the laboratory. Please contact the team at bsa@berkeley.edu to schedule the inspection.

In addition to the initial inspection, the biosafety team will visit each laboratory annually to verify that it is continuing to work safely, and to provide any guidance that may be needed or requested.

Committee Review and Approval of Applications

CLEB Meeting Dates

CLEB meetings are held the first week of every month, including summer break. To determine when the meeting will take place, please see the [EH&S website](#).

Preliminary Review

The biosafety staff will conduct a preliminary review of the BUA application form prior to distribution to CLEB members. If additional information is required, the PI will be notified by email and asked to resubmit the application with additional information.

Multiple Committee Approvals

Final committee approval may depend on the approval of other institutional

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committees associated with specific research experiments including, but not limited to, the Animal Care and Use Committee (ACUC) and the Committee for the Protection of Human Subjects (CPHS). Please note that BUA application approval is required before ACUC approval. Ensure that appropriate committee-concurrent approval is listed on page 1 of the BUA application, with comments on the status of the other committee approvals.

Approval at a Convened Meeting

All BUA applications must be reviewed at a convened meeting with a quorum prior to approval. CLEB does not have a “designated member review” process. A quorum consists of the following: the Chair, the Biosafety Officer, a subject matter expert in the area of the experiment being reviewed, and one community member.

Faculty members are permitted to speak to the committee during the review of their applications with permission and approval of the committee chair. To request an invitation to the CLEB meeting, contact the EH&S biosafety program staff at bsso@berkeley.edu.

Post-committee Approval

Investigators will be notified promptly of the result of committee voting. If any corrections or clarifications need to be made, the EH&S biosafety program staff will contact the applicant to discuss what actions are requested. Once all action items are addressed and meet the standards of the biosafety program, an approval letter will be sent to the PI. CLEB approvals are valid for a maximum of three years. After three years, the BUA must be renewed by resubmitting a BUA application, or terminated if the research previously described is complete.

If the laboratory is classified BSL2 or higher, the PI will be sent a PDF of the Biohazard door sign in addition to the approval letter. The blank sections (emergency contact, location and date) must be filled out, and once complete, the sign must be printed and posted at the entrance to any BSL2 laboratory spaces.

PIs must immediately notify the EH&S biosafety program staff when the

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experiments are complete. A close-out inspection or consultation may also be necessary.

Amending the BUA

If any change is made to research covered by a BUA, an amendment to the BUA must be filed. CLEB follows an amendment process that allows for changes and modifications without completion of a new BUA application. Changes that can be made using the amendment form include location changes, additions of new agents or recombinant DNA experiments, or modifications to the Scope of Work. The [amendment form](#) can be found online, or requested from bs@berkeley.edu. Excepting the form, the process for submitting an amendment is identical to the initial BUA application review and approval.

Any questions in relation to amending the BUA may be directed to the EH&S biosafety staff at bs@berkeley.edu.

To update personnel, the PI may email bs@berkeley.edu with the changes including what agents each individual is working with, as well as their training dates.

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Section III.

Risk Assessment

Risk assessment, as described in the *Center for Disease Control's Biosafety in Microbiological and Biomedical Laboratories, 5th Edition (BMBL)*, is a process that determines the appropriate level of containment for biological agents. The use of any agent that may be transmitted via inhalation or exposure to mucous membranes of infectious aerosols is of primary concern, as well as contact with blood, bodily fluids, and other potentially infectious material. These experiments fall under regulatory standards in California via the [Cal/OSHA Airborne Transmissible Disease Standard](#) and [Bloodborne Pathogen Standard](#). This type of work involves known hazards to laboratory workers, the environment, and the general public. An assessment of risk must be performed prior to the initiation of any biological experiment to ensure that adequate safeguards are implemented to minimize risk.

Classification of Agents by Risk Group

The WHO and the NIH have similar systems for assessing risk by placing agents into one of four risk groups. These risk groups, as defined by the NIH, are summarized in the table 1 below:

TABLE 1 – NIH RISK GROUPS

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 be</i> 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 <i>usually</i> available (high individual risk and high community risk).

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Microorganisms that are RG1 require standard laboratory facilities and microbiological practices, whereas those in RG4 require maximum containment facilities. Many of the agents likely to be handled experimentally at Berkeley are RG1 or RG2 pathogens, designated as low and moderate hazard, respectively. RG2 and RG3 agents typically require more sophisticated engineering controls (e.g., facilities and equipment) than standard laboratories, as well as special handling and decontamination procedures.

Microorganisms classified as RG2 or higher have been reported to cause infection and disease in otherwise healthy adults. Many RG2 agents have been associated with laboratory-acquired infections. The progression from invasion to infection to disease following contact with an infectious agent depends upon the route of transmission, inoculum, invasive characteristics of the agent, and resistance of the person exposed (whether innate or acquired). Not all contacts result in infection and even fewer develop into clinical disease. Even when disease occurs, severity can vary considerably. It is prudent to assume virulence and handle such agents at the prescribed biosafety level (outlined in Section IV).

There are currently no laboratories working with Risk Group 4 agents at UC Berkeley, and therefore, not appropriate spaces or work procedures are established on campus.

Recombinant DNA

Recombinant DNA (rDNA) work is covered by the NIH [Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules](#) (NIH Guidelines). The NIH Guidelines are designed to outline the practices for constructing and handling rDNA, both natural and synthetic, as well as cells, organisms, and viruses that contain these molecules. The following table outlines the various levels of work defined in the NIH Guidelines as well as the level of approval necessary to initiate the work.

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TABLE 2 – rDNA WORK LEVELS ACCORDING TO NIH

LEVEL	APPROVAL/REVIEW	REQUIREMENTS
III-A	NIH Director, RAC, CLEB†	A drug-resistant gene transferred into a (new) microorganism.
III-B	NIH/OBA, CLEB†	The formation of rDNA containing genes for the biosynthesis of toxin molecules with LD50 < 100 ng/kg.
III-C	RAC, IRB, CLEB†	rDNA (or DNA or RNA derived from rDNA) transferred into humans.
III-D	CLEB†	rDNA transferred to or from whole animals, whole plants (high risk work), experiments involving >10 Liters of culture, influenza viruses, in the presence of a helper virus, agents listed in Risk Groups 2, 3, 4, or restricted agents, or infective eukaryotic viruses in cell culture.
III-E	CLEB§	rDNA involving eukaryotic viruses (not more than 2/3 genome) in cell culture, whole plants (low risk work), BSL1 rodents, or any work not covered in the other categories (most non-pathogenic rDNA work).
III-F	CLEB	rDNA involving nucleic acids not in organisms/viruses, not capable of penetrating cellular membranes, indigenous plasmids/ viruses propagated in original host, specific exemptions from NIH (including the creation of transgenic BSL1 mice through breeding and the use of E. coli K12 for cloning.)

† - Requires approval prior to initiation of work

§ - Requires submission of work to CLEB prior to initiation of work

Often this type of work will involve a classification of risk group, with an emphasis on analysis of how the rDNA may alter overall risk of the agents. The risk group of any parent agents for the specific nucleic acids involved is a foundation for determining containment levels. From there an analysis of risk factors will be used to determine the appropriate containment level which may be higher or lower than the parent agent's risk group.

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Risk Factors of the Agents

A risk assessment of biological agents should take into account both the intrinsic hazards of the agent as well as considerations of individuals handling the agents, addressing the potential for emergent hazards from rDNA work, and hazards associated with the vectors used for insertions.

Agent hazards are those risks that are intrinsic to the agent being handled such as the following:

- Capability to infect and cause disease in a susceptible human host
- Severity of the disease
- Infectious dose
- Availability of preventative measures
- Availability of effective treatments
- How the agent is transmitted (i.e. route of exposure)
- Quantity, concentration, and total volume used
- Stability in the environment
- Zoonotic concerns
- Allergenicity

Whenever a risk assessment process includes reducing the containment level for a particular agent, a diagnostic test should be performed to ensure that virulence factors are no longer present. Care should be taken with newly attenuated pathogens not to assume a lower risk level.

Exposure Sources

Exposure sources in the laboratory are hazards that could result in the infection of researchers or the public through work with biological agents. Some of the more common hazard considerations include the following:

- Aerosol generation (e.g. pipetting, mixing, blending, grinding, sonicating,

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vortexing, centrifuging, shaking)

- Manipulation with sharps
- Animal handling
- Contact with blood, bodily fluids, or other potentially infectious material
- Ingestion of agents via contaminated work areas
- Eye-splashes from liquid nitrogen storage

When performing a risk assessment of laboratory procedures, all potential routes of exposure should be addressed. Most laboratory-acquired infections have resulted from inhalation of aerosols, splashes or sprays, and needlesticks. It is good practice to look for potential exposures via ingestion, inoculation, inhalation, and contamination of skin and mucous membranes.

Any specimens from human and animal sources may contain infectious agents. When handling these agents, researchers should follow universal precautions. The use of **universal precautions** is an approach to infection control in which all potentially infectious material is treated as if known to be infectious for bloodborne pathogens.

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Section IV.

Containment of Biological Agents

Although the most important aspect of biohazard control is the awareness and care taken by personnel in handling infectious materials, certain features of laboratory design, ventilation, and safety equipment can prevent dissemination of pathogens should accidental release occur.

Biosafety Levels

Biosafety Levels consist of combinations of laboratory practices and procedures, safety equipment, and laboratory facility design features appropriate for operations performed within the laboratory, and are based on the potential hazards imposed by the agents used and for the specific laboratory activity. It is the combination of practice, equipment, and facility that form the basis for physical containment strategies for infectious agents.

There are four biosafety levels, with Biosafety Level 1 (BSL-1 or BL1) being the least stringent and Biosafety Level 4 (BSL-4 or BL4) being the most stringent. Generally speaking, the BSL-1 is assigned to work with nonpathogenic microorganisms, BSL-2 is recommended for disease agents transmitted by direct contact (percutaneous inoculation, ingestion, or mucous membrane exposure), BSL-3 is recommended for disease agents with a potential for aerosol transmission, and BSL-4 is recommended when total separation between the infectious agent and investigator is critical.

Risk Group designations often correlate directly with the physical containment level appropriate for a given research activity. This manual is designed to focus on Biosafety Level 2, but a brief description of the correlation between Risk Group and Biosafety Level and the facility design features appropriate for laboratories operating at the various biosafety levels is presented in the Tables 1 and 2.

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TABLE 3 - RELATIONSHIP OF RISK GROUPS TO BIOSAFETY LEVELS, PRACTICES, AND EQUIPMENT

RISK GROUP	BIOSAFETY LEVEL	LABORATORY PROCEDURES	SAFETY EQUIPMENT	EXAMPLES OF LABORATORIES
1	Basic – BSL-1	GMT ^a	None required; open bench work	Basic teaching
2	Basic – BSL-2	GMT plus protective clothing; biohazard sign	Open bench plus BSC ^b for activities with aerosol-potential	Primary health services; primary level hospital; diagnostic, teaching, and public health
3	Containment – BSL-3	As BSL-2 plus special clothing, controlled access, directional air flow	BSC and/or other primary containment for all activities	Special diagnostic; Regional Biocontainment Laboratory
4	Maximum Containment – BSL-4	As BSL-3 plus airlock entry, shower exit, special waste disposal	Class III BSC or positive pressure suits, double-ended autoclave, HEPA-filtered air	Dangerous pathogen units

^a GMT, Good Microbiological Technique

^b BSC, Biological Safety Cabinet

Practices and Procedures

The following practices, corresponding to BSL-2, are important for the prevention of laboratory infection and disease, as well as for the reduction of the potential for contamination of experimental material. These practices and procedures provide the foundation for the more restrictive containment of RG₃ organisms. If you are considering research with a RG₃ organism, contact the Biosafety Program for any additional precautions that may be required.

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Personal Hygiene

- a. Do not eat, drink, chew gum, use tobacco, apply cosmetics, or handle contact lenses in the laboratory.
- b. Do not store food for human consumption in laboratory refrigerators.
- c. Wash hands frequently after handling infectious materials. Also wash hands after removing potentially contaminated latex/nitrile gloves and protective clothing, and before leaving the laboratory.
- d. Keep hands away from mouth, nose, eyes, face, and hair.
- e. Do not store personal items such as coats, boots, bags, and books in the laboratory.
- f. Do not remove personal protective equipment (such as cloth laboratory coats) from the laboratory.

Laboratory Procedures for Handling Infectious Substances

- a. A laboratory biosafety manual must be assembled outlining activities and defining standard operating procedures. In most cases, your laboratory's BUA and this Institutional Biosafety Manual will provide you with the necessary information to complete the laboratory safety manual. A template for the manual can be found in Appendix 1.
- b. If working with recombinant DNA, or with agents at BSL-2 or higher, approval must be obtained from CLEB.
- c. Principal Investigators are responsible for training employees and ensuring that all personnel are informed of hazards, though they may delegate the task to a laboratory supervisor or representative.
- d. Plan and organize materials and equipment before starting work.
- e. Keep laboratory doors closed; limit access to laboratory personnel.
- f. When RG2 (or higher) pathogens are used in long-term studies, post a biohazard sign at the laboratory entrance identifying the agents in use and the appropriate emergency contact information. Electronic versions of these biohazard signs will be generated by the biosafety program based

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upon the information provided in the BUA for each laboratory.

- g. BSL-2 laboratories should contain a sink for hand washing, an eyewash station in which the eyewash is tested/flushed monthly, be relatively free of clutter, and be easy to clean.
- h. Wear a fully fastened laboratory coat when working with infectious agents. Wear protective gloves whenever handling potentially hazardous materials, including human blood and body fluids.
- i. Remove and leave all protective clothing, including gloves, within the laboratory before exiting. If transport of research materials through public spaces is required, then wash hands, don a clean glove to carry materials, and use an ungloved hand to handle public equipment (door handles, elevator buttons, etc.). Laboratory coats may be carried.
- j. Never mouth pipette; use mechanical pipetting devices.
- k. When practical, perform all aerosol-producing procedures such as pipetting, shaking, grinding, sonicating, mixing, and blending in a properly operating biological safety cabinet (BSC). Note that placement of certain equipment within the BSC may compromise cabinet function by disturbing the air curtain. BSC certification and recertification should be performed with these items inside the BSC.
- l. Centrifuge materials containing infectious agents in durable, shatter-resistant, closable tubes. Use a centrifuge with sealed heads or screw-capped safety cups. After centrifugation, open the tubes within a BSC.
- m. Try to avoid using needles, syringes, razor blades, and other sharps when possible. When necessary, discard used syringe-needle units in a sharps container without removing or recapping the needles.
- n. Cover countertops where hazardous materials are used with plastic-backed disposable paper to absorb spills and dispose of them daily or following a spill.
- o. Wipe work surfaces with an appropriate disinfectant after experiments and immediately after spills.
- p. Decontaminate all contaminated or potentially contaminated materials by appropriate methods before disposal (See Chapter D in this section of the Manual).

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- q. Report all accidents and spills to the laboratory supervisor. All laboratory personnel should be familiar with the emergency spill protocol and the location of cleanup equipment.
- r. Good housekeeping practices are essential in laboratories engaged in work with infectious microorganisms. At a minimum, the laboratory should be cleaned weekly. Do not forget to routinely decontaminate all shared equipment and equipment in common areas.
- s. Be sure to advise custodial staff of hazardous areas and places they are not to enter. Use appropriate biohazard signs.

Engineering Controls

Laboratory Design

The more virulent an organism, the greater the degree of physical containment required. Proper safety equipment provides primary containment; laboratory design provides secondary containment. The biosafety staff at EH&S is available for consultation on these matters.

Laboratory Ventilation

To control containment, it is important that laboratory air pressure be lower than that in the adjacent spaces. This negative air pressure differential ensures that air will enter the laboratory and not egress to the hallway. To maintain negative room pressure, laboratory doors must be kept closed.

Exhaust air from biohazardous laboratories should not be recirculated in the building. It should be ducted to the outdoors and released from a stack located remotely from the building air intake. In certain special situations, including many BSL-3 laboratories, air exhausting from a containment facility should be filtered through HEPA (high efficiency particulate air) filters, which are capable of capturing microorganisms.

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Biological Safety Cabinets

Biological safety cabinets (BSCs) are the primary means of containment developed for working safely with infectious microorganisms. When functioning correctly and used in conjunction with good microbiological techniques (GMT), BSCs are very effective at controlling infectious aerosols. BSCs are designed to provide personnel, environmental, and product protection when appropriate practices and procedures are followed.

The following are brief descriptions of BSC types and guidelines for their use. For more in-depth descriptions, including diagrams of airflow and more detailed usage parameters, please visit this site: <http://safety.uchicago.edu/pp/labsafety/biosafety/cabinets.shtml>.

Biological Safety Cabinet Types

Three kinds of biological safety cabinets, designated as Class I, II, and III, have been developed to meet varying research and clinical needs.

CLASS I cabinets are manufactured on a limited basis and have largely been replaced by Class II cabinets. A Class I cabinet is essentially a HEPA-filtered chemical fume hood in which all of the air that enters the cabinet is exhausted into the room or ducted to the outside.

CLASS II cabinets are the most utilized class of BSC on campus. Two varieties of Class II BSCs are used here. Both are adequate for manipulations of RG2 or RG3 pathogens.

CLASS II TYPE A cabinets recirculate 70% of the internal air and exhaust 30% of filtered air into the laboratory.

CLASS II TYPE B cabinets either recirculate 30% of internal air and exhaust 70% of filtered air through a duct to the outside atmosphere, or exhausts 100% of the air.. Because of the greater safety margin, small amounts of nonvolatile chemical carcinogens or radioactive materials can be used in this cabinet.

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Horizontal laminar flow clean benches are not biological safety cabinets and should never be used for work with potentially hazardous materials, whether biological or chemical. These devices help ensure a sterile workspace in the cabinet but have no protection for the worker or the environment. Similarly, chemical fume hoods are not biological safety cabinets. They draw air in, potentially protecting the worker, but do not protect the material in the cabinet (your sample), and exhaust aerosolized material and vapors/gases into the environment.

The ultraviolet lamps within some biological safety cabinets provide only limited ability to inactivate microbes. Efficacy is limited to exposed surfaces and penetration of organic material is poor. Note that effectiveness decreases as the lamp ages. Furthermore, exposure to the ultraviolet light may cause eye damage. Therefore, ultraviolet lamps are not recommended to be the sole source of decontamination of BSC surfaces.

Biological Safety Cabinet Operation

Startup

1. Turn on blower and fluorescent light; be sure that the drain valve is closed.
2. Wait at least two minutes before loading equipment. This is to purge the BSC of contaminated air.
3. Check grills for obstructions
4. Disinfect all interior work surfaces with a disinfectant appropriate for the agent in use.
5. Adjust the sash to proper position; NEVER use above the 8-inch mark.
6. RESTRICT traffic in the BSC vicinity. To ensure proper functioning of a BSC, it is best to place them away from high-traffic areas and doorways to common areas.

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Loading Materials and Equipment

1. Load only items needed for the procedure.
2. Ensure that the rear or front exhaust grills are free from obstruction.
3. Disinfect the exterior of all containers prior to placing them in the BSC.
4. Arrange materials to minimize movement within the cabinet.
5. Arrange materials within the cabinet from CLEAN to DIRTY (or STERILE to CONTAMINATED).
6. Materials should be placed at least six inches from the front BSC grill.
7. Never place non-sterile items upstream of sterile items.
8. Maintain the BSC sash at proper operating height, approximately level with a researcher's armpits.

Prescribed Work Technique

1. Wash hands thoroughly with soap and water before and after any procedure.
2. Wear gloves and laboratory coat; use aseptic technique.
3. Avoid blocking front grill. Work only on a solid, flat surface; ensure that any chair in use is adjusted so armpits are at elevation of lower window edge.
4. Avoid rapid movement during procedures, particularly within the BSC, but in the vicinity of the BSC as well.
5. Move hands and arms straight into and out of work area; never rotate either out of the work area during a procedure.

Final Purging and Wipe-down

1. After completing work, run the BSC blower for two minutes before unloading materials from the cabinet.

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2. Disinfect the exterior of all containers BEFORE removal from the BSC.
3. Decontaminate interior work surfaces of the BSC with an appropriate disinfectant.

Maintenance

To function adequately, the cabinet airflow must be closely regulated and the HEPA filters must be verified as leak-proof. UC Berkeley requires that all BSCs be certified annually. Technical Safety Services (TSS) is the vendor approved by campus to perform BSC certifications. Please contact them directly to schedule an appointment for the certification of your cabinet. Additionally, EH&S will pay for the annual certification of BSCs used at BSL2 or higher levels. Be sure to notify TSS to charge UC Berkeley's "EH&S blanket PO" when arranging for the certification.

In addition to annual recertification of the BSC, the cabinet must be recertified anytime it is relocated. EH&S does not cover the cost of recertification in the case of such an event.

Waste Disposal

There are two primary streams for waste used in biological research: **white bag** and **red bag waste**. White bag waste is material contaminated with BSL1 agents, such as culture plates, pipettes, gloves, and other contaminated waste. Red bag waste is biohazardous waste, meaning that it is contaminated with BSL2 agents or higher.

White Bag Waste

Waste that has been contaminated with recombinant DNA, or any other BSL1 material, should be considered white bag waste. White bag waste is regulated by the NIH and requires the inactivation and decontamination of recombinant material prior to disposal. Therefore, all white bag waste is autoclaved prior to disposal in the general trash if solid, or down the drain if liquid.

Liquid BSL1 cultures can be mixed with bleach to a final concentration of 10% bleach, sit for 20 minutes, and then disposed of down the drain, or autoclaved prior to drain disposal.

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Red Bag Waste

Red bag waste is all waste that would be considered biohazardous and regulated under the California Department of Public Health's Medical Waste Management Act. At UC Berkeley, all BSL2 waste is classified as red bag waste.

To remove biohazardous waste from campus, Berkeley uses a contractor, Stericycle, to disinfect the material and properly dispose of the waste offsite. Therefore, BSL2 waste does not need to be autoclaved.

All solid BSL2 waste should be disposed of in solid wall containers lined with red bags labelled with a biohazard symbols. Additionally, the rigid walled container must have a closing lid and clearly display biohazard symbols on all sides.

All red bag waste should be removed from the laboratory and taken to the Medical Waste Consolidation room, if available, weekly. Each laboratory will need to affix a specific barcode to the barrel. To create an account for Stericycle pickup, please contact hwp@berkeley.edu.

For laboratories in buildings without a Medical Waste Consolidation room, contact the EH&S Hazardous Waste Program, hwp@berkeley.edu, to schedule pickup. Removal of the waste still must occur weekly.

For more information on medical waste, please see the [EH&S fact sheet](#).

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Section V.

Emergency Plans and Reporting

Spill Cleanup Procedures

If a biological material spills in the laboratory, it is important to not panic and to use common sense. If there is no injury involved, please follow the instructions below on how to clean the spill. If anyone is injured, it is always important to tend to the injured person first.

Spill of Biological Agents in a Biological Safety Cabinet

1. Keep the biological safety cabinet running.
2. Don personal protective clothing including gloves, goggles, and a laboratory coat.
3. Put absorbent paper down on the spill area.
4. Apply an effective disinfectant (e.g., 1:10 dilution of bleach) along the walls, work surfaces and trough. Avoid splashing the agent while applying the disinfectant. Do not attempt to clean the HEPA filters or other internal parts!
5. Allow 10-20 minutes for the disinfectant to work.
6. Wipe or absorb all excess disinfectant and place the absorbent material into a red bag. This waste must be handled as medical waste.
7. Reapply disinfectant to all exposed surfaces.
8. In the event of liquid spillage into the catch basin, add an equal volume of disinfectant and wait for 20 minutes to clean up the sterilized material. Process the absorbed material as medical waste.
9. Disinfect all materials used in the biosafety cabinet by wiping the surface with an effective disinfectant. Do not attempt to disinfect contaminated cardboard or other paper items that absorb liquid. Dispose of these

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items as medical waste.

10. If the biosafety cabinet was exposed to excessive amounts of biological contamination, STOP. Call the EH&S biosafety program at (510) 642-3073 to coordinate further cleanup with the biosafety cabinet contracted vendor.

Spill Outside of a Biological Safety Cabinet in a BSL 2 Laboratory

1. Ensure that any other people in the vicinity are notified that a spill has occurred and that the area should be avoided.
2. Don appropriate protective clothing, which includes the following recommended items:
 - a. Laboratory coat
 - b. Gloves
 - c. Face shield, or safety glasses in conjunction with a surgical mask for mucous membrane protection.
 - d. Shoe covers (if available)
3. Cover the spill with absorbent material, such as towels or paper wipes.
4. Carefully pour an effective disinfectant such as a solution of 10% bleach first around the edges of the spill, then onto the spill. Avoid splashing or generating aerosols.
5. Allow disinfectant to remain in contact with spill for the manufacturer's recommended contact time (20 minutes for 10% bleach).
6. Carefully pick up any broken sharp material using either tongs or a dustpan.
7. Work concentrically to clean up the absorbent material. Always work from the outer edge of the spill toward the center.
8. Clean spill area with fresh towels soaked in disinfectant.
9. Place all towels or absorbent materials into a designated container for biohazardous waste.

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10. Remove and segregate protective clothing for disposal or cleaning.
11. Wash hands prior to leaving the area.
12. Immediately report the incident to the laboratory manager or PI. Additionally, the biosafety program staff must be notified within 8 working hours by sending an email to bsa@berkeley.edu.
13. Discuss potential solutions with fellow laboratory members to prevent similar incidents from occurring in the future.

Spill Outside of a Biological Safety Cabinet in a BSL 1 Laboratory

1. Don appropriate protective clothing, which includes the following recommended items:
 - a. Laboratory coat, waterproof apron
 - b. Rubber or nitrile gloves (for chemical disinfectants)
 - c. Safety glasses
2. Place paper towels or other absorbent material over the spill area.
3. Carefully pour an effective disinfectant (10% solution of bleach, unless the area is stainless steel or capable of being corroded) around the edges of the spill, and then onto the spill. Avoid splashing or generating aerosols.
4. Allow disinfectant to remain in contact with spill for 20 minutes.
5. Apply more paper towels or absorbent material to wipe up spill.
6. Clean spill area with fresh towels soaked in disinfectant.
7. Place all towels or absorbent materials into a white bag.
8. Remove protective clothing and segregate for disposal or cleaning.
9. Wash hands prior to leaving the area.

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Exposure Response Protocols

In the event of an injury or exposure to a biological agent, please seek medical attention. During business hours (Mon—Fri, 8 am—5 pm), please visit Urgent Care at the Tang Center. When you are seen by a medical professional, let them know that this incident occurred in a laboratory setting and that the Occupational Health Physician should be notified. Please be sure to provide the medical professional with as much detail as possible such as the agent you were working with and whether it has been genetically modified.

If the injury or exposure occurs outside of business hours, please seek medical attention at Alta Bates Hospital and be sure to state that you were injured or exposed in a laboratory at UC Berkeley.

If you or someone else in the laboratory needs immediate medical attention, please call 911 from any campus phone. If you are calling from a cell phone while on campus, call (510) 642-3333.

Reporting

In the event of a spill or injury, the EH&S biosafety program staff must be notified. In the event of an injury, EH&S should be notified immediately by calling (510) 642-3073. If a spill of biological material has occurred, please notify the EH&S biosafety program staff within eight working hours of the spill by sending an email to bs@berkeley.edu.

When notifying the group of the spill, please include a description of how the spill occurred, how it was cleaned up and what actions will be taken to prevent a future spill. Additionally, please include the species of agent spilled and whether it had been genetically altered. The biosafety program staff will follow up if any additional information is needed, and may require completion of a report for NIH.

Spill reporting very rarely results in any punitive actions and can be a valuable educational tool for the individual labs as well as for the institution. Lessons learned from these incidents can be used to prevent others from occurring and the biosafety staff will cooperate with laboratory personnel to ensure that appropriate training, if needed, is completed post-incident.

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Appendix 1: Working with Viral Vectors

Introduction

Viruses and viral vectors have become a staple of the molecular biology community. As such, it is important for users to understand the origins of these tools and potential implications of their use. Expanded sections for each virus contain information on virology, clinical features, epidemiology, treatment, laboratory hazards, personal protective equipment (PPE), disinfection, and use with animals.

Containment

Suggested biosafety containment levels are provided for each vector system. Use of a higher-level containment facility may be required in some cases, depending on the specific properties of the vector or insert. Special care should be given to the design and handling of virus vectors containing genes that make growth-regulating products, products released into the circulation, products that may have a general effect on the host-immune system.¹ Work with any viral vector requires approval from the Committee for Laboratory and Environmental Biosafety. Additional approval from the Animal Care and Use Committee (ACUC) is required for research involving viral vectors and animals.

Click on link at end of each section for additional virus specific information.


1. **Adenovirus:** Adenoviruses are infectious human viruses which often cause mild respiratory illness, pink eye or gastroenteritis. Rare cases of severe disease can occur, and its use as a genetic vector therefore requires the use of adequate containment equipment and practices. Biosafety Level 2 (BSL-2) is appropriate for many constructs. Particular care should be given to vectors containing genes that make products similar to those of the deleted adenovirus genes. [Additional Adenovirus information](#)
2. **Adeno-associated virus (AAV):** These are infectious human viruses with no known disease association. Some AAV types are common in the general population, and these viruses have the ability to integrate into the host chromosome. The NIH Guidelines ([Appendix B](#)) state that “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” can in most cases be handled at biosafety level 1 (BSL-1). This level of containment is modified by other considerations (see above General Comments). [Additional AAV information](#)
3. **Retrovirus:** These are infectious viruses which can integrate into transduced cells with high

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frequency, and which may have oncogenic potential in their natural hosts. Retrovirus vector systems are typically based on murine viruses - most commonly, these systems include ecotropic viruses (which can infect only murine cells), amphotropic viruses (which can infect human cells) or pseudotyped viruses, when vector particles express glycoproteins (GPs) derived from other enveloped viruses (which can also infect human cells). The most common GP currently used is VSV-g, however there are newer pseudotypes being derived from viruses such as measles (Rubeola), Ebola and Marburg. Pseudotyping vectors often results in a higher required biosafety level. Containment for vectors with the ability to infect human cells (amphotropic) will usually be recommended at BSL-2, whereas for ecotropic vectors with no ability to infect human cells, BSL-1 containment may be appropriate.

- a. **MMLV:** The host range of recombinant MMLV vectors is dependent on the specificity of the viral envelope. The ecotropic env gene produces particles that infect only rodent cells. Amphotropic env gene allows infection of murine and nonmurine cells, including human cells. VSV-G envelope allows infection in a wide range of mammalian and non-mammalian cells. Biosafety Level 2 (BSL-2) is appropriate for many constructs, while higher levels may be required depending upon the construct. [Additional MMLV information](#)
- b. **Lentivirus:** Lentiviruses are a subset of retroviruses, with the ability to integrate into host chromosomes, and to infect non-dividing cells. These viruses can cause severe immunologic and neurologic disease in their natural hosts. Lentivirus vector systems can include viruses of non-human/non-primate origin (feline immunodeficiency virus, equine infectious anemia virus) as well as simian viruses (simian immunodeficiency virus) and human viruses (HIV). The more recent generation vectors have been designed to significantly diminish the possibility for recombination to occur resulting in a wild-type potentially infectious virus. Typical lentivirus vectors are packaged using pseudotyped enveloped proteins. The most common envelope protein used for this purpose is from vesicular stomatitis virus (VSV). It is usually recommended that work with lentiviruses be conducted at BSL-2. [Additional Lentivirus information](#)
4. **Baculovirus:** Non-mammalian virus vectors that infect insects, these are very stable and may remain dormant in the environment for years before infecting insects. Work is mostly done at the BSL-1 level.
5. **Rabies virus:** Rabies virus is a member of the Rhabdoviridae family and is a common zoonotic infection from bats and other wild mammals. Infection results in encephalitis or paralysis, and is often fatal. Due to its neuronal tropism, pseudotyped rabies virus vectors can be used to study neuronal trafficking or express endogenous genes efficiently in neurons. Biosafety Level 2 (BSL-2) is appropriate for many constructs. [Additional Rabies virus information](#)

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6. **Sendai virus:** Sendai virus (SeV) causes respiratory disease in rodents and sometimes swine. There is limited evidence of zoonotic transmission to humans, but the virus is capable of infecting human cell lines, and is similar to human parainfluenza virus type 1. For these reasons, SeV work is usually classified as BSL-2. [Additional Sendai virus information](#)



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Adenovirus ⁽²⁾

Virology: Adenoviruses are medium-sized (90-100 nm), non-enveloped icosahedral viruses containing double-stranded DNA. There are more than 49 immunologically distinct types (6 subgenera: A through F) that can cause human infections. Adenoviruses are unusually stable to chemical or physical agents and adverse pH conditions, allowing for prolonged survival outside of the body.

The adenovirus infection cycle can be clearly divided into two phases, which are separated by viral DNA replication. The first or “early” phase covers the entry of the virus into the host cell and the entry of the virus genome to the nucleus. The late genes are transcribed from the major late promoter. The “late” phase is involved in making gene products that are related to production and assembly of capsid proteins.

TABLE 4 - ADENOVIRAL COMPONENTS

ADENOVIRAL GENES	FUNCTION
Early genes (E): E1A, E1B, E2, E3, E4	Adenoviral gene transcription, replication, host immune suppression, inhibition of host cell apoptosis
Delayed early genes: IX, IVa2	Packaging
Major late Unit (L)	

Viruses packaged by transfecting HEK 293 cells with adenoviral-based vectors are capable of infecting human cells. These viral supernatants could, depending on the gene insert, contain potentially hazardous recombinant virus. Similar vectors have been approved for human gene therapy trials, attesting to their potential ability to express genes *in vivo*. For these reasons, due caution must be exercised in the production and handling of any recombinant adenovirus.

The probability of producing replication competent adenovirus (RCA), although low, increases with each successive amplification. RCA is produced when adenoviral DNA recombines with E1-containing genomic DNA in HEK 293 cells. It is suggested to use early amplification stocks when needed to produce additional quantities of adenovirus.

Clinical features: Adenoviruses most commonly cause respiratory illness; however, depending on the infecting serotype, they may also cause various other illnesses, such as gastroenteritis, conjunctivitis, cystitis, and rash-associated illnesses. Symptoms of respiratory illness caused by adenovirus infection range from the common cold syndrome to pneumonia, croup, and bronchitis. Patients with compromised immune systems are especially susceptible to severe complications from adenovirus infection that can cause more systemic diseases (e.g. hepatitis).

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Epidemiology: Although epidemiologic characteristics of the adenoviruses vary by type, all are transmitted by direct contact, fecal-oral transmission, and occasionally waterborne transmission. Some types are capable of establishing persistent asymptomatic infections in tonsils, adenoids, and intestines of infected hosts, and shedding can occur for months or years. Some adenoviruses (e.g., serotypes 1, 2, 5, and 6) have been shown to be endemic in parts of the world where they have been studied, and infection is usually acquired during childhood. Other types cause sporadic infection and occasional outbreaks; for example, epidemic keratoconjunctivitis is associated with adenovirus serotypes 8, 19, and 37. Epidemics of febrile disease with conjunctivitis are associated with waterborne transmission of some adenovirus types. ARD is most often associated with adenovirus types 4 and 7 in the United States. Enteric adenoviruses 40 and 41 cause gastroenteritis, usually in children. For some adenovirus serotypes, the clinical spectrum of disease associated with infection varies depending on the site of infection; for example, infection with adenovirus 7 acquired by inhalation is associated with severe lower respiratory tract disease, whereas oral transmission of the virus typically causes or mild disease, if any.

Treatment: Most infections are mild and require no therapy or only symptomatic treatment. Because there is no virus-specific therapy, serious adenovirus illness can be managed only by treating symptoms and complications of the infection.

Laboratory hazards: Ingestion; droplet exposure to the mucous membrane.

Susceptibility to disinfectants: Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, 0.25% sodium dodecyl sulfate

Use in Laboratory: BSL-2

Use with Animals: ABSL-2 housing post injection/exposure of animals.

Adenovirus MSDS

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Adeno-associated Virus ⁽³⁾

Virology: Adeno-associated virus gets its name because it is often found in cells that are simultaneously infected with adenovirus. AAV are Parvoviridae; icosahedral, 20-25 nm in diameter, single stranded DNA genome with a protein capsid. AAV is dependent on the presence of wild type adenovirus or herpesvirus for replication; in the absence of these helper viruses, AAV will stably integrate into the host cell genome. Co-infection with helper virus triggers a lytic cycle as do some agents which appropriately perturb host cells. Wild type AAV integrates preferentially into human chromosome 19q13.3-qter; recombinant vectors lose this specificity and appear to integrate randomly, thereby posing a theoretical risk of insertional mutagenesis.

Clinical features: No known pathology for wild type AAV serotype 2.

Epidemiology: Not documented definitively. Infection appears to occur via mouth, esophageal, or intestinal mucosa.

Treatment: No specific treatment.

Laboratory hazards: Ingestion, droplet exposure of the mucous membrane, direct injection; insertional mutagenesis; integration and expression of oncogenes or potential oncogenes.

Susceptibility to disinfectants: Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, 0.25% sodium dodecyl sulfate

Use in Laboratory: BSL-1; BSL-2 in the presence of helper virus

Use with Animals: ABSL-2 practices for infections with subsequent ABSL-1 housing; ABSL-2 housing in the presence of helper virus.

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Lentivirus⁽⁵⁾

Virology: The genus of the family Retroviridae consists of non-oncogenic retroviruses that produce multi-organ diseases characterized by long incubation periods and persistent infection. Five serogroups are recognized, reflecting the mammalian hosts with which they are associated. HIV-1 is the type species.

1. **Bovine lentiviruses** (e.g. Bovine immunodeficiency virus, Jembrana disease virus)
2. **Equine lentiviruses** (e.g. Equine infectious anemia virus)
3. **Feline lentiviruses** (e.g. Feline immunodeficiency virus)
4. **Ovine/caprine lentivirus** (e.g. Caprine arthritis-encephalitis virus, Ovine lentivirus, Visna virus)
5. **Primate lentivirus group**
 - a. Human immunodeficiency virus (HIV) types 1 - 3
 - b. Simian AIDS retrovirus SRV-1
 - c. Human T-cell lymphotropic virus type I and II Simian immunodeficiency virus

Most of the lentiviral vectors presently in use are HIV-derived. The cis- and trans-acting factors of lentiviruses are often on separate plasmid vectors, with packaging being provided in trans. The vector constructs contain the viral cis elements, packaging sequences, the Rev Response Element (RRE), and a transgene (6).

Lentiviral Pseudotyping

Replacement of the HIV envelope glycoprotein with VSV-G provides a broad host-range for the vector and allows the viral particles to be concentrated by centrifugation. Lentiviruses can also be pseudotyped with other envelope proteins, such as the envelope of rabies virus.

Clinical Features(4,7) : In terms of the pathogenesis of lentivirus, some key properties are listed below:

1. Lentiviruses persist lifelong. This is a function both of their ability to integrate into the host chromosome and of their ability to evade host immunity. This ability to evade host immunity may be related both to the high mutation rates of these viruses, and to their ability to infect immune cells (macrophages, and in the case of HIV, T-cells).
2. Lentiviruses have high mutation rates. Lentiviruses replicate, mutate and undergo selection by host immune responses.

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3. Infection proceeds through at least three stages.
 - a. Initial (acute) lentivirus infection is associated with rapid viral replication and dissemination, which is often accompanied by a transient period of disease.
 - b. This is followed by a latent period, during which the virus is brought under immune control and no disease occurs.
 - c. High levels of viral replication then resume at some later time, leading to disease.

Acute infection with human lentiviruses can appear as non-specific “flu-like” and “mononucleosis-like” symptoms, including myalgia, arthralgia, diarrhea, nausea, vomiting, headache, hepatosplenomegaly, weight loss and neurological symptoms.

Epidemiology: Transmitted from person to person through direct exposure to infected body fluids (blood, semen), sexual contact, sharing unclean needles etc.; transplacental transfer can also occur.

Treatment: Specific measures for the opportunistic diseases that result from AIDS; multidrug treatment for HIV.

Laboratory Hazards: Direct contact with skin and mucous membranes of the eye, nose and mouth; accidental parenteral injection; ingestion; hazard of aerosols exposure unknown; insertional mutagenesis; integration and expression of oncogenes or potential oncogenes.

Susceptibility to disinfectants: Susceptible to many disinfectants - 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, ethanol

Use in Laboratory: BSL-2

Use with Animals: For use of third generation (or higher), four plasmid Lentiviral vector systems -In rodents without human cells present: ABSL-2 practices for infections with a subsequent restriction of 48 hours prior to downgrade to ABSL-1 housing (approved SOP required for downgrade).

Lentivirus/Human Immunodeficiency Virus MSDS

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Moloney Murine Leukemia Virus (MoMuLV or MMLV) ⁽⁸⁾

Virology: Retroviridae; subfamily oncovirinae type C, enveloped, icosahedral core, virions 100 nm in diameter, diploid, single stranded, linear RNA genome. MoMuLV integrates into the host genome and is present in infected cells as a DNA provirus. Cell division is required for infection. Virus is not lytic.

Data suggests a pathogenic mechanism in which chronic productive retroviral infection allowed insertional mutagenesis leading to cell transformation and tumor formation. The nature of a transgene or other introduced genetic element may pose additional risk.

The host range of recombinant MoMuLV vectors is dependent on the specificity of the viral envelope. The ecotropic env $\rightarrow\rightarrow$ gene produces particles which infect only rodent cells. The amphotropic env gene allows infection of rodent and non-rodent cells, including human cells. VSV-G envelope allows infection in a wide range of mammalian and non-mammalian cells.

Clinical features: None to date.

Epidemiology: MoMuLV only infects actively dividing cells. In mice, the virus is transmitted in the blood from infected mother to offspring. Transmission may also occur via germline infection. In vivo transduction in humans appears to require direct injection with amphotropic or pseudotyped virus.

Treatment: No recommended treatment.

Laboratory Hazards: Contact with feces or urine from infected animals for 72 hours post infection. Contact with tissues and body fluids of infected animals. Direct injection, insertional mutagenesis; integration and expression of oncogenes or potential oncogenes.

Susceptibility to disinfectants: Susceptible to many disinfectants - 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, ethanol

Use in Laboratory: BSL-1 (ecotropic), BSL-2 (amphotropic, VSV-g pseudotyped, contain toxin or oncogene)

Use with Animals: ABSL-1 housing for ecotropic, ABSL-2 for amphotropic or pseudotyped vector

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Rabies virus ⁽⁴⁾

Virology: Family Rhabdoviridae, genus Lyssavirus; bullet-shaped, enveloped virus; approximately 75nm in diameter by 180 nm in length; single-stranded, negative-sense RNA genome.

Recombinant rabies virus vectors: Replication-deficient rabies vectors can be useful tools for investigation into neuronal trafficking or targeted expression in neurons. SADdG-mCherry/EnvA-SADdG is an example of a modified rabies virus. This modified version of the rabies virus forces neurons it infects to produce a red fluorescent protein called mCherry. mCherry makes the infected cells glow red so they are visible under a microscope. The benefit is the ability to trace a neural circuit on the cellular level as only connected/attached neurons are affected.

Initial deletion: This modification deletes a gene which encodes the rabies virus envelope B19-glycoprotein (RG) and which is required for the production of competent or infectious viral particles from the virus genome in transduced cells. As a result, the mutant virus cannot spread to any other surrounding cells from the originally infected cells.

If the B19-glycoprotein is (intentionally) over-expressed as a transgene in a defined group of infected cells, the virus can trans-synaptically transport to adjacent cells only (single-step) and never go beyond.

Second modification: This alters the tropism of the virus so that it cannot infect any mammalian cells except those that express a genetically-specified neuronal population transgene which encodes the envelope receptor (TVA) of this pseudotyped virus. Since mammalian neurons do not express TVA, the injected virus cannot infect wild-type human neurons.

If the virus is able to infect a TVA-positive neuron, it can replicate and strongly label the first-order (initially infected) neurons, but since its genome lacks the B19 glycoprotein, it cannot infect other neurons by itself.

In short, the risk for infection is specified by transgene expression and retrograde transport is limited to a single synapse. Thus the resultant virus becomes a “mono-synaptic” transneuronal tracer and significantly reduces the biohazard risk because the virus has no potential to infect or trans-synaptically transport to any mammalian cells, including human and mice.

In general, as the rabies virus is a negative-strand RNA virus, it does not integrate into the cell genome and has no chance to produce a G protein RNA template. Therefore, there is essentially no risk to generate replication competent rabies virus.

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Pseudotyped rabies virus: Rabies virus in which the rabies envelope gene is deleted can be pseudotyped with a number of different envelope genes, including EnvA, VSV-g, avian sarcoma leucosis virus glycoprotein, or HIV env. This pseudotyping alters the cell tropism of the virus and can be useful for specific experimental purposes.

TABLE 5 - COMMONLY USED RABIES VIRUS TERMINOLOGY

Rabies virus	Wild-type rabies virus
Mutant rabies virus	Rabies virus that has been mutated from the original wild-type sequence
Pseudotyped rabies virus	Rabies virus in which the envelope gene has been replaced with the envelope gene from another virus
Pseudorabies virus	NOT A RABIES VIRUS; A herpesvirus that predominantly infects swine, but can also infect a range of other mammals, including rodents

Clinical Features: Initial symptoms of rabies include fever, headache, malaise, and upper respiratory and gastrointestinal tract disorders, which can last 4-10 days. Specific symptoms develop as either encephalitis or paralysis.

Epidemiology: The risk for rabies transmission varies in part with the species of biting animal, the anatomic site of the bite, and the severity of the wound. Although risk for transmission might increase with wound severity, rabies transmission also occurs from bites by some animals (e.g., bats) that inflict rather minor injury compared with larger-bodied carnivores, resulting in lesions that are difficult to detect under certain circumstances. Any penetration of the skin by teeth constitutes a bite exposure. All bites, regardless of body site or evidence of gross trauma, represent a potential risk. For the past several decades, the majority of naturally acquired, indigenous human rabies cases in the United States have resulted from variants of rabies viruses associated with insectivorous bats. The contamination of open wounds or abrasions (including scratches) or mucous membranes with saliva or other potentially infectious material (e.g., neural tissue) from a rabid animal also constitutes a non-bite exposure. Two cases of rabies have been attributed to probable aerosol exposures in laboratories, and two cases of rabies have been attributed to possible airborne exposures in caves containing millions of free-tailed bats (*Tadarida brasiliensis*) in the Southwest. However, alternative infection routes cannot be discounted.

Treatment: Wash the wound with a soap solution, followed by 70% ethanol or an iodine con-

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taining solution. Following wound care, a clinician must decide whether to begin passive and/or active immunization. There is no established treatment for rabies once symptoms have begun, but supportive therapy may include intubation, sedation, mechanical ventilation, fluid and electrolyte management, nutrition, and management of intercurrent illnesses and complications. Incubation period of 1-3 months is typical, although incubation more than 1 year has been reported in humans. Administration of rabies post-exposure prophylaxis is a medical urgency, not a medical emergency, but decisions must not be delayed. Prophylaxis is occasionally complicated by adverse reactions, but these reactions are rarely severe. Therefore, when a documented or likely exposure has occurred, post-exposure prophylaxis should be administered regardless of the length of the delay, provided that compatible clinical signs of rabies are not present in the exposed person. Rabies virus is inactivated by desiccation, ultraviolet irradiation, and other factors and does not persist in the environment. In general, if the suspect material is dry, the virus can be considered noninfectious. Non-bite exposures other than organ or tissue transplants have almost never been proven to cause rabies, and post-exposure prophylaxis is not indicated unless the non-bite exposure met the definition of saliva or other potentially infectious material being introduced into fresh, open cuts in skin or onto mucous membranes.

Vaccination: Consultation is available to determine if vaccination with the Rabies vaccine is appropriate for personnel using rabies.

Laboratory Hazards: Parenteral injection, droplet or aerosol exposure of mucous membranes or broken skin with infectious fluids or tissues.

Susceptibility to disinfectants: Susceptible to 70% ethanol, phenol, formalin, ether, trypsin, β -propiolactone and some other detergents.

Use in Laboratory: BSL-2

Use with Animals: ABSL-2 procedures and housing

Rabies virus MSDS

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Sendai virus ⁽⁹⁻¹¹⁾

Virology: Sendai virus, or murine parainfluenza virus type 1, is an enveloped, 150-200nm in diameter, single strand, negative-sense RNA virus that is part of the Paramyxoviridae family. It typically infects rodents and swine, and causes a highly transmissible respiratory tract infection. Sendai virus replication occurs in the cytoplasm of infected cells.

Sendai viral vectors: Sendai viral vectors have been developed that are able to express up to four exogenous genes, and are used to create iPSCs for use in cell reprogramming and stem cell research. Sendai virus does not integrate into the genome. Co-infection of Sendai-transduced cells transplanted into animals with wild-type Sendai may lead to expression of exogenous genes in animal models.

Clinical features: No known pathology for Sendai virus.

Epidemiology: Not documented definitively. Infection appears to occur via aerosol and contact. Capable of infecting human cells in tissue culture

Treatment: No specific treatment.

Laboratory hazards: Droplet exposure of the mucous membrane, direct injection.

Susceptibility to disinfectants: Susceptible to 1% sodium hypochlorite, 70% ethanol, formaldehyde

Use in Laboratory: BSL-2

Use with Animals: ABSL-2

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5. University of Chicago, Guidelines for Handling Pathogenic Microorganisms and Other Potentially Infectious Materials at Biosafety Level 2 (BSL2)
http://researchadmin.uchicago.edu/docs/ibc/UC_Biosafety_Manual.pdf
6. University of Fribourg, Research: Lentivirus
<http://www.unifr.ch/biochem/index.php?id=137>
7. Kenyon College, What are Lentiviral Vectors
<http://biology.kenyon.edu/slonc/gene-web/Lentiviral/Lentiviz.html>
8. UC San Diego School of Medicine, Moloney Murine Leukemia virus Safety Data Sheet
<https://healthsciences.ucsd.edu/som/pediatrics/research/labs/miyanohara-lab/safety/Pages/moloney-murine.aspx>
9. Charles River Laboratories, Technical Sheet: Sendai Virus
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10. University of Illinois, Urbana, Sendai Virus
<https://research.illinois.edu/files/upload/sendai.pdf>
11. Life Technologies, User Guide: CytoTune – iPS 2.0 Sendai Reprogramming Kit
http://tools.lifetechnologies.com/content/sfs/manuals/cytotune_ips_2_o_sendai_reprog_kit_man.pdf

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Appendix 2: Important Contact Information

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Appendix 3: Bloodborne Pathogen Considerations

All laboratories that work with human blood, human cells, or human cell lines need to participate in the bloodborne pathogen program as outlined in the UC Berkeley Exposure Control Plan. Please review the criteria in the exposure control plan to determine whether your research requires participation in the program.

[UC Berkeley Exposure Control Plan](#)

Title 8, CCR, Section 5193, the California Bloodborne Pathogen Standard

<http://www.dir.ca.gov/title8/5193.HTML>

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Appendix 4: Useful Resources

1. [The NIH Guidelines for Research involving recombinant or synthetic nucleic acid molecules](#)
2. [CDC's Biosafety in Microbiological and Biomedical Laboratories, 5th Edition](#)
3. [CalOSHA Bloodborne Pathogen Standard](#)
4. [California Aerosol Transmissible Disease Standard](#)
5. [American Biosafety Association](#)
6. [World Health Organization Laboratory Biosafety Manual](#)
7. [Pathogen Safety Data Sheets](#)

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