

BIOSAFETY MANUAL FOR MOLECULAR ONCOLOGY LABORATORY

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Section I: General Guidelines for Biosafety Level-2 Lab Practices

Biosafety Level 2 (BSL-2) vs. Biosafety Level 1 (BSL-1):

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is neither required nor generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

Biosafety Level 2 is similar to Biosafety Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It **differs** from BSL-1 in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; (3) extreme precautions are taken with contaminated sharp items; and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

The Molecular Oncology Laboratory is a BSL-2 facility. The following standard and special practices apply to agents assigned to Biosafety Level 2:

A. Standard Microbiological Practices:

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.
4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. Policies for the safe handling of sharps are instituted.
6. All procedures are performed carefully to minimize the creation of splashes or aerosols.
7. Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern.
8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated off-site from the facility are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.

B. Special Practices:

1. Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.
2. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.
3. A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate information to be posted includes the agent(s) in use, the biosafety level, the required immunizations, the investigator's name and telephone number, any personal protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.
4. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).
5. 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.
6. Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory director. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

7. The laboratory director ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.
8. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
 - a. *Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.*
 - b. *Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.*
 - c. *Syringes which re-sheath the needle, needleless systems, and other safety devices are used when appropriate.*
 - d. *Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.*
9. Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.
10. Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.
11. Spills and accidents that result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
12. Animals not involved in the work being performed are not permitted in the lab.

C. Safety Equipment

1. Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:
 - a. *Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonate eggs.*
 - b. *High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.*
2. Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.

3. Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.
4. Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

D. Laboratory Facilities:

1. Provide lockable doors for facilities that house restricted agents.
2. Each laboratory contains a sink for handwashing.
3. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.
4. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
5. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
6. Install biological safety cabinets in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. Locate biological safety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the biological safety cabinets' air flow parameters for containment.
7. An eyewash station is readily available.
8. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
9. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

For additional information, please read the NIH guidelines for handling Biosafety Level 2 biohazardous materials (<http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm>).

Section II: Construction and Use of Recombinant Adenoviruses

The adenovirus used for the AdEasy system is replication-defective. However precautions must be observed. Fully active adenoviruses most commonly cause respiratory illness although they may also cause various other illnesses, such as gastroenteritis, conjunctivitis, cystitis, and rash illness. 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 of adenovirus infection. Although

epidemiological characteristics of the adenoviruses vary by type, all are transmitted by direct contact, fecal-oral transmission, and occasionally waterborne transmission.

Many mishaps can be avoided by being familiar with the protocols before undertaking experiments. Please read the Molecular Oncology Laboratory Protocol Books located in room J-611 prior to working with the recombinant adenovirus system.

In addition to the guidelines for handling Biosafety Level 2 biohazardous materials outlined in **Section I**, the following precautions apply at all times:

- a. *Always wear gloves whenever handling cell and tissue culture containers and the virus itself. Double gloves may be needed in the case of injecting viruses into animals.*
- b. *Use the laminar air flow tissue culture hood whenever handling cell and tissue cultures or pipetting the virus.*
- c. *Disinfect contaminated tips and waste with Wiscodye or bleach (e.g., waste such as used pipettes, pipette tips, microfuge tubes, cell culture flasks will be first soaked in a Wiscodye or bleach containing-beaker for 30 min. and then disposed to biohazard waste container for autoclaving. The work surface area will be disinfected by wiping with Wiscodye or bleach spray, followed by cleaning with 70% ethanol).*
- d. *Clean the pipet aid and pipetman used for the experiment;*
- e. *Properly dispose the disinfected waste into the biohazard bags.*
- f. *Animals which have been injected with adenoviruses should be transported in a isolation container.*
- g. *The virus itself will only be handled in a biosafety level 2 cabinet as outlined in the IACUC protocol. At this time the virus will be in a syringe handled only by the investigators. The used syringe and needle will be placed into biohazard and sharps containers by the investigators.*

Additionally, the following frequently asked questions may help you better understand recombinant adenovirus.

What disease could adenovirus cause? Adenovirus is the same virus as the common cold. The virus used in these experiments is deficient in viral replication.

How is it spread naturally? It is spread by aerosols in the air.

When is it present in the animal facility? It will only be in biohazard containers with the exception of intraoperative injections.

How could I be exposed? By needle sticks and actively inhaling aerosols.

What do I do to protect myself? Standard needle stick precautions and careful handling of sharps. Masks or laminar air flow hoods should be utilized when the virus is being handled.

What is an exposure? Direct inhalation of aerosols of virus or a needle stick.

What do I do if I am exposed? Please contact Dr. He or Dr. Haydon.

Is it treatable? It is only as treatable as the common cold. Possible illness is very self-limited. 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.

Should I be tested for it before or after an exposure? No. Adenoviral infections are self-limited in most instances.

Is there anyone who should not go into a room with this agent? Yes, Severely immunocompromised individuals. For example, individuals with advanced AIDS, transplant patients on large doses of steroids or other anti-immune medications.

NOTE: The following document is the Material Safety Data Sheet of Recombinant Adenovirus Prepared by UCSD Program in Human Gene Therapy, which is recommended to you as a supplemental reading on recombinant adenovirus biosafety.

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

INFECTIOUS AGENT

Name: Adenovirus type C strain 5 and recombinant vectors based on Adenovirus 5.

Characteristics: Adenoviridae; non-enveloped, icosahedral virions, 75-100 nm diameter, double stranded, linear DNA genome. Virus is lytic.

Biosafety Level: NIH BSL 2

HEALTH HAZARD

Pathogenicity: Wild type Adenovirus infection varies in clinical manifestation and severity; symptoms include fever, rhinitis, pharyngitis, cough and conjunctivitis. The risk from infection by defective recombinant adenoviral vectors depends both on the dose of virus and on the nature of the transgene. Adenovirus does not integrate into the host cell genome but can produce a strong immune response.

Host Range: Humans are the natural reservoir for wild type Adenovirus 5. Recombinant Adenovirus vectors infect a variety of mammalian cell types.

Mode of Transmission: Wild type virus is spread directly by oral contact and droplet spread; indirectly by handkerchiefs, eating utensils and other articles freshly soiled with respiratory discharge of an infected person. In the laboratory, care must be taken to avoid spread of infectious material by aerosol, direct contact or accidental injection.

Incubation Period: From 1-10 days.

VIABILITY

Drug susceptibility: No specific anti-viral available.

Susceptibility to Disinfectants: Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde. Recommend fresh solution of 10% bleach for 30 minutes.

Physical Inactivation: Sensitive to heat; 1 hour at 56°C is used to inactivate virus.

Survival Outside of Host: Adenovirus has been reported to survive 3-8 weeks on environmental surfaces at room temperature.

MEDICAL

Surveillance: Pre-employment serum samples banked. Monitor for symptoms; confirm infection by serological analysis or viral culture.

First Aid/Treatment: For splashes to the eye of material containing virus, rinse eye at eyewash for 15 minutes then report to hospital emergency room for evaluation. A serum sample should be taken as soon as possible (at UCSD this is done by Occupational Health). In the case of accidental injection of material containing virus, wash area well with soap and water then contact office of Occupational Health for advice, evaluation and serum sample. At UCSD, notify supervisor and EH&S as soon as possible after exposure. Supportive therapy is indicated for symptoms of suspected infections.

Immunization: None available.

Prophylaxis: None available.

LABORATORY HAZARDS

Laboratory-acquired infections: Rare cases reported in laboratories working with clinical specimens.

Sources/Specimens: Respiratory secretions. Theoretical risk from exposure to laboratory cultures of wild type virus or recombinant virus.

Primary Hazards: Ingestion, droplet exposure of the mucous membranes, direct injection.

Special Hazards: Contact with feces or urine from infected animals for 72 hours post infection.

RECOMMENDED PRECAUTIONS

Containment Requirements: Biosafety level 2 plus UCSD Adeno special practices and BSL 2 containment facilities for all activities involving the virus, recombinant virus vectors, and potentially infectious body fluids or tissues.

Protective Clothing: Laboratory coat, gloves, goggles.

HANDLING INFORMATION

Spills: Allow aerosols to settle for 15 minutes; wear protective clothing and gently cover the spill with adsorbent paper towel and apply freshly prepared 10% sodium hypochlorite starting at the perimeter and working towards the center; allow at least 30 minutes contact time before clean up.

Disposal: Decontaminate all wastes before disposal; steam sterilization, incineration, chemical disinfection. At UCSD contaminated material may be sealed in labeled, doubled, red biohazard bags and transported in covered, leak proof containers to BFI disposal bins for eventual incineration.

Storage: In sealed containers that are appropriately labeled and in approved locations for BSL2 materials at -70C.

Transport: Material must be sealed in primary and secondary containers, appropriately labeled.

TRANSGENES AND OTHER FOREIGN GENETIC ELEMENTS

Considerations: What is the replication status of your vector? In general, recombinant Ad 5 vectors produced in the Vector Development laboratory are replication incompetent. What is the nature of the transgene/s - are any potentially hazardous transgenes expressed, i.e., toxins, oncogenes? Have any foreign elements been introduced which alter the specificity, host range, stability, or titer of the resulting vector? It is imperative that those handling recombinant vectors consider both the nature of the virus used as a vector and the effects of any transgene, introduced genetic elements, or other modification.

Section III: Construction and Use of Retroviral Vectors

Retroviral vectors are becoming standard tools in cell biology as well as potential therapeutic agents for human disease. Many investigators have come to believe that retroviral vectors are safe, but current biosafety guidelines and distributors of vectors both recommend using the vectors under biosafety level-2 containment (BSL-2). The safety of retroviral vectors for both introduction into humans and for use in basic research continues to be an important issue.

Production of retroviral vectors has a common strategy, although details may vary. Retroviruses package RNA molecules into virus particles. Normally, the double stranded RNA retrovirus genome is packaged into virions, but retrovirus packaging cell lines (also known as helper cells) are constructed in order to package other RNA molecules. These RNA molecules have limited retroviral sequences and commonly express a messenger RNA of interest (the "vector sequence") as well as a selectable marker such as a drug-resistance gene. A typical packaging cell line is stably transfected with two partial (split) retroviral genomes. One construct contains the gag/pol region that encodes proteins required for virus particle assembly and reverse transcription (copying the double stranded RNA insert into DNA), and the second construct contains the env gene that encodes the proteins needed for virus binding to, and entry into, target cells. The viral RNA encoding these functions is not packaged into virus particles because the RNA sequences needed for binding to gag proteins (the packaging signal, or Ψ) have been deleted. The vector sequence containing the packaging signal is transfected into the packaging cells, and inclusion of the packaging signal in the construct insures that the vector sequence is packaged into virus particles.

Virus particles are harvested from packaging cell lines transfected with a vector sequence, and these particles are used to "transduce" the vector sequence (as well as the retrovirus RNA) into target cells bearing the appropriate receptors for the retroviral or other viral envelope expressed on the virus particles. "Transduction" is in essence a one time infection since the viral particles

are infectious, but their genetic information is insufficient to generate new infectious virus unless some rare rescue event takes place. Because none of the retrovirus genomes expressed in packaging cell lines is intact, no replication competent viruses are produced unless a rare and specific recombination event generates an intact retroviral genome. The virus particles are "infectious" for only one replication cycle. They can bind and enter target cells expressing appropriate receptors, although very low levels of virus entry may occur in the absence of specific receptor binding. The vector sequence is reverse transcribed into DNA, and the two retroviral LTR and the viral integrase mediate integration of the vector sequence into the target cell DNA. The integrated vector DNA becomes a permanent part of the target cell genome, and it is thus possible that rescue of RCR by recombination with endogenous retroviral elements can occur many years after the initial transduction of target cells.

Generation of replication competent retroviruses (RCR) in target cells or tissues is the primary risk associated with the use of retroviral vectors. Assessment of this risk is the primary task in determining the safety of retroviral vectors. The target cell range of the vector is also a safety issue. Incorporation of a virus envelope that can infect cells from multiple species increases the risk of both RCR generation and the potential danger of any resulting virus, which could spread from one species to another.

Another consideration in risk assessment is the nature of the vector coding sequence. Marker genes such as green fluorescent protein (GFP) pose no special risk (unless one is concerned with turning the proverbial green thumb into a literal green thumb). However, vectors that include genes involved in oncogenesis, growth regulation, innate or adaptive immunity, or infectious diseases obviously carry a greater risk. A strong oncogene (e.g., *ras*) in a vector that is later rescued into an RCR by recombination events would recreate a human version of mouse leukemia viruses. The widespread availability of retrovirus vectors for laboratory use and the generation of "safer" vectors appear to have resulted in a sense of false security, particularly amongst first time users of vectors. Thus, Biosafety level 2 containment and careful monitoring of long-term or animal experiments for the emergence of vector-derived RCR is essential to ensure safety.

In the **Molecular Oncology Laboratory**, we will only work the widely used and commercially available retroviral vectors (e.g., MoMLV, or Moloney Murine Leukemia Virus, from Clontech). To minimize the possible generation of RCRs, we will use the two plasmid transfection system. Briefly, HEK293 cells will be transfected with the retroviral shuttle vectors containing the transgenes and the retroviral packaging vector, pAmpho. As a result, amphotropic retroviral vectors will be produced so that they can be used to infect both rodent and human cells. No retroviral vectors will be used for direct injections into animals.

Many mishaps can be avoided by being familiar with the protocols before undertaking experiments. Please read the Molecular Oncology Laboratory Protocol Books located in room J-611 prior to working with the retroviral vector system. In addition to the guidelines for handling Biosafety Level 2 biohazardous materials outlined in **Section I**, the following precautions apply:

- a. *Always wear gloves whenever handling cell and tissue culture containers and the virus itself. Double gloves may be needed in the case of injecting viruses into animals.*
- d. *Use the biosafety cell/tissue culture hood whenever handling cell and tissue cultures or pipetting the virus. The virus itself will only be handled in a biosafety level 2 cabinet.*
- e. *Disinfect contaminated tips and waste with Wiscodye or bleach (e.g., waste such as used pipettes, pipette tips, microfuge tubes, cell culture flasks will be first soaked in a Wiscodye*

or bleach containing-beaker for 30 min. and then disposed to biohazard waste container for autoclaving. The work surface area will be disinfected by wiping with Wiscodye or bleach spray, followed by cleaning with 70% ethanol).

- d. Clean the pipet aid and pipetman used for the experiment;*
- e. Properly dispose the disinfected waste into the biohazard bags.*

Additionally, the following frequently asked questions may help you better understand retroviral vectors.

What disease could retrovirus cause? MoMLV is a murine retrovirus, and no known human diseases have been associated with this virus. However, the risks involving in the use of the retroviral vectors largely depend on the transgenes to be expressed. If potential oncogenic genes are used, special precautions have to be given how to handle the viral vectors.

How is it spread naturally? By direct contact or blood-borne.

When is it present in the animal facility? No retroviral vectors will be directly injected into animals. Rather, only the retroviral vector-transduced cells will be injected into animals.

How could I be exposed? By direct contact with the skin, or internal exposure through cuts or needle sticks.

What do I do to protect myself? Always wear gloves; Handle sharps carefully; Masks or biosafety laminar air flow hoods should be utilized when the virus is being handled.

What is an exposure? Direct contact with the skin, or any internal exposure through cuts or needle sticks.

What do I do if I am exposed? The affected areas will be thoroughly washed with soap and water. Notify Dr. He or Dr. Haydon.

Is it treatable? No virus-specific therapy is available although serious illness can be managed by treating symptoms and complications of the infection.

Should I be tested for it before or after an exposure? No standard tests and/or surveillance methods are available to determine possible retroviral vector infection.

Is there anyone who should not go into a room with this agent? Yes, Severely immunocompromised individuals. For example, individuals with advanced AIDS, transplant patients on large doses of steroids or other anti-immune medications.