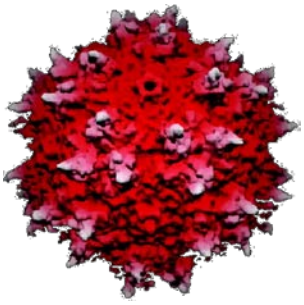
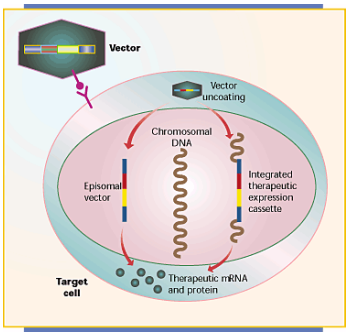
Laboratory Class 2 Biological Safety Manual





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Principles of Biological Safety

The term “containment” is used in describing safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.

Both good microbiological technique and the use of appropriate safety equipment provide primary containment, the protection of personnel and the immediate laboratory environment from exposure to infectious agents. The use of vaccines may provide an increased level of personal protection. Secondary containment, the protection of the environment external to the laboratory from exposure to infectious materials, is provided by a combination of facility design and operational practices. Therefore, the three elements of containment include laboratory practice and technique, safety equipment, and facility design. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements.

Laboratory Practice and Technique

The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or potentially infectious materials must be aware of potential hazards, and must be trained and proficient in the practices and techniques required for handling such material safely. The director or person in charge of the laboratory is responsible for providing or arranging for appropriate training of personnel.

Each laboratory should develop or adopt a biosafety or operations manual that identifies the hazard that will or may be encountered, and which specifies practices and procedures designed to minimize or eliminate risks. Personnel should be advised of special hazards and should be required to read and to follow the required practices and procedures. A scientist trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling infectious agents must direct laboratory activities.

Laboratory personnel, safety practices, and techniques must be supplemented by appropriate facility design and engineering features, safety equipment, and management practices.

Safety Equipment (Primary Barrier)

Safety equipment includes **biological safety cabinets (BSCs),** enclosed containers, and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The biological safety cabinet (BSC) is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. Three types of biological safety cabinets (Class I, II, III) used in microbiological laboratories are described and illustrated in Appendix A. Open-fronted Class I and Class II biological safety cabinets are primary barriers which offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques. The Class II biological safety cabinet also provides protection from external contamination of the materials (e.g., cell cultures, microbiological stocks) being manipulated inside the cabinet.

An example of another primary barrier is the **safety centrifuge cup**, an enclosed container designed to prevent aerosols from being released during centrifugation. To minimize this hazard, containment controls such as BSC's or centrifuge cups must be used for handling infectious agents that can be transmitted through the aerosol route of exposure.

Safety equipment also may include **items for personal protection such as gloves, coats, gowns, shoe covers,** boots, respirators, face shields, safety glasses, or goggles. Personal protective equipment is often used in combination with biological safety cabinets and other devices that contain the agents, animals, or materials being worked with. In some situations in which it is impractical to work in biological safety cabinets, personal protective equipment may form the primary barrier between personnel and the infectious materials. Examples include certain animal studies, animal necropsy, agent production activities, and activities relating to maintenance, service, or support of the laboratory facility.

Facility Design (Secondary Barrier)

The design of the facility is important in providing a barrier to protect persons working inside and outside of the laboratory within the facility, and to protect persons or animals in the community from infectious agents that may be accidentally released from the laboratory. Laboratory management is responsible for providing facilities commensurate with the laboratory's function and the recommended biosafety level for the agents being manipulated.
The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. For example, the exposure risks for most laboratory work in Biosafety Level 1 and 2 facilities will be direct contact with the agents, or inadvertent contact exposures through contaminated work environments. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave), and hand washing facilities.
As the risk for aerosol transmission increases, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features could include specialized ventilation systems to assure directional air flow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks as laboratory entrances, or separate buildings or modules for isolation of the laboratory.

Biosafety Levels

Four biosafety levels (BSLs) are described which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed, the documented or suspected routes of transmission of the infectious agents, and for the laboratory function or activity.
The recommended biosafety level(s) for the organisms represent those conditions under which the agent can ordinarily be safely handled. The laboratory director is specifically and primarily responsible for assessing risks and for appropriately applying the recom-

mended biosafety levels. Generally, work with known agents should be conducted at the biosafety level recommended. When specific information is available to suggest that vir-ulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, or other factors are significantly altered, more (or less) stringent practices may be specified.

Biosafety Level 2 practices, equipment, and facilities are applicable to clinical, diagnostic, teaching, and other facilities in which work is done with the broad spectrum of indigenous moderate-risk agents present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low. Hepatitis B virus, Salmonellae, and *Toxoplasma* spp. are representative of microorganisms assigned to this containment level. Biosafety Level 2 is appropriate when work is done with any human-derived blood, body fluids, or tissues where the presence of an infectious agent may be unknown. (Laboratory personnel working with human-derived materials should refer to the Bloodborne Pathogen Standard for specific, required precautions).

Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme precaution with contaminated needles or sharp instruments must be emphasized. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or devices such as a BSC or safety centrifuge cups. Other primary barriers should be used, as appropriate, such as splash shields, face protection, gowns, and gloves.

Secondary barriers such as hand washing and waste decontamination facilities must be available to reduce potential environmental contamination.

Biological Safety Level 2

The following is from *Biosafety in Microbiological and Biomedical Laboratories*, 1999, HHS publication No. (CDC) 93-8395, Centers for Disease Control & Prevention/National Institutes of Health

Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in four ways.

1. Laboratory personnel must have specific training in handling pathogenic agents and should be directed by competent scientists.
2. Access to the laboratory must be limited when work is being conducted.
3. Extreme precautions must be taken with contaminated sharp items.
4. Certain procedures in which infectious aerosols or splashes may be created must be conducted in biological safety cabinets or other physical containment equipment.

The following standard and special practices, safety equipment, and facilities apply to the use of 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 and animals, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, the use of tobacco products, handling contact lenses, and applying cosmetics are not permitted in the work areas. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated for that purpose only.
4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. All procedures are performed carefully to minimize the creation of splashes or aerosols.
6. Work surfaces are decontaminated at least once a day and after any spill of viable material.
7. All cultures, stocks, and regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leak-proof container and closed for transport from the laboratory. Materials to be decontaminated off-site from the laboratory are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.
8. An insect and rodent control program is in effect.

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 be unusually hazardous are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at 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.

2. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet specific entry requirements (e.g., immunization) enter the laboratory or animal rooms.
3. When the infectious agent(s) in use in the laboratory require special provisions or entry (e.g., immunization), a hazard warning sign incorporating the universal biohazard symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the infectious agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates the special requirement(s) for entering 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. A Biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and to follow instructions on practices and procedures.

7. Laboratory 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. 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.
   a. 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, preferable by autoclaving.
   b. Syringes that re-sheathe the needle, needle-less systems, and other safe devices should be used when appropriate.
   c. 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, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.
10. Laboratory equipment and work surfaces should be decontaminated with an appropriate 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. Equipment must also be decontaminated before removal from the facility when it must be packaged for transport. Packaging and shipment shall be in accordance with applicable local, state, or federal regulations,.
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 work being performed aren't permitted in the lab.

C. Safety Equipment (Primary Barriers)

1. Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used under the following conditions.
    a. Use Biological Safety Cabinets whenever 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 eggs.
    b. Use Biological Safety Cabinets whenever 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 guards) is required to prevent 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, and administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; personnel should never take it home.
4. Gloves are required when handling infected animals and when hands may contact infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate; if a spill or splatter occurs, the hand will be protected after the contaminated glove is removed. Gloves are disposed of when contaminated, removed when work with infectious materials is completed, and are never worn outside the laboratory. Disposable gloves are not washed or reused.

D. Laboratory Facilities (Secondary Barriers)

1. Each laboratory contains a sink for hand washing.
2. The laboratory is designed so that it can be easily cleaned. Walls and floors must be constructed of water impervious material that will stand up to harsh disinfectants. Carpeting is not allowed in laboratory facilities.
3. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
4. Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning. No cloth or fabric seating is permitted.
5. If the laboratory has windows that open, they are fitted with fly screens.
6. A method for decontamination of infectious or regulated laboratory wastes is available (e.g., autoclave, chemical disinfection, incinerator, or other approved decontamination system).
7. An eyewash/safety shower facility is readily available.
8. All laboratories require single pass air that is not then recirculated to any other area of the facility. Laboratories should be negative pressure to surrounding areas to prevent accidental spread of potentially infectious or recombinant agents.
9. 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.

Training and record keeping

All students and staff who work with the biohazardous material must be fully appraised of the nature of the biohazardous material as well as the risks associated with the handling of such material. This information should be included in the risk assessment document that should be compiled by the person who has responsibility for working with the biohazardous material.

All users must go through a training session about rules and use of the laboratory and disposal and handling of biohazard waste. Once trained, their names will be written in the LOG BOOK with the date of the inclusion. These records will be kept for at least 10 years.

Any university facility where work on BHM is carried out should be restricted to individuals who have training in the handling, storage and disposal of biohazardous material.

Prior to outsiders accessing the facility, all surfaces should be disinfected and the biohazardous material safely stored in lockable refrigerators, freezers or incubators located within the facility. Maintenance staff, contractors and visitors should be informed of the nature of the BHM and the steps necessary to avoid contamination. Also, access to the facility should be under supervision of the laboratory safety officer.

Complete dated laboratory notes should be kept to record receipt, propagation, experimental procedures and disposal of biohazard material. Any accidents involving the handling of biohazard material should be documented according to the university safety procedures in the LOG ON BOOK, which should be available for inspection

Biological Waste Disposal Policy

This policy is intended to provide guidance and insure compliance with the NIH/CDC guidelines.

Categories

1) Infectious/potentially infectious/R-DNA

a) human pathogens
b) animal pathogens
c) plant pathogens
d) recombinant DNA
e) human and primate blood, blood products and other body fluids
f) human and primate tissue
g) any material containing or contaminated with any of the above (test tubes, needles*, syringes, tubing, culture dishes, flasks, etc.)

needles, razor blades, scalpels, broken glass → must be packaged in plastic sharps boxes
plastic long pipettes → must be within a box or other puncture proof container before adding to waste.
This waste must be inactivated prior to disposal. The preferred method is steam sterilization (autoclaving), although chemical inactivation or incineration may be appropriate in some cases. Storage of non-inactivated waste is restricted to within the generating laboratory. The material may not be stored longer than 24 hours prior to inactivation.

2) Non-infectious waste

All trash is consider infected in type 2 class

3) Mixed radioactive/biohazardous waste

The biohazardous component of mixed radioactive/biohazardous waste shall be inactivated prior to its release to Radiation Safety for disposal as radioactive waste. Steam-sterilization or chemical inactivation shall be employed as above. Although many radioactive materials can be autoclaved safely, please check with the Radiation Safety Office regarding the best method to employ with any given radionuclide.

4) Mixed chemical/biohazardous waste

The biohazardous component of mixed chemical/biohazardous waste shall be inactivated prior to its release for chemical disposal. Precautions should be taken to prevent the generation and release of toxic chemicals during the inactivation process. In general, autoclaving is not recommended because flammable or reactive compounds should not be autoclaved due to the explosion hazard. Please check with the Biological Safety Office for guidance regarding particular chemicals.

5) Animal carcasses and materials

The disposal of animal carcasses and other animal materials shall be through the Animal Care Services incinerators or the Veterinary Medicine tissue digesters only. No animal bodies or material shall be disposed of as regular trash or through the biomedical waste receptacle.

Packaging

1) Autoclave bags

These are used for the initial collection of certain biological wastes.
These bags must be placed in metal boxes to be autoclaved prior to disposal.

2) Sharps

Needles, scalpels and razor blades are required to be containerized in yellow plastic sharps containers. These are provided by the department. All other sharps (broken glass and plasticware, pipettes, etc.) shall be containerized in puncture-resistant boxes.

3) Biohazard containers

These are used to place the autoclave bags for the collection of the biological waste.
They are metal containers clearly labeled with eth Biohazard symbol and the sign Class 2 . They are provided by the department.

Transport

The transport of biohazardous waste outside of the laboratory (i.e., to an autoclave or incinerator) must be in a closed, leak-proof container that is labeled “biohazard”. Only trained personnel may transport biomedical waste. Labeling may be accomplished by use of a metal waste box with the universal biohazard symbol.
Any container leaving the room should be first sprayed with virkon 1% and wipe with a cloth.
Transportation of autoclave-bagged waste must be in closed, leak-proof containers, properly labeled as “Biohazards.” Movement of regulated/biological waste through public corridors, along carpeted hallways, and on public elevators must be avoided. Any leakage/spills from these containers must be immediately reported to the Safety Committee. Signs must be displayed to prevent tracking of the spills to other areas.

Training

All employees who handle biological waste shall be trained annually regarding its proper handling. All new employees shall be trained before they are allowed to handle biological waste.
Autoclave plastic bags shall be used for collection of biohazardous tissue culture items, petri dishes, and other non-sharp items. A secondary container (metal box) is required to prevent leakage, however, absorbent material must be added to prevent the presence of any liquid and fluid. The bag should be fold over the edges of the secondary container (see below). When full close the bag as shown below:

1. Seal bag by twisting and taping.
2. Double upper section over and tape to double seal the bag.
3. Write initials and date.

The biohazardous waste items must be inactivated by autoclaving in 5 days of accumulation. After autoclaving, bag will be then placed into a black bag and disposed as non-hazardous trash.
Biohazard bags should never be handled by non-research staff or placed in the hallway. Full, untreated autoclave bags shall be stored only in the lab where accumulation occurred. Full autoclave bags must be treated within 5 days.

Autoclave Use and Testing

Mondays and Fridays are reserved for autoclaving goods from the class 2 lab.

1. Write your name and phone extension in the log-in book
2. The autoclave should have water covering 2-3 cm over the bottom tray.
3. Place all trash in t bags loosely close
4. Close all screws of the autoclave and open the venting-valve
5. Set up the timer on the wall for 2 hours.
6. Set up a hand timer for 10-15 minutes, after that time temperature is over 80C and the venting-valve should be closed
7. After autoclave is finished wait until the pressure indicator is close to zero, then open the venting-valve and empty the autoclave.
8. Trash can be then non hazardous, placed in black bags and left in the room for further disposal. The animal cages should be placed in the cleaning facility.

AUTOCLAVE GUIDELINES

STERILIZATION TIMES (Drying Time Not Included)
121 C / 250 F and 15 p.s.i.

BIO WASTE IN AUTOCLAVE BAGS, *LOOSELY TIED*

MULTIPLE BAGS --- 100 MINUTES OR LONGER
SINGLE BAG (FULL) --- 90 MINUTES
PARTIAL BAG --- 60 MINUTES

DRY GOODS

GLASSWARE, EMPTY, INVERTED --- 15 MINUTES
INSTRUMENTS, WRAPPED --- 30 MINUTES
UTENSILS, WRAPPED --- 30 MINUTES

LIQUIDS (Bottles with vented caps 1/2 FULL)

75ml --- 25 MINUTES
250ml --- 30 MINUTES
500ml --- 40 MINUTES
1000ml --- 45 MINUTES
1500ml --- 50 MINUTES
2000ml --- 55 MINUTES

The above times should be used as a guide in determining the length of time items should be autoclaved in order to achieve sterilization.

Disinfectants

"Disinfectant" refers to an agent that is applied to treat (usually) inanimate objects to render them free of pathogenic or infectious microorganisms. In contrast, the term, "sterilant", refers to an agent that renders items free of **all** microorganisms. The two are not the same and should not be confused.
Disinfectants are used in laboratory and chemical settings to 1) treat a surface or an item before or after routine use, or 2) to treat a surface or an item after a spill or "contaminating event".
Because disinfectants are antimicrobial, they may, by their nature, also have a toxic effect to the user. Therefore, Material Safety Data Sheets and other manufacturer's product information should be available and thoroughly reviewed before using these products.
There are many different types and formulations of disinfectants. The researcher or clinician should ensure that the proper product, one that is effective against the specific microorganism being studied, is used.
Whenever a disinfectant or sterilant is used, proper safety precautions must be followed. Appropriate clothing (gloves, safety goggles, aprons) must be worn. In addition, these compounds must be used in well-ventilated areas.
Following is a discussion of general categories of disinfectants. Please note that there are several different products and different formulations in each category.

Alcohols

The most commonly used alcohols, ethanol and isopropanol, are most effective at concentrations of 70% in water. Both higher and lower concentrations are less effective. Alcohols are active against vegetative bacteria, fungi, and lipid viruses but not against spores. They are only moderately effective against nonlipid viruses. Alcohols are difficult to use as contact disinfectants because they evaporate rapidly and do not penetrate organic matter well. When using alcohols, it is best to clean an object, then submerge it in alcohol for the appropriate time. Alcohols are often used in concert with other disinfectants such as formaldehyde (but see toxicity warning below) or chlorine (2000 ppm chlorine in alcohol). Alcohol is NOT a registered tuberculocidal or HIV listed disinfectant.

Virkon

It is most often sold as pink tablets or powder, which dissolve readily in water. It is intended to be mixed with water to form a 1% to 3% solution (by weight, i.e. 10g to 30g per litre). The pink colour is useful in that in helps gauge the concentration when preparing the Virkon, and importantly, as the Virkon ages it discolours, making it obvious when it needs to be replaced. The solution is generally stable for five to seven days.

Virkon has a faint lemon odor, but the scent is still considered unpleasant by some. It is relatively safe in terms of skin contact, but can cause eye damage and should not be used as a hand-washing liquid.

Virkon's ingredients are:

potassium peroxymonosulfate (21.5%) — disinfecting/cleansing agent
sulphamic acid — disinfecting/cleansing agent,
malic acid — disinfecting/cleansing agent,
sodium dodecyl benzene sulphonate — detergent
sodium chloride (1.5%) — disinfecting/cleansing agent,
sodium hexametaphosphate — buffering agent
Amaranth dye — an indicator colour
Lemon extract — odorant

Hydrogen peroxide

Hydrogen peroxide is usually available as a 30% solution. It may be diluted 1:5 for use as a disinfectant. It is active against a wide array of microorganisms. However, it is an oxidizing agent and should not be used on aluminum, copper, zinc, or brass. Hydrogen peroxide is unstable at high temperatures and in light.

Chlorine compounds

The most commonly used and generally effective disinfectant is sodium hypochlorite (common household bleach). It is a strong oxidizing agent and therefore can be corrosive to metal. A 1:50 dilution, supplying 1000 ppm available chlorine, of the common household product (e.g. chlorox) is very effective as a general laboratory disinfectant and a 1:10 dilution supplying 5000 ppm available chlorine is effective against spills involving blood or other organic material. Please note that the presence of high concentrations of protein can inactivate the action of chlorine products. Dilute hypochlorite solution must be prepared daily to be maximally effective. There are more concentrated sodium hypochlorite solutions available for industrial use, so please read the product information carefully to determine the proper dilution.

Table 1: Dilutions of Household Bleach

Volume Of Bleach	Volume of Water (mg/L)	Dilution Ratio	Sodium Hypochlorite %	Available Chlorine
Undiluted	0	1:1	5.25	50,000
1	9	1:10	0.5	5,000
1	99	1:100	0.05	500

Laboratory Spills

A spill of biological materials that takes place in the open laboratory may create a serious problem. Every effort should be taken to prevent spilling materials.
A spill poses less of a problem if it happens inside a biological safety cabinet, provided splattering to the outside of the cabinet does not occur. Direct application of concentrated liquid disinfectant and a thorough wipe down of the internal surfaces of the cabinet will usually be effective for decontaminating the work zone, but gaseous sterilants will be required to disinfect the interior sections of the cabinet and HEPA filter(s).

Spill in the Open Laboratory

Advance preparation for management of a spill is essential. A “spill kit” may include the following:

- Leak-proof containers
- Forceps
- Paper towels
- Disinfectant
- Respirators, if necessary
- Rubber gloves and other personal protective equipment
- Absorbent powder or other material
- Autoclavable bag

If potentially hazardous biological material is spilled in the laboratory, avoid inhaling any airborne material by holding your breath and leaving the laboratory. Warn others in the area by posting signs and go directly to wash or change room area. If clothing is known (or suspected) to be contaminated, remove the clothing with care, folding the contaminated area inward. Discard the clothing into a bag or place the clothing directly in an autoclave. Wash all potentially contaminated body areas as well as the arms, face and hands. Shower in the fith floor facilities. Reentry into the laboratory should be delayed for a period of thirty minutes to allow reduction of the aerosol generated by the spill.
Protective clothing should be worn when entering the laboratory to clean the spill area. Rubber gloves, autoclavable or disposable footwear, an outer garment, and a respirator equipped with a HEPA filter should be worn. If the spill was on the floor, do not use a surgical gown that may trail on the floor when bending down. Take the “ spill kit” into the laboratory room, place a discard container near the spill, and transfer large fragments of material into it; replace the cover. Using a hypochlorite containing 5000 ppm (10% by volume) available chlorine, iodophor solution containing 1600 ppm iodine, or other appropriate EPA registered disinfectant, carefully pour the disinfectant around and into the visible spill. Avoid splashing and work from the outside toward the center. Allow 30 minutes' contact time. Use paper or cloth towels to wipe up the disinfectant and spill, working toward the center of the spill. Discard all towels and other clean up materials into a discard container as they are used. Wipe the outside of the discard containers, especially the bottom, with a towel soaked in a disinfectant. Place the discard container and other materials in an autoclave and sterilize.

Alternately, place all materials in the appropriate biomedical waste system for incarceration. Remove shoes or shoe covers, outer clothing, respirator, and gloves and sterilize by autoclaving. Wash hands, arms and face, or if possible, shower.
If gaseous decontamination of the room is required, contact the Environmental Health and Safety Division.

Spill in a Biological Safety Cabinet

A spill that is confined to the interior of the biological safety cabinet should present little or no hazard to personnel in the area. However, chemical disinfection procedures should be initiated at once **while the cabinet ventilation system continues to operate** to prevent escape of contaminants from the cabinet.
Spray or wipe walls, work surfaces, and equipment with a disinfectant. A disinfectant with a detergent has the advantage of detergent activity that will help clean the surfaces by removing both dirt and microorganisms. Virkon 1% is appropiate. The operator should wear gloves during this procedure. Use sufficient disinfectant solution to ensure that the drain pans and catch basins below the work surface contain the disinfectant. Lift the front exhaust grill and tray and wipe all surfaces. Wipe the catch basin and drain the disinfectant into a container. The disinfectant, gloves, wiping cloth and sponges should be discarded into an autoclave pan and autoclaved.
The above procedure will not disinfect the filters, blower, air ducts, or other interior parts of the cabinet. If the entire interior of the cabinet needs to be sterilized, contact the Safety Division for the name of the current contractor

Decontamination

Surface Decontamination

All containers and equipment should be surface decontaminated and removed from the cabinet when work is completed. At the end of the workday, the final surface decontamination of the cabinet should include a wipe-down of the work surface, the cabinet's sides and back, and the interior of the glass. If necessary, the cabinet should also be monitored for radioactivity and decontaminated when necessary. Investigators should remove their gloves and gowns and wash their hands as the final step in safe microbiological practices.

Small spills within the BSC can be handled immediately by removing the contaminated absorbent paper toweling and placing it into the biohazard bag. Any splatter onto items within the cabinet, as well as the cabinet interior, should be immediately wiped with a towel dampened with decontaminating solution. Gloves should be changed after the work surface is decontaminated and before placing clean absorbent toweling in the cabinet. Hands should be washed whenever gloves are changed or removed.

Spills large enough to result in liquids flowing through the front or rear grilles require more extensive decontamination. All items within the cabinet should be surface decontaminated and removed. After ensuring that the drain valve is closed, decontaminating solution can be poured onto the work surface and through the grille(s) into the drain pan.

Thirty minutes is generally considered an appropriate contact time for decontamination, but this varies with the disinfectant and the microbiological agent. Manufacturer's directions should be followed. The spilled fluid and disinfectant solution on the work surface should be absorbed with paper towels and discarded into a biohazard bag. The drain pan should be emptied into a collection vessel containing disinfectant. A flexible tube should be attached to the drain valve and be of sufficient length to allow the open end to be submerged in the disinfectant within the collection vessel. This procedure serves to minimize aerosol generation. The drain pan should be flushed with water and the drain

Gas Decontamination

BSCs that have been used for work involving infectious materials must be decontaminated before HEPA filters are changed, internal repair work is done or before a BSC is relocated. The most common decontamination method uses formaldehyde gas, although more recently, hydrogen peroxide vapor has been used successfully. This environmentally benign vapor is useful in decontaminating HEPA filters, isolation chambers, and centrifuge enclosures. Call the the current BSC vendor who does the gas decontaminations and certifications. All BSCs must be re-certified following any gas decontamination, maintenance or relocation.

Ultraviolet Lamps

Ultraviolet (UV) lamps are not required in BSCs. If installed, UV lamps must be cleaned weekly to remove any dust and dirt that may block the germicidal effectiveness of the ultraviolet light. The lamps should be checked periodically with a meter to ensure that the appropriate intensity of UV light is being emitted. UV lamps must be turned off when the room is occupied to protect eyes and skin from UV exposure, which can burn the cornea and cause skin cancer. Do not depend on UV lamps to disinfect the area.
Virucidal action correlates with shorter wavelengths of the UV spectrum, 250-260 nm.
Mechanism of UV radiation injury attributed to absorption by and resultant damage to nucleic acids. Due to low energy, the power of penetration is poor. Dust and thin layers of proteins on surfaces reduce the virucidal activity

HEPA Filters

HEPA filters, whether part of a building exhaust system or part of replacement when they become so loaded that sufficient air flow maintained. Filters must be decontaminated before removal.

ANEXE I

ADENOVIRUS/ADENOVIRAL VECTORS: STANDARD OPERATING PROCEDURE

Containment Level

Adenoviral vectors may be contained at varying biosafety levels, dependent on the nature of the inserted genes and its replication competence. Generally, adenovirus is classified as a risk group 2 (RG2) organism.

Approvals

Experiments using recombinant adenovirus require approval from the University Biosafety Committee (IBC) before initiation of experiments.

Precautions

1. Adenovirus is a pathogen of respiratory and gastrointestinal mucous and eye membranes, and does not have to be replication-competent to cause corneal and conjunctival damage. Goggles should be worn when working with the agent/vector.

2. The replication-defective virus may become complemented in vivo. Complementation may cause replication and spread of an otherwise replication-defective vector.

3. Adenovirus (unlike HIV), is relatively stable outside the host. After having been extracted with ether, and/or chloroform, it can still be infective.

4. Biohazard warning signs and labels must be used to indicate each area where adenovirus is used or stored (including Biosafety cabinets, incubators, refrigerators, laboratory entrance doors, etc.).

Laboratory Practices

1. Personnel must have prior experience with adenovirus or must be provided with suitable and sufficient information, instruction and training on working with the agent prior to initiating work.

2. The room should be balanced negative in relation to surrounding spaces, including corridors

3. No work with Adenovirus is permitted on the open bench.

4. A certified biosafety cabinet must be used for all manipulations including (but not limited to):

   a. pipetting

   b. harvesting infected cells for RNA

   c. loading and opening containers

5. Centrifugation must be done in closed containers and using sealed rotors.

6. All vacuum lines must be fitted with a HEPA filter (an example is the "Vacushield™" in-line hydrophobic filter, available through laboratory supply catalogs).

Personnel Protective Equipment

1. Gloves (nitrile, latex, etc)

2. Wrap around outer clothing when introducing vector into animals or performing necropsies. Lab coats are adequate for tissue culture manipulations.

3. Goggles (not to be confused with safety glasses)

Decontamination – * Note - This is not to be performed for personnel exposure

1. The most effective disinfectant (with a minimum 15 min. contact time) is a freshly prepared 10:1 dilution of sodium hypochlorite (bleach) solution (stock is 5% solution).

2. Autoclaving for 1 hour at 121°C (15 lbs per square inch of steam pressure)

Animal Use

Concurrent approvals are needed from the Institutional Biosafety Committee (IBC) and the University Committee on the Use and Care of Animals (UCUCA).

If animals are administered adenovirus intravenously, you must work under the assumption that animals may shed the recombinant adenovirus, and take appropriate precautions as described in this section.

1. Perform inoculations carefully to reduce the possibility of creating splashes or aerosols.

2. When animals are administered adenovirus/adenoviral vectors intravenously, an Animal will remain in the Biosafety Level-2 (ABSL2) area for the first 72 hours after administration.

3. Infected animals may excrete adenovirus. Precautions must be taken not to create aerosols when emptying animal waste material and when washing down cages, or cleaning the room with pressure hoses. After this time, the animals must be changed to a clean cage and can then be moved to a standard animal housing facility.

4. Special training must be given to all animal husbandry personnel on adenovirus, the hazards associated with the work, required practices and procedures and proper handling of bedding, cage washing, and all other husbandry materials associated with the experiment.

5. Arrangements must be made with OSEH for proper disposal of animal carcasses.

Employee Exposure

1. Eye Exposure from splash or aerosols - rinse a minimum of 15 minutes in eye wash or flush area with water and report the incident to your supervisor immediately after flushing. Follow up at the university's occupational medical provider.

2. Needlestick and/or Sharps Exposure – Contaminated skin should be thoroughly washed using soap and water for approximately 20 minutes. Report the incident to your supervisor immediately after washing. Seek medical attention at the university's occupational medical provider.

Symptoms

Acute Respiratory Illness (cold-like symptoms); pneumonia. Conjunctival infection (or red eye), corneal inflammation leading up to scarification.

ANEXE II

Biosafety Considerations for Research with Lentiviral Vectors

Recombinant DNA Advisory Committee (RAC) Guidance Document

Background: The use of lentiviral vectors has been increasing because the vector system has attractive features; however, such research also raises biosafety issues. The NIH Office of Biotechnology Activities has received frequent questions regarding the appropriate containment for lentiviral vectors, particularly those derived from HIV-1. Because the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) do not explicitly address containment for research with lentiviral vectors, the RAC was asked to provide additional guidance for institutional biosafety committees (IBCs) and investigators on how to conduct a risk assessment for lentiviral vector research. At the March RAC 2006 meeting (webcast), the RAC offered the following findings and recommendations.

Risks of lentivirus vectors: The major risks to be considered for research with HIV-1 based lentivirus vectors are

- potential for generation of replication-competent lentivirus (RCL), and
- potential for oncogenesis.

These risks can be mitigated by the nature of the vector system (and its safety features) or exacerbated by the nature of the transgene insert encoded by the vector.

General criteria for risk assessment of lentivirus vectors: Decisions about containment should take into account a range of parameters/considerations including:

- the nature of the vector system and the potential for regeneration of replication competent virus from the vector components,
- the nature of the transgene insert (e.g., known oncogenes or genes with high oncogenic potential may merit special care)
- the vector titer and the total amount of vector,
- the inherent biological containment of the animal host, if relevant,
- negative RCL testing (*see section below*)

General containment considerations: Either BL2 containment or enhanced BL2 containment is often appropriate in the laboratory setting for research involving the use of advanced lentivirus vector systems that have multiple safety features and that segregate vector and packaging functions onto four or more plasmids. Enhanced BL2 containment may include in addition to attention to sharps (and use of safety needles where feasible), the use of personal protective equipment intended to reduce the potential for mucosal exposure to the vector. In most such research, these levels of containment are also expected to be appropriate even when producing large volumes of HIV-1 vectors (>10 L).

The appropriate containment level for specific lentivirus vector research is, of course, determined following a complete risk assessment and local IBC review. The following sections discuss some considerations which should form an important part of the biosafety assessment for research involving lentivirus vectors.

Potential for generation of replication competent lentivirus (RCL) from HIV-1 based lentivirus vectors: The potential for generation of RCL from HIV-1 based lentivirus vectors depends upon several parameters, the most important of which are

• the number of recombination events necessary to reassemble a replication competent virus genome and

• the number of essential genes that have been deleted from the vector/packaging system.

On this basis, later generation lentivirus vector systems are likely to provide for a greater margin of personal and public safety than earlier vectors, because

• they use a heterologous coat protein (e.g., VSV-G) in place of the native HIV-1 envelope protein (However, the use of the certain coat proteins, such as VSV-G, may broaden the host cell and tissue tropism of lentivirus vectors, which should also be considered in the overall safety assessment by the IBC),

• they separate vector and packaging functions onto four or more plasmids and

• they include additional safety features (e.g., they do not encode Tat, which is essential for replication of wild-type HIV-1).

In contrast, earlier vector systems (such as two-plasmid vector systems) may have a higher potential for generation of RCL.

RCL testing: The National Gene Vector Laboratory (NGVL) has produced over 60 liters of HIV-1 vector and has screened supernatant and cells from different vector systems, using different assays, without detecting RCL (K. Cornetta, personal communication of unpublished data). This suggests that the frequency of RCL generation using lentivirus vectors is very low. It may not, however, be zero. There is a need for continued investigation of RCL generation using lentivirus vectors, in order to inform and advance the field of lentivirus vector technology.

The FDA requires that lentiviral vector stocks used in human clinical trials be tested for RCL. Individual research laboratories conducting preclinical research often use only small volumes (e.g., a few milliliters) of lentivirus vectors expressing lower risk transgenes such as GFP. While these laboratories are not mandated to characterize vector stocks, such testing should be encouraged. However, RCL testing requires expertise with the appropriate assays and such expertise may not be available in laboratories that do not work regularly with infectious lentiviruses. In such laboratories, the use of a positive control may increase risk to the investigator as compared to use of the test material. IBCs may make containment assignments without requiring such testing by undertaking a risk assessment that considers the nature of the specific vector system being used and overall past experience with the system.

Animal studies: Some animals, such as wild-type mice, cannot support replication of infectious HIV-1. As a result, the potential for shedding of RCL from such animals is very low (even if RCL were present in the original vector inoculum). IBCs may consider the biosafety issues associated with animal husbandry and housing *after* the initial injection separately from the initial inoculation itself. In general, the initial delivery of vector should be performed under BL2 or BL2-N, according to the animal model, or under enhanced BL-2/BL2-N containment (see "*General containment considerations*"), so as to minimize the risk of autoinoculation by the investigator.

However, it may be permissible to reduce the containment level at some point following vector delivery. For example, if there is no expectation of infection (see below), the site of inoculation has been thoroughly cleansed, and the bedding changed, it may be acceptable to consider reducing containment from BL2/BL2-N to BL1/BL1-N within a few days (the specific time period can be specified by the local IBC, and may vary anywhere from 1-7 days depending on local and experimental considerations). Animals engrafted with human cells or animal hosts that are permissive for HIV-1 replication constitute a special case, in light of their potential to support replication of infectious HIV-1. Use of lentivirus vectors in these animals requires a higher level of containment.

Other lentivirus vectors: Some non-human lentivirus vectors (e.g., FIV, SIV, EIAV, etc.) are also in use. Of these, the most frequently encountered are feline immunodeficiency virus (FIV) vectors. In the Appendix B-V of the *NIH Guidelines*, a containment level appropriate for Risk Group 1 agents is recommended for use of certain animal viral etiologic agents not associated with disease in healthy human adults. However, replication-defective vectors in which a heterologous envelope (such as VSV-G) is used for vector packaging may require BL2 containment in the laboratory setting, since these vectors have the potential to transduce human cells, and thus have the potential to cause insertional mutagenesis. Under circumstances in which mice are not permissive hosts for FIV replication, BL1 containment may be acceptable for mouse housing and husbandry when dealing with mice that have received FIV vectors (subject to the considerations noted above).

Summary: A comprehensive risk assessment and determination of containment for research with lentiviral vectors should consider the nature of the vector system, transgene insert, and type of manipulations involved. For many experiments, either BL-2 or enhanced BL-2 will be appropriate. Examples of biosafety considerations and risk assessments for three different scenarios are included below.

ANEXE III

Containment for animals injected with recombinant virus (retrovirus, lentivirus, adenovirus, AAV, Sindbis virus, pseudorabies virus, Herpes simplex virus)

Please note the containment conditions for animal work are conditioned on proof that each batch of virus used is substantially free of replication competent virus.

In general:

• Handling and direct injection of viral vectors should be done at BL2
• Transfer of cells transfected with replication defective vectors is safer than direct vector injection
• Limit down-grading of containment for housing animals to the use of vector systems in which the potential for replication-competent revertants is very low, or to situations where that replication-competent revertant would be incapable of replicating anyway
• Most fully replication competent viruses are cleared within two to three weeks from immunocompetent hosts

Virus-specific guidelines:

Retrovirus and Lentivirus

• > 0 risk, though urine and feces are not major routes of transmission
• Membrane-bound viruses, so tend not to hang around unless there is a continued source of new virus
• Replication-competent retrovirus or lentivirus is the main risk
• Handle vector in lab at BL2
• Clean injection site with virucidal agent
• Animal housing: Animals injected with retroviruses or lentiviruses need to be kept at BL2-N until the first cage change, assuming there is no RCR/RCL.

Adenovirus

• Humans given vector through IV showed none in biological fluids after 48 hours
• Wild-type form of adenovirus only permissive in humans so unlikely it would be worse for animals
• Handle vector in lab at BL2
• Clean injection site with virucidal agent
• Animal housing: BL1-N containment acceptable, unless virus administered intravenously – then keep at BL2-N for 3 days/72 hours.

Adeno-associated virus (AAV)

• May keep transduced animals in a clean facility alongside non-transduced animals, with transduced animals in cages with filters built into lids
• Handle vector in lab at BL2
• Clean injection site with virucidal agent
• Animal housing: BL1-N containment acceptable

Sindbis virus

• Mosquito-borne virus

• No mouse-to-mouse or mouse-to-human transmission
• Virus not shed in urine or feces
• No need to handle bedding or cages in any special way
• Handle vector in lab at BL2
• Clean injection site with virucidal agent
• **Animal housing: BL1-N containment acceptable**

Pseudorabies virus (PRV)
• PRV infected animals do not secrete PRV in feces or urine
• PRV strains used for tracing experiments are attenuated; most are based on live vaccine strains. They are not virulent strains.
• Isolate infected animals from uninfected animals, in separate cages or even better, separate rooms
• Handle vector in lab at BL2
• Clean injection site with virucidal agent
• **Animal housing: BL1-N**

Herpes simplex virus (HSV)
• Handle vector in lab at BL2
• Clean injection site with virucidal agent
• **Animal housing: BL2-N until first scheduled cage change, then BL1-N**
• **Replication-competent HSV1: viral shedding over in 7-8 days, so BL2-N housing should last for one week.**

ANEXE IV

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

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

Characteristics: Adenoviridae; non-enveloped, icosahedral virions, 75-80 nm diameter, double stranded, linear DNA genome. Virus is lytic.

Biosafety Level: NIH BSL 2

SECTION II - 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.

SECTION III - 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 oC is used to inactivate virus.

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

SECTION IV - 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. 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.

Immunization: None available.

Prophylaxis: None available.

SECTION V - 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.

SECTION VI - 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, N-95 mask, goggles.

SECTION VII - 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 BSL 2 materials at -700C.Transport: Material must be sealed in primary and secondary containers, appropriately labeled.

SECTION VIII - 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.

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

Name: Adeno-associated Virus (AAV) serotype 2 recombinant vectors.

Characteristics: Parvoviridae; icosahedral, 20-25 nm in diameter; single stranded DNA genome with protein capsid. AAV is dependent for replication on presence of wild type adenovirus or herpesvirus; in the absence of helper virus, AAV will stably integrate into the host cell genome. Co-infection with helper virus triggers lytic cycle as do some agents which appropriately perturb host cells. Transduction does not appear to require cell division.

Biosafety Level: NIH BSL 2

SECTION II - HEALTH HAZARD

Pathogenicity: No known pathology for wild type AAV serotype 2. Wild type AAV integrates preferentially into human chromosome 19q13.3-qter; recombinant vectors lose this specificity and appear to integrate randomly, thereby posing a theroretical risk of insertional mutagenesis.

Host Range: Human, will infect a range of mammalian cells.

Mode of Transmission: Not documented definitively. Infection apparently via mouth, esophageal, or intestinal mucosa.

Incubation Period: No symptoms apparent.

SECTION III - VIABILITY

Drug susceptibility: No specific anti-viral available.

Susceptibility to Disinfectants: Susceptibile to 5% phenol, 10% bleach, or 10% Wescodyne. Recommend fresh solution of 10% bleach for 30 minutes.

Physical Inactivation: Resistant to inactivation by pH 3-9 or to 60 oC for 1 hour. Recommend treatment of items for disposal (chemically decontaminated or not) by incineration (BFI at UCSD) or by steam sterilization for 60 minutes.

Survival Outside of Host: AAV's resistance to physical inactivation permits survival outside host organisms for up to several weeks under normal environmental conditions.

SECTION IV - MEDICAL

Surveillance: Pre-employment serum sample.

First Aid/Treatment: Because of the possibility of adenovirus contamination,

recommended treatment is the same as for adenovirus exposure. 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. 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. Notify supervisor and Safety and health committee as soon as possible after exposure.

Immunization: None available.

Prophylaxis: None available.

SECTION V - LABORATORY HAZARDS

Laboratory- acquired infections:

Sources/Specimens: Theoretical risk from exposure to laboratory cultures of recombinant virus.

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

Special Hazards: Contact with feces or urine from infected animals for 72 hours post infection. Because residual helper virus may not be completley inactivated during AAV purification, helper virus risks must be assessed. The Vector Development laboratory uses Adenovirus 5 as a helper virus.

SECTION VI - 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, N-95 mask, goggles; as for Adenovirus.

SECTION VII - HANDLING INFORMATION

Spills: Allow aerosols to settle 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, 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 BSL 2 materials at -700C.

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

SECTION VIII - TRANSGENES AND OTHER FOREIGN GENETIC ELEMENTS

Considerations: What is the replication status of your vector? In general, recombinant AAV 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.

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

REPLICATION-DEFECTIVE LENTIVIRAL VECTORS (Biosafety Level 2)

Cultures of replication defective lentiviral vectors are non-infectious and are not hazardous materials as defined by OSHA 1919.1200. However, these materials are produced in cells where there is the possibility of recombination to form wild type virus. As such, they should be handled as potentially infectious material.

Description:

Lentiviral vectors consist of recombinant transgene sequences (e.g., marker or human genes), and viral packaging and regulatory sequences which are then flanked by lentiviral long terminal repeats (LTRs). The removal of the viral structural genes renders the vector replication defective and dependent upon a helper vector(s) or packaging cell line. Lentiviruses are enveloped viruses and upon leaving the producer cell line, the viral capsid becomes enclosed in a lipid by layer derived from the host cell. The vectors' LTRs are self-inactivating (SIN), thus restricting mRNA production from integrating vectors to the internal promoter, severely reducing full-length vector transcripts. By default, the lentiviral vectors are pseudotyped with the VSV-G Indiana envelope serotype; however the envelope protein can be customized as desired.

Lentiviral cultures are provided as either low concentration (>1x10^{6} infectious units/ml) virus in tissue culture media, or as high concentration, purified (>1x10^{9} infectious units/ml) virus in phosphate buffered saline. Trace components present in the purified virus include, but are not limited to, inorganic salts, vitamins and other nutrients, and human cellular proteins, carbohydrates, amino acids, and fats. The material is normally shipped and stored frozen. Further vector application and handling is described in the following publication:

Kafri, Tal. (2004). Gene delivery by lentivirus vectors an overview. Methods Mol Biol. 2004; 246:367-90. Review.

SECTION I

Hazardous Ingredients

None

SECTION II

Physical Data

Liquid or frozen particle suspensions

SECTION III

Health Hazards

Replication-defective lentiviral vectors are not known to cause any diseases in humans or animals. However, lentiviruses can integrate into the host cell genome and thus pose some risk of insertional mutagenesis.

SECTION IV

Fire and Explosion

None

SECTION V

Reactivity

Not chemically reactive. Will enter permissive mammalian cells and interact or react with cellular components.

SECTION VI

Method of Disposal

Spill: Contain spill and decontaminate the area using a disinfectant such as chlorine bleach (10% f.c.), Wescodyne, or detergent-based disinfectant.

Waste Disposal: Dispose of viral stocks by autoclaving at 121 ^{o}C for 30-45 minutes

Dispose of infected liquid cultures by decontamination with chlorine bleach (10% f.c.) for 10 minutes and then dispose of in sink.

Dispose of infected animal carcasses or tissues by incineration

Follow all Federal, State, and Local regulations.

SECTION VII

Special Protective Information

Handle as biohazardous material under Biosafety Level 2 containment

SECTION VIII

Special Precautions or Comments

It is recommended that all Lentiviral vectors and cultures be handled by qualified microbiologists using appropriate safety procedures and precautions. Upon accidental exposure to Lentiviral vectors, seroconversion towards HIV-1 viral proteins could result and health provider should be contacted.

Information on the classification of human etiologic agents on the basis of hazard can be found as Appendix B in the NIH **Guidelines for Research Involving Recombinant DAN Molecules** at http://www.grants.nih.gov/grants/policy/recombinentdnaguidelines.htm

The above information is accurate to the best of our knowledge. All materials and mixtures may present unknown hazards and should be used with caution. The user should exercise independent judgment as to the hazards based on all sources of information available. The Gene Therapy Center shall not be held liable for any damage resulting from the handling or use of the above product.

ANEXE V

Animals in the class 2 lab:

Animals should remain in the Scantainer all times. Check always that the ventilation is ON and sufficient.

Animals should be monitored daily by the responsible researcher to check health status and availability of food and water.

48-72 hours post-surgery the animals are considered clean. They can be transferred to the 5th floor.

1. Bring a new cage and top metal grid into the lab. Transfer the animals to the new cage. (Always wear mask, glasses and hat for this process).

2. All used items: cage and contents, metal top, food, water and bottle; should be put into autoclave bags and sterilized. Sorted in different bags: i) cage +bedding and ii) bottles+lids. Close bags, initial and date are written outside

3. Clean cages with animals are wiped outside with Virkon 1% and placed on the transport cart with deep trays.

4. Transport them to the stables in the 5th floor and then add food and water bottle.

5. Communicate to the stable's responsible about the arrival of new animals.

ANEXE VI

Animals surgery in the class 2 lab:

Wear always mask, hat and protective glasses. Write your name, date and type of surgery in the LOG-ON book.

When handling viral vectors do it in the hood or over the inverted flow table as close as possible to the surface.

When eppendorfs are opened over the table use a tissue to stop possible spills. Wipe your hands with Virkon after the handling.

Sharps are ALWAYS trashed into the yellow containers. Do NOT recap used needles!

When finished decontaminate your tools with ethanol 70 % and then water and soap. Clean tools will be placed in the oven and sterilize them 1 hour at 150C.

Area of surgery must be thoroughly cleaned with Virkon 1% and after it ethanol 70%. Stereotaxic and delicate surfaces only ethanol 70%.

All trash will be put into an autoclave bag and this will be closed and left in the metal container until sterilization. The researcher who generates the trash is responsible of disposing it not later than 5 days.
Sign-out in the LOG-ON book.

ANEXE VII

Entrance to the class 2 lab:

1. Within the clean area cover your shoes with blue plastic booties.
2. Enter the classified area and wear gloves.
3. Finally wear a gown, and if necessary, hat and mask.

Exit from the class 2 lab:

1. Wipe your gloves with Virkon 1%.
2. Take off mask and hat, and the gown and hang them on the hooks or discard them into the autoclave trash bin.
3.Take of the blue booties and step immediately into the clean area.
4. Finally, take off gloves and discard them into the autoclave trash bin.
5. Wipe your hands with the hand cleaner from the dispenser by the door

Transport instructions:

Transport of any biologically active material from the Class 2 lab will be done inside of a double container (bag+ box) and on the transport cart with deep trays. Within the second container absorbent material in appropiate ammount should be added. The outside surface will be disinfected with Virkon, or 70% ethanol and then put on the transport cart (third container). Any other material leaving the room (cages, tools…) should be disinfected by using a towel soaked in a Virkon 1% to wipe all surfaces.

ANEXE VIII

If a spill occurs:

1. **Mark the area clearly and inform the personnel nearby.**
2. **Protective clothing should be worn to clean the spill area. Rubber gloves, shoe covers, disposable gown and a mask should be worn.**
3. **Take the " spill kit" from the laboratory room, place the autoclave bag in a container near the spill, and transfer large fragments of material into the bag.**
4. **Using a hypochlorite containing 5000 ppm (10% by volume), or Virkon 1%, carefully pour the disinfectant around and into the visible spill. Avoid splashing and work from the outside toward the center. Allow 30 minutes' contact time.**
5. **Use paper or cloth towels to wipe up the disinfectant and spill, working toward the center of the spill. Discard all towels and other clean up materials into the autoclave bag as they are used.**
6. **Wipe the outside of the discard containers, especially the bottom, with a towel soaked in a disinfectant.**
7. **Remove shoes or shoe covers, outer clothing, respirator, and gloves and put into the autoclave bag.**
8. **Place the discard container and other materials in an autoclave and sterilize.**
9. **Wash hands, arms and face, or if possible, shower.**
10. **Inform the safety committee (Marina at 5554).**

ANEXE VIX

POLICIES FOR THE USE OF SHARPS (ANIMAL WORK):

	**Step 1** Place the cap on a flat surface, and then remove your hand from the cap.
	**Step 2** With one hand, hold the syringe and use the needle to "scoop up" the cap.
	**Step 3** When the cap covers the needle completely; use the other hand to secure the cap on the needle hub. Be careful to handle the cap at the bottom only (near the hub).

When performing animal work, the following general practices should always be employed if contaminating agents are being used:

- Work only with restrained and/or unconscious animals.
- Have a sharps container right on hand to dispose of used needles.
- Have a clean work area and don't have any distractions around.
- Never pile up sharps.
- If you are doing surgery of any kind, keep all of your scalpels, knives, etc pointing in the same direction if they are in a box or container (that way, if you reach in to pick them up, you can grasp the "safe" end of the stack).

Many accidental needle sticks occur when staff is recapping needles. Recapping is a dangerous practice: if at all possible, dispose of needles immediately without recapping them.

If it does become necessary for you to recap a needle (for example, to avoid carrying an unprotected sharp when immediate disposal is not possible), do not bend or break the needle and do not remove a hypodermic needle from the syringe by hand.

To safely recap needles, use the "**one-hand**" technique: