BIOLOGICAL SAFETY MANUAL (DRAFT)

CHULABHORN RESARCH INSTITUTE

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I. INTRODUCTION

A. Scope

The Chulabhorn Research Institute (CRI) has gained recognition as an excellent research institute in the country. This is a highly commendable achievement and one that could not have been realized without the continued support and dedication of researchers, staff members, and employees. Similar unfailing cooperation and support is necessary for the institution to be equally successful in its development of a comprehensive occupational health and safety program for protection of CRI personnel, students, and visitors. An important part of this program is concerned with safety in research studies and safe disposal of laboratory biohazardous wastes in compliance with relevant legislation and standard practices.

The purpose of this manual is to describe the operation of the biological safety program and to provide guidelines for all CRI personnel for the safe operation of laboratories and performance of experiments involving biological agents.

Activities which are those specifically addressed are those involving:

- work with recombinant DNA (rDNA)
- various bacterial, fungal, and parasitic agents
- Live viruses
- experimentally infected research animals, animal fluids and tissues
- Human blood, other body fluids and tissues
- Receipt, handling, and disposal of biological materials

The Manual does not address issues of radiation or chemical safety. For radiation and chemical safety issues please refer to radiation and chemical safety manuals.

B. Regulation of Biological Agents at the Chulabhorn Research Institute

The CRI Safety Committee has responsibility for the approval of CRI regulations and other actions to ensure regulatory compliance, safe working conditions, and a professional laboratory environment which is conducive to research and teaching activities, as it relates to the management of Biological Agents in the institute premises.
The CRI Biological Safety Sub-committee has responsibility for providing guidance and advice to the Safety Committee for the development and promulgation of safety standards for the conduct of research and teaching activities involving potentially hazardous biological agents by CRI personnel.
The Safety officer, under the direction of the Safety Committee, is responsible for administering the Biosafety program on a day-to-day basis, for providing

technical advice on safety procedures and equipment, conducting laboratory compliance reviews, providing biological safety training, and providing guidance and information related to compliance with pertinent regulations.

C. The Biological Safety Program at the Chulabhorn Research Institute

The Biosafety program at CRI developed from the Institute's commitment to employee safety with regard to biohazardous materials and to address and comply with the Biosafety Guidelines developed by the National Center for Genetics Engineering and Biotechnology regarding safe research with rDNA and biotechnology, and based on internationally accepted standard practices regarding safe research with associated viral materials, human blood, body fluids and tissues and other potentially biohazardous materials. In order for these rules and procedures to be effective, it is important to have a structured administrative format in place that defines the roles and responsibilities of each person or administrative office.
The Biological Safety Sub-committee provides technical
The key components of the program are:

- The Safety Committee
- The Biological Safety Sub-committee
- Office of Research
- The Laboratory head
- The Principal Investigator (PI)
- The Researcher or User
- Safety officer

The roles and responsibilities of each are described below:

1. The Safety Committee

- Oversee CRI's biological, chemical, and radiation safety programs
- Respond to and review employee complaints related to safety issues including health hazards
- Review safety training plans to ensure compliance with any national or international safety practices and CRI safety policies
- Report progress in the area of safety management at CRI to the Vice-president for Research annually
- Investigate work-related incidents such as injuries, including reviewing the incident report and determining a strategy for abatement for submission to the Vice-president for Research
- Coordinate with the Occupational Health and Safety unit on occupational health and safety issues directly relating to biosafety

2. The Biological Safety Sub-committee

The Biological Safety Sub-committee is one of the CRI Safety Sub-committees with memberships appointed by the CRI President. The Sub-committee shall be

made up of 6 voting members, including the chairperson. There should be representative(s) from laboratory in the biological sciences. Collectively, the membership shall have expertise in research with microbial pathogens, chemical toxicology, and rDNA, and be cognizant of any potential risks to public health and the environment.

- Review all projects/protocols involving the use of rDNA, infectious disease agents, and other potentially biohazardous materials
- Recommend approvals to the Safety Committee in accordance with CRI biosafety guidelines
- Monitor the progress of the work through safety officer to ensure that this is conducted under safety guidelines and report non-compliance to the Safety Committee
- Recommend training for PI and laboratory personnel as required for compliance with safety guidelines corresponding to the biohazard level involved
- The Biological Safety Sub-committee will meet twice a year or more often as necessary or as directed by the committee chairperson, to review related projects/protocols or other issues related to biosafety

It is important for personnel to understand that certain information in Committee files may be subjected to public scrutiny under a disclosure provision of the current National Center for Genetics Engineering and Biotechnology guidelines. These may include documents such as project registration documents, research related accidents, and facilities inspection reports.

3. Office of Research

The Office of Research is responsible for
• Promoting the importance of safety in all activities
• Supporting a broad-based research safety program that will protect CRI laboratory personnel, visitors, and students from ill-health effects and injuries associated with the use of hazardous agents in use in CRI facilities
• Assigning responsibility for the program components to appropriate individuals, task forces or committees and identifying and implementing clear lines of authority
• Providing facilities that meet the Institute requirements for working with hazardous materials

4. The Laboratory Head

Laboratory Head is responsible for
• Receives from Office of Research the material for registration of biological research
• Ensures that the Application for Biosafety Approval (ABA) form is completed by each PI conducting applicable research
• Submits completed ABA and annual registration forms to Office of Research
• Taking appropriate measures to assure that their laboratory activities comply with all relevant CRI research safety policies and guidelines.

• Ensuring that personnel, students and visitors have had training and instruction in laboratory safety and security procedures appropriate for their assignments.
• Ensuring that emergency response plans are in place for their areas and facilities of responsibility.
• Providing Safety officer with the name of the designated laboratory safety coordinator for their respective unit(s)
• Ensures that laboratory personnel receive any necessary medical surveillance

5. The Principal Investigator

The Principal Investigator (PI) is directly and primarily responsible for full compliance with the policies and procedures described in the Biosafety Manual.
• Completes an ABA form for all research proposals involving the use of biological materials or agents
• Accepts direct responsibility for the health and safety of those working with biological materials in his/her project(s)
• Ensures proper lab orientation, training, and instruction for laboratory personnel in safe practices and protocols, including, instruction in good microbiological techniques and practices needed to work safely with the biological agents and materials involved
• Ensures compliance by laboratory personnel with the relevant CRI regulations, guidelines, and policies
• Ensures biosafety cabinets are certified as needed and personal protective equipment is provided and used
• Reports immediately to Safety Officer and Laboratory Head any violations of the National Center for Genetics Engineering and Biotechnology Guidelines, problems with containment and any significant research - related accidents or illnesses

6. The Researcher or User

The researcher or user includes researchers, assistant researchers, students, visiting scholars, and laboratory aids. Laboratory staff members are the most critical element in maintaining a safe working environment. Each person must consider their own safety and that of their co-workers. The laboratory staff's responsibilities include, but are not limited to the following:
• Conscientiously follow lab-specific biosafety and security practices and procedures
• Becomes familiar with all biological agents being used in the lab and the potential risks associated with exposure
• Know all emergency procedures established by the laboratory head or PI
• Participates in appropriate training and instruction
• Follows all laboratory practices guidelines provided in this manual and complies with all CRI safe research policies
• Report violations in procedure to safety officer and the laboratory head or PI.
• Notify the Safety Officer and the laboratory head or PI of major exposure events, or spills as soon as they occur

7. Safety officer

- Collaborates with the Biological safety Sub-committee in implementation of the CRI biosafety program
- Oversees periodic inspections to ensure that all CRI activities related to biological agents are complied with the biosafety manual or best standard practices
- Provides general biosafety training
- Coordinates with Office of Research to arrange and ensure periodic updates to biohazardous agents inventory and the certification of equipment (such as biosafety cabinets) and the training of personnel as required
- Conducts a biosafety assessment and prepares an annual report. Reviews, investigates and maintains a file on biosafety assessments, all accident reports, and complaints, and reports to the biological safety sub-committee and office of research
- Maintains such files of reports, biosafety inspections, reviews and certifications as may be necessary to document compliance with safety standards as imposed

II. BIOHAZARDOUS RESEARCH PROJECT REGISTRATION AND REVIEW

A. Introduction

Each Principal Investigator (PI) is responsible for the completion of an ABA form (Appendix__ and also available at www.cri.or.th/...//) for all research involving biological materials or agents including the assignment of the required Biosafety Level to the proposed research. This includes research involving:

- rDNA, including experiments that are specifically exempt under the National Center for Genetics Engineering and Biotechnology and CDC-NIH, USA Guidelines
- Bacterial, fungal, parasitic, or other potentially infectious agents
- Live viruses
- Human blood, other body fluids and tissue

The Biological Safety Sub-committee will review all submitted ABA form; confirm, where applicable, that exempt status is appropriate for certain rDNA work; and recommend approval for those applications, that are complete and which provide for safe handling of potentially biohazardous materials under the appropriate Biosafety Level, to the Safety Committee.

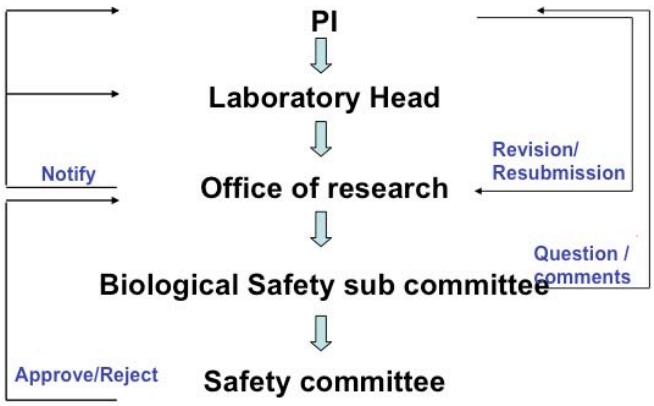
B. Registration and approval Process

The registration and approval process begins with submission of the complete application for ABA form by PI through laboratory head to Office of Research. Office of Research distributes the applications to members of the Biological Safety Sub-committee. Once a month the Biological Safety Sub-committee meets and reviews the applications submitted within the last month. At this time the application will either be recommended to the Safety Committee for consideration of approval, or will be sent back with comments and/or questions

to Office of Research to be given back to the PI. The PI can submit the same application after revision as suggested by the Sub-committee or made scientifically argument for review.

The renewal or amendment of the approved application shall be submitted to the biological safety sub-committee for recommendation to the Safety committee for final consideration of approval.

The chart provided below indicates the steps involved in this process:



C. List of Select Agents and Toxins

1. Genetic Elements, Recombinant Nucleic Acids, and Recombinant Organisms
    - Nucleic acids that can produce infectious forms of any of the select agent viruses.
    - Recombinant nucleic acids that encode for the functional form(s) of any of the select agent toxins if the nucleic acids:
        - can be expressed *in vivo* or *in vitro*, or
        - are in a vector or recombinant host genome and can be expressed *in vivo* or *in vitro*.
    - Select agents that have been genetically modified.
2. Human blood, other fluids, and tissue
3. Animal blood, other fluids, and tissue

More of selected agents are listed in Appendix A.

III. WORKING SAFELY WITH BIOLOGICAL MATERIALS

A. Exposure Control

Biosafety program is the containment of potentially harmful biological agents. The term "containment" is used in describing safe methods, facilities and equipment 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 people, and the outside environment to potentially hazardous agents.

The three elements of containment include laboratory practice and technique, safety equipment, and facility design.

1. 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 infected 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 laboratory head or PI is responsible for providing or arranging for the appropriate training of personnel.

Each laboratory head or PI should identify specific hazards that will or may be encountered, and consider specifies practices and procedures designed to minimize or eliminate risks. Personnel should be advised of special hazards and are expected to follow the required practices and procedures. For example, a scientist, trained and knowledgeable in appropriated laboratory techniques, safety procedure, and hazards associated with infectious agents or materials. The individual should consult with any biosafety or other health and safety professionals with regard to risk assessment.

The PI is responsible for selecting additional safety practices, when standard laboratory practices are not sufficient to control the hazards associated with a particular agent or laboratory procedure.

2. Safety Equipment (Primary Barriers and Personal Protective Equipment)

Safety equipment includes biological safety cabinets (BSCs), enclosed containers, and other engineering controls designed to remove or eliminate or minimize exposures to hazardous biological materials. The BSC is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures.

Safety equipment may also include items for personal protection such as personal protective clothing, respirators, face shields, safety glasses or goggles. In some situations, personal protective clothing 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 laboratory facility.

3. Facility Design and Construction (Secondary Barriers)

The design of a facility is important in providing a barrier to protect persons those working inside and outside the laboratory and to protect people or animals in the community from infectious agents which may be accidentally released from the laboratory. The laboratory head or PI is responsible for providing facilities commensurate with the laboratory's function and the recommended biosafety level for the agent being manipulated.

The secondary barrier(s) recommended will depend on the risk of transmission of specific agents. For example, all Chulabhorn Research Institute falls within Biosafety Levels 1 and 2 (BSL-1 and BSL-2) (see B. Biosafety Levels below) and the exposure risks involve direct contact with the agents, or inadvertent contact through contaminated work environments. Secondary barriers in these laboratories include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave) and hand washing facilities.

When the risk of infection by exposure to an infectious aerosol is present, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features include specialized ventilation systems to ensure directional air flow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, air locks as laboratory entrances, or separate buildings or modules to isolate the laboratory.

B. Laboratory Biosafety Levels

CDC-NIH, USA Guidelines has established **four levels of biosafety**, based on the degree of hazard associated with an organism, to describe the combination of laboratory practices and techniques, safety equipment, and facilities needed to protect against exposure. These four biosafety levels (BSL) require successively more restrictive practices and facilities as work moves from the least restrictive BSL-1 to work with the highest hazard level of BSL-4. Exposure to biohazardous agents is intended to be prevented or limited by establishing and following the appropriate biosafety level practices and conditions. Research in the CRI is currently limited to BSL-1 and BSL-2.

BSL-1 applies to the basic level of containment and essentially represents good microbiological practice with no special primary or secondary barriers required. This applies to work with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans. This includes such organisms as the bacteria (*Vibrio harveyi*), infectious canine hepatitis virus (*Bacillus subtilis and Naegleria gruberi*), or host/vector strains of *E. coli* and yeast *Saccharomyces cerevisiae*.

BSL-2 applies to work with a broad spectrum of indigenous moderate-risk agents that are generally present in the community and associated with human disease of varying severity. These agents can be use safety in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low. The work is done with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent (bloodborne pathogen) may be unknown.

All of the viral agents used in this institute, such as adenovirus, cytomegalovirus, and other herpes viruses fall within the BSL2 level of work. Other microorganisms assigned to this containment level include *Salmonella* spp., *Toxoplasma* spp., Hepatitis B virus, and HIV. With the use of good microbiological techniques, much of this work can be done on open bench tops

as long as there is limited potential for splashes and aerosol creation. **In addition to BSL-1 conditions,** this level of work also requires that:

-Laboratory personnel have specific training in handling any pathogenic agents used and accessed to the laboratory is limited when BSL-2 work is being done.

-Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials.

-Personal protective equipment should be used as appropriate, such as splash shields, face protection, gowns, and gloves.

-Extreme precautions are taken with contaminated needles or sharp instruments.

-Biosafety cabinets are used when there is potential for splash or aerosol creation

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

BSL-3 applies to work with indigenous or exotic agents with a potential for respiratory transmission, and may cause serious and potentially lethal infection. *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetti* are assigned to this level. **In addition to BSL-1 & BSL-2 conditions,** this level of work also requires that:

-Laboratory personnel have specific training in handling any pathogenic agents used and accessed to the laboratory is limited when BSL-3 work is being done.

-Primary hazards to personnel working in these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols.

-Personnel are placed in primary and secondary barriers in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols.

BSL-4 applies to work with dangerous and exotic agents of high individual risk of life-threatening disease, and may be transmitted via the aerosol route and there is no available vaccine or therapy. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at this level. **In addition to BSL-3 conditions,** this level of work also requires that:

-Laboratory personnel have specific training, experience and procedures in handling special characteristics of the agents used.

-Access to the laboratory is controlled when BSL-4 work is being done, and are completely isolated from aerosolized infectious materials.

-Primary hazards to personnel working in these agents relate to autoinoculation,

ingestion, and exposure to infectious aerosols.

-Personnel work in a Class III BSC or in a full-body, air-supplied positive-pressure personnel suit.

-The BSL-4 facility is generally a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems to prevent release of viable agents to the environment.

BSL-3 or BSL-4 is not currently being done at CRI. A good summary of requirements at each laboratory biosafety level can be found at (See CDC Guideline: Section IV Laboratory Biosafety Level Criteria for an outline of good practices).

http://www.cdc.gov/od/ohs/biosfty/bmbl5toc.htm.
http://www4.od.nih.gov/oba/rac/guidelines/guidelines.htm.

SUMMARY OF RECOMMENDED BIOSAFETY LEVELS FOR INFECTIOUS AGENTS

BSL	AGENTS	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Not known to consistently cause diseases in healthy adults	Standard Microbiological Practices	None required	Laboratory bench and sink required
2	• Agents associated with human disease • Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers: • Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials PPEs*: • Laboratory coats; gloves; face protection as needed	BSL-1 plus: • Autoclave available
3	• Indigenous or exotic agents with potential for aerosol transmission • Disease may have serious or lethal consequences	BSL-2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of laboratory clothing before laundering • Baseline serum	Primary barriers: • Class I or II BSCs or other physical containment devices used for all open manipulation of agents PPEs: • Protective laboratory clothing; gloves; respiratory protection as needed	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhaust air not recirculated • Negative airflow into laboratory
4	• Dangerous/exotic agents which pose high risk of life-threatening disease • Aerosol-transmitted laboratory infections have occurred; or related agents with unknown risk of transmission	BSL-3 practices plus: • Clothing change before entering • Shower on exit • All material decontaminated on exit from facility	Primary barriers: • All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decontamination systems • Other requirements outlined in the text

* PPE – Personal Protective Equipment

Guidelines for Good Laboratory Practices at BSL-1 and BSL-2*

Currently, CRI does not have BSL-3 and BSL-4 facility. For research project required BSL-3 and BSL-4 facility, please consults the safety committee.

*Indented and bulleted items indicate additional requirements for work at BSL2.

1. Immediately notify the laboratory head or PI in case of an accident, injury, illness, or overt exposure associated with laboratory activities. As appropriate, seek for any necessary medical surveillance and/or treatment.

2. CRI personnel working with potential biological hazardous agents must have general biosafety training and other appropriate training(s) pertinent to their research.

3. Be aware that access to the laboratory is limited or restricted at the discretion of the laboratory head when experiments or work with cultures or specimens is in progress. Laboratory should have doors to control access.

4. Understand that the PI and/or laboratory head must ensure that all laboratory personnel receive appropriate initial training, necessary on-going training, and supervision regarding on hazards associated with the agents involved; the necessary precautions to prevent exposures; and exposure evaluation procedures.

5. Understand that personal health status may impact an individual's susceptibility to infection or necessary medical surveillance and any conditions in this regard should be discussed with laboratory head and healthcare personnel (specify healthcare service) as appropriate.

*Only personnel advised of the special hazards and meeting any specific entry requirements, i.e., appropriate immunizations, serum sampling, are permitted in the laboratory. Understand and follow all biosafety procedures provided by the PI and/or laboratory head.

*Be aware that any possession or use of select biological agents or toxins requires restricted lab access; written and strictly followed safety and security plans; personnel background checks and training; accurate records and/or reporting of agent use, transfer, loss, or destruction. Any plans for obtaining such materials must be discussed with the Biological safety sub-committee and approved by the Safety Committee.

*Ensure that when infectious agents are in use in the laboratory, a biohazard sign is posted on the lab access door. This sign identifies the agent(s) in use, the biosafety level, any required immunizations, the PI's name and telephone number, and any personal protection equipment (PPE) that must be worn in the laboratory.

6. Wash hands frequently and always after handling viable material or animals, after removing gloves, and before leaving the laboratory. A sink for handwashing is present in each laboratory.

*Consider foot, knee, or automatically operated handwashing sinks.

*Know the location of a readily accessible eyewash station.

7. Do not eat, drink, smoke, chew gum, handle contact lenses, or apply cosmetics in the laboratory. Persons wearing contact lenses in the laboratory should also wear goggles or a face shield.

8. Do not bring any food, medications, or cosmetics, into the laboratory for storage or later use. Food is stored outside the work area in cabinets or refrigerators designated specifically for that purpose.

9. Do not bring animals unrelated to experimental work into the laboratory.

10. Do not pipette by mouth; only mechanical pipetting devices are permitted.

11. Perform all procedures carefully to minimize the creation of splashes or aerosols.

12. Establish and follow policies for safe handling of sharps. Use a high degree of caution when handling any contaminated sharp item, such as needles and syringes, slides, pipettes, capillary tubes, and scalpels. Substitute plasticware for glass whenever possible. Handle broken glassware with brush and dustpan, tongs, or forceps - not directly with hands.

13. Do not bend, shear, break, recap, or remove used needles from disposable syringes or otherwise manipulate such units by hand before disposal. Dispose of needles and syringes in the puncture resistant container provided in the laboratory for this purpose. Place full containers in an autoclave bag and sterilize before disposal in medical waste boxes.

> *Restrict needles and syringes or other sharp instruments in the laboratory for use only when there is no alternative, such as for parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles.
>
> *Use only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) for injection or aspiration of infectious material.

14. Use of lab coats, gowns, or other designated laboratory uniform is recommended to prevent contamination or soiling of street clothing.

> *Wear lab coats, gowns, smocks, or other provided protective garments while working with hazardous materials. When leaving the lab, remove and leave coats and other protective clothing in the lab for either disposal or laundering.

15. Wear gloves if the skin on the hands is broken or if a rash is present. Protective eyewear should be worn for procedures that involve anticipated splashes of microorganisms or other hazardous materials to the face.

> *Wear gloves when manipulating infectious materials or agents or when hands must otherwise contact contaminated surfaces. Remove and change gloves when overtly contaminated or when torn or punctured. Do not wear contaminated gloves outside the lab. Do not wash or reuse disposable gloves. Consider alternatives to latex gloves to prevent allergic response.

*Wear appropriate face protection (goggles, mask, face shield or other splatter guard) for anticipated splashes or sprays of infectious materials to the face when agents **must** be handled outside the BSC. Persons wearing contact lenses should also wear eye protection.

16. Decontaminate equipment and work surfaces at completion of work, at the end of the day, and following spills of viable materials. If a spill occurs, cover the spill with paper towels and soak the towels with a 1 to 10 dilution of chlorine bleach or other suitable disinfectant. Allow the material to soak for approximately 20 minutes before discarding materials in biohazard bag. Bench tops are impervious to water and resistant to solvents, acids, alkalis, and chemicals used for surface decontamination. Laboratory surfaces and spaces between fixtures are designed to be easily cleaned; no carpets or rugs.

17. Work on open bench tops is permitted; use of special containment equipment such as a biological safety cabinet (BSC) is not generally required for agents assigned to BSL1.

*Work in the open laboratory is permitted, except that a properly maintained biological safety cabinet is required 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 embryonate eggs.

High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in 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.

Be aware that air sampling studies have shown that most of the common manipulations of bacterial and viral cultures in research laboratories release aerosols of viable organisms. This must be considered when evaluating need for use of the biological safety cabinet or other physical containment device.

18. Dispose of all regulated medical wastes (potentially biohazardous) and associated wastes as outlined in the provided Laboratory Waste Streams charts (see section F) developed for all laboratories using biological materials.

*Cover containers of all cultures, tissues, specimens of body fluids, or other potentially infectious waste to prevent leakage during collection, handling, processing, storage, transport, or shipping.

C. Animal Biosafety Levels

It should be noted that all work conducted in the CRI Laboratory Animal Unit (LAU), including all work with infectious agents, must be submitted to the CRI

Institute Animal Care and Use Committee (IACUC) for prior review. This can be done through the submission of a completed "protocol format", which may be requested from the CRI LAU office located on the Mezzanine floor of the Biomedical Research Building (Ms. Saowanee Sattayadit, ext 3110). Where CRI LAU safety standards, particularly those related to biosafety, are similar to any of the guidelines below, the one that is more stringent must be followed at all times.

In general, there are four animal standard biosafety levels (ABSL) described for activities involving infectious disease work with commonly used experimental animals (note: animal research work at CRI's LAU is currently limited to that in rats and mice). These four combinations of practices, safety equipment and facilities are designated Animal Biosafety Levels 1, 2, 3 and 4, providing increasing levels of protection to personnel and the environment.

This guidance is provided for the use of experimentally infected animals housed in indoor research facilities, and is also useful in the maintenance of laboratory animals that may naturally harbor zoonotic infectious agents. In both instances, the institutional management must provide facilities, staff, and established practices that reasonably ensure appropriate levels of environmental quality, safety, security and care of laboratory animals. Laboratory animal facilities are a special type of laboratory. As a general principle, the biosafety level (facilities, practices and operational requirements) recommended for working with infectious agents *in vivo* and *in vitro* are comparable.

The animal room can present unique problems. In the animal room, the activities of the animals themselves can present unique hazards not found in standard microbiological laboratories. Animals may generate aerosols, they may bite and scratch, and they may be infected with a zoonotic agent. The co-application of Biosafety Levels and the Animal Biosafety Levels are determined by a protocol driven risk assessment and should involve the active participation of the CRI Laboratory Animal Unit (LAU) chief, a veterinarian, the Biosafety Sub-committee, and the PI. Note: The LAU chief is currently Dr. Wantanee Rattanasak, who is a veterinarian.

The CRI LAU facilities, operational practices and quality of animal care are based on the *Guide for the Care and Use of Laboratory Animals*. The CRI Occupational Health and Safety unit addresses the potential hazards associated with the conduct of laboratory animal research. The Occupational Health and Safety Unit may consult with the Biosafety Sub-committee and vice versa on details regarding biosafety and how it relates to occupational health in the context of research carried out in the LAU.
Facilities for laboratory animals used in studies of infectious or non-infectious disease should be physically separate from other activities, such as animal production and quarantine. Traffic flow to minimize the risk of cross contamination has been incorporated into the facility design (i.e. one-way workflow from clean corridors, through animal rooms to dirty corridors) but must be observed and maintained at all times.

The CRI LAU Program Description covers the issues of the pest management program as well as the appropriate disposal of wastes from the animal room, including animal tissues, carcasses and bedding. The CRI LAU Program Description is available at the LAU office on the Mezzanine floor in the Biomedical Research Building.

The recommendations detailed below describe four combinations of practices, safety equipment, and facilities for experiments with animals involved in infectious disease research and other studies that may require containment. These 4 combinations provide increasing levels of protection to personnel and to the environment and are recommended as minimal standards for activities involving infected laboratory animals. The 4 ABSLs describe animal facilities and practices applicable to work with animals infected with agents assigned to Biosafety Levels 1-4 (see Section IIIB), respectively. Investigators that are inexperienced in conducting these types of experiments should seek help in designing their experiments from individuals who are experienced in this special work.

Facility standards and practices for invertebrate vectors and hosts are not specifically addressed in this section.

ABSL-1 is suitable for work involving well-characterized agents that are not known to cause disease in immuno-competent adult humans, and present minimal potential hazard to personnel and the environment. Facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Personnel must have specific training in animal facility procedures and must be supervised by an individual (i.e. PI) with adequate knowledge of potential hazards and experimental animal procedures. The following standard practices, safety equipment and facility requirements apply to ABSL-1:

(a) Standard microbiological practices

- Access to the animal and procedure rooms must be controlled.
- Workers/researchers must wash their hands prior to entering and leaving animal and procedure rooms.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics and storing food for human consumption must not be permitted in laboratory areas.
- Mouth pipetting is prohibited; mechanical pipetting devices must be used.
- Policies for the safe handling of sharps, such as needles, scalpels, pipettes and broken glassware, must be developed and implemented, e.g. needles should not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers for sharps disposal; non-disposable sharps must be placed in hard walled containers for transport to a designated processing area for decontamination; broken glassware should not be

handled directly but removed using a brush, dustpan and tongs/forceps.

- Always try to minimize the creation of splashes and aerosols.
- Decontaminate work surfaces after completion of work and after spills/splashes of potentially infectious material with appropriate disinfectant (see Table X on page Y for a list of appropriate disinfectants).
- Decontaminate all cultures, stocks and other potentially infectious materials before disposal using an effective method (see Table X on page Y for effective decontamination methods).
- Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/or animals are housed or manipulated.
- A sign incorporating the universal biohazard symbol must be posted at the entrance to the animal and procedural room when infectious agents are present. This sign may include the name of the agent(s) in use, and the name and phone number of the PI or other responsible personnel. Agent information should also be posted in accordance with institutional policy.
- Workers/researchers must receive appropriate training for their duties, including on the necessary precautions to prevent exposures, and exposure evaluation procedures. Workers/researchers must also receive pertinent updates and additional training where available. All personnel are recommended to submit a completed "health surveillance form" to the CRI Occupational Health and Safety Unit. Activities that are considered to pose a health risk may require health surveillance.

(b) Special practices – none required

(c) Safety equipment (primary barriers and personal protective equipment)

- a risk assessment should determine the appropriate type of PPE to use. This will be determined in coordination with the CRI Occupational Health and Safety Unit based on the information submitted in the completed "health surveillance form".
- Protective laboratory coats, gowns or uniforms, which are provided by the Institute, are recommended to prevent contamination of personal clothing. These must not be taken and worn outside the facility.
- Protective eyewear, which is provided by the Institute, must be worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous material.
- Gloves must be worn to protect hands from exposure to hazardous material (alternatives to latex gloves should be available). Gloves do not preclude the need to wash hands

prior to entering and leaving the animal/procedure rooms. Gloves should be changed when contaminated or compromised, and prior to leaving the room. Gloves should NOT be reused.

(d) Laboratory facilities (secondary barriers)

- The animal facility should be separated from areas that are open to unrestricted personnel traffic within the building. External doors to the facility should be self-closing and self-locking.
- Doors to areas where infectious materials/exposed animals are housed should open inward, be self closing, and kept closed when experimental animals are present.
- Sinks for hand washing must be accessible and in working condition, e.g. sink traps filled with water.
- Coordinate with the CRI LAU office regarding cleaning of the animal/procedural rooms (in general, I think rooms with potentially hazardous agents must be cleaned by the researcher/person designated and trained by PI rather than the general LAU staff).
- Chairs/tables used in procedural rooms must be covered with non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant (see Table X on page Y for a list of appropriate disinfectants).
- Ventilation should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*. No recirculation of exhaust air should occur.
- Cage washers should have a final rinse temperature of at least 180 degrees F (will check and add).
- Illumination should be adequate for all activities, avoiding reflections and glare that could impede vision.
- Emergency eyewash and shower are readily available (will check and add).

ABSL-2 builds upon the practices, procedures, containment equipment and facility requirements of ABSL-1. ABSL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure. ABSL-2 requires that (1) access to the animal facility is restricted, (2) personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of pathogenic agents, (3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures, and (4) procedures involving the manipulation of infectious materials, or where aerosols or splashes may be crated, should be conducted in BSCs or by use of other physical containment equipment. Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Implementation of employee occupational health programs through the CRI Occupational Health and Safety Unit should be considered.

(a) Standard microbiological practices (same as ABSL-1)

- The IO/IACUC/facility manager establishes and enforces policies, procedures and protocols for institutional policies and emergency situations. Worker safety and health concerns must be addressed as part of the animal protocol review.
- A safety manual specific to the animal facility is prepared and adopted in consultation with the IO/IACUC/facility manager, and must be available and accessible. Consideration should be given to the specific biohazard unique to the animal species and protocol in use.
- Access to animal/procedural rooms must be controlled. All persons, including facility personnel, service workers and visitors, are advised of the potential hazards and instructed on the appropriate safeguards.
- Protective laboratory coats, gowns or uniforms, which are provided by the Institute, are recommended to prevent contamination of personal clothing. These must not be taken and worn outside the facility
- Gloves must be worn to protect hands from exposure to hazardous material (alternatives to latex gloves should be available). Gloves do not preclude the need to wash hands prior to entering and leaving the animal/procedure rooms. Gloves should be changed when contaminated or compromised, and prior to leaving the room. Gloves should NOT be reused.
- Eye and face and respiratory protection should be used in rooms containing infected animals as dictated by the risk assessment. This assessment will be conducted by the CRI Occupational Health and Safety Unit from the completed "health surveillance" form.
- Workers/researchers must wash their hands prior to entering and leaving animal and procedure rooms.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics and storing food for human consumption is not permitted in laboratory areas.
- All procedures are carefully performed to minimize creation of aerosols of infectious materials and waste.
- Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
- Policies for the safe handling of sharps, such as needles, scalpels, pipettes and broken glassware, must be developed and implemented, e.g. needles should not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers for sharps disposal. Non-disposable sharps must be placed in hard walled containers for transport to a designated processing area for decontamination. Broken glassware should not be

handled directly but removed using a brush, dustpan and tongs/forceps.

- Always try to minimize the creation of splashes and aerosols.
- Decontaminate work surfaces after completion of work and after spills/splashes of potentially infectious material with appropriate disinfectant (see Table X on page Y for a list of appropriate disinfectants).
- Animals and plants not associated with the work being performed are not permitted in the areas where infectious materials and/or animals are housed or manipulated.
- Decontaminate all potentially infectious materials before disposing (see Table X on page Y for a list of appropriate decontamination methods).
- A sign incorporating the universal biohazard symbol must be posted at the entrance to the animal and procedural room when infectious agents are present. This sign may include the name of the agent(s) in use, and the name and phone number of the PI or other responsible personnel. Agent information should also be posted in accordance with institutional policy.
- Workers/researchers must receive appropriate training for their duties, including on the necessary precautions to prevent exposures, and exposure evaluation procedures. Workers/ researchers must also receive pertinent updates and additional training where available. All personnel should also register with the CRI Occupational Health and Safety Unit for health surveillance

(b) Special practices

- All persons entering the animal/procedural room must be advised of the potential hazards and meet specific entry/exit requirements as jointly set by the PI, Safety Committee and CRI LAU chief.
- All involved persons must submit a completed "health surveillance" form at the CRI Occupational Health and Safety unit for medical surveillance, and appropriate immunizations. Baseline serum samples should be stored.
- A laboratory-specific biosafety manual must be prepared and adopted as policy, and must be available and accessible.
- The PI must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents (how to monitor compliance – training records?).
- Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within the CRI LAU.
- Laboratory equipment should be routinely decontaminated by staff properly trained and equipped to work with infectious material, as well as after spills, splashes and other potential contamination, and before repair, maintenance or removal from the animal/procedural rooms.

- Incidents that may result in exposure to infectious materials must be immediately evaluated and addressed according to procedures described in the biosafety manual. All such incidents must be reported to the PI and also to the Occupational Health and Safety unit.
- Animals and plants not associated with the work being performed are not permitted in the laboratory
- All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment device. Consideration should be given to the use of animal restraint devices and practices (physical or chemical) that reduce the risk of exposure during animal manipulations.
- Autoclaving of content prior to incineration is recommended.
- Equipment, cages, racks should be handles in a manner that minimizes contamination of other areas.

(c) Safety equipment (primary barriers and personal protective equipment)

- Properly maintained BSC's (preferably Class II), or other appropriate personal protective equipment (PPE), or other physical containment devices must be used whenever: procedures with a potential for creating infectious aerosols or splashes are conducted (e.g. animal necropsy, harvesting infected tissues from animals and intranasal inoculation of animals) or high concentrations or large volumes of infectious agents are used (centrifugation must be done using sealed rotor heads or centrifuge safety cups).
- Where appropriate, animals should be housed in a primary biosafety containment equipment appropriate for the species, such as solid wall and bottom cages covered with filter bonnets for rodents.
- Protective laboratory coats, gowns, or uniforms designated for facility use must be worn while working with hazardous materials, and removed BEFORE LEAVING the work area (should NOT be taken home).
- Eye and face protection is used for anticipated splashes or sprays of infectious or other hazardous materials when the agent must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse.
- Gloves must be worn to protect hands from exposure to hazardous material (alternatives to latex gloves should be available). Gloves do not preclude the need to wash hands prior to entering and leaving the animal/procedure rooms. Gloves should be changed when contaminated or compromised, and prior to leaving the room. Gloves should NOT be reused.

- Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment (IACUC/Occupational Health and Safety)

(d) Laboratory facilities (secondary barriers)

- The animal facility should be separated from areas that are open to unrestricted personnel traffic within the building. External doors to the facility should be self-closing and self-locking.
- Doors to areas where infectious materials/exposed animals are housed should open inward, be self closing, and kept close when experimental animals are present.
- Sinks for hand washing must be accessible and in working condition, e.g. sink traps filled with water
- Coordinate with the CRI LAU office regarding cleaning of the animal/procedural rooms (in general, I think rooms with potentially hazardous agents must be cleaned by the researcher/person designated and trained by PI rather than general LAU cleaning staff).
- Chairs/tables used in procedural rooms must be covered with non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant (see Table X on page Y for a list of appropriate disinfectants).
- Ventilation should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*. No recirculation of exhaust air should occur.
- Cage washers should have a final rinse temperature of at least 180 degrees F.
- Illumination should be adequate for all activities, avoiding reflections and glare that could impede vision.
- Emergency eyewash and shower should be readily available.

The CRI Laboratory Animal Unit is not currently equipped or designed for work with BSL-3 or BSL-4 agents. No work with BSL-3 or BSL-4 agents is currently allowed.

IV. LABORATORY PROCEDURES AND EQUIPMENT

A. Biological Safety Cabinets (BSCs)

Types of BSCs

BSCs are classified as Class I, Class II or Class III cabinets. When properly maintained and operated, they effectively contain and capture microbial contaminants and infectious agents using HEPA (High Efficiency Particulate Air) filters. (See Figure 1.) Biosafety cabinets should not be confused with clean benches which only protect the material being worked with and are not suitable for work with infectious or toxic material. (Although clean benches, like BSCs, have HEPA-filtered air, with clean benches the air flows over the experimental material toward the user rather than being drawn away.) BSCs

should also not be confused with conventional fume hoods that do not filter microorganisms.

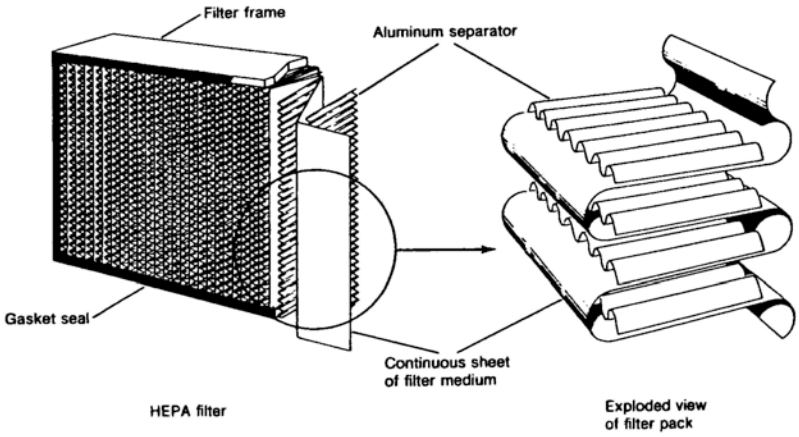
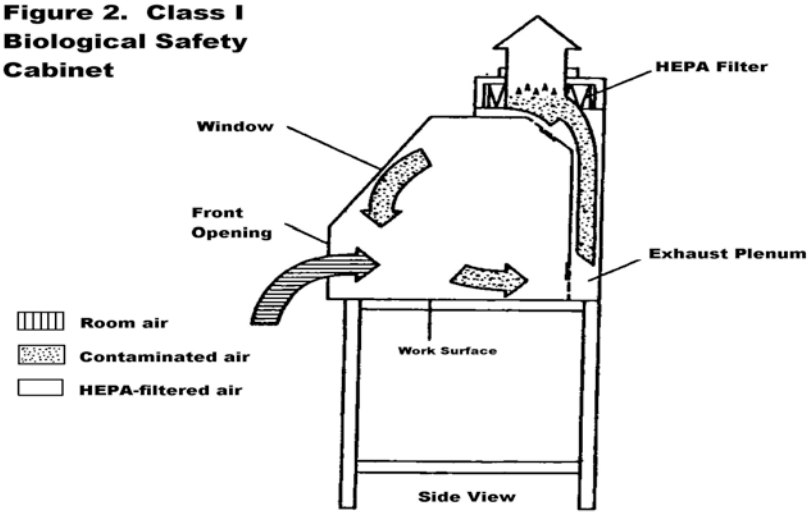


Figure 1. Diagram of HEPA filter. These filters are typically constructed of continuous sheets of paper-thin filter medium, pleated to increase surface area, divided by aluminum separators, and affixed to a frame.

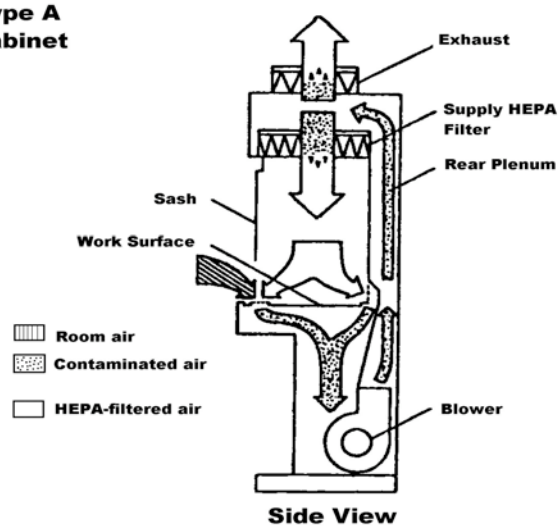
Class I BSCs provide personnel and environmental protection, but not product protection (See Figure 2).

Figure 2. Class I Biological Safety Cabinet



Class II BSCs are the most commonly used BSC on campus. These cabinets provide personnel, environmental and product protection (See Figure 3). Only those, which are hard ducted to the outside and provide a face velocity of 80 to 125 feet per minute should be used when working with volatile chemicals. Additionally, cabinets are not designed to prevent ignition of volatile flammable chemicals.

Figure 3. Class II, Type A Biological Safety Cabinet



Working in a BSC

1. Turn the cabinet on for at least 10 - 15 minutes prior to use, if the cabinet is not left running.
2. Disinfect work surface with 70% alcohol or other suitable disinfectant.
3. Consider the materials necessary for the planned work in the cabinet.
4. Place items into the cabinet so that they can be worked with efficiently without unnecessary disruption of the air flow, working with materials from the clean to the dirty side.
5. Wear appropriate personal protective equipment. At a minimum, this will include a buttoned laboratory coat and gloves.
6. Adjust the working height of the stool so that the worker's face is above the front opening.
7. Delay manipulation of materials for approximately one minute after placing the hands/arms inside the cabinet.
8. Minimize the frequency of moving hands in and out of the cabinet.
9. Do not disturb the airflow by covering any of the grillwork with materials.
10. Work at a moderate pace to prevent the air flow disruption that occurs with rapid movements.

11. Wipe the bottom and side of the hood surfaces with disinfectant when work is completed.

NOTE: Be very careful when using small pieces of materials such as kimwipes in the hood. These can be blown into the hood and disrupt the motor operations.

Certification of the BSC

Certification is a series of performance tests on the BSC to confirm that it will provide the user and experimental material the protection for which it is designed. The air flows, filters, and cabinet integrity are checked to ensure that the cabinet meets minimum performance standards. Certification is arranged through the Laboratory head/PI and provided by an outside vendor.

BSCs intended for user protection must be certified:

- After they are received and installed (before use with infectious materials)
- After filter changes
- Annually

Biological safety cabinets intended only for protection of the experimental material are certified at the discretion of the Laboratory head/PI.

BSC decontamination (using the paraformaldhyde gas production process) is also provided by an outside vendor and needs to be done:

- Before any maintenance work requiring disassembly of the air plenum, including filter replacement
- Prior to cabinet recertification
- Before moving the cabinet to a new laboratory

B. Decontamination

Definitions

Decontamination is a process or treatment that renders an instrument or environmental surface safe to handle. A decontamination procedure can be as simple as clean-up with detergent and water or as thorough as sterilization. Sterilization, disinfection, and antisepsis are all forms of decontamination.

Sterilization is the use of physical or chemical processes to destroy all microbial life, including highly resistant forms, such as bacterial spores.

Disinfection is the elimination of essentially all pathogenic non-spore forming microorganisms but not necessarily all microbial forms from work surfaces and equipment. Effectiveness is influenced by a number of factors, including: types and numbers organisms; amount of organic matter; the object being disinfected; the disinfectant being used; exposure time, temperature and concentration.

Antisepsis is the application of a liquid antimicrobial to skin or other living tissue to inhibit or destroy microorganisms. Examples include hand washing with germicidal solutions or swabbing skin before an injection.

When to Decontaminate

All materials and equipments contaminated with or containing potentially infectious agents should be decontaminated:

- Upon completion of procedures involving the use of biologically-active materials
- In the event of spills of such materials
- At least daily
- Before being washed, stored, or discarded

Decontamination is accomplished by steam heat sterilization in an autoclave, or by surface application of or placement in a chemical disinfectant solution, such as 1:10 bleach solution or its equivalent.

Autoclave Use

Autoclaving (saturated steam under pressure of approximately 15 psi to achieve a chamber temperature of at least 121°C for a designated time) is the preferred and most convenient method to rapidly destroy all forms of microbial life. However, to do this, the autoclave process must reach proper temperature and time and also prevent the entrapment of air in the bag or container of treated material.

- Material to be sterilized must come into contact with live steam.
- Bags or containers should be left open during autoclaving or water (~200ml) should be added to sealed bags to generate steam.
- Heat indicator tape should be used with each autoclave load to indicate that sterilization has been completed.
- Autoclave sterility monitoring should be conducted on a regular basis using biological indicators (such as *B. stearothermophilus* spore strips) placed among treated materials and at locations throughout the autoclave. The spores, which are more resistant to heat than most microbials, provide validation of general microbial destruction when they are effectively inactivated (121°C for 13 minutes) by autoclave operation.

Chemical Disinfectant Use

The most practical use of chemical disinfectants is for surface decontamination and, when used in sufficient concentration, as a decontaminant for liquid wastes prior to final disposal down the drain.

GENERAL RECOMMENDATIONS:

Liquid Decontamination

- Add liquid chlorine bleach to provide a final 1:10 dilution
- Let stand at least 20 minutes
- Discard down the drain

Surface Decontamination

- Wipe with 1:10 dilution of chlorine bleach, or
- Wipe with iodophor disinfectant (per label concentration), or
- Wipe with 70% alcohol

See Appendix B for further information on disinfectants.

C. Exposure to Infectious Agents

In the event of an exposure to an infectious agent or material, the following guidelines should be used:

Intact skin

- Remove contaminated clothing
- Vigorously wash contaminated skin for 1 minute with soap and water

Broken, cut or damaged skin or puncture wound

- Remove contaminated clothing
- Vigorously wash contaminated skin for 5 minutes with soap and water
- Seek medical attention

Eye

- Immediately flush eyes for at least 15 minutes with water, preferably using an eyewash; if no eyewash is available, pour water on the eye(s) for 15 minutes, rinsing from the nose outward to avoid contamination of the unaffected eye.
- Hold eyelids away from your eyeball and rotate your eyes so that all surfaces may be washed thoroughly.
- Seek medical attention

Ingestion or Inhalation

- Seek medical attention
- Do not induce vomiting unless advised to do so by a health care provider

D. Biological Material Spills

Spills and Preparing for Them

In the event of a spill of biological material, the individual(s) who caused the spill is responsible for the clean-up. CRI does not have a spill response team.

- Minimize the consequences of any spill of biological material by performing work on plastic-backed liner, when possible, to absorb spills

- Have a simple spill kit on hand including:
    - Chlorine bleach or some other concentrated disinfectant
    - A package or roll of paper towels or absorbent pads
    - Autoclavable bags
    - Rubber gloves
    - Forceps for pick-up of broken glass

Spills Inside a Biological Safety Cabinet

1. LEAVE THE CABINET TURNED ON
   While wearing gloves, spray or wipe cabinet walls, work surfaces, and equipment with disinfectant equivalent to 1:10 bleach solution. If necessary, flood the work surface, as well as drain pans and catch basins below the work surface, with disinfectant for a contact time of at least 20 minutes.
2. Soak up disinfectant and spill with paper towels. Drain catch basin into a container. Lift front exhaust grill and tray and wipe all surfaces. Ensure that no paper towels or solid debris are blown into the area beneath the grill.
3. Autoclave all clean-up materials before disposal in the biohazard waste container. Wash hands and any exposed surfaces thoroughly after the clean-up procedure.

Small Spill of Material Outside of a Biological Safety Cabinet (Spill that can be covered by a few paper towels)

1. Wearing gloves and a lab coat, cover the spill with paper towels and gently apply disinfectant, proceeding from the outer edge of the spill to its center. Leave in place for 20 minutes.
2. Pick up the towels and discard into a biohazard container. Pick up any pieces of broken glass with forceps and place in sharps container.
3. Re-wipe the spill area with disinfectant and thoroughly wash hands after glove removal.
4. Autoclave all clean-up materials before disposal in the biohazard waste container.

Large Spill of BL2 Material (>500ml) Outside of a Biological Safety Cabinet

1. Hold your breath and leave the room immediately.
2. Warn others to stay out of the spill area to prevent spread of contamination; post a sign stating: "DO NOT ENTER, BIOHAZARD SPILL", contact Safety Officer (name and phone #) for information.
3. Remove any contaminated clothing and put into a biohazard bag for later autoclaving.
4. Wash hands and exposed skin and inform your Laboratory head or PI about the spill. (Laboratory head or PI will report the incidence to Safety officer)

5. Put on protective clothing (lab coat, gloves and, if indicated, surgical mask, eye protection, shoe covers) and assemble clean-up materials.
6. Wait 30 minutes before re-entering the contaminated area to allow dissipation of aerosols.
7. Cover the spill with paper towels and gently apply disinfectant, proceeding from the outer edge of the spill to its center. Leave in place for 20 minutes.
8. Collect all treated materials and discard in a biohazard container. Pick up any broken glass with forceps and place them into a sharps container.
9. Re-wipe the spill area with disinfectant and wash hands thoroughly at completion of clean-up.

E. Biological Waste Handling

Biohazardous Waste
Some wastes associated with biological materials must be disposed of in special ways because they may have been contaminated with infectious organisms or agents. These wastes include the following:

- All sharps, e.g. glass implements, needles, syringes, blades, etc. coming from facilities using infectious materials
- Biologically-cultured stocks and plates, human blood or tissues

For disposal of these wastes, the lab personnel:

1. Sterilize or disinfect waste materials associated with viral, bacterial or other agents infectious to humans (by autoclave or chemical treatment equivalent to 1:10 bleach solution). See appendix A for selected infectious agents.
2. Place all biohazardous wastes, except for sharps, directly into the red bag marked with the Biohazardous waste symbol  ; these bags must be secondarily contained in a puncture resistant outer container and covered with a lid. Biohazard sticker must be present on both the container and the lid.
3. Place sharps into labeled sharps containers which when filled are placed into the medical waste box. It is extremely important to remember NOT to clip, bend, shear or separate needles from syringes and do not recap needles – these are the times that you are most likely to get injured.
4. When the biohazardous waste box is filled, seal the bag liner and put in autoclave resistant container for decontamination by autoclaving before disposal as regular waste. Alternatively, keep filled biohazardous waste bag in secure designated area awaited for pick up outsource professional waste treatment company.

Exemptions: Tissue and cell culture materials that are not known or suspected of being infected are permitted to be disposed as non-biohazardous waste. Note that these materials should be inactivated with an appropriate disinfectant to avoid contamination elsewhere in the laboratory.

Other wastes generated in the Institute that are not contaminated with biological agents or materials are not treated as biohazardous and may be discarded in the regular trash container, with recyclables, or into other specially designated waste containers. These include such items as recyclable and non-recyclable waste glass, gloves, unused plates or tubes, fly media or embryo plates, etc.

In order to clarify how these various wastes are to be handled in laboratories using biological materials, the waste stream charts have been developed and put into use.

LABORATORY BIOLOGICAL WASTE STREAMS

CATEGORY	DESCRIPTION	CONTAINER	LOCATION	HANDLING
Broken Glass, Sharp Plastic, Non-recyclable Glass	Non-contaminated* broken glass, plastic serological pipettes, plate glass, pyrex, light bulbs	Tall cardboard/plastic container with heavy plastic liner	Corridor and/or Lab	Removed by custodial staff when full and treated as Solid Waste (not recyclable)
Empty Chemical Containers	Intact, clean triple-rinsed glass and plastic (#1 and #2) containers; recyclable without cap	Special plastic bucket with lid	Corridor	Removed by custodial staff when full and treated as Recyclable waste
Lab Trash	Non-contaminated* gloves, bench paper, packaging materials, foil, plastic bags, paper towels, weighing boats, bottle caps, fly media, fly plates (with or without media); tubes (with or without media), filter flasks	Standard waste can with liner	Lab	Removed by custodial staff when full and treated as Solid Waste
Regulated Biohazardous Waste				
All sharps	All Pasteur and other glass pipettes, needles, syringes, scalpel blades, razor blades, slides, coverslips	Labeled sharps container	Lab	Placed in sharp biohazardous waste boxes by lab occupants for professional waste management pick up
Other Biohazardous Waste	Experimentally cultured stocks, plates or other materials ** Ethidium bromide gels	Standard biohazardous waste box with red plastic liner	Lab	Sealed and placed in secured area by lab occupants nd treated as Biohazardous Waste or wait for professional waste management pick up
Liquid Waste	Liquid medium used for growth of microorganisms or in contact with infectious agents or potential biohazards	Glass bottle	Lab	Steam heat sterilization in an autoclave, or by surface application of or placement in a chemical disinfectant solution, such as 1:10 bleach solution or its equivalent. Final disposal of sterilized liquid waste down the drain using running water.
Reusable glass wares and plastic wares	Glass wares and plastic wares in contact with infectious agents or potential biohazards	Autoclavable plastic bag	Lab	Steam heat sterilization in an autoclave. Regular washing procedure.
Animal Bedding Waste		Animal bedding waste bag	Animal care facility	???
Animal Carcasses		Biohazard bag with red plastic liner	Animal care facility freezer	Professional waste pick up

* Non-contaminated applies to any material not having been in contact with an infectious agent. The New Jersey Regulated Medical Waste regulations define infectious agent as “any organism (such as a virus or a bacteria) capable of being communicated by invasion and multiplication in body tissues and capable of causing disease or adverse health impacts in humans”.
** Other Regulated Medical Waste - Any solid waste generated in the diagnosis, treatment, or immunization of human beings or animals, in research pertaining thereto, or in the production or testing of biologicals, in the following categories: cultures and stocks (of infectious agents and associated pathological wastes, human blood and blood products, sharps (used or unused), animal waste (contaminated animal carcasses and animal bedding exposed to agents infectious in humans).

Additional biohazardous waste:

Mix waste: A mixture of biohazardous and chemical waste is categorized as harzardous chemical waste. biohazardous and radioactive waste is categorized as radioactive waste. A Mixture of biohazardous, chemical and radioactive waste is categorized as radioactive waste. Those mix waste must be subjected to waste treatment as applicable to their categories

F. Packaging and Shipping Biological Materials

Definitions

Packaging and shipping of biological materials must be done in a way that ensures the contents will not leak and that the package will arrive in good condition.

The definitions below apply to the packaging and shipping instructions that follow:

Etiologic agent means a viable microorganism or its toxin, which causes, or may cause, human disease.

Specimen means any human or animal material including, but not limited to, excreta, secretion, blood and its components, tissue, and tissue fluids, etc., which is reasonably believed to contain an etiologic agent, and is being shipped for purposes of research.

Biological product means a biological prepared and manufactured in accordance with regulations that govern the manufacture of vaccines, reagents, etc.

Packaging

Packaging

All biological materials including specimens and biological products that may contain an etiologic agent must be packaged to withstand leakage of contents, shocks, pressure changes, and other conditions incident to ordinary handling and transportation (passage through cancellation machines, sorters, conveyors, etc). Biological materials must be packaged according to the triple packaging principle depicted in Figure --. The three elements of triple packaging include: primary receptacle, leak-proof secondary container, and durable outer container. Contents should not leak to the outside of the shipping container, even if leakage of the primary container occurs.

All biological materials including specimens and biological products that may contain an etiologic agent must be packaged to withstand leakage of contents, shocks, pressure changes, and other conditions incident to ordinary handling and transportation (passage through cancellation machines, sorters, conveyors, etc). Biological materials must be packaged according to the triple packaging principle depicted in Figure --. The three elements of triple packaging include: primary receptacle, leak-proof secondary container, and durable outer container. Contents should not leak to the outside of the shipping container, even if leakage of the primary container occurs.

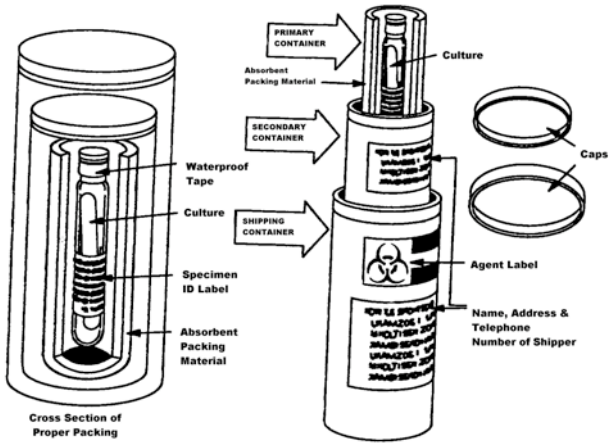


Figure 4 – The triple packaging for biological material transport.

Packaging with Dry Ice

1. If used, place dry ice between the secondary biological material container and outside shipping container.

2. Place shock absorbent material between primary and secondary containers so as to prevent the biological material containers from becoming loose inside the outer container as the dry ice sublimates.

Labeling

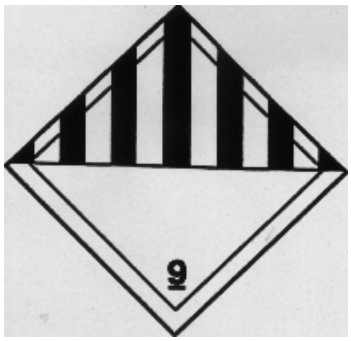
The outer shipping container of all biological materials which are being shipped must be clearly labeled and bear a biohazard symbol.

G. Shipping and Transportation Methods and Requirements

Registered mail or the equivalent

1. For such shipments, internationally or domestically, follow the International Air Transport Association (IATA) Dangerous Goods Regulations.

2. Apply appropriate labels to the outer shipping container for packages containing dry ice and/or infectious substances as shown in Figures below.



Dry ice

Infectious substance

3. Contact the specific carrier's dangerous goods agent prior to shipment for any additional packaging and labeling requirements.

Importation/Exportation of Etiologic Agents

For importation and exportation of infectious agents, etiologic agents and vectors that may contain them, follow the International Air Transport Association (IATA) Model Regulations on the Transport of Dangerous Goods regulation.

APPENDIX A

List of Select Agents and their Biosafety Level

Bacterial Agents	BSL
Actinetobacter calceticus	2
Actinobacillus sp.	2
Actinomyces sp.	2
Aeromaonas sp.	2
Arachnida propionica	2
Bacillus alvei	2
Bacillus anthracis*	2
Bacteroides sp.	2
Bartonella sp.	3
Bordetella sp.	2
Bordetella pertussis	2
Borrelia sp.	2
Brucella sp.*	2/3
Campylobacter fetus var. jejuni	2
Camplobacter sp.	2
Chlamydia psittaci	2
Chlamydia pneumoniae	2/3
Chlamydia trachomatis	3
Clostridium botulinum*	2/3
Clostridium tetani	2
Corynebacterium diphtheriae	2
Corynebacterium equi	2
Corynebacterium haemolyticum	2
Corynebacterium pseudotuberculosis	2
Corynebacterium pysogenes	2
Corynebacterium renale	2
Enterobacteriaceae all other	2
Erysipelothrix rhusiopathiae	2
Escherichia coli	2
Escherichia coli K12 derivative	1
Francisella tularensis*	2/3
Fusobacterium sp.	2
Haemophilus sp.	2
Klebsiella sp.	2
Legionella pneumophilia	2/3
Leptospira interrogans all servars	2
Listeria sp.	2
Moraxella sp.	2
Mycobacterium avium	2
Mycobacterium bovis	3

Mycobacterium leprae	2
Mycobacterium sp.	2
Mycobacterium tuberculosis	2/3
Mycoplasma sp.	2
Neisseria gonorrhoreae	2/3
Neisseria menegitidis	2/3
Nocardia sp.	2
Pasteurella sp.	2
Pseudomonas mallei	2/3
Neisseria gonorrhoeae	2/3
Pseudomonas testoserone	2
Rotococcus (Coryne.) equi	2
Salmonella sp.	2
Salmonella typhi	2/3
Shigella sp.	2
Staphylococcus sp.	2
Streptococcus sp.	2
Streptocacillus moniliformis	2
Streptomyces somaliensis	2
Treponema pallidum	2
Vibrio sp.	2
Yersinia pestis*	2/3

Fungal Agents	**BSL**
Blastomyces dermatitides	2
Coccidioides immitis*	2/3
Cryptococcus neoformans	2
Epidermophyton - pathogenic sp.	2
Histoplasma capsulatum	2/3
Microsporum - pathogenic sp.	2
Paracoccidioides brasilienisis	2
Sporothrix schenckii	2
Trichophyton - pathogenic sp.	2
Candida albicans	2
Miscellaneous Molds	2

Parasitic Agents	**BSL**
Anaplasma sp.	2
Ascaris sp.	2
Coccidia sp.	2
Cryptosporidia sp.	2
Echinococcus Granulosus	2

Ehrlichia sp.	2
Entamoeba sp.	2
Enterobius sp.	2
Fasciola sp.	2
Giardia sp.	2
Haemobartonella sp.	2
Hymenolepsis nana	2
Leishmania sp.	2
Leukocytozoon sp.	2
Naegleria sp.	2
Plasmodium sp.	2
Sarcocystis sp.	2
Schistosoma sp.	2
Strongyloides sp.	2
Taenia solium	2
Toxocara canis	2
Toxoplasma sp.	2
Trichinella spiralis	2
Trypanosoma sp.	2

Rickettsial Agents	**BSL**
Coxiella burnetii*	2/3
Rickettsia akari	2/3
Rickettsia australis	2/3
Rickettsia canada	2/3
Rickettsia conorii	2/3
Rickettsia prowazekii*	2/3
Rickettsia rickettsii*	2/3
Rickettsia siberica	2/3
Rickettsia tsutsugamushi	2/3
Rickettsia typhi (R. mooseri)	2/3
Rochalimaea quintana	2
Rochalimaea vinsonii	2
Spotted Fever Group - other	2/3

Viral Agents	**BSL**
Adenoviruses	2
Adenoviruses - animal - all	2
Aleutian Disease Virus	2
Arboviruses - certain	2
Arboviruses - certain	3
Arboviruses - certain	4

Arenaviruses - certain	3
Arenaviruses - certain	4
Avian Erthyroblastosis Virus	2
Avian Leucosis Virus	2
Avian Lymphomatosis Virus	2
Avian Myeloblasotosos Virus	2
Bovine Encephalomyelitis Virus	2
Bovine Leukemia Virus	2
Bovine Respiratory Syncytial Virus	2
Bovine Rhinotracheitis (IBR)	2
Cache Valley Virus	2
Canine Hepatitis Virus	2
Canine Distemper Virus	2
Caprine Arthritis	2
Coxsackie A & B Viruses	2
Cytomegaloviruses	2
Encephalomyelitis Virus*	2
Echovirus	2
Dengue Virus	2
Encephalomyocarditis Virus	2
Epidemic Diarrhea Infant Mice	2
Epstein-Barr Virus	2
Feline Leukemia Virus	2
Feline Sarcoma Virus	2
Filoviruses	2
Flanders Virus	2
Gibbon Ape Lymphosarcoma	2
Hart Park Virus	2
Hemorrhagic Fever Agents*	2
Hepatitis A Virus, Hepatitis E Virus	2
Hepatitis B Virus, Hepatitis C Virus, Hepatitis D Virus	2
Herpesvirus - other	2
Herpesvirus ateles	2
Herpesvirus saimir	2
Herpesvirus Simiae (B-virus)	3
Human Herpesviruses	2
Hog Cholera Virus	2
Human T-Cell Leukemia Virus I & II	2
Infectious Bronchitis Virus	2
Influenza Virus	2
Influenza Virus Virulent Avian	3

K (Rate) Virus	2
Lactic Dehydrogenase Elevating	2
Langat Virus	2
Laryngotracheitis Virus	2
Lassa Virus*	4
Low Risk Oncogenic Viruses	2
Lymphocytic Choriomeningitis Virus	2/3
Marburg Virus*	4
Measles Virus	2
Memingopneumonitis Virus	2
Mouse Encephalomyelitis Virus	2
Mouse Hepatitis Virus	2
Mouse Leukemia Virus	2
Mouse Pneumonia Virus	2
Mumps Virus	2
Myxomatosis Virus	2
Newcastle Disease Virus	2
Newcastle Disease Virus (VVND)	2
Non-Defective Adenovirus 2SV40 HYB	2
Papilloma Virus Shope	2
Parainfluenza Virus	2
Poliovirus - all types	2
Polyoma Virus	2
Poxvirus alastrim	2
Poxvirus monkey pox	3
Poxvirus - Smallpox*	
Poxvirus sp.	2
Pseudorabies Virus	2
Rabies Virus	2/3
Reovirus sp.	2
Respiratory Syncytial Virus	2
Retroviruses, including HIV & SIV	2/3
Rhinovirus sp.	2
Rous Sarcoma Virus	2
Rubella Virus	2
Simian Virus - other	2
Simian T-Cell Leukemia Virus	2
Sindbis Virus	2
Slow Viruses	2
Tensaw Virus	2
Tick-Borne Encephalitis Complex	4

Transmissible Spongiform Encephalopathies (Creutzfeldt-Jakob, kuru, and related agents	2
Turlock Virus	2
Vaccinia Virus	2
Venezuelan Equine Encephalitis*	3
Vesicular Stomatitis - lab adapted	2
Vesicular Somatitis Virus	3
Woolly Monkey Fibrosarcoma	3
Yaba Virus	2
Yellow Fever Virus 17D Strain*	2
Yellow Fever Virus Except 17D*	3

* - Select agents

APPENDIX B

Disinfectants

Table 1 Disinfectant Activity

Disinfectants			Practical Requirements				Inactivates				
Type	Category	Use Dilution	Contact Time (min)	Lipovirus, Broad Spectrum	Temp (degree C)	Relative Humidity (%)	Vegetative Bacteria	Lipovirus	Nonlipid virus	Mycobactria	Bacterial Spores
Liquid	Quat. Ammon. Cpds	0.1%-2.0%	10	NE			+	+			
	Phenolic Cpds	1.0%-5.0%	10	NE			+	+	B		
	Choringe Cpds	500 ppm*	10	30			+	+	+	+	+
	Iodophore	25-1600 ppm*	10	30			+	+	+		
	Alcohol, Ethyl	70%-85%	10	30			+	+	B		
	Alcohol, Isopropyl	70%-85%	10	30			+	+	B		
	Formaldehyde	0.2%-8.0%	10	30			+	+	+	+	+
	Glutaraldehyde	2%	10	30			+	+	+	+	+
Gas	Ethylene Oxide	8-23 g/ft^3	60	60	37	30	+	+	+	+	+
	Paraformaldehyde	0.3 g/ft^3	60	60	>23	60	+	+	+	+	+

NE=not effective
B=Variable results dependent on virus
*=Available halogen (1:100)

Table 2 Important Characteristics

Disinfectants		Important Characteristics										
Type	Category	Effective Shelf Life > 1 week (A)	Corrosive	Flammable	Explosion Potential	Residue	Inactivated by Organic Matter	Compatible for Optics (D)	Skin Irritant	Eye Irritant	Respiratory Irritant	Toxic (E)
Liquid	Quat. Ammon. Cpds	+					+	+	+	+		+
	Phenolic Cpds	+	+			+			+	+		+
	Choringe Cpds		+			+	+		+	+	+	+
	Iodophore	+	+			+	+		+	+		+
	Alcohol, Ethyl	+		+						+		+
	Alcohol, Isopropyl	+		+						+	+	
	Formaldehyde	+				+			+	+	+	+
	Glutaraldehyde	+				+		+	+	+	+	+
Gas	Ethylene Oxide	N/A		+ (B)	+ (B)			+	+	+	+	+
	Paraformaldehyde	N/A		+ (C)	+ (C)			+	+	+	+	+

N/A=not applicable
(A)=Protected from light and air
(B)=Neither flammable nor explosive in 90% CO2 or fluorinated hydrocarbon, the usual form
(C)=At concentrations of 7%-73% by volume in air, solid-exposure to open flame
(D)=Usually compatible, but consider interferences from residues and effects on associated materials such as mounting
(E)=By skin or mouth, or both. Refer to manufacturer's literature and the Material Safety Data Sheet

Table 3 Disinfectant Applications

Disinfectants		Disinfectant Applications										
Type	Category	Work Surfaces	Dirty Glassware	Large Area	Air Handling	Portable equip. Surface decon	Portable Equip. Penetrating Decon	Fixed Equip. Surface Decon	Fixed Equip. Penetrating Decon	Optical & Electronic Inst.	Liquid & Discard	Book, Paper
Liquid	Quat. Ammon. Cpds	+	+			+		+				
	Phenolic Cpds	+	+			+		+				
	Choringe Cpds	+	+			+		+			+	
	Iodophore	+	+			+		+				
	Alcohol, Ethyl	+	+			+		+				
	Alcohol, Isopropyl	+	+			+		+				
	Formaldehyde	+	+			+		+				
	Glutaraldehyde	+	+			+		+				
Gas	Ethylene Oxide					+	+			+		+
	Paraformaldehyde			+	+	+	+		+	+		

APPENDIX C

Application for Biosafety Approval (ABA) form

SUBMIT TO OFFICE OF RESEARCH
2009 version

FOR OFFICIAL USE ONLY
APPLICATION ID:_____
DATE OF APPROVAL:_____
EXPIRES:_____

THE CHULABHORN RESEARCH INSTITUTE

THE SAFETY COMMITTEE

APPLICATION FOR BIOSAFETY APPROVAL (ABA)

For research involving biohazardous include recombinant DNA, human blood or tissues and may include animal blood or tissues.
If you are not sure if this form applies to your work please check with the Biological Safety Sub-committee

---

Principle Investigator:_____ Date:_____
Title:_____ Phone Number:_____
Laboratory/Unit:_____ E-mail Address:_____

Title of Research Project:_____
Duration of Research Project: From: _____To:_____
Funding Source of this Project:_____

BIOHAZARDOUS AGENT(S) USED

ITEM NO.	BIOHAZARDOUS AGENT	BIOLOGICAL SAFETY LEVEL
1.	_____	_____
2.	_____	_____
3.	_____	_____
4.	_____	_____

PERSONNEL

The list of personnel should include all those who will physically handle the biohazardous agents or recombinant DNA molecules and are conceivably at risk from research procedures involving the use of these biological materials. Approval of the proposed experiment is given only for the identified personnel listed below. The Biosafety Officer must be notified if any new personnel are added. List additional personnel on a copy of this sheet as needed.

NAME	TITLE	LABORATORY/UNIT	E-MAIL	TELEPHONE
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____

LOCATION OF EXPERIMENTS, STORAGE OF AGENTS, AND AUTOCLAVE

Approval of the proposed experiments is given only for the location listed below.

	BUILDING	ROOM NUMBER	BS LEVEL	SHARED ROOM
LOCATIONS EXPERIMENTS CONDUCTED	_____	_____	_____	YES NO
	_____	_____	_____	YES NO
	_____	_____	_____	YES NO
	_____	_____	_____	YES NO
LOCATIONS AGENTS STORED	_____	_____	_____	YES NO
	_____	_____	_____	YES NO
	_____	_____	_____	YES NO
	_____	_____	_____	YES NO
NEAREST AUTOCLAVE	_____	_____		
	_____	_____		

PHYSICAL CONTAINMENT EQUIPMENT

BUILDING	ROOM NO.	Biosafety Cabinet MANUFACTOURERE	MODEL	SERIALS #	DATE OF CERTIFICATION
_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____

Do NOT use a non-vented biosafety cabinet if you plan to use volatile radioactive isotopes in work involving viable biohazardous agents at Biosafety Level 2 or above. Consult the Biosafety Manual or contact safety officer for recommended practices.

DESCRIPTION OF THE EXPERIMENT

Provide a short summary of the project in lay language and a technical description of the project, explaining the goal(s) and method **s to be used. List experimental procedures and assays that will be used to enhance biosafety; describe procedures that may create bio**hazards (i.e., aerosol generation from centrifugation, FACS analysis, exposure to sharps, etc). If animal work is included, state ex perimental procedures to be used. Provide information concerning potential biohazard shedding during the animal model and any model specific hazards. Continue on a separate sheet if necessary.

1. Does proposed reserch involved rDNA? Yes No

If no, proceed to Question 2.

If vector is plasmid based, describe plasmid and insert, or nature of synthetic nucleic acid, using maps if available. Provide source of plasmid material (e.g., made in lab A, purchased from Company X, gift from Dr. Y)

If vector is viral in origin, complete following:

Adenovirus Adeno Associated Virus Alphavirus (e.g., SFV, SIN)

Herpesvirus Poxvirus (e.g., Vaccinia) Other_____

Retrovirus

Murine Strain_____

Lenti Vector Backbone (e.g. HIV, SIV, etc)_____

Helper Plasmids

Provide strain, vector backbone:_____

Wild type deletions:_____

_____

Replication status:_____

Envelope packaging system(s):_____

Include source of vector (e.g., made in lab A, purchased from Company X, gift from Dr. Y).

Describe host cells into which rDNA will be introduced. Include source of host cells.

Provide information concerning nature of insert (specific gene(s), class of gene, source of insert, gene function, etc.).

2. Does proposed research involve infectious agents? Yes No

If yes, answer below questions.

Name of agent(s) and Biosafety level. Include source of agent.

Provide antibiotic/antiviral drug resistance profile for specific strain of agent(s) to be used in project.

Concentration and volumes of agents generated. Will volumes in excess of 10 liters be generated? Yes No

List target cells/animals to be used. If animals used, describe biosafety precautions to be taken.
Include housing conditions and methods of animal transport, if appropriate.

Indicate if you will be following CRI's recommended procedures for the following
(if not, provide information on substitute procedures to be used):

Biohazardous agents will be stored in secondary containment Yes No

All equipment used with biohazards agents will be with Biohazard labels Yes No

All biohazards agents will be placed in secondary containment prior to transport within the Institute. Containers will be labeled with Biohazard stickers. Yes No

Decontamination will be performed using 0.5% sodium hypochlorite (1:10 dilution of bleach). Yes No
If bleach is not appropriate (e.g., corrosive to equipment) provide name of disinfectant(s), concentration to be used and contact time.

3.Describe precautions to be taken when handling materials.

PPE: Check all that apply

Mask Gloves Lab coat Shoe covers Disposable Gown Safety Sharps

Head cover Respirator (provide type_____) Other_____

4. Describe risk of infection, clinical symptoms, and any recommended medical surveillance and preventive laboratory practices to be used.

5. Indicate training status of all listed personnel:

CERTIFICATION TO BE FILLED OUT BY PRINCIPAL INVESTIGATOR

I confirm that I have read and understood the relevant Guidelines and that this project confirms in all respects with those guidelines.

Principal Investigator ..... Date: .....

(_____)

CERTIFICATION OF Laboratory head:

Laboratory Head ..... Date: .....

(_____)

THE BIOLOGICAL SUB-COMMITTEE ASSESSEMENT OF THIS APPLICATION:

Recommended for Approval

Return to PI for revision:

Comments and/or Questions

Biological Safety Sub-committee, Chair ..... Date: .....

(_____)

THE SAFETY COMMITTEE ASSESSMENT OF THIS APPLICATION

Approved

Approved with recommendations

Not Approved

Safety Committee, Chair ..... Date: .....

(_____)