LOUISIANA STATE UNIVERSITY
Occupational and Environmental Safety

Guidelines for Laboratories Culturing Risk Group 3 Arboviruses, and/or Handling Animal Tissues, Fluids or Blood Potentially Infected with RG-3 Arboviruses

September, 2004

Introduction

Louisiana State University (LSU) follows federal guidelines in the conduct of research and other activities which might place personnel in direct contact with animal carcasses, blood, tissues, or body fluids that could potentially contain infectious zoonotic pathogens, including risk group 3 arboviruses. Additional requirements for working with human samples apply (BBP, 29 CFR 1910.1030), and are not included in this document. Contact OES-Biosafety at 578-4658 for guidance on the use of human tissues or fluids.

Risk group 3 (RG-3) agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available. Many RG-3 agents may infect through the respiratory route. Risk group 3 agents generally are handled and cultivated utilizing BSL-3 containment facilities and practices. However, certain procedures may be done in BSL-2 facilities using BSL-3 practices. For a more complete listing of risk groups (or BSL) for arboviruses, see the most current editions of the CDC/NIH publications *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), and the *NIH Guidelines for Research Involving Recombinant DNA Molecules* which are available at http://lsu.edu/ehs.

Like most other viruses, a spectrum of disease is observed in naturally acquired RG-3 arbovirus infections. Most cases are sub-clinical or mild self-limiting infections. Severe or fatal cases are most often associated with underlying predisposing factors such as age, immune status, and genetic components such as the *Flv* allele which influences susceptibility to West Nile virus infection in mice. For St. Louis encephalitis virus, susceptibility increases with age for humans, and the epidemiology of West Nile virus fatalities suggests the same may be true for this virus.

In general, the BSL-2 level facilities with application of "universal precautions" (see below) procedures are appropriate for most diagnostic applications involving RG-3 arboviruses. Some enhancements of work practices are more consistent with BSL-3 containment guidelines to ensure the safety of researchers, notably in the areas of waste management and personal protective equipment. Laboratory cultivation of larger quantities of RG-3 arboviruses requires BSL-3 laboratory facilities and practices. In the conduct of both BSL-2 and BSL-3 laboratory activities, strict adherence to sharps management procedures is critical because most laboratory-acquired arbovirus infections are due to accidental perenteral inoculations. Beyond the required and recommended laboratory practices, there are record keeping, training and health monitoring requirements and recommendations. This document breaks each section into requirements and recommendations. All requirements must be strictly adhered to in the conduct of research.

Blood and unfixed tissues of animal origin can contain a variety of potential pathogens that can infect humans. The biological safety office of LSU takes the position that all blood, human and animal, should be handled as if it is known to contain pathogens. Prudent safety practices, such as adherence to the universal precautions will protect researchers and others from research-acquired infections.

Universal Precautions

The universal precautions are a set of guidelines developed in the 1980's to minimize the risk of health care workers to HIV infection. The universal precautions are developed around the idea that all blood and certain body fluids should always be treated as though infected with HIV and other bloodborne pathogens. All researchers and health care workers should routinely use appropriate barrier precautions to prevent skin and mucous membrane exposure during contact with any blood or body fluids.

The Centers for Disease Control and Prevention in 1989 issued guidelines for the application of the universal precautions, primarily aimed at health care facilities, but applicable to a variety of work settings including laboratories. These guidelines recommend following the steps outlined below:

1) All work activities should be classified according to the potential for exposure. For those tasks with greater potential for exposure, there is greater need for appropriate protective equipment. As a minimum, gloves, protective outer clothing and eye protection should be worn at all times when there is a reasonable potential for exposure.

2) A detailed work practice program should be developed that includes standard operating procedures (SOPs) for all activities having the potential for exposure. Once these SOPs are developed, a worker education program is implemented to assure familiarity with work practices for potentially exposed workers. No worker should engage in tasks or activities that potentially put them at risk of exposure before receiving training pertaining to the SOPs, work practices and protective equipment required for that task.

3) The employer should monitor workers to insure that the required work practices are observed and that protective clothing and equipment are provided and properly used. Records documenting the procedures and criteria used to assess the risk of job activities should be maintained. Copies of all standard operating procedures (SOPs) for tasks or activities involving predictable or unpredictable exposure to tissue, blood, or other body fluids should also be kept on file, and made accessible to employees. In addition, training records including the dates of training sessions, the content of the sessions and the name and qualifications of the individuals providing the training should also be kept.

The following is a brief explanation of the components of the exposure control plan:

Engineering Controls

Louisiana State University biological safety office advocates the use of available technology to minimize, isolate or otherwise neutralize the risk of hazards to laboratory workers. Since parenteral exposure due to percutaneous injuries is the main risk posed by bloodborne pathogens, a major emphasis is on the prevention of sharps-related injuries. The majority of infections resulting from laboratory exposures are due to percutaneous injuries; most are from injuries involving hollow-bore needles.

Work Practices

The way tasks are carried out can minimize the potential for exposure to infectious pathogens in the laboratory. Standard microbiological practices have been recommended by the CDC and by the NIH for all laboratory containment levels. These practices have been designed to prevent indirect transmission of infectious material from environmental surfaces to the hands and from the hands to the mouth or mucous membranes. Such practices include the prohibition of mouth-pipetting, eating, drinking, smoking, applying cosmetics or handling contact lenses while in the laboratory. The practices also are intended to minimize environmental contamination.

Personal Protective Equipment (PPE)

Exposure to laboratory workers can be minimized by the appropriate and effective use of personal protective equipment (PPE). What constitutes appropriate PPE is determined by the procedure being conducted and the type, duration and extent of exposure.

Training

The responsibility for safety rests with the principal investigator / lab director. No person shall be required to perform hazardous tasks without proper training to do so. Formal safety training should be conducted upon initial hiring and annually thereafter. Training must be conducted by an individual who is knowledgeable about biological safety and laboratory techniques.

Employees must be educated about their risk and methods, equipment and practices to control these risks. Training must be made available during working hours and be understood by the employee. The topics required to be covered by training include:

- Epidemiology, transmission and symptoms of the pathogens to which the employee may be exposed.
- Universal precautions, engineering controls, safe work practices and personal protective equipment.
- Emergency and post-exposure management; occupational health program (if any).

For more information or assistance with training, contact the LSU Occupational and Environmental Safety, Biosafety office at 578-5640 or 578-4658.

Medical Care and Monitoring

The exposure control plan is primarily intended to prevent accidental infections, but it also contains specific requirements for post-exposure medical care. The use of preventive strategies will not completely eliminate the possibility of exposure to infectious materials. For researchers who handle blood, cultures, or other infectious materials, the plan requires a confidential medical evaluation, follow-up and documentation of any exposure incident. An exposure incident is defined as "***specific eye, mouth, other mucous membrane, non-intact skin, or parenteral contact with blood or other potentially infectious materials that results from the performance of an employee's duties***." The LSU Student Health Center works with OES to manage post-exposure control.

RG-3 Arbovirus Exposure Control Plan for Laboratories

September, 2004

I. Applicability

This plan applies to all research labs engaged in research on RG-3 bloodborne arbovirus pathogens, including but not limited to West Nile and St. Louis encephalitis viruses. The plan also applies to all labs conducting research in which contact with suspected or potentially infected animal blood, serum, unfixed tissues (e.g., brain) or primary cell culture, cerebrospinal fluid, or infected arthropods.

II. General Precautions

1) Precautions for the field collection of dead birds and other animals:

All LSU personnel or students involved in collecting dead birds and other animals shall follow the recommended precautions of the CDC / NIOSH available at http://www.cdc.gov/niosh/topics/westnile/recout.html. Further guidance may be obtained in the document *Epidemic/Epizootic West Nile Virus in the United States: Guidelines for Surveillance, Prevention, and Control, 2003*. These precautions include:

- precautions for mosquito avoidance (i.e. wearing long sleeved shirts, full length trousers, socks, light colored clothing, high boots) and the use of effective mosquito repellants (i.e. 20-30% DEET)
- when possible, minimize outdoor activities in places and at times when (e.g. dusk, night, dawn) mosquitoes are most likely to be encountered
- bare-handed contact is avoided when handling dead animals and birds and precautions must be taken to avoid direct contact with blood or excretions: rubber / nitrile / latex / vinyl / PVC gloves shall be used to collect dead birds
- cut-resistant gloves should be considered – these may be worn under rubber / nitrile / latex / vinyl / PVC gloves to avoid cuts or puncture wounds from bills, claws, or instruments during handling and field dissection of birds and other animals
- hands should be washed after handling dead birds and removal of gloves; if hand-washing in the field is not possible, the use of an antimicrobial gel should be considered

2) Precautions for the handling of suspect specimens in animal necropsy suites:

- where practical, all small animal carcasses should be manipulated in a certified biological safety cabinet (BSC)

- larger carcasses (e.g., horses) should be manipulated using equivalent protective measures (e.g. splash protection on eyes, protective solid-front gowns with tight fitting wrists, rubber / latex / vinyl / PVC gloves, and respiratory protection (NIOSH certified N-95 to N-100 respirator)

3) Precautions for the handling of human and animal (including avian) suspect clinical specimens (including blood, serum, CSF, arthropods, and tissues):

- potentially infected human and animal clinical specimens and small volume cultures grown for diagnostic purposes may be handled in a BSL-2 facility ***using BSL-3 operational practices*** as described in this document*

  **** A small volume culture of RG-3 arbovirus is up to four six-well plates, or two 25 cm^2 flasks, or equivalent monolayer surface area. Small volume cultures for diagnostic purposes may be grown and manipulated in a BSL-2 laboratory inside a BSC, but larger cultures must be grown and manipulated only within a BSL-3 laboratory.***

- blood collection should be carried out using standard universal precautions (e.g. wearing gloves, hand washing, using care to avoid accidental needle sticks)

- sorting of mosquitoes for species identification may be performed in a BSL-2 facility; when handling live mosquitoes, effective repellent should be worn and a BSC should ***not*** be used

- certified biological safety cabinets (preferably class II) should be used for laboratory manipulations of suspect clinical specimens

III. Laboratory Specific Safety Plans

Requirements:

Each laboratory working with any RG-3 arbovirus agent or blood, fluids or tissues from suspect animals shall develop a laboratory specific safety plan. It is the principle investigator's responsibility to develop the plan, but the task may be delegated to a qualified employee. If delegated, the PI will review and approve the final plan. The safety plan must do the following:

1) Hazard Identification. Identify tasks to be used in the conduct of research which could potentially place employees in contact with pathogens.

2) Task Ranking. Prioritize the hazardous tasks according to the potential for exposure to pathogens during the activity. Ranking should be based on the likelihood of infectious agents being present, the specific manipulations that might place workers at risk for transmission (for example, using sharps), and the quantities or concentration of infectious materials being used.

3) Standard Operating Procedures. Standard operating procedures (SOPs) for all hazardous tasks or activities must be written and followed with the goal of minimizing the risk of worker exposure during the performance of the tasks.

4) Sharps Management Program. If sharps are used for tasks involving infectious substances, a sharps injury prevention program must be developed, elements of which should include:

- training workers in the safe use and disposal of sharps
- modifying work practices that pose a sharps injury hazard to make them safer
- promoting safety awareness in the laboratory
- establishing procedures for reporting and follow-up of sharps-related injuries
- evaluating the effectiveness of injury-prevention efforts

5) Hazard Communication. Ensure that all workers are made aware of the specific hazards of the tasks and the standard operating procedures in use within the lab before engaging in hazardous tasks or activities.

It is the principle investigator's responsibility to ensure that all employees have a sufficient knowledge base to allow them to safely conduct the activities within their responsibilities. It is also the principle investigator's responsibility to monitor those individuals under their supervision to ensure strict compliance with laboratory safety procedures; where necessary, the PI must take appropriate corrective action in the event of non-compliance. Corrective action might include reprimand, mandated additional training, reassignment to non-hazardous tasks, or dismissal. The action taken would depend on the severity of the infraction and whether there is a pattern of habitual disregard on the part of the employee.

IV. Engineering Controls

Requirements:

1) Sharps disposal containers. Sharps disposal containers must meet the following criteria:

- able to be tightly closed and puncture resistant
- leak-proof on sides and bottom
- labeled with biohazard symbol and color-coded (red or orange)
- easily accessible and located close to the area where sharp instruments are used
- autoclavable

2) Broken Glass Management. Broken glassware must never be handled directly. Instead, it is to be removed by mechanical means such as tongs, dustpan and broom. Broken glassware not contaminated with infectious materials should be discarded in appropriate hard-sided containers; contaminated broken glassware must be discarded in biohazard sharps containers.

3) Biological Safety Cabinets. A properly maintained and certified biological safety cabinet (BSC) must be used for work with a high potential for creating aerosols or droplets. Examples of

manipulations that shall be done in a BSC include:

- blending, chopping or other mechanical homogenization of tissues
- sonication of fluids, cells or tissues
- necropsy of potentially infected birds or other small animals
- opening of pressurized or vacuum vials of potentially infectious materials
- opening of cell cultures potentially infected with arboviruses

Open flames are not permitted within the BSC because of damage incurred to the filters by flames and the potential to disrupt air flow within the BSC. If inoculating loops need to be used within the BSC, then disposable sterile loops or ceramic loop incinerator devices should be employed.

Note: *All open work with infectious material must be performed in a BSC (BSL-3 practices). No work with potentially infectious materials or infected animals may be conducted on the open bench.*

4) Vacuum lines. Vacuum lines must be protected with liquid disinfectant traps and high-efficiency particulate air (HEPA) filters or filters of equivalent or superior efficiency. The traps and filters must be checked routinely and maintained or replaced as necessary.

Recommendations:

1) Sharps Use Policy. Research laboratory safety plans should restrict the use of needles and other sharp instruments in the laboratory for use only during tasks when there is no alternative.

2) Capillary Tubes. If capillary tubes are used, such as for micro-hematocrit measurements, then the tubes should be of unbreakable plastic or glass coated with plastic to minimize the risk of broken tubes.

3) Plastic ware. Eliminate the use of glass vessels, test tubes, pipets and other laboratory ware as much as possible. Where possible, substitute safer devices into all laboratory procedures in place of more hazard-prone ones.

4) Safety Needles and Related Devices. Where possible, use safety products which are designed to reduce the risk of percutaneous injuries due to blood collection or manipulation of blood, tissues or pathogen cultures. If needles must be used, the suitability of self-sheathing needles or needle-less devices should be investigated. If other types of sharps are used, such as scalpels, safer alternatives should be explored. The following are examples of safety-engineered alternatives:

- shielded, self-blunting or retracting needles
- plastic vacuum / specimen tubes resistant to breakage
- retracting lancets
- unbreakable plastic capillary tubes for hematocrit determination

- rounded-tip, retracting or shielded scalpel blades
- disposable scalpels or quick-release scalpel blade handles
- vacuum blood tube devices for safe stopper removal

V. Laboratory Work Practice Precautions

Requirements:

1) General Lab Practices. A ***minimum*** of BSL-2 practices will be used at all times. Eating, drinking, smoking, applying cosmetics or lip balm, and handling contact lenses are prohibited in work areas where there is a reasonable possibility of exposure to pathogens. All procedures involving blood or other potentially infectious materials shall be performed in such a manner as to minimize splashing, spraying, spattering and the production of droplets. Mouth pipetting is prohibited. No food or drink will be stored in refrigerators, freezers, shelves, cabinets or on counter tops or benches within the laboratory or other areas where infectious materials may be present. Garments, gloves or other personal protective equipment shall be removed and replaced immediately or as soon as feasible if they become contaminated with blood or other infectious materials. ***All personal protective equipment is removed before leaving the laboratory***.

2) Hand-washing. Frequent hand-washing will be practiced whenever the hands become visibly contaminated with material, after the completion of work tasks involving the handling of infectious materials, before leaving the laboratory, and after removing gloves. After exiting the lab, hands should be again washed before eating or handling contact lenses. Strict adherence to hand-washing practice will prevent contact transfer to mucous membranes of infectious agents. If cuts, scrapes or other lesions are present on the skin, gloves must be worn all times to prevent contamination of the non-intact skin. Workers with skin lesions or dermatitis on hands or wrists will not perform procedures with potentially infectious materials even if they are wearing gloves.

The proper procedure for hand-washing is as follows:

a. Use a hands-free sink if possible. If not, open faucets to create a stream of warm running water.
b. Wet hands under running warm water, then use soap. Preferably, anti-bacterial soap should be used. Hibiclens® soap is an excellent anti-microbial soap.
c. Lather well beyond the wrists. Work all surfaces thoroughly including the wrists, palms, back of the hands, fingers and under the fingernails. Rub hands together for at least 15-20 seconds.
d. Rinse thoroughly with clean water. Be sure not to touch the side of the sink.
e. Dry hands completely. If a hands-free sink is not used, cover the faucet handle with a paper towel when turning off the water to protect your clean hands from pathogens that might be present on the handle.

3) Opening of tubes and containers. Cover pressurized or vacuum containers during opening or when needles are removed from pressurized vials. Use gauze that has been soaked with

alcohol or cut-out squares absorbent lab matting. This minimizes the possibility of aerosols being created during these manipulations. If the material in the container is infectious or potentially infectious, such activities shall be done ***only*** within the BSC.

4) Transport of specimens. Infectious substances and specimens shall be transported to other labs or areas using leak-proof containers within a secondary container to safely manage spills if they occur. Test tubes or other small samples should be transported within a rack placed within a secondary container (such as a modified tackle box) labeled with a biohazard symbol. Capillary tubes should be transported in a solid-walled secondary container, for example a plastic snap or screw-top tube. Transport of cultures or hemacytometers from the BSC to the microscope or other areas within the lab shall make use of trays or other secondary containers.

Field specimens being transported to the lab shall be double-bagged, with the outer layer a biohazard bag, and placed into leak-proof secondary containers of the appropriate size. Collection equipment, including containers used for transport, shall be routinely disinfected.

5) Routine Cleaning and Disinfection. Routine cleaning of work surfaces with disinfectant must be done after completion of each procedure and at the end of each work day, and additionally as necessary when spills occur. Disinfection and cleaning can be accomplished with a variety of agents, including iodophors registered as hard-surface disinfectants, phenolics, alcohol or diluted bleach. When using alcohol, use at 70% and leave in wet contact with surfaces for ***at least*** 15 minutes. When using bleach, dilute to 10% (v/v) with water and prepare fresh daily. Equipment which is to leave the lab for repair, calibration or discard must be decontaminated before it leaves the laboratory or labeled as to the biohazard involved. Bleach is corrosive, and should probably not be used for this purpose. Routine cleaning, such as sweeping, mopping and dusting shall not be done by housekeeping personnel, but rather by laboratory workers who have completed training in the specific hazards present in the lab.

6) Spill Cleanup. A small volume culture of RG-3 arbovirus is less than or equal to four six-well plate, two 25 cm^2 flasks, or equivalent monolayer surface area. Small volume cultures for diagnostic purposes may be grown and manipulated in a BSL-2 laboratory, but larger cultures must be grown and manipulated only within a BSL-3 laboratory. Small-volume and large-volume spills are managed differently according to the following procedures:

A. Small volume spill cleanup:
Decontamination shall be done following spills of all infectious or potentially infectious materials. Cleanup of small spills involves the following steps:

a. If broken glass is present, pick it up with tongs or other mechanical device.
b. Flood the spill with an appropriate disinfectant. Potentially infectious material may be treated with an approximate one tenth volume of undiluted bleach to achieve a final 10% concentration. Absorb the spill with either paper towels or absorbent lab “diaper” material or granular material impregnated with disinfectant.

c. Carefully scrape up the absorbent materials and discard in the biohazard waste.
d. Clean the area with soap and water.
e. Decontaminate with an appropriate fresh disinfectant.

B. Large volume spill cleanup (BSL-3 Lab):
Large volume spills and spills where the titer of infectious virus is suspected of being high require greater precautions. The procedure for such spills is modified as follows:

a. Leave the lab area immediately. Remove PPE and discard to the biohazard trash. Overtly contaminated PPE should be removed during exit from the BSL-3 lab and left inside the lab.
b. Allow at least 30 minutes for aerosols to settle and air changes in the lab to reduce the risk of inhalation exposure before re-entering the lab. Wear a properly fit-tested respirator (NIOSH N-95 or N-100) when re-entering the lab.
c. If broken glass is present, pick it up with tongs or other mechanical device.
d. Flood the spill with an appropriate disinfectant. Potentially infectious material may be treated with an approximate one tenth volume or more of undiluted bleach to achieve a minimum 10% (v/v) concentration. Absorb the spill with either paper towels or absorbent lab "diaper" material or granular material impregnated with disinfectant.
e. Carefully scrape up the absorbent materials and discard in the biohazard waste.
f. Clean the area with soap and water.
g. Decontaminate with an appropriate fresh disinfectant.

All spills in the BSL-3 laboratory should be reported to the facility coordinator.

7) Disposal of Wastes. Solid wastes shall be collected into biohazard bags suitable for autoclaving. Two layers of biohazard bags used for collection shall be placed inside a suitably sized leak-proof secondary container, such as a large plastic bucket fitted with a lid. When full but not overflowing, the bags are closed and autoclaved. If waste is to be removed to a common-use autoclaving facility, it should be transported to the facility while still inside a closed secondary container. ***Never*** attempt to manually compact solid infectious wastes. The bags are removed from the secondary container only when they are placed in the autoclave or into a leak-proof tub in preparation for autoclaving. Sharps containers are tightly closed and autoclaved.

All laboratory wastes from RG-3 arbovirus research labs and animal rooms must be decontaminated by autoclaving before disposal (BSL-3 practices). After autoclaving, decontaminated solid waste can be appropriately packaged for disposal with other biohazard wastes, but is not to be discarded with ordinary trash.

Fluid wastes (such as culture supernatants) may be discarded after autoclaving by carefully pouring down the drain with water rinse. Liquid and solid pathogen culture materials must always be autoclaved before disposal by other methods.

8) Access. Laboratory doors shall remain closed at all times when work is in progress, and entry to the area is restricted. The principle investigator will establish specific written entry requirements and policies whereby only those individuals who have a need to enter and have been made aware of the potential hazards within are allowed access. Infectious materials stored within the lab, in freezers or refrigerators, should be secured in leak-proof double containers.

A biohazard warning sign must be posted at all entrances to the laboratory with:

(1) the name of the infectious agent,
(2) special requirements for entry, and
(3) name and phone number of the laboratory director or other responsible person.

Additional access requirements must be met before access to the BSL-3 lab can be obtained. Call OES/Biosafety office at 578-4658 for more information.

Recommendations:

1) Absorbent Lab Matting. The use of absorbent lab matting will help reduce splatters on lab benches and other work surfaces, such as within biological safety cabinets, and is recommended whenever potentially infectious materials are in use. Lab matting should be changed frequently and whenever obvious spills of potentially infectious materials occur. Lab matting should always be discarded to the biohazard trash, even when not obviously contaminated.

2) Designation of laboratory areas. Certain areas of the laboratory may be designated as "clean" or "dirty" to help minimize the possibility of inadvertent contamination. Infectious substances should only be opened or handled in "dirty" areas. If more than one BSC is available, one should be designated as "dirty" and the other reserved for "clean" activities.

3) Equipment and Facility Maintenance. Laboratory and field equipment should be checked routinely for contamination and appropriately decontaminated. Laboratories working with pathogens should be kept scrupulously clean and tidy. Sweeping and mopping of the floors should be regularly scheduled, and should be done at least once a week, more often if floors become contaminated with infectious materials. Disinfectant such as bleach can be combined with an anionic detergent (but ***NOT*** cationic detergents) for mopping floors. The presence of blood and other organic material can limit the effectiveness of any disinfectant, requiring higher concentrations and longer contact times to compensate.

VI. Personal Protective Equipment (PPE)

Requirements:

1) Gloves. Gloves are required whenever hand contact with blood or other potentially infectious materials, mucous membranes or non-intact skin is likely or anticipated. Gloves are also required to be worn when handling or touching contaminated items or surfaces and for

performing vascular access procedures. Gloves must be worn when handling clinical specimens, infected animals or potentially contaminated equipment. In research laboratories, gloves should be worn for ***all*** procedures, cleaning spills, and handling wastes.

With the exception of heavyweight utility dishwashing gloves used for heavy cleaning, gloves must *never* be washed or disinfected for re-use. Detergents, alcohol or other disinfectants may compromise the ability of the glove to resist penetration by infectious substances. Gloves must be changed when visibly contaminated, torn or whenever tasks are completed. All layers of gloves shall be removed before handling telephones, doorknobs or "clean" equipment.

Gloves are to be removed for discard "inside out" to keep the "dirty" side inward and thus prevent inadvertent contamination of laboratory surfaces or equipment. Hands are to be washed with soap and warm water immediately after glove removal.

2) Protective Clothing. Shorts and sandals are not appropriate attire in an infectious disease laboratory due to the risk of exposure through exposed skin, and are not allowed. Laboratory coats, gowns or aprons are required at all times in the laboratory. The BSC is not a substitute for protective clothing.

When a potential for splashes exists, solid front fluid-resistant gowns are necessary. If anticipated exposure potentially involves splashes, other protective clothing must be added: hoods or caps, face protection (mask and goggles or face shield) and disposable shoe covers. Lab coats, gowns or aprons must be removed prior to exit from the laboratory, and are either disposable or laundered on the premises. Alternatively, lab coats may be bagged and autoclaved, and then removed off-site for laundering. Plastic buttons may melt in the autoclave, so coats should be fitted with heat-resistant closures.

For RG-3 arboviruses, if work is outside the BSC and splashes are anticipated, a fit-tested NIOSH N-95 or N-100 respirator must be worn.

3) Eye protection. Even though a BSC has a face shield, eye protection must be worn in the laboratory. Safety glasses with side-splash protection are recommended; even if the worker wears corrective eyeglasses safety glasses should be worn over them. Goggles or a full-coverage face shield may substitute for safety glasses, and must be used if procedures that could generate splashes have to be performed outside the BSC, such as during necropsy of a horse or other large animal that may have died from encephalitis.

Recommendations:

1) Choice of Gloves. Latex gloves are effective for the routine prevention of skin exposure to infectious materials. Gloves are not intended to prevent puncture wounds from needles or other sharps. However, a needle-stick through glove-protected skin has been documented to be less likely to result in infection due to a "wiping" effect which may remove some infectious material

from the external surfaces of the needle. This effect is magnified by increasing the number of layers of gloves, so two or more pair of gloves should be donned for tasks involving the use of needles. Other gloves, such as chain mail, are available which resist punctures and protect against cuts due to scalpels or other sharp instruments. Nitrile gloves are more puncture resistant than latex or vinyl gloves, and are equivalent to latex for dexterity. Heavyweight utility dishwashing gloves should be used over latex gloves for heavy cleaning and instrument decontamination, but should be discarded if they develop leaks.

For handling of infectious materials, a double layer of gloves is recommended. When working in a BSC handling infectious materials, the outer layer of glove should be removed and discarded before leaving the hood. This requires the strategic placement of a biohazard bag within the BSC.

VII. Training

Requirements:

1) Laboratory/Agent-Specific Training. Training in laboratory-specific standard operating procedures to minimize exposure is part of the laboratory safety plan, and as such is the responsibility of the principle investigator. Additional training is also required for all employees working in a BSL-3 research laboratory or a laboratory that falls under bloodborne pathogen regulatory control.

2) Timetable for Training. Training shall be provided at the time of assignment to hazardous tasks and annually afterwards. All training must be documented.

Recommendations:

1) Topic Recommendations. The following topics should be considered the minimum of those covered:

(1) *Pathogen overview*. A brief description of the major arbovirus pathogens likely to be encountered, epidemiology, common routes of laboratory-acquired infection, signs of disease and available treatments is covered.

(2) *Standard precautions and controls*. A brief summary of the standard precautions is provided, along with descriptions of engineering controls, work practice controls and personal protective equipment appropriate for tasks in a research laboratory.

(3) *Medical management*. The reporting and management of exposures with follow-up is discussed.

2) Training Resources. Training for BSL-2, BSL-3, bloodborne pathogen, safe BSC operation, and other topics is available from the Occupational and Environmental Safety office. Call 578-4658 to arrange training.

VIII. Medical Care and Exposure Management

Researchers and other employees or potential employees in laboratories where RG-3 arboviral pathogens are studied or may be present are encouraged to consult with their private physicians, or with physicians at the LSU Student Health Center, about any concerns they may have regarding the possible impact of their job responsibilities on their health. In particular, employees or potential employees who may have an underlying medical condition that could impact their risk of disease in the event of an exposure are encouraged to discuss these issues with a medical doctor. Since the severity of disease resulting from exposure to these agents is strongly correlated with immune system function and age, employees who believe they may be at increased risk of illness resulting from exposure may wish to consider alternative assignments.

Requirements:

1) **Post-Exposure Evaluation**. Immediate action and subsequent follow-up documentation are required if an employee is occupationally exposed; this means any eye, mouth, mucous membrane, non-intact skin, or parenteral contact with blood or other potentially infectious materials that results from the performance of an employee's duties. Cuts or needlesticks are to be immediately washed with soap and hot water. Medical treatment should be sought immediately. In the case of splashes of infectious materials to the nose, mouth, or eyes, the affected area is to be flushed extensively with water, saline or sterile irrigating solution. Medical attention should be sought as soon as possible. If exposure to infectious materials occurs during normal working hours, medical attention should be provided by the LSU Student Health Center. If exposure occurs at other times, attention should be sought at the nearest hospital emergency room or after-hours clinic.

2) Reporting of Exposures. All exposures must be reported to the laboratory principle investigator, who will document them. An Occupational Accident or Illness Report must be filled out and signed by the supervisor of the exposed worker and forwarded to LSU Risk Management within five days of the exposure (see PS-90 or go to the risk management web site at http://appl003.lsu.edu/pubsafety/riskmgt.nsf/index for specific instructions). The Workers Compensation Coordinator can be reached at 578-3285. When an exposure occurs, the principle investigator will also contact the Biological Safety Manager at 578-4658, who will complete an incident report. The incident report should contain as much of the following information as possible:

a. a description of the exposed person's duties that relate to the exposure incident,
b. the route of exposure and detailed circumstances of the exposure,
c. the infectious, or potentially infectious material to which the employee was exposed; blood, culture fluid, etc.

3) Follow Up: The exposed employee will be offered follow-up medical attention at the LSU Student Health Center, including:

a. a confidential medical evaluation
b. as medically indicated, post-exposure treatment
e. medical evaluation of subsequent illness resulting from the exposure

Where possible, the infectious or potentially infectious material to which the individual has been exposed will be evaluated to determine the virus levels, which may provide a useful indicator of the risk of infection.

The physician conducting the post-exposure evaluation and follow-up will be provided with a copy of the exposure incident report, information about the source of infectious material, if available, and all medical records relevant to the appropriate treatment of the employee (subject to consent for release by the employee). All medical information shall remain confidential.

4) Documenting of Employee / Student Risk Acceptance. The principle investigator, upon decision to offer technical or student employment to an individual, shall require the potential employee to sign a statement that they have been made aware of the risks involved in the employment, accept the employment with knowledge of the risks, and agree to follow the laboratory and university safety and incident reporting policies.

Recommendations:

1) Baseline Serum Collection. It is recommended that baseline serum samples be collected from all researchers potentially exposed during the conduct of their assigned tasks.

2) Medical Surveillance / Conditional Assignment Policies. After documenting a risk assessment of the hazardous tasks to be performed in the laboratory, the principle investigator may conclude that a medical surveillance program and / or conditional assignment policy should be made available to employees. Both the surveillance program and / or a conditional assignment policy, if either or both are adopted, must be documented as written laboratory policies. These policies, if adopted by the principal investigator, must be explained and written copies made available to potential employees prior to final acceptance of employment. Potential employees must also be provided with a detailed disclosure, preferably both orally and in writing, of the possible biological hazards inherent in the tasks required in the conduct of the work.

A laboratory surveillance program may consist of offering employees a pre-employment physical and/or medical testing for prior exposure to pathogens or other underlying medical conditions that might place the employee at risk of serious disease in the event of an exposure. Before offering a medical surveillance program, the principle investigator should seek guidance from the following LSU offices: Human Resource Management, Risk Management, Student Health Center and the Occupational and Environmental Safety. All costs of surveillance programs must be borne by the laboratory, department, or college.

A conditional assignment policy is intended to minimize the potential of exposure for those employees who believe or know themselves to be at greater risk of disease from an occupational exposure. Accordingly, principle investigators may wish to consider requests from employees to be assigned only to tasks that do not have associated high risks of exposure to potentially infectious materials. Employees who may wish to request conditional assignment include those who have an underlying medical condition that might place that individual at increased risk of disease in the event of exposure to a pathogen being studied or potentially present in the laboratory. Additionally, the principle investigator may restrict any individual who has consistently shown disregard for safety policies and procedures from conducting hazardous tasks.

Prepared by
Matthew S. Philpott, Ph.D.
Biological Safety Manager
Louisiana State University