



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

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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://oes.lsu.edu>.

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

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.

- 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 cm² 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

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

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

- 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:

- Use a hands-free sink if possible. If not, open faucets to create a stream of warm running water.
- Wet hands under running warm water, then use soap. Preferably, anti-bacterial soap should be used. Hibiclens® soap is an excellent anti-microbial soap.
- 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.
- Rinse thoroughly with clean water. Be sure not to touch the side of the sink.
- 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

other areas within the lab shall make use of trays or other secondary containers.

Feld 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

- 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 (per TSA (808 da) wrk ((69 tr 5) L N 9 6) collect 3 code ad 8 W I g 1 5 TD

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.

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

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

(2)

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:

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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