

Guidelines for Research Involving Viral Vectors: Flavivirus vectors

Flaviviruses (family *Flaviviridae*) are enveloped, positive-sense, single-stranded RNA viruses that are normally transmitted between vertebrate hosts by insect vectors. Vertical transmission from mother to offspring in utero or through breastmilk has also been noted for some viruses and sexual transmission is of concern for Zika virus specifically. The flavivirus genome consists of a single long open reading frame that encodes both structural and non-structural proteins. Upon entry into the cell cytoplasm the viral RNA can serve as mRNA and is translated to form a single long polyprotein that is cleaved by cellular and viral proteases to form individual proteins. As such, viral vectors based on the flaviviruses require heterologous genes to be inserted in frame with the viral coding region and flanked by protease cleavage sites. Replication defective vectors are generated by replacing the structural protein genes with the heterologous gene of interest. As an alternative, genes can be inserted in non-coding regions with assistance from an internal ribosomal entry site sequence.

Flaviviruses and flavivirus vectors replicate to moderate titers (greater than 1×10^8 particle forming units/ml in some cases) in both vertebrate and invertebrate cells. Infection of vertebrate cells are typically lytic though it takes several days to a week for cytopathic effects to be recognized. Infection of insect cells is persistent. Flavivirus replication occurs in the cell cytoplasm thus flavivirus vectors are suited for transient expression of a gene of interest in targeted cells.

Potential Health Hazards

Human infections with flaviviruses are most often subclinical but can also present as mild to severe flu-like symptoms. Severe cases can result in encephalitis, hepatitis, and hemorrhagic fever depending on the virus and prior immunity. Other viruses have been associated with congenital malformations including microcephaly in children born to infected mothers.

Modes of Transmission

Wild-type flaviviruses are normally transmitted between vertebrate hosts by insect-vectors including mosquitoes and ticks depending on the virus. Direct person-to-person transmission has not been documented except for Zika virus where sexual transmission has been implicated. Humans can serve as an amplifying host for some flaviviruses, such as dengue viruses, Zika virus and yellow fever virus, and can infect mosquitoes when fed upon.

Laboratory Acquired Infections

Laboratory acquired infections have been documented for some flaviviruses. Exposure to aerosols, contact with broken skin or contaminated animal bedding, and accidental auto-inoculation as well as the bite of experimentally infected mosquitoes have all been implicated in laboratory acquired infections.

Host Range

Flaviviruses can infect a wide array of vertebrate and invertebrate species depending on the specific virus. Vertebrate species include dogs, cattle, horses, pigs, deer, rodents, killer whale, alligators, frogs, bats and birds, in addition to humans and other non-human primates. Invertebrate species include both *Aedes* and *Culex* species of mosquito; and *Ixodes* species of tick depending on the virus.

Survival

Some viruses, for example dengue viruses, have been reported to be stable in dried blood for as much as 9 weeks at room temperature.

Laboratory Practices

Work with dengue viruses and Zika virus which are not associated with severe disease in humans upon primary infection can be conducted at Biosafety Level -2 (BSL-2). Flaviviruses associated with severe infections in humans such as Japanese encephalitis virus, Murray Valley encephalitis virus, Powassan virus, St. Louis Encephalitis virus, West Nile virus, and Yellow fever virus are classified as Risk Group-3 (RG-3) pathogens and require BSL-3 containment and work practices. Live attenuated vaccines of Japanese encephalitis virus and Yellow fever virus have been licensed and should be considered for further protection of laboratory workers.

Work with replication competent flavivirus vectors must be done according to the RG assignment of the parental viruses. Work with replication defective replicons based on RG-3 flavivirus vectors and containing two-thirds or less of the viral sequence can be handled at BSL-2, only after the absence of replication competent virus has been documented using validated methods.

Biosafety Level 2 practices include:

- Use Universal Precautions. Assume that all human and animal body fluids and tissues are infectious.
- All activities with infectious material should be conducted in a biological safety cabinet (BSC) or other appropriate primary containment device in combination with personal protective equipment.
- Centrifugation of infected materials must be carried out in closed containers placed in sealed safety cups, or in rotors that are loaded or unloaded in a biological safety cabinet.
- **Mouth pipetting is strictly prohibited**; mechanical pipetting devices will be used. Pipettes should have cotton-plugged tops. Pipette tips may or may not require filters depending on the biological or application. Care must be taken not to contaminate

hand-held or automatic pipettors and other instruments with toxic or infectious materials.

- Biohazard markings will be on all contaminated waste and waste disposal containers; in addition to any equipment used for work or for storage of biological hazards.
- Chewing gum, eating, drinking, or applying cosmetics are not permitted within any laboratory.
- Using tobacco products is not permitted within any University building.
- Personal reading materials such as magazines, newspapers, schoolwork and other personal materials are prohibited in the laboratory.
- Animals and plants not involved in the studies being performed are not permitted in the laboratories.
- Flammable liquids will be stored in flammable storage cabinets, when feasible. Ethers will only be stored in rated refrigerators if it is required by the SDS and only in refrigerators designed and clearly marked “flammables safe”. Only bring into the BSL-3 adequate supplies for short-term inventory. Excess inventory will be stored in the storage room outside the facility.

Biosafety Level 3 practices in addition to those for BSL-2 include:

- The work surfaces of isolation laboratories and suite area benches will be decontaminated with an appropriate liquid disinfectant solution at least once a day when the laboratory is in use and after any spill of viable material.
- Personal protection is partially dependent upon the carefully balanced directional airflows within the containment facility. Doors will be opened only enough to allow passage in or out. Doors will never be needlessly held open and **never** propped open when working with infectious or toxic agents.
- The use of needles, syringes, and other sharp objects should be strictly limited.
- An insect and rodent control program is in effect. This program will include a regular placement of traps and poison bait.
- With the exception of small alcohol lamps that are used within a biosafety cabinet to heat-fix slides and are only used for short periods (minutes), open flames are not permitted in the BSL-3 unless pre-approved by the University Fire Marshal.
- Open wounds, cuts, scratches, and grazes should be covered with waterproof dressings.

Personal Protective Equipment

Personal protective equipment for BSL-2 laboratory work includes a dedicated laboratory coat, eye protection, and disposable gloves at a minimum.

Personnel entering the BSL-3 laboratory should remove street clothing and jewelry; and change into dedicated laboratory clothing and shoes before donning two pairs of booties, two pairs of gloves and respiratory protection (i.e. PAPR unit). Additional protection must be worn over laboratory clothing when infectious materials are directly handled, such as solid-front gowns with tight fitting wrists at a minimum.

Precautions When Using Animals

Animals infected with flaviviruses or flavivirus vectors will be housed according to the RG assignment of the specific virus or vector. Live attenuated vaccine strains of RG-3 viruses which have been exempted by the CDC and/or USDA are classified as RG-2 pathogens and may be manipulated using ABSL-2 facilities and work practices. Work with replication defective replicons based on RG-3 flavivirus and containing two-thirds or less of the parental viral sequence can be handled at ABSL-2, only after the absence of replication competent virus has been documented using validated methods.

Some flavivirus are shed in the animal's excreta thus cages and bedding should be handled as a biohazard. Avoid creating aerosols when emptying animal waste materials and decontaminate the bedding and cages via chemical treatment or autoclaving.

For work in the ABSL-3:

- Retractable or safety hypodermic needles and syringes will be used when feasible for injections and aspirations of fluids from lab animals and diaphragm bottles.
- Extreme caution must be used whenever handling needles and syringes to avoid self-inoculation and the generation of aerosols during use and disposal.
- Needles may not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The used needles and syringes will be placed in a puncture-resistant sharps container and autoclaved before disposal in the proper medical waste sharps container. If removal of the needle from the syringe is critical to the procedure, then the needle will be held with a hemostat or mechanical device during removal, using only a one-handed technique.
- To insert a needle into a diaphragm bottle, place the bottle on the work surface and then stick the needle through the diaphragm. DO NOT hold the bottle while inserting the needle as this can easily cause self-inoculation. In general, use a one-handed technique and all steps must be performed in a BSC whenever practical when working with infectious material.
- Retractable scalpels will be used when possible. Straight blades will be used with a holding device, never handle cutting edges with fingers.
- Additional PPE may be required to minimize bites and scratches; and to provide additional protection against auto-inoculation.

Recombinant Flavivirus Research

All protocols involving recombinant flavivirus vectors, regardless of the funding source, must comply with the NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid; and must be approved by the Institutional Biosafety Committee before any work is initiated.

Employee Exposure

Eye Exposure- remove PPE if necessary, proceed to the eyewash station in the laboratory, and rinse eyes with cold running water for 15 min.

Skin Exposure- remove PPE if necessary. If there are no cuts or abrasions, wash the affected area with soap and water.

Accidental Needlestick Injury or exposure of cuts/abrasions- remove PPE and wash the affected area with soap in the laboratory sink. Allow the wound to bleed if bleeding is not excessive and irrigate the wound for 15 min.

Report Incidents and Seek Treatment- actual or suspected exposure incidents should be reported immediately to the supervisor and biological safety officer. The responsible official (RO) and/or alternate responsible official (ARO) should also be notified in the event of injuries/exposures involving RG-3 agents. The individual should go to the University Physicians Group (normal working hours) or to the emergency room at USA Medical Center. The attending physician will arrange consultation by a USA infectious disease physician.

Spills and Disposal Procedures

In the case of an accident outside a BSC or other primary containment device, whereby a container holding an infectious substance breaks or spills, the following steps must be taken:

- Exit the vicinity of the spill inform any coworkers within the lab to do the same.
- Notify the supervisor and the RO/ARO if the work involves RG-3 pathogens. The BSO should also be notified if the spill involves work with recombinant/synthetic nucleic acids. If the work involves select agents, the RO/ARO will contact Safety and Environmental Compliance along with appropriate Federal, State and local authorities as may be appropriate.
 - A risk assessment will be performed in collaboration with Environmental health and Safety as needed to determine how best to address the spill.
- Post a sign notifying others in the facility of the situation. **DO NOT ATTEMPT TO CLEAN THE SPILL!**
- Exit the laboratory removing PPE at the threshold of the door.
 - If the incident involves RG-3 pathogens, shower immediately with soap for 3 to 5 min.
- Wait at least 30 minutes after the spill before entering the room. *In the event of an emergency/injury involving personnel, immediately proceed with spill management.*
- The use of additional PPE beyond what is normally worn on entry to the laboratory will be determined during the risk assessment and communicated to the response team prior to clean-up.
- In most situations the spill can be handled as follows:
 - Add premeasured decontaminant (e.g. Bleach, Roccal, Micro-Chem Plus) to the water provided in the spill kit.
 - Gently cover the spill with paper towels or Red-Z powder.

- Apply the decontaminant solution starting at the perimeter of the spill and working towards the center.
 - Allow 30 minutes' contact time with the decontaminant solution before cleanup, except in emergencies (i.e. injury).
 - Remove paper towels or Red-Z to a biohazard bag along with any paper towels used to wipe the area dry. If the decontaminant solution was used on metal, wipe the area with 70% ethanol.
 - Discard protective clothing into the biohazard bag and autoclave.
- Additional procedures/decontamination (chemical decontamination of surfaces or VHP) will be determined during the risk assessment and communicated to the response team as necessary.
 - Confirm that the spill has been reported, and that the clean-up and all necessary paperwork have been completed.

Small spills (less than 1 ml) within a BSC can be handled by covering the spill with a paper towel soaked in disinfectant and allowing an appropriate contact time before collecting the paper towel and processing in the normal waste stream. For larger spills, the following steps must be taken.

- Stop what you are doing and secure any remaining stocks in the cabinet. It is never appropriate to continue working in a BSC that is grossly contaminated with infectious agents.
- If working with RG-3 pathogens, remove wrap around gown into the BSC or tear off arms into the BSC. If working with RG-2 pathogens decontaminate sleeves of laboratory coat by saturating with appropriate disinfectant before exiting cabinet.
- Remove outer gloves and exit the BSC.
- Replace outer gloves
- Report the spill to the supervisor and the RO/ARO if RG-3 viruses are involved. Allow the air to settle in the BSC (approximately 10 min.)
- Don a new PPE and return to the BSC with additional cleaning supplies.
- Cover the spill with paper towels and pour decontamination solution over the paper towels working from the outside towards the center. Never use a spray bottle to apply decontamination solution after a spill.
- Use a paper towel soaked in decontamination solution to wipe down any other items in the BSC and allow 30 min. contact time for the decontamination solution to work.
- After the appropriate contact time, bag all waste and remove all items from the BSC, wiping them down with a disinfectant soaked paper towel while removing. Depending on what equipment was in the hood these might need to be bagged for storage until a decontamination cycle (VHP, etc.) can be scheduled to decontaminate internal parts.
- Once emptied, decontaminate all surfaces in the BSC with an appropriate decontamination solution using a Swiffer (or equivalent) to reach hard to reach spaces. Allow for an appropriate contact time for effective decontamination.

- Work can resume after the BSC has been properly decontaminated. If there is another BSC in the laboratory it can be used during the cleaning and decontamination process.

Disinfectants

Flaviviruses are subject to inactivation with many common disinfectants including 10% bleach (1% sodium hypochlorite), 70% ethanol, 2% glutaraldehyde, quaternary ammonium compounds and phenolics. Physical inactivation includes exposure to heat (moist or dry) and exposure to UV.

Decontamination

All solid laboratory waste should be decontaminated by autoclaving for a minimum of 30 min. at 121°C using a gravity cycle. Liquid waste can either be chemically inactivated by adding disinfectant to the appropriate dilution and allowing a minimum of 30 min. contact time before disposable, or autoclaved on a liquid cycle at 121°C for 30 min.

Vaporous hydrogen peroxide or other appropriate fumigant can be used to decontaminate major equipment within the BSL-3/ABSL-3 facility or in the event of gross contamination because of a spill.

Transport Requirements

Infectious materials must be appropriately contained in sealed leak proof secondary containers when moved between laboratories within a facility. Whenever possible, a cart should be used to transport the materials to avoid accidental spills. Materials must be appropriately contained and labeled for transport within the University. Shipping infectious substances, diagnostic specimens, and/or shipping with dry ice off-campus require training and certification. See Shipping and Packaging Biological Materials posted on the [USA Biosafety training website](#) for additional information.

Materials to be transported between facilities within the University system should be appropriately contained and labelled. Shipments of infectious substances off-campus must be done according to the regulations of the International Air Transport Association by individuals who are trained and certified in the transport of dangerous goods. Shipment involving select agents requires pre-notification of the CDC and or USDA and should be handled as described in the USA Laboratory of Infectious Disease Security Plan.