



Guidelines for Research Involving Viral Vectors: Alphaviruses and Alphavirus Vectors

Alphaviruses (family *Togaviridae*) are enveloped, positive sense, single stranded RNA viruses that are normally transmitted between vertebrate hosts by mosquito vectors. The alphavirus genome is divided into two segments encoding the structural and nonstructural proteins. The nonstructural proteins are encoded within the 5' third of the genome and are translated directly from the viral RNA upon entry into the cytoplasm. The alphavirus structural protein genes correspond to the 3' third of the viral genome and are transcribed from a sub-genomic RNA promoter. Viral vectors based on the alphaviruses can take advantage of this genome organization in multiple ways including replication competent vectors wherein a second sub-genomic promoter driving a gene of interest is inserted between nonstructural and structural gene regions or downstream of the structural protein genes. Alphavirus replicons in which the structural protein genes have been replaced by a gene of interest are replication defective and must be packaged by supplying the structural proteins in trans or may be delivered directly to a cell as DNA under the control of a DNA dependent RNA polymerase promoter.

Alphaviruses and alphavirus vectors replicate to high titers (greater than 1×10^9 particle forming units/ml in some cases) in both vertebrate and invertebrate (mosquito) cells. Infection of vertebrate cells are typically lytic, but infection of mosquito cells is persistent. Alphavirus replication occurs in the cell cytoplasm and protein expression from the sub-genomic promoter can exceed 80% of total cellular protein in infected cells thus alphavirus vectors are ideal for transient expression of high levels of a gene of interest.

Potential Health Hazards

Human infections with alphaviruses are most often subclinical but can also present as mild to severe flu-like symptoms. Severe cases involving wild alphaviruses such as chikungunya virus (CHIKV) are most often associated with a debilitating arthritis; whereas new world alphaviruses such as eastern, western and Venezuelan equine encephalitis viruses (EEEV, WEEV & VEEV, respectively) are associated with severe neurological symptoms leading to significant morbidity and mortality.

Modes of Transmission

Wild-type alphaviruses are normally transmitted between vertebrate hosts by mosquito vectors. Direct person-to-person transmission has not been documented. Humans can serve as

an amplifying host for some alphaviruses, such as certain strains of VEEV, and can infect mosquitoes when fed upon. VEEV, EEEV and WEEV have been demonstrated to be infectious via the aerosol route when delivered in high concentrations in the laboratory setting.

Laboratory Acquired Infections

Laboratory acquired infections have been documented for some alphavirus including at least two deaths. Exposure to aerosols, contact with broken skin or contaminated animal bedding and accidental autoinoculation

- x 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.
- x 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.
- x Mouth pipetting is strictly prohibited mechanical pipetting devices will be used. Pipettes should have capped/plugged tops. Pipette tips may or may not require filters depending on the biological or application. Care must be taken to not contaminate hand-held or automatic pipettors and other instruments with toxic or infectious materials.
- x 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
- x

Personal Protective Equipment

Personal protective equipment for BSL₂ laboratory work includes a dedicated laboratory coat, eye protection, and disposable gloves at a minimum.

Personnel entering the BSL₂ 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

Recombinant Alphavirus Research

All protocols involving recombinant alphavirus 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 **CR3** 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. If the injury/exposure involves a select agent, the RO/ARO will immediately notify the CDC and follow up with a Form 3 within seven calendar days.

Spills and Disposal Procedures

- x 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 cleanup.
- x In most situations the spill can be handled as follows:
 - o Add premeasured decontaminant (e.g. Bleach, Roccal, ~~Mobam~~ Plus) to the water provided in the spill kit.
 - o Gently cover the spill with paper towels or ~~RZ~~ powder.
 - o Apply the decontaminant solution starting ~~at~~ the perimeter of the spill and working towards the center.
 - o Allow 30 minutes' contact time with the decontaminant solution before cleanup, except in emergencies (i.e. injury).
 - o Remove paper towels or ~~RZ~~ 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.
 - o Discard protective clothing into the biohazard bag and autoclave.
- x Additional procedures/decontamination (chemical decontamination of surfaces or VHP) will be determined during the risk assessment and communicated to the response team as necessary.
- x Confirm that the spill has been reported, and that the ~~clean~~ and all necessary paperwork have been completed.
- x If the spill involves ~~R3~~ select agents, ~~lab~~ personnel in the vicinity of spill at the time of occurrence will be required to give a formal report to the RO/ARO who in turn will notify the CDC.

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.

- x 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.
- x If working with ~~R3~~ pathogens, remove wrap around gown into the BSC or tear off arms into the BSC. If working with ~~R2~~ pathogens decontaminate sleeves of laboratory coat by saturating with appropriate disinfectant before exiting cabinet.
- x Remove outer gloves and exit the BSC.
- x Replace outer gloves
- x Report the spill to the supervisor and the RO/ARO if ~~R3~~ viruses are involved. Allow the air to settle in the BSC (approximately 10 min.)
- x Don a new PPE and return to the BSC with additional cleaning supplies.
- x 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.
- x 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.
- x After the appropriate contact time, bag all waste and remove all items from the BSC,

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