

Room 233 Vaccinia Virus SOP

Animal Protocol Number & Title: 2010-239 “Cell-mediated immune responses to poxviruses”

Room Contacts:

1st contact: Mansour Haeryfar Office: 82488 After Hours: 519-282-3260
Additional contacts: pending

EMERGENCY PROCEDURES:

In the event of a skin exposure, puncture, or needle-stick injury, beginning immediately, hold the affected area under cold water and disinfect the wound area with a bleach scrub brush for 15 minutes, while encouraging the site to bleed by massaging. Bleach scrub brushes are available behind the sink in Room 233.

In the event of an eye-splash, beginning immediately, remove all Personal Protective Equipment, walk to the east sink in Room 234 and flush eye in eyewash station for 15 minutes.

Immediately report all incidents to Dr. Haeryfar and to Employee Health by telephone (ext 82047). Complete a Workplace Accident Incident Form and send to Rehabilitation Services immediately.

PROCEDURES (Level 2+ work practices in a Level 2 area):

Ensure that experiments are well-planned such that all equipment & reagents needs are gathered in advance. Work slowly and cautiously, taking care not to create spills, droplets or aerosols.

- 1) Responsibilities of research staff: identification of cages and all associated waster with biohazard labels, animal monitoring for overall health and for post-vaccination events (on days prescribed by animal protocol), animal monitoring records, all animal manipulations, treatments, euthanasia and tissue harvest, double-bagging and removal of carcasses from room, husbandry room checks (on the days that they enter the room), all husbandry, including cage changes, food and water bottle changes, mop floor, transfer of all materials to autoclave or incineration stations, all autoclaving and subsequent disposal of materials.
- 2) Responsibilities of ACVS staff: none required.
- 3) Room 233 will be posted with “Vaccinia Virus In Use: DO NOT ENTER” signage and the room door locked when work with vaccinia virus is ongoing. Only vaccinated individuals may enter the room while this signage is displayed. The sign can be removed only after the procedure is complete; biohazard bags removed from the room; and the biosafety cabinet, benches, centrifuge and other non-disposable equipment are disinfected by wiping down with 10% bleach.

- 4) The following Personal Protective Equipment (PPE) must be donned in Room 233 prior to working with vaccinia virus: gown, protective sleeves, hair net, mask, double gloves, safety goggles. The outer pair of gloves and protective sleeves **MUST BE REMOVED** prior to withdrawing hands from the BSC after coming in contact with vaccinia virus.
- 5) A layer of towels moistened in 10% bleach must be placed on the floor of the BSC prior to any procedure, including either *in vitro* and *in vivo* work. A layer of dry paper towels may be placed over the moistened towels as a working surface, such that mice do not contact the bleach. These paper towels must be discarded in a biohazard bag maintained inside the BSC.
- 6) All equipment required for performing a particular procedure, including a box of gloves, should be transferred to the BSC prior to beginning a particular procedure.
- 7) Disposable items that contact the virus during either *in vitro* or *in vivo* procedures, such as pipettes and pipette tips, must be discarded into biohazard bags stationed inside the BSC. Prior to disposal, the biohazard bag must be tied loosely, wiped with 10% bleach, removed from the BSC, and transferred to metal tray for transport to the Government Certified autoclave in SDRI 16.1. Alternatively, biohazard bags may be transferred to a second biohazard bag, transferred to metal tray and transported (using a cart) to the incineration area in Room M605.
- 8) Virus-containing fluids must not be vortexed, because of the possibility of creating aerosols.
- 9) Liquid waste must be discarded into a disposable bottle, which is filled to the 10% level with concentrated bleach (i.e. 50 ml bleach in a 500 ml bottle). At the end of the procedure and following a 20 minute inactivation period, the outside of the container must be wiped with 10% bleach, then liquid contents discarded in the sink in Room 233. The container must be discarded in the biohazard bag-lined garbage pail stationed beside the BSC.
- 10) *Needles must be used with extreme caution as needle-sticks are the most common cause of laboratory-related injuries.* After use, needles **SHOULD NOT** be re-capped, rather immediately transferred to a sharps container stationed inside the BSC. Researchers are strongly encouraged to anesthetize mice for injections. An appropriate anesthetic for this short injection procedure is isoflurane:propylene glycol (30:70), as per SOP 354. At the end of each procedure, sharps containers must be closed, wiped down with 10% bleach prior to removing from the BSC.
- 11) Non-disposable equipment, such as pipette-aids, pipettors, tube racks, ice buckets, necropsy equipment, etc., must be designated and labeled "For use in Room 233 ONLY", and must never be removed from this room. After each procedure, all non-disposable equipment, including ice buckets and tube racks, must be wiped with 10% bleach. Any autoclavable equipment, such as scissors and forceps, must be immediately placed in autoclave pouches and then transferred a metal tray prior to transport to the designated autoclave in SDRI Room 16.1.

- 12) Disposable flasks or other labware containing infectious materials, such as cell cultures, must be labeled with a biohazard sticky label, well sealed, then wiped with 10% bleach prior to removing from the BSC. The items must be placed in a secondary container (such as a clear plastic Tupperware container), which is wiped with 10% bleach prior to transferring both primary and secondary containers out of Room 233 and to the incubator in Room 234. All PPE, with the exception of inner pair of gloves must be removed prior to exiting Room 233 with the cultures. The incubator must be labeled with a sign, "CAUTION: VACCINIA VIRUS".
- 13) All centrifugation steps should be performed in the centrifuge in Room 233, if possible. Tubes must be tightly sealed and wiped with 10% bleach prior to the removal from the BSC. In the event that plates must be centrifuged (ie. need to use Alegra centrifuge in Room 234), they must be sealed with parafilm prior to removal from the BSC. The items must be placed in a secondary container (such as a clear plastic Tupperware container) prior to transferring to the centrifuge in Room 234.
- 14) All samples destined for further analysis in Room 234, such as flow cytometry, must be inactivated in the BSC in Room 233 using a fixative such as $\geq 1\%$ paraformaldehyde. Following inactivation, cultures must be transferred to clean containers, such as cytometry tubes, held in a clean tube rack. The outside of the tubes and the tube rack must be wiped down with 10% bleach prior to removal from the BSC. The items must be placed in a secondary container (such as a clear plastic Tupperware container) prior to moving to the cytometer room off of Room 234.
- 15) BCU cages are to be opened within the BSC only. After mouse procedures are complete, BCU must be taped shut (attach lid to cage bottom), entire cage re-wiped with 10% bleach-moistened towel, then cage may be removed from the BSC. Cages should be then transferred to the small bench on the north wall of Room 233, and marked with a Biohazard sticker, labeled "Vaccinia Virus". This bench should be wiped with 10% bleach at the completion of each mouse experiment.
- 16) Research staff will euthanize mice by cervical dislocation at the termination of the study, or in the event of ill health (as defined in the animal study protocol). Carcasses must be transferred to a biohazard bag, which must be sealed and wiped with 10% bleach prior to removal from the BSC. Biohazard bags are then transferred to a metal tray and transported (using a cart) to the MSB incineration area in M605.
- 17) Sick mice, as defined in SOP 321, will not be treated nor will ACVS be notified, rather they will be immediately euthanized as described above.
- 18) Cage bottoms with soiled bedding and water bottle are changed on a 7, 10 or 14-day schedule, when 4, 3 or 2 mice occupy the cage, respectively. Cage tops, wires with remaining food and mouse houses will be transferred from the old cage to the new cage. Cage wires will be replenished with fresh food and water bottle. The inside and outside of the cage will be wiped with 10% bleach-moistened towel prior to removing from BSC. BCU cages containing dirty bedding will then be taped shut (attached lid to cage bottom), entire cage re-wiped with a 10% bleach-moistened towel, transferred to a biohazard bag, then removed from BSC. Cage will be transferred to a second biohazard bag, which is sprayed down with 10% bleach, prior to transferring (using a cart) to the Government

Certified SDRI autoclave in Room 16.1. Autoclaved cages are then transferred by the research staff to the DSB 6th floor cool room and cage washing facility, respectively. Cages may be washed using normal procedures by AC staff.

- 19) Prior to leaving Room 233, the gown and protective sleeves must be transferred to a biohazard bag which is then tied shut, wiped down with 10% bleach, removed from the BSC, and transferred to metal tray for transport to the designated autoclave in SDRI 16.1. Safety goggles should be stowed in a designated drawer, and hair net, mask and inner pair of gloves must be discarded in the biohazard bag-lined garbage can stationed beside the hood.
- 20) Autoclaving must be performed in the Government Certified autoclave in SDRI 16.1 (autoclave #4) and according to the UWO Autoclaving SOP, posted on the side of the autoclave. Scheduling of runs must be made in conjunction with SDRI autoclave staff. A sign "Vaccinia Virus" placed on the autoclave during the run. Research or SDRI autoclave staff will immediately begin an autoclave run of 121oC at 15 psi for 60 minutes. At the completion of the run, metal trays will be transported back the Room 233 BSC using a cart and contents may be transferred to the normal biohazard waste stream. Cages will be transported using a cart to the ACVS cage-washing station. Note that according to Government Certification Policy, the autoclave must be certified with a spore ampule test every 6 days.
- 21) All records documenting *in vitro* and *in vivo* procedures, as well as animal health and spore test records will be maintained by research staff in Room 232. Any paper materials taken into Room 233 (ie. protocols for a particular procedure) must be discarded in the biohazard bags inside Room 233; or alternatively, may be placed in a clean biohazard bag, tied shut, then autoclaved prior to transferring the paper material to lab notebooks.