

Protocol Description:

The two known cases of laboratory-acquired rabies infection are thought to have resulted from exposure to high-titered infectious aerosols. One case was due to a fixed Challenge Virus Standard and the other to an attenuated strain derived from the Street Alabama Dufferin strain. We wish to demonstrate the levels of virus in aerosols that will result in infections in dogs (vaccinated and not) that are exposed to these aerosols. Dogs will be exposed to aerosols containing various concentrations of virus and humanely euthanized eight to ten days after exposure. Postmortems and tissue evaluations will be used to determine if the dogs have been infected by the virus and the route of infection (lungs, ethmoid process, etc.).

Safety Plan:

Risk group assessment:

Risk Group 2 (RG2) agents are associated with human disease that is rarely serious and for which preventive or therapeutic interventions are *often* available.

Risk Group 3 (RG3) agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available.

- Risk Group 2 (RG2) for fixed virus and Risk Group 3 (RG3) for street virus
- BSL-2 and ABSL-2

Characteristics of virus:

- Single strand RNA virus (Rhabdovirus). The virus is excreted in the saliva; transmission is mainly due to a bite or salivary contamination of a wound or skin abrasion. The virus, when introduced into a muscle or subcutaneously, infects muscle fibers and muscle spindles after binding to the nicotinic acetylcholine receptors. It then enters the peripheral nerves and travels to the CNS where it multiplies in glial cells and neurons. The incubation period in humans is usually 2-8 weeks but can be as short as 10 days or as much as a year. The mode of transmission by inhalation is not yet known.
- The virus is inactivated on exposure to ultraviolet light, by heat (1 hour at 50° C) and by lipid solvents. It does not survive for long period outside the host unless protected in a cool, dark place.
- The virus is susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, 70% ethanol, and formaldehyde.

Containment:

- Laboratory practices. All viral manipulations will be performed in Biosafety cabinets that have exhaust air flow vented to the outside. Preparation of the viral

containing aerosols and exposure of the animals to the aerosols will occur in the same room. Dogs will be placed in cabinets wherein the viral aerosol will be introduced through masks that cover the dog's nose and mouth. All air from these cabinets will be vented to the outside. Post mortems will be performed using a downdraft necropsy table. Tissue handling will occur in a biosafety cabinet.

Engineering Controls:

- Biosafety cabinets (vented directly to the outside) and specially designed cabinets for exposure will be utilized.
- Necropsy table (down drafted), the airflow from which is vented directly to the outside.
- The virus will be stored frozen in an adjacent laboratory. Transfer of the frozen viral samples will be in 2-layered containers.

Personal Protective Equipment (PPE):

- The following PPE will be utilized during the steps indicated:
 - Virus preparation and aerosol preparation: double gloves, gowns, NIOSH approved N95 dust/mist respirators (or full face N95 respirators without the goggles), shoe covers.
 - Aerosol preparation: double gloves, gowns, NIOSH approved N95 dust/mist respirators (or full face N95 respirators without the goggles), shoe covers.
 - Animal handling (pre- and post-exposure): double gloves, gowns, NIOSH approved N95 dust/mist respirators (or full face N95 respirators without the goggles), shoe covers.
 - Exposure of animals: double gloves, gowns, NIOSH approved N95 dust/mist respirators (or full face N95 respirators without the goggles), shoe covers.
 - Necropsy: double gloves, gowns, NIOSH approved N95 dust/mist respirators (or full face N95 respirators without the goggles), shoe covers.
 - Tissue handling: double gloves, gowns, NIOSH approved N95 dust/mist respirators (or full face N95 respirators without the goggles), shoe covers.

- Cabinet cleanup: double gloves, gowns, NIOSH approved N95 dust/mist respirators (or full face N95 respirators without the goggles), shoe covers.
- Dog housing cleanup: double gloves, gowns, NIOSH approved N95 dust/mist respirators (or full face N95 respirators without the goggles), shoe covers.
- Small spill clean up: double gloves, gowns, NIOSH approved N95 dust/mist respirators (or full face N95 respirators without the goggles), shoe covers.

Storage

- Viral specimens will be stored at -70° C.
- Prepared aerosol solutions will be maintained for no longer than the day that they are prepared. The solutions will be mixed with 1% sodium hypochlorite solution for disinfection and discarded down the drain.
- Cultures will be stored at 40° C within incubation ovens. Cultures will be treated with 1% sodium hypochlorite solution

Transport

Viral specimens will be transported from room to room in a sealed, leak proof primary container that is securely positioned in a secondary leak proof, closable container having a clearly visible biohazard symbol on the outside. Specimens will not leave the laboratory suite area.

Dogs will be housed in a Class II biosafety cabinet during aerosol exposure. The biosafety cabinet is located in the Animal Biosafety Level 2 room assigned to this project. Consequently, dogs will not be transported to another location for aerosol exposure.

Dogs will be euthanatized within the ABSL2 facility, placed in plastic bags and a secondary leak proof container for transport to the necropsy facility. After the post mortem the dogs will be placed in biohazard bags and boxes. The boxes will be appropriately marked with the biohazard symbol and labeled for transport as infectious waste per university policies.

Spill Management:

A biological spill kit will be available in all rooms in which viral specimens are in use. All personnel in the laboratory will be trained in spill containment procedures. The kit will contain bleach (4.5% sodium hypochlorite solution for preparation of solutions,

absorbent material (cat litter), waste containers (biohazard bags), and mechanical tools (forceps, dustpan and broom). For spills in a hood or biosafety cabinet, a standard operating procedure (SOP) has been developed for the potentiality. Basically, the spill is covered with absorbent material, disinfected with 1% sodium hypochlorite solution (freshly prepared from the stock solution); the spilled container and the absorbent material is transferred to a biosafety bag; the area is then disinfected with bleach solution, rinsed with water, and dried. Sharps are handled with the forceps. For spills outside a hood or safety cabinet, the room will be evacuated immediately for one half hour. The same procedures as above will then be applied. Dress and PPE are as above. For spills on a person, contaminated clothing will be removed and placed in a biohazard bag; contaminated skin will be flushed with water and then disinfected with 70% alcohol. For large spills, the area will be evacuated. A no-entry sign will be posted and EHS (2-1284) will be contacted immediately.

Waste Disposal:

All material coming in contact with the virus will be disinfected with freshly prepared 1% sodium hypochlorite solution, placed in a biohazard bag, and then placed in a biohazard burn box for disposal. Spills clean up will be treated the same way.

Bloodborne Pathogens Compliance:

All individuals coming in contact with animal carcasses, tissues or body fluids must comply with the OSHA Bloodborne Pathogen Standard (i.e., Exposure Control Plan, Universal Precautions and Annual Training).

Medical Surveillance:

All persons in the laboratory will be enrolled in a medical surveillance plan covering the research. The individuals will be or have a history of being vaccinated with the human diploid cell vaccine (HDCV) and have titers drawn every year during the research. Appropriate follow up at the University's Employees Health Services has been discussed with the occupational physician there. If UEHS is closed, the exposed individual will go to the hospital's Emergency Department for follow up treatment. Pregnant or immunosuppressed individuals will be discouraged from participating in the research.

Animal Husbandry:

Carcasses will be disposed by wrapping in absorbent material, bagging and boxing. The boxes will then be appropriately labeled and transported offsite for incineration. Animal waste will be flushed down the drain during daily cleaning of the dog runs. Individuals will wear PPE as indicated above.

The principal zoonosis of interest is rabies. These will be conditioned dogs that have been checked for disease by ULAR veterinarians prior to entering the laboratory.

Disinfection:

Chlorine compounds. A solution of 1% sodium hypochlorite will be used for disinfection. The minimum contact time is five minutes. Contact times will be proportionately increased relative to concentration of organic matter present.

NOTE: Sodium Hypochlorite solutions should be made up daily. Typical household bleach is 4.5% sodium hypochlorite. Steam sterilization may be used but all materials must be placed into a red bag and biohazard burn box following sterilization. The biohazard boxes will be shipped offsite for incineration.

Training and Experience:

All members of the laboratory have attended the Laboratory Safety Course offered by Environmental Health and Safety. "Worker's Right to Know" training is completed yearly or when a new individual joins the group. Respirator evaluations are combined with the annual visit to Employee Health Service for all individuals. Respirator training is completed by EHS annually. Bloodborne Pathogen training is completed annually also. Training on SOPs for the Laboratory Standard is completed annually. All individuals have completed the ULAR Animal course as directed by the USDA. Training materials are checked annually with members of EHS, ULAR or the hospital safety team.