Suspected rabies specimen submission and processing.

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This protocol describes the biosafety procedures involved in the referral of rabies-positive or rabies-suspected biological specimens. These recommendations are based on those proposed by Cornell University College of Veterinary Medicine (https://ahdc.vet.cornell.edu/samples/rabies.cfm) and the National Working Group on Rabies Prevention and Control published by the Centers for Disease Control and Prevention (CDC)[1].

Rabies transmission routes

The most common mode of rabies virus transmission is through the bite and virus-containing saliva of an infected host. Though transmission has been rarely documented via other routes such as contamination of a fresh wound with infected saliva, contamination of mucous membranes (i.e., eyes, nose, mouth), aerosol transmission, and corneal and organ transplantations. The virus cannot invade intact skin. Exceptionally, respiratory and oral transmission can occur. Personnel working in rabies laboratories are at risk of rabies infection through accidental injection or contamination of mucous membranes with rabies virus contaminated material and by exposure to aerosols of rabies infected material. When working with infected animals, the highest viral concentrations are present in central nervous system (CNS) tissue, salivary glands, and saliva, but rabies viral antigens may be detected in all innervated tissues. BSL-2 and/or ABSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious materials or animals.

Rabies virus is fragile outside the host and is not viable for long. Environmental contamination is of very little significance, other than aerosol contamination in bat caves. Rabies virus is inactivated by exposure to sunlight and temperature above 56°C. Rabies virus is inactivated by exposure to 70% ethanol, phenol, formalin, ether, trypsin, β-propiolactone, and some other detergents [2].

Biosafety measures

1) All persons involved in handling or processing of potentially rabies-infected specimens should receive pre-exposure immunization with regular serologic tests and booster immunizations as indicated (CDC, MMWR, 48: 1-22, 1999). Unimmunized individuals should not enter laboratories where work with rabies-suspected samples is conducted.

2) Rabies vaccination also is recommended for all individuals entering or working in the same room where lyssaviruses or infected animals are used.

3) Prompt administration of postexposure booster vaccinations is recommended following recognized exposures in previously vaccinated individuals per current guidelines.

4) All rabies-suspected tissues must be disposed of as medical waste and all activities related to the handling of animals and samples for rabies diagnosis should be performed using appropriate biosafety practices to avoid direct contact with potentially infected tissues or fluids (CDC and National Institutes of Health, Biosafety in Microbiological and Biomedical Laboratories, 4th edition, U.S. Government Printing Office, 1999).

5) All manipulations of tissues and slides should be conducted within a Class II Biological Safety Cabinet and in a manner that does not aerosolize liquids or produce airborne particles.

6) Barrier protection is required for safe removal of brain tissue from animals submitted for rabies testing and sampling.

7) At a minimum, barrier protection during necropsy should include the following as Personal Protective Equipment (PPE): heavy rubber gloves, laboratory gown and waterproof apron, boots, surgical masks, protective sleeves, and a face shield.

8) Care should be taken to protect eyes and hands during manipulation of specimens and during clean up of the working and surrounding area.

9) Because of the risk of breakage, glass vials and tubes are unacceptable for specimen storage.

10) The most likely sources for exposure of laboratory and animal care personnel are accidental parenteral inoculation, cuts, or needle sticks with contaminated laboratory equipment, bites by infected animals, and exposure of mucous membranes or broken skin to infectious tissue or fluids. Infectious aerosols have not been a demonstrated hazard to personnel working with routine clinical materials or conducting diagnostic examinations.

11) BSL-2 and/or ABSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious materials or animals.

12) Additional primary containment and personnel precautions, such as those described for BSL-3, are indicated for activities with a high potential for droplet or aerosol production, and for activities involving large production quantities or high concentrations of infectious materials.

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Acceptable specimens include only

1) Rabies vector species showing clear signs of rabies infection, from an area with or without previous confirmed case.

2) A mammal not commonly recognized as a rabies vector, but showing clear signs of rabies infection.

3) A domestic animal that dies or is euthanized under the care of a veterinarian for which rabies is part of the differential diagnosis of neurologic disorder.

4) Specimens associated with laboratory-approved enhanced surveillance programs associated with wildlife vaccination programs.

5) When working with infected animals, the highest viral concentrations are present in central nervous system (CNS) tissue, salivary glands, and saliva, but rabies viral antigens may be detected in all innervated tissues.

Unacceptable specimens include

1) Live animals will not be accepted by the laboratory.

2) Intact or entire heads of suspected animals will not be accepted.

3) Whole brainstem and cerebellum specimens.

Bagging, transport and shipping of gross anatomy specimens

1) It is not always feasible to open the skull or remove the brain of an infected animal within a BSC, but appropriate methods and personal protection equipment, including dedicated laboratory clothing, heavy protective gloves to avoid cuts or sticks from cutting instruments or bone fragments, and a face shield or PAPR to protect the skin and mucous membranes of the eyes, nose, and mouth from exposure to tissue fragments or infectious droplets should be used.

2) Specimens should be submitted to local rabies surveillance lab in at least **TWO** separately sealed plastic bags and kept refrigerated (at least 4 ºC and optimally frozen at <12 ºC) in a plastic cooler.

3) Plastic bags should not leak during transport.

4) Suspected specimens must be accompanied by a complete rabies specimen history form.

5) Specimen transit time should be as short as possible, preferably within 48 hours. A fresh, unfixed brain sample is critical to a rapid and accurate diagnosis of rabies. Refrigeration will preserve a sample for at least 48 hours. Freezing of the sample for transit will not reduce the sensitivity of the test, but may introduce additional testing delays and impede recognition of proper gross anatomy.

6) Substantial green color, liquefaction, desiccation, or an unrecognizable gross anatomy are indicative of an unsatisfactory sample.

Sampling of gross anatomy specimens

1) Gross specimens should only be opened and handled by previously immunized personnel with experience in the handling of potentially infected animals.

2) Gloves, respiratory protection (at least N95 respirators) and lab coat must be worn during sampling of gross anatomy specimens.

3) Rabies virus normally affects the whole brain of most animals, but because spread may be unilateral, especially in larger animals, therefore both hemispheres should be sampled.

4) Left cerebral hemisphere samples not wider than 5 mm in width should be placed in a previously labelled cryovial (Sample A) previously loaded with 1.8 mL of VTM (see associated protocol).

5) Right cerebral hemisphere samples not wider than 5 mm in width should be placed in a previously labelled cryovial (Sample B) previously loaded with 1.8 mL of VTM.

6) Cerebellum samples not wider than 5 mm in width should be placed in a previously labelled cryovial (Sample C) previously loaded with 1.8 mL of VTM.

7) Brain stem samples not wider than 5 mm in width should be placed in a previously labelled cryovial (Sample D) previously loaded with 1.8 mL of VTM. Although the hippocampus was once the tissue of choice for histologic tests for Negri bodies, hippocampus is of limited additional value when brain stem and cerebellum are examined.

8) After placing in cryovials, plastic bags should be sealed again and sprayed with 0.5%hypochlorite solution. Allow for contact time of at least 5 minutes and no more than 10 minutes.

9) Spray cryovials with 0.5% hypochlorite solution after closing and before placing inside bag or cardboard box for transport. Allow for contact time of at least 5 minutes and no more than 10 minutes.

10) Spray transport cooler or box with 0.5% hypochlorite solution after placing cryovials inside and before loading into vehicle. Allow for contact time of at least 5 minutes and no more than 10 minutes.

In-lab manipulation or processing of rabies-infected specimens

1) Personnel entering the laboratory should remove street clothing and jewelry, and change into dedicated laboratory clothing and shoes, or don full coverage protective clothing (i.e., completely covering all street clothing). Additional protection may be worn over laboratory clothing when infectious materials are directly handled, such as solid-front gowns with tight fitting wrists, gloves, and respiratory protection. Eye protection must be used where there is a known or potential risk of exposure to splashes.

2) Once inside the final testing laboratory external coolant or transport box should be sprayed with 0.5% hypochlorite solution before entering the BSL2+ laboratory and allowed for contact time of at least 5 minutes and no more than 10 minutes.

3) Outer transport container or coolant should only be opened inside BSL2+ laboratory and the inner cryovial container opened and handled only inside a BSC within the BSL2+ laboratory.

4) Once inside BSL2+ lab, full personal protective equipment including outer overall, faceshield, PAPR or respirators and double gloves should be worn.

5) Cryovials should again be sprayed with 0.5% hypochlorite solution and allowed a 5-minute contact time before drying and re-labelling. They should then be placed in the BSL3 -80 ºC ultrafreezer for permanent storage.

6) Personal protective equipmente should be sprayed with 0.5% hypochlorite solution and allowed a 5-minute contact time before drying and hanging or discarding.

7) 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.

8) 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.

9) The use of needles, syringes, and other sharp objects should be strictly limited.

10) Decontaminate all materials for disposal by steam sterilisation, chemical disinfection, and/or incineration

Decontamination and disinfection

1) Rabies virus can be inactivated by sodium hypochlorite, 45-75% ethanol, iodine preparations, quaternary ammonium compounds, formaldehyde, phenol, ether, trypsin, β-propiolactone and other detergents.

2) It is also inactivated by a very low pH (below 3) or very high pH (greater than 11).

3) This virus is susceptible to ultraviolet radiation. It is rapidly inactivated by sunlight and drying, and (in dried blood and secretions) it does not survive for long periods in the environment.

References

1. Hanlon CA, Smith JS, Anderson GR. Recommendations of a national working group on prevention and control of rabies in the United States. Article II: Laboratory diagnosis of rabies. The National Working Group on Rabies Prevention and Control. J Am Vet Med Assoc. 1999 Nov 15;215(10):1444-6. PubMed PMID: 10579039.

2. Bleck, T. P. (2006). Rabies. In R. L. Guerrant, D. H. Walker & P. F. Weller (Eds.), Tropical Infectious Diseases: Principles, Pathogens, and Practice (2nd ed., pp. 839-851). Philadelphia, PA: Elsevier Churchill Livingston.

3. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition

Revision history

1.0 Original document.