Prion Research Procedures

Introduction

All protocols for working with prions must be approved by the Institutional Biosafety Committee (IBC) before work is started. Submit SOPs and an application form to the committee, http://www.research.umn.edu/ibc/forms.html. Approved protocols are effective for three years with annual review.

Note: Laboratory space assignment and animal housing biosafety levels must be included as part of the IBC approval process.

There are no known effective treatments or vaccines for prion (also known as Transmissible Spongiform Encephalopathies or TSEs). It is necessary to handle prions with extreme caution, both for worker protection and for environmental protection. The highest concentrations of prions in infected animals are in the central nervous system and its coverings. Studies indicate that it is likely that high concentrations of prions may also be found in spleen, thymus, lymph node, and lung tissue.

Scrapie is a prion disease that affects sheep and goats. Chronic Wasting Disease (CWD) is a prion disease that affects deer, moose, and elk. There has been no documented evidence that either scrapie or CWD can be transmitted to humans. Bovine Spongiform Encephalopathy (BSE) is a prion disease that normally affects cattle.

Cruetzfeldt-Jakob Disease (CJD) is a rare human prion disease. A form of CJD called variant Creutzfeldt-Jakob disease (vCJD) has been diagnosed since 1996 and is thought to be linked to the consumption of meat products derived from BSE-infected cattle.

A USDA permit is required to receive and work with animal prions.

Note: Import includes crossing state lines.

Exposure Control Methods

- Personnel working with prions cannot have contact with any other animal colonies or with susceptible animal species. Prion animal colonies must have separate air supply and exhaust systems (per USDA).
- Follow Biosafety Level 2 requirements as outlined in the Biosafety Level 2 Procedures Fact Sheet.
- The principle investigator or lab supervisor must train all workers in specific handling procedures for prions. Written Standard Operating Procedures (SOPs) must be available for worker reference.
- Lab access must be limited to trained individuals. If non-trained individuals need to enter the lab they must be accompanied by a trained individual.
- One of the main precautions to be taken when working with prions is to avoid puncture of the skin. Therefore, sharps and glass should not be used unless it has been determined that there is no other alternative.
- All work surfaces should be protected with disposable bench covers that are handled as low level prion waste.
• All manipulations with prions should be done in a biological safety cabinet. Cabinet needs to be certified annually.
• Open centrifuge rotors in a biological safety cabinet.
• Transport of all prion materials outside a biological safety cabinet requires secondary containment.
• All workers should wear appropriate personal protective equipment:

1. Double gloves
2. Lab coat (disposable back closing preferred)
3. Goggles to prevent touching of face
4. Full face protection if it is necessary to handle prion material outside the biological safety cabinet

**Spill Clean-up and Disinfection**

Prions are characterized by extreme resistance to conventional inactivation procedures including irradiation, boiling, dry heat, and many chemicals (formalin, betapropriolactone, alcohols). Fixation with alcohol, formalin, or glutaraldehyde strongly stabilizes the infectivity of prions and makes them more difficult to inactivate. Formalin-fixed and paraffin-embedded tissues, especially of the brain, remain infectious. As a consequence, contaminated materials should not be exposed to fixation reagents, and should be kept wet between the time of use and disinfection by immersion in chemical disinfectants. Fixed material that contain or may contain prions must be disposed of as "prion waste".

**Note:** Be sure to decontaminate all equipment prior to maintenance or service work.

**Effective Disinfectants**

- 40% household bleach, per USDA requirements (1 part 5.25% bleach plus 1.5 parts water to produce 40% bleach solution, which equals to 20,000 ppm) for 1 hour
- Freshly made 2N NaOH for 1 hour

In addition to the above disinfectants, prions can be inactivated by:

- Steam autoclaving at 134°C for 1 hour or
- Incineration

**Disinfection of Surfaces and Laboratory Equipment**

- Flood with 2.0 N NaOH or 40% household bleach and let stand for 1 hour. Mop up and rinse with water.
- If possible, autoclave the equipment at 134°C for 1 hour
Spill Clean-up

- Notify other lab workers that a spill has occurred.
- It is important to keep contaminated surfaces moist until decontamination is complete as the infectious agents become even more resistant to chemical inactivation when dry.
- Cover spill with paper towels or other absorbent material.
- Saturate with 2N NaOH or 40% bleach and let sit for at least 1 hour. If possible, leave the lab to avoid prolonged breathing of fumes. Direct other workers not to enter the lab.
- Dispose of absorbent material and any other clean-up material in yellow waste bag.
- Report all spills to lab supervisor.

Waste Handling

The following procedures are to be followed for disposing of prion waste. Other methods of waste disposal must first receive IBC approval.

Non-Tissue Low Level Solid Prion Waste (Includes Animal Bedding)

- Place waste in yellow bag.
- Place in yellow barrel for incineration.
- Call Facilities Management (5-6481) to arrange to have a yellow barrel delivered to the lab and for pick-up / replacement of yellow barrels.

Liquid Prion Waste

- Dispose in the Tissue Digester in the Veterinary Diagnostic Laboratory (St.Paul campus). Call 625-0255 to make arrangements.
- Autoclave at 134°C for 1 hour.
- Treat with final concentration of 40% bleach or 2N NaOH for 1 hour. Neutralize with acid until the pH is between 5 and 8 and dispose of down the drain or hold for chemical hazardous waste disposal.

Sharps & Histology Slides

Place sharps in a puncture proof red sharps container. When container is no more than 3/4 full, close and seal the container. Place container in a yellow waste bag for incineration.

Note: Small quantities of sharps may be placed in other small, sealed, puncture-proof containers and put in yellow bag.

Infected Animal Carcasses and Tissue
Dispose of all infected carcasses and tissue in the tissue digester adjacent to Vet Diagnostics on the St. Paul campus. Call 625-0255 to make arrangements.

**Accident Response**

- Needle sticks or laceration: gently encourage bleeding, wash with warm soapy water, rinse, dry and cover with a waterproof dressing.
- Accidental contamination of skin: wash with warm soap and water for 15 minutes. (May clean briefly, 1 minute, with 0.1N NaOH or 10% bleach, followed by washing with warm soap and water for 15 minutes.)
- Splashes to the eye or mouth: irrigate with copious amounts of water for 15 minutes.
- For all injuries and biological exposures, call 911 or immediately seek medical treatment if lab personnel is overtly exposed to Prions.
  - For urgent care employees may go to HealthPartners Occupational and Environmental Medicine (M/F day time or Urgent Care after hours), or UMMC-Fairview Hospital (24 hrs). You may seek medical attention at the closest available medical facility or your own healthcare provider.
  - Follow-up must be done by HealthPartners Occupational and Environmental Medicine.
- Report the incident to your supervisor as soon as possible, fill out the appropriate documentation.
  - [Employee First Report of Injury](#)
  - [Supervisor Incident Investigation Report](#)
- Send [Incident Report Form](#) to the IBC if exposure has occurred during work on an IBC protocol.
- Report all biohazard exposures to the Office of Occupational Health and Safety (612-626-5008) or uohs@umn.edu.

**Note:** It is important to fill out all of the appropriate documents to be eligible to collect workers compensation should any complications from the hazardous exposure arise in the future.

**References**


*BL2 TSE Laboratory Standard Operating Procedures*, U.S. National Institutes of Health (NIH), Rocky Mountain Laboratories (RML). Hamilton, Montana