

HARVARD BRAIN TISSUE RESOURCE CENTER

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[1-800-272-4622] <http://www.brainbank.mclean.org>

Restless Legs Syndrome (RLS) Protocol

Important notes

1. Please call the Brain Bank 1-800-272-4622 before removing the brain.
 2. Do not embalm the decedent until after the brain and other tissue have been removed.
 3. Please place the decedent into mortuary refrigeration as soon as possible, but within 6 hours after death.
 - If mortuary refrigeration is not available, the whole fresh brain should be removed immediately and prepared following *Whole Fresh Brain Tissue Protocol* as listed below and then kept refrigerated until the proper shipping arrangements can be made.
 4. Complete the postmortem as soon as possible after death, preferably within 12 hours after death.
 5. The Brain Bank will send the proper shipping materials to your location
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Tissue preparation

1. Complete the pathologist time record sheet provided.
2. **CSF:** Before removing the brain from the skull, obtain a 5-10 ml sample of CSF from the lateral ventricles. Freeze the sample in one or two labeled plastic sample tubes.
3. **Brain:** Remove the entire brain, including the brain stem and cerebellum and as much of the cervical as possible on initial sectioning (see below). Please follow the *whole fresh brain tissue protocol*
4. **Spinal Cord:** Removal of the entire length of spinal cord is optimal, but may not be possible.
 - Full Cord:**
 - ♦ Cut the cord into its three main anatomic subdivisions: cervical, thoracic, and lumbar.
 - ♦ Fixed cord sample: Cut a 2.5-cm (1-inch) thick cross-section segment from the rostral portion of each cord region and place these segments in 10% formalin.
 - ♦ Frozen cord sample: Place the remaining segments into plastic bags labeled with the region each contains and freeze.
 - Cervical Cord Only:** If removal of the full cord is not possible, after removing the brain from the cranium, reach through foramen magnum with forceps, grasp arachnoid of cord, cut the denticulate ligaments with scissors (long curved scissors are optimal), and then cut across the cord as caudal as possible. Evaluation of cervical cord is critical to the evaluation of restless leg syndrome, and obtaining this section is very much appreciated.
5. **Serum:** Freeze 5-10 ml sample in plastic sample tubes.
6. **Peripheral Nerve:** Cut two 2x1x0.2-cm.samples from the upper and lower extremities
 - ♦ Place one piece from each extremity in a labeled cassette and immerse in 10% formalin.
 - ♦ Place one piece from each extremity in a labeled, sealed bag and freeze at -70°C, or on crushed ice
7. **Dorsal root ganglia:** Remove as many dorsal root ganglia as possible

- ♦ Place two ganglia in labeled, sealed bags and freeze at -70°C, or on crushed ice
- ♦ Place remaining ganglia in labeled cassettes and immerse in 10% formalin.

8. Label all specimens clearly with the decedent's name.

Fresh Brain Preparation

1. Place whole fresh brain into double plastic bags. ADD NO SOLUTION. Seal each bag securely.
2. Place double-bagged fresh brain into the gallon plastic pail. Fill pail with cold water.
3. Pituitary gland (optional) after removing the brain, dissect out the intact pituitary gland, place it into a labeled, small plastic bag, seal bag securely, and then place it into the gallon plastic pail.
4. Press lid on firmly to seal the pail.
5. Label pail with the donor's name.

Packaging

6. Keep Styrofoam cooler in the plastic bag-lined cardboard shipping box. This shipping box assembly will be used for transporting the brain to the Brain Bank.
7. Securely seal the pail within the plastic bag.
8. Place the bagged pail into the center of the plastic-lined Styrofoam shipping cooler.
9. Fill the Styrofoam cooler with chipped ice or ice cubes (wet ice) so that the pail is completely surrounded by ice.
10. Securely seal the inner plastic bag. Replace the lid on the cooler box. Securely seal the outer plastic bag.
11. Enclose a copy of the donor consent, pathology time record sheet, and any available medical records along with the tissue. Securely tape the cardboard box closed for shipping

Shipping

The Brain Bank will send the proper shipping instruction and materials to your location. Call the brain bank when the tissue is ready for shipping. To facilitate transportation, we will need your exact location and the time the shipping box will be ready. Courier arrangements will be made to pick up the box from your facility

Thank you for carefully following this preparation procedure. Your generous assistance in providing valuable tissue for research is highly valued and very much appreciated.