**Purpose**

Occasionally, a brain biopsy or autopsy is performed in order to establish a diagnosis of Creutzfeldt-Jakob disease (CJD). Tissue containing the agent causing CJD and other prion diseases remains infectious following formalin fixation, standard tissue processing, and paraffin embedding. Therefore, special sampling and processing is required in order to render the tissue non-infectious. Avoid extra handling of tissue, and never put it in a cryostat.

**Procedure**

**Contact the neuropathologist on-call to review how the specimen is to be handled.** Generally, one portion of the specimen is frozen fresh for western blot, and the remainder fixed for microscopic examination, storing and labeling all containers as CJD biohazards.

The following steps outline how the tissue is to be processed and saved:

* Cover work area with a water resistant barrier (blue pad, white absorbent side up). Use Universal Precautions and cut-resistant gloves.

* Ideally two separate containers will be sent to pathology, each with unfixed gray and white matter on saline-soaked Telfa. (Also, they often send dura in a separate container, not useful to r/o CJD, but helpful with r/o vasculitis.) If only one brain biopsy specimen is sent, it should be divided initially to obtain two separate pieces. One piece will then be processed for shipping to the National Prion Surveillance Center (see #3 below) and the other piece processed for light microscopic examination (see #4 below). It is recommended that the House Officer who begins a CJD tissue process it through to the point that the tissue is safe (From step 1 -6 of this protocol).

You will see from the instructions below that each of these two pieces will again be bisected for proper processing if there is sufficient tissue available. If not, just go with two pieces: One to freeze and send, and one for formalin-FORMIC ACID-formalin described below.

* For **western blot**, the National Prion Disease Pathology Surveillance Center (NPDPSC) at Case Western requests at least 10 mg of tissue (pea-sized or 0.5 cm diameter) for biopsies. Put tissue into a ½ inch diam plastic vial (same vial used in room for flow cytometry) and screw on the cap (no OCT needed). Put the vial into a biohazard bag and close bag.

If there is enough tissue, split the fresh tissue into 2 containers before freezing, in case the 1st container is lost in shipping or the case turns out not to be CJD but a metabolic or genetic disease. The size of enough tissue would be the size of a kidney bean or about 1.5 x 1 x 0.5 cm.

Store initially in the – 70 freezer in autopsy room, noting the exact freezer drawer it is in. Note this in the log and on a note taped onto your Residents’ morgue-computer screen to Diana French, who will assist with packaging. (If Diana is out of town, her pager will forward to her back-up.) Leave a copy of the surgical pathology specimen sheet which has enough information to identify the case and the patient’s name and numbers, so Diana can print out a history. Our Morgue Staff are specially trained to handle CJD. To avoid risk to other staff, it is important that they handle this material.

Check with Diana the very next morning to see what help she needs. If necessary, remind her that Dr. McKeever's, Camelo-Piragua or Lieberman’s CJD cases do NOT require approval to go to CaseWestern, and it must be shipped on dry ice and properly labeled so that the overnight service will take it.
* Fix ALL remaining tissue for **light microscopic examination**, in formalin for at least 24 hours first. Write on the container the time and date that each reagent was started.

After at least 24 hours in formalin, proceed. If there is sufficient tissue, split this tissue in half and separate halves into two containers. The size of enough tissue would be the size of a lima bean or about 2 x 1.2 x 0.5 cm. Both containers need gray and white matter. If the fixed specimen was too small to divide, this second piece must be skipped. Treat tissue in the first container with undiluted formic acid (minimum 95 % FA) for 1 hour. There is a small space in the isolation room of the morgue for processing CJD tissue where the FA, cut gloves, chemical waste container for FA, and other items are stored. Transfer FA-treated tissue into fresh formalin and fix for at least 48 hours.

Submit for paraffin processing in our histology lab, room 2F341, explaining the agent and its disinfection with FA to our histotechnologist. Until we know that CJD has been ruled out, ONLY FA-treated tissue goes to our histotechnologists.

If there was a second container with tissue not treated with FA, keep it in standard formalin until CJD is either diagnosed or ruled out. This standard wet tissue is for cases that prove not to be prion disease, but something different that requires a labile stain, other procedure from paraffin, or post-fixing in glutaraldehyde for electron microscopy.

* **Decontamination of work area:** Soak instruments in undiluted sodium hypochlorite bleach for 1 hour. Place blue pad in medical waste. Clean surfaces with undiluted sodium hypochlorite bleach.

* **Store and label all containers as biohazards.** Contact the neuropathologist: The frozen western blot specimen and FA treated paraffin sections are done first. If the diagnosis of a prion disease like CJD is made, excess tissue must be safely discarded or donated to the NPSC.

\*\*Footnote:

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Phone and verify the address before sending. Include a brief clinical history and a copy of the gross or preliminary pathologic diagnosis with the specimen.

**References**

Johnson R, Gibbs C. CJD and related transmissible spongiform encephalopathies. New Engl J Med 339: 1994-2004, 1998.

Rosai J. Ackerman's Surgical Pathology, eighth ed. St. Louis, Missouri 1996. Mosby-Year Book, pp. 2267-8.

Castellani, et al. Biopsy diagnosis of CJD by Western blot. Human Pathol 28:623-6, 1997.

Brown P, et al. A simple and effective method for inactivating virus infectivity in formalin-fixed tissue samples from patients with CJD. Neurology 40:887-890, 1990.