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Protocol status: Working
We use this protocol and it's working

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Autopsy and Brain Collection and Fixation

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ABSTRACT

Protocol for autopsy and brain collection and fixation in the Kordower Laboratory.

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MATERIALS

- Shoe covers
- Impermeable gown
- Nitrile Gloves
- N95 Respirators
- Hair Covers
- Face Shields
- Autopsy Head Block
- Scalpel Handles
- #21 Scalpel Blades
- #61 Scalpel Blades
- Toothed Thumb Forceps
- Allis Tissue Forceps
- Skull Breaker
- Needle Holder
- Curved Scissors
- Postmortem Needle
- Postmortem Thread
- Autopsy Saw and Blade
- 12" Autopsy Knife
- Plastic Cutting Board
- Disposable Absorbent Pads
- Plastic 1 gal Bucket with lid
- 4L of Buffered 4% Paraformaldehyde of Buffered Saline
- Liquid Nitrogen
- Aluminum Foil
- 0.1 M phosphate buffered saline (PBS; pH 7.4) containing 2% dimethyl sulfoxide (DMSO) and 10% glycerol
- 0.1 M phosphate buffered saline (PBS; pH 7.4) containing 2% dimethyl sulfoxide (DMSO) and 20% glycerol
- Cryoprotectant (0.1 M PBS, pH 7.4, containing 30% sucrose and 30% ethylene glycol)
- 30-50 mL Centrifuge Tubes
- Lab Marker
- Digital Camera

Autopsy

1 Everyone present for autopsy must don Personal Protective Equipment (impermeable gown, shoe covers, hair cover, N95 respirator, face shield, two pairs of nitrile gloves).

2 Place body on table in dorsal recumbency.

3 Place absorbent pads under head and over face. Place head block under absorbent pad supporting head.

4 With #21 scalpel, make a coronal incision in the scalp from the top of one ear to the top of the other ear. Bias the incision a few centimeters dorsal to the coronal midline so that the incision can be hidden by a pillow.

5 Retract the anterior scalp with forceps while separating the subcutaneous attachments with scalpel. Evert the scalp over the eyes and nose until the brow ridge is exposed.

6 With autopsy saw, cut through the skull from ear to ear, just superior to the brow ridge. Avoid cutting too deeply and damaging the cortex. Use skull breaker to separate bones by inserting into gap and twisting.

7 Roll the body into ventral recumbency and retract the posterior scalp in the same manner until the occipital bun is exposed.

8 With autopsy saw, cut through the skull from ear to ear, across the occipital bun. Avoid cutting too deeply and damaging the cortex. Use skull breaker to separate bones by inserting into gap and twisting.

9 Using curved scissors, separate the dura from the skull cap either by cutting or blunt dissection. Set the skull cap aside.

10 Remove the remaining dura from the top of the brain with curved scissors, being careful not to tear the cortex. Blunt dissection will help separate the dura in the longitudinal fissure.

11 Cut the dura around the cerebellum with curved scissors and pull the cerebellum forward to expose the spinal cord.

12 Reach through the foramen magnum and cut the spinal cord as far down as possible with curved scissors.

13 Cut the cranial nerves and vessels with curved scissors. Cut the vessels close to the brain, leaving room for them to be clamped during arterial embalming.

14 Place the brain in bucket half filled with buffered saline (if collecting fresh-frozen tissue) or buffered 4% paraformaldehyde, then fill bucket the rest of the way and cap with lid and store at 4ºC until processing.

14.1 If post-mortem interval (PMI) is less than 6 hours, can collect fresh-frozen tissues from one hemisphere immediately following autopsy. If PMI is more than 6 hours, fix entire brain. Store in buffered 4% paraformaldehyde at 4ºC for 24 hours prior to processing.

15 Blot any remaining fluids inside the skull with gauze or towels. These can be left in the skull to continue absorbing fluids. Replace the skull cap and revert the skin.

16 Using Postmortem needle and thread, suture the skin in a simple interrupted pattern.

Tissue Processing

17 Remove brain from saline or fixative and blot dry. Weigh whole brain, record weight, and take photos.

18 Place the brain on the cutting board ventral side down and with 12" autopsy knife hemisect the entire brain, including the brainstem and cerebellum. Use caution to avoid cutting the medial cortex.

19 Place each hemisphere on the cutting board lateral side down. Using #61 scalpel, cut and separate the brainstem and cerebellum at a line from under the pineal body to the optic chiasm. Do the same for the other hemisphere.

Fresh-Frozen Tissue

20 If post-mortem interval (PMI) is less than 6 hours, collect fresh-frozen tissues from one hemisphere as follows.

21 Place the hemicerebrum to be frozen on the cutting board ventral side down and with 12" autopsy knife section the entire brain coronally in 1 cm increments, taking care to keep cuts perpendicular to the midline. Use long strokes of the knife rather than a sawing motion.

22 Take photos of each slab, then wrap in aluminum foil and number sequentially from rostral to caudal.

23 Wrap the brainstem and cerebellum in one piece in aluminum foil.

24 Place each wrapped slab in liquid nitrogen for 5 min then store all frozen slabs in a plastic tub at -80°C.

Fixed Tissue

25 Place the hemicerebrum to be fixed on the cutting board ventral side down and with 12" autopsy knife section the entire brain coronally in 2 cm increments, taking care to keep cuts perpendicular to the midline. Use long strokes of the knife rather than a sawing motion.

26 Take photos of each slab, then immersion fix all slabs in buffered 4% paraformaldehyde for five days at 4ºC.

27 After fixation, transfer slabs to 0.1 M phosphate buffered saline (PBS; pH 7.4) containing 2% dimethyl sulfoxide (DMSO) and 10% glycerol for two days at 4ºC.

28 Next, transfer slabs to 0.1 M PBS (pH 7.4) containing 2% DMSO and 20% glycerol for at least two days at 4ºC. Store in this solution until sectioning.

29 Sequentially number 18 centrifuge tubes and fill each halfway with cryoprotectant (0.1 M PBS, pH 7.4, containing 30% sucrose and 30% ethylene glycol).

30 Block tissue areas to be sectioned and then section at 40 µm thickness on a freezing sliding microtome. Collect serial sections into sequentially-numbered centrifuge tubes such that each tube contains every 18th section throughout the tissue.

31 Store tissue sections in cryoprotectant (0.1 M PBS, pH 7.4, containing 30% sucrose and 30% ethylene glycol) at -20ºC long term.