Clinical Diagnosis: Dementia:

(i) Alzheimer's disease

(ii) Dementia with Lewy bodies

(iii) Normal adult brain

Protocol: Block taking, histology and immunohistochemistry

SLICING:

The first incision is normally the one separating the hindbrain (brainstem and cerebellum) from the cerebral hemispheres. This should be made by a clean transverse cut across the midbrain, at right angles to the long axis of the brain stem.

For coronal slices, make the first cut through the mamillary bodies passing through the anterior margin of the thalamus. Place the anterior part of the cerebrum, cut surface down, between a pair of 0.5cm guides and lay out posterior surface upward, with the right side on the right. The procedure is repeated for the posterior part. Blocks for paraffin wax embedding are cut at 0.5cm.

Block taking

1. As soon as the brain or hemi-brain is well-fixed it should be sliced and blocks taken. Antigenicity is reduced by the length of time tissue is in formalin.
2. Sufficient blocks should be taken to meet the CERAD criteria (see notes in Mirra et al., 1991 below). In addition, a representative sample of blocks should be taken for archiving or to demonstrate special pathology.
3. Blocks, in general, should be taken from the same hemisphere and should fit easily on a 3" x 1" standard slide. The block should, where possible, include the complete depth of a sulcus and be marked for the face to be cut prior to embedding.

Blocks required for **CERAD neuropathological diagnosis of Alzheimer's disease** (See notes in Mirra et al., 1991 below)

1. Middle frontal gyrus at the level of the genu of the corpus callosum.
2. Superior and middle temporal gyri at the level of the lateral geniculate body.
3. Hippocampus and parahippocampal gyrus at the same level as (2).
4. Inferior parietal lobule at the level of the splenium of the corpus callosum.

BrainNet Europe II

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Content

5. Hemi-midbrain at the level of the third nerve.

Blocks for archiving

6. Superior frontal gyrus at the level of the genu of the corpus callosum and including the corpus callosum.
7. Calcarine sulcus approximately 2cm from the occipital pole.
8. Striatum at the level of the anterior commisure to include the nucleus basalis of Meynert.
9. Amygdala.
10. Thalamus and subthalamic nucleus.
11. Pons, rostral part through the mid-point of the locus coeruleus.
12. Medulla through the maximum diameter of the inferior olive.
13. Cerebellum, a slice through the superior cerebellar peduncle and including the dentate nucleus.
14. Spinal cord (when available: C4, T4, L4 and selected PRGs).

Stains. In the first instance, request the following on the 5 CERAD blocks.

1. Haemotoxylin and eosin (7µ sections).
2. Modified Bielschowsky (14µ sections).

Additional blocks and stains should only be requested after the standard set described above has been examined and where this is likely to produce evidence of other pathological findings such as the distribution of micro infarcts significant on white matter pallor.

Protocol: Processing fresh brain

Safe practice in handling fresh human material

Treat all fresh material as infectious.

1. Always wear gloves when handling fresh human material, even when frozen.
2. Gloves and other soiled material should be disposed of in bags labelled clinical waste and incinerated.
3. Instruments should be left to soak for 24 hours and working surfaces washed down with hypochlorite (2.5% chloros) and left at least overnight, otherwise instruments should be autoclaved at 120°C for not less than 15 minutes.
4. Sharps should be disposed of in the sharps container.

For a more detailed description of safe practices in the clinical laboratories and postmortem room refer to the Laboratory Guidelines (available in Room 507).

Note: These procedures are an outline of those described in "Code of Practice for the Prevention of Infection in Clinical Laboratories and Postmortem Rooms", HMSO 1987.

Basic Rules

The autopsy of the nervous system should be performed without delay. Some parts of the central nervous system are very rapidly altered by postmortem autolysis. As a general guide, for rapid freezing of brain tissue, the postmortem should be carried out without delay, preferably within 24 hours. It may be possible, however, to use material for freezing up to 72 hours postmortem.

Removal of the brain

Turn the body face up. Cut the scalp with a scalpel along a coronal plane from one pinna to the other. Free the scalp and reflect forwards to the supraorbital ridges and backwards to the external occipital protuberance. Open the cranial cavity with an electric saw. Remove the skull cap by pulling from front to back using the wedge of the autopsy hammer inserted in the centre of the frontal bone cut. Normally, the dura should remain intact. Cut the dura first longitudinally, about 2cm on either side of the midline from front to back, and then in a semicircle along the edge of the cut bone. Cut the anterior attachment of the falx cerebri.

Gently raise the fronal lobes by hand and cut the anterior connecting structures (optic nerves, internal carotid arteries and pituitary stalk) with scissors.

Cut the tentorium cerebelli along its attached edge to the upper body of the petrous bone.

Supporting the brain in the left hand, section the posterior connecting structures (cranial nerves and vertebral arteries). With a long, thin scalpel transect the upper cervical cord. Finally, cut the dura of the posterior fossa to deliver the brain.

Before removing the brain insert a 20ml syringe into the third ventricle and gently draw up CSF. Keep on ice.

Then place the brain carefully in a plastic bag and put in a watertight container holding crushed ice (4°C).

Brain weight

Weigh the whole brain. After separating the hind-brain (brainstem and cerebellum) from the cerebral hemispheres, by making a clean transverse cut across the midbrain, weigh the brainstem and cerebellum. Record the weights in the AUTOPSY REPORT (See below).

Cerebrospinal Fluid (CSF)

Some CSF can be obtained when removing the brain (see Removal of the Brain above). This is not always possible, however. In the mortuary, or later in the dissecting room, CSF can often be removed from the third ventricle using a 20ml syringe alone, or from the lateral ventricles using a 20ml syringe and needle. Note the volume recovered and its condition (eg. Blood-stained). Spin down the CSF in a “universal” tube in the centrifuge in Room 507).

Gross examination of the brain

1. The brain should be carefully examined and any abnormal or interesting features recorded and photographed whenever possible.
2. If the brain is found to have a major lesion, other than that consistent with dementia, or if the postmortem delay is greater than 72 hours, the brain should be suspended by the arteries at the base of the brain in 10% buffered formal saline for at least one month.

3. Place the dissecting board on an aluminium tray in the Microbiological Safety Cabinet, Room 617. For the dissection use a disposable brain knife and scalpel. Separate the left and right cerebral hemispheres by a cut through the midline. One hemisphere is placed, flat surface downwards, in a pot containing 10% buffered formal saline. The other hemisphere is sliced fresh for rapid freezing. Make coronal slices at 1cm intervals throughout the cerebrum. Place the anterior part of the cerebrum, cut surface down, between a pair of centimetre guides and lay out posterior surface upward, with the right side on the right. The procedure is repeated for the posterior part of the hemisphere. One cerebellar hemisphere from the same side as the sliced cerebrum is sliced at 0.5cm with the first cut through the superior cerebella peduncle and perpendicular to the folia. Subsequent slices are make parallel to this first slice and frozen. The centralateral cerebellar hemisphere is fixed. One hemibrainstem is frozen and the other fixed for histology.

Rapid freezing and fixing of brain slices

Coronal slices throughout the cerebral hemisphere, cerebellum, and brainstem are either frozen rapidly on a brass plate (18" x 12" x 1/2") and stored permanently at -70°C or fixed in 10% buffered formal saline. Alternate hemispheres may be fixed or frozen and a record made, in the AUTOPSY REPORT (see below) under the appropriate heading (FROZEN MATERIAL or FIXED MATERIAL).

Brain slices, once frozen, are placed in "self-seal" plastic bags, labelled, and placed in an airtight plastic container and stored in a -70°C freezer.

AUTOPSY REPORT

Enter data concerning the brain material as the brain is being processed. The data is entered in the report in a format that can readily be transferred to the brain bank computer database.

The report is divided into a number of sections:

Information	Database file name
Autopsy data	AUTOPSY
Frozen material	FZBRAIN
Fixed material	FXBRAIN
Cerebrospinal fluid	CSF

Neuropathological Examination

Details of the macroscopic examination and findings on slicing the brain are entered in the AUTOPSY REPORT as they are encountered.

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