Clinical Diagnosis: MOTOR NEURONE DISEASE

Protocol: Fresh Brain Dissection

General

1. Tissue should be removed and processed as quickly as possible after death, preferably within 24 hours.
2. Alternate cerebral hemispheres should be used for block taking.
3. The brainstem should be removed by a high horizontal cut through the midbrain at a level anterior to the third nerve. The medulla oblongata at the junction with the pons is removed by a horizontal slice and the **full length of the medulla is then fixed**. The pons and midbrain are divided in the midline; one side is fixed and the other side rapidly frozen by laying the medial surface face down on the brass plate at -70°C.
4. The **precentral gyrus** should be marked with **Indian ink** on the hemisphere to be sliced fresh.
5. One cerebral hemisphere is sliced at 1cm thick intervals and slices are rapidly frozen by contact with a brass plate kept at -70°C in the freezer.
6. Cerebrospinal fluid should be withdrawn from the ventricles, spun down to remove red blood cells, and frozen.

Block Taking for Freezing and Fixation

Site

1. Midbrain: one side frozen, one side fixed.
2. Pons: one side frozen, one side fixed.
3. Medulla oblongata to be fixed for its full length.
4. Cerebellum: Slice through the superior cerebellar peduncle to reveal the dentate nucleus and make parallel 0.5cm slices. Freeze the slices.

BrainNet Europe II

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5. Spinal Cord:

Formalin fixation Level	Freezing Level
Upper cervical to C4	
	C5-C6
C6-C8	
	T1
T2-T4	
	T5
T6-T8	
	T9
T10-T12	
	L1-L4
L5 and remainder	

Once the precentral gyrus has been marked with Indian ink and is dry, slice the hemisphere in approximately 1cm thick slices. Freeze the slices by contact with the brass coronal plate at -70°C.

Other nerves and muscles to be fixed except where marked*.

If possible take at least 3cm of muscle.

1. Dorsal root ganglia

Nerves:

2. Femoral nerve at the level of the inguinal ligament
3. Sural nerve

Muscles:

4. Digastric muscle, anterior and posterior parts
5. Hypoglossal muscle, tongue
6. Intercostal muscle
7. Diaphragm
8. Quadriceps muscle *(half frozen, half fixed).
9. Olfactory bulbs (for fixation).

Clinical Diagnosis: MOTOR NEURONE DISEASE

Protocol:
Dissection of formalin-fixed brain (10% buffered formal saline)

Protocol for block taking:

1. Remove the brainstem and cerebellum by a cut through the midbrain.
2. Mark the precentral gyrus with Indian ink (to identify motor cortex after slicing).
3. Slice the whole hemisphere in 5mm slices.

Block taking

For routine processing tissue blocks should fit on a 1" slide.

Blocks	Sites
	Spinal cord:
1,2	Cervical level (2 blocks)
3,4	Thoracic level (2 blocks)
5,6	Lumbar level (2 blocks)
7	Medulla oblongata (at mid olive to include the XII nerve nucleus)
	Precentral gyrus:
8	Superior
9	Lateral
10	Frontal lobe. Superior frontal gyrus at the level of the genu of the corpus callosum
11	Temporal lobe. Superior temporal gyrus at the level of the lateral geniculate nucleus.
12	Hippocampus at same level as Block 11
13	Occipital lobe including the calcarine fissure.
14	Basal ganglia at the level of the anterior commissure to include the nucleus basalis of Meynert.
15	Amygdala
16	Midbrain at level of the third nerve
17	Pons to include the locus coeruleus
18	Cerebellum including dentate nucleus
19	Thalamus at the level of the mammillary bodies
20	Olfactory bulbs

Clinical Diagnosis: MOTOR NEURONE DISEASE

Protocol: Histology and immunohistochemistry

Section thickness	Stain/Immunohistochemistry
7µm	Haematoxylin and eosin
	Immunohistochemistry
14µm	Luxol fast blue/Nissl
	Modified Bielschowsky

Minimal blocks and stains for a rapid diagnosis:

Blocks	Sites	Stains
1,2	Spinal cord. Cervical level (2 blocks)	H & E, LFB/N and Ubiquitin
3,4	Spinal cord. Thoracic level (2 blocks)	" "
5,6	Spinal cord. Lumbar level (2 blocks)	" "
7	Medulla	" "
10	Superior frontal gyrus	" "
12	Hippocampus	" "
16	Midbrain	" "

Additional blocks and stains should **only** be requested if a diagnosis cannot be soundly based on the minimal blocks.

Blocks	Sites	Stains
	Spinal cord:	
1,2	Cervical level (2 blocks)	H & E, LFB/N, and ubiquitin
3,4	Thoracic level (2 blocks)	" "
5,6	Lumbar level (2 blocks)	" "
7	Medulla oblongata (at mid olive to include the XII nerve nucleus)	" "
	Precentral gyrus:	
8	Superior	" "
9	Lateral	" "
10	Frontal lobe. Superior frontal gyrus at the level of the genu of the corpus callosum.	H&E, LFB/N, Modified Bielschowsky
11	Temporal lobe. Superior gyrus at the level of the lateral geniculate nucleus.	" "
12	Hippocampus at same level as Block 11	H&E, LFB/n, mod.Biel. and ubiquitin
13	Occipital lobe including the calcarine fissure.	H&E, LFB/N, mod. Biel.
14	Basal ganglia at the level of the anterior commissure to include the nucleus	H&E. LFB/N and basalis of Meynert ubiquitin
15	Amygdala	" "
16	Midbrain at level of the third nerve	" "
17	Pons to include the locus coeruleus	" "
18	Cerebellum including dentate nucleus	" "
19	Thalamus at the level of the mammillary Bodies	" "
20	Olfactory bulbs	" "

Disorder: Motor Neurone Disease/ Amyotrophic Lateral Sclerosis

Diagnostic criteria: El Escorial

Under the auspices of the WFN, a workshop was held in El Escorial, Spain, May 26-28 1989, funded by the Spanish ALS Association (ADELA), the ALS:MND Research Foundation (Switzerland) and the Motor Neuron Disease Association).

Operational criteria to make the diagnosis of Amyotrophic Lateral Sclerosis (ALS)* are required to ensure uniform inclusion of patients with ALS and uniform exclusion of other diseases. The criteria are sensitive to the fact that features of ALS may initially affect different regions of the central nervous system (cortical, brainstem, cervical, thoracic and/or lumbosacral spinal cord motor neurons), yet manifest in their course in multiple segments within a region and in multiple regions of the central nervous system.

1. The diagnoses of ALS requires the **presence** of
   - a Lower motor neuron signs
   - b Upper motor neuron signs
   - c Progression, and...
2. The **absence** of
   - a Sensory signs
   - b Sphincter abnormalities
   - c Parkinson's disease
   - d Alzheimer's disease
   - e Other causes of ALS-like syndromes
   - f Anterior visual system abnormalities.
3. The diagnosis is **supported** by
   - a Fasciculations in one or more regions or abnormalities in...
   - b Isokinetic/isometric strength tests
   - c Pulmonary function tests
   - d Speech tests
   - e Swallowing studies
   - f Muscle biopsy
   - g Normal nerve biopsy

4. The division into **Definite, Probable, Possible, and Suspected cases** defined below is based on the grouping of clinical signs from 1a and b in one or more regions (brainstem, cervical, thoracic, lumbosacral) of the central nervous system, together with 1c and 2.

   Clinical signs:

   Definite UMN and LMN signs in 3 regions (brainstem, cervical, lumbosacral) eg. Charcot ALS.

   Probable UMN and LMN signs in 2 different regions and UMN signs rostral to the LMN spinal; eg. UMN bulbar, LMN spinal; UMN and LMN bulbar, LMN spinal; etc.

   Possible UMN and LMN in 1 region or UMN in 2 or 3 regions, eg. Monomelic ALS; progressive bulbar-palsy.

   Suspected LMN in 2 or 3 regions or other motor syndromes.

5. The diagnosis of Proven, Definite, Probable, Possible and Suspected ALS is subject to the results of electrophysiological, laboratory and pathological tests and may be confirmed, excluded or upgraded and downgraded as defined below:

   Electrophysiological, laboratory or pathological tests

   Proven Confirmed by post mortem examination alone requiring the **presence** of:

   LMN and UMN neuronal loss and neuronal atrophy,

   loss of Nissl substance and cortico-spinal tract degeneration

   and the **absence** of:

   other major CNS abnormalities, extensive central chromatolysis, active neuronophagia, Alzheimer's neurofibrillary changes, abnormal storage material, significant spongiform-change and extensive inflammation.

   Definite Electrophysiological and laboratory tests are supportive or exclusionary; raise to Proven is confirmed by post-mortem examination.

Probable Electrophysiological and laboratory tests are supportive or exclusionary; raised to Proven if confirmed by post-mortem examination; raised to Definite by clinical or electrophysiological spread. If appropriate correction of laboratory abnormality does not stabilize or improve condition and progression occurs then laboratory exclusion is rescinded.

Possible If appropriate correction of laboratory abnormality does not stabilize or improve condition and progression occurs then laboratory exclusion is rescinded.

Suspected raised Electrophysiological and laboratory tests are supportive or exclusionary; to Proven is confirmed by post-mortem examination; raised to Possible, Probable or Definite by clinical or electrophysiological spread.
If appropriate correction of laboratory abnormality does not stabilize or improve condition and progression occurs then laboratory exclusion is rescinded.

6. 'ALS-like Syndromes' present with the ALS phenotype as defined by 1-5 and must be excluded by appropriate laboratory tests:

a) Sponsylotic myelopathy

b) Vascular

c) Lymphoma

d) Non-tumor endocrine abnormalities

e) Acute infection

f) Post-infectious

g) Monoclonal gammopathy

h) Dysimmune

i) Exogenous toxins

j) Physical injury

k) Genetic/acquired enzyme defects

7. 'ALS Variants; comprise clinical syndromes where the predominant presentation is that seen in sporadic ALS, but which includes one or more additional features such as:

   a) Geographic (Western Pacific, Guam, Kii, etc.)

   b) Dementia

   c) Extrapyramidal signs

   d) Cerebellar degeneration

   e) Autonomic nervous system involvement

   f) Objective sensory abnormalities.

*'Amyotrophic lateral sclerosis' is used interchangeably world-wide as a specific type of progressive neurodegenerative disorder of the motor system and as a general group of these conditions with upper, lower or mixed upper and lower motor neuron degeneration. The term 'motor neuron disease, motor neurone disease, etc' is used in the United Kingdom and elsewhere to denote the use of 'amyotrophic lateral sclerosis' described above. For historical reasons, we agree to use the term 'amyotrophic lateral sclerosis' or 'ALS' throughout this document.

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