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### **Muscle Biopsy Service: Neuropathology Department, John Radcliffe Hospital, Oxford.**

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## 1. Introduction

The Neuropathology Department is based at the John Radcliffe Hospital site in Oxford and is located in the West Wing which is alongside the Children's Hospital. We are a small department and prior notification of biopsies is essential in order for us to organise staff and workloads. Ideally we would prefer 24 – 48 hours notice.

**(a) Investigations for mitochondrial disease, respiratory chain studies and metabolic disorders** require tissue to be snap frozen within 20 minutes of being taken. It is our policy not to receive fresh tissue from outside of Oxford for these investigations.

If these investigations are required, you should contact Dr David Hilton-Jones to arrange for the patient to be referred and seen at the ORH muscle clinic.

Tel number: (01865 (2)31854

E-mail address: [David.Hilton-Jones@clneuro.ox.ac.uk](mailto:David.Hilton-Jones@clneuro.ox.ac.uk)

Alternatively the tissue can be prepared and frozen by your local cellular pathology / histopathology laboratory and then sent on to us packed in dry ice in a polystyrene box.

**NB:** Not all cellular pathology departments have access to reagents necessary for the optimal preparation of muscle biopsies. Before requesting this service from the pathology laboratory ensure they have the experience and are able to access the required reagents.

**(b) Investigations for inflammatory conditions** such as Inclusion Body Myositis (IBM), Dermatomyositis, and Polymyositis do not require rapid freezing and we can accept these samples 1-2 hours after being taken (see later for details).

## 2. Service Availability & Contacts

Please note our laboratory service is available from 8.30 am until 5 pm, Monday to Friday with no Bank Holiday cover. Please contact us if you have any queries or require advice.

Dr Waney Squier (Consultant Neuropathologist)

Tel: 01865 234932

E-mail: [waney.squier@clneuro.ox.ac.uk](mailto:waney.squier@clneuro.ox.ac.uk)

Dr David Hilton-Jones (Consultant Neurologist)

Tel: 01865 231854

E-mail: [David.Hilton-Jones@clneuro.ox.ac.uk](mailto:David.Hilton-Jones@clneuro.ox.ac.uk)

## Service Availability & Contacts Continued.....

Louise Young (Biomedical Scientist)  
E-mail: [louise.young@orh.nhs.uk](mailto:louise.young@orh.nhs.uk)

Tel: 01865 234417

Nicky Sullivan (Biomedical Scientist)  
E-mail: [nicky.sullivan@orh.nhs.uk](mailto:nicky.sullivan@orh.nhs.uk)

Tel: 01865 234417

### 3. Sampling Advice for Surgeons

#### (a) Size of the Biopsy

The size of the sample depends on the investigations requested, but generally a sample of muscle measuring 1.5 x 1.0 x 1.0 cm is adequate.

#### (b) Suggested Protocol for the Surgeon

Muscle should **NEVER** be fixed in formalin, placed in saline or wrapped in cling film.

The usual muscles for biopsy are posterior body of the deltoid or vastus lateralis. Other muscles may be biopsied if there is a clinical indication. The site of the biopsy must always be stated on the request form together with contact details of the surgeon and clinician in charge of the case (who is usually not the surgeon).

EMG findings, CK level and clinical history are required for reporting.

If further advice is required please contact: Dr Waney Squier (Consultant Neuropathologist) on 01865 234932.

### 4. Sending Fresh Muscle Biopsies Directly to Neuropathology, Oxford.

#### a) Letting us know.

- i. Contact the Neuropathology laboratory as soon as you know of a biopsy (should be at least 24 hours notice). This enables us to obtain relevant clinical information about the sample ensuring it is dealt with correctly.
- ii. We will fax information on how to send fresh muscle samples, an address label, as well as directions of how to get to our department (which you can pass on to your delivery person).

**b) Time of biopsy.**

It is important for biopsies to be arranged for morning sessions. If this is not possible the sample should be delivered directly to us no later than 4pm.

**c) Request card and information required.**

Request cards must accompany the sample. Please ensure the following information is included:

- Patient's full name and date of birth
- Hospital or NHS number if they have one
- Patients consultant
- Date / time of biopsy
- Site of biopsy
- Consent for use of tissue or images
- Clinical details
- Name of doctor arranging biopsy, their contact number / bleep
- Where copy of report should be sent

**NB:** If no request card or insufficient clinical details accompany the sample, reporting of the case will be held up.

**d) Sending fresh muscle samples.**

- i. The fresh sample should be placed in a clean universal container with **nothing else added**. (This means **NO** saline, gauze, wax, etc. The sample **MUST NOT** be wrapped in cling film).
- ii. The sample should be kept cool at all times but not placed directly on ice.
- iii. The sample should be sent to us immediately, without delay and directly to our department using the hospitals mode of transport for urgent samples.
- iv. (Please ensure your chosen transport can deliver directly to us at the John Radcliffe Hospital and not via another hospital's transport system).
- v. Ideally the sample should arrive in our laboratory within 1-2 hours of being taken.
- vi. Attach address label to package (see Appendix 2)

**e) Keeping us informed.**

Please call when the specimen is on its way, and give us an approximate time of arrival.

Call either Louise Young or Nicky Sullivan on 01865 234417.

## Appendix 1

### Laboratory sampling, freezing and sending of frozen samples (including staff training).

#### Personnel Requirement:

Sampling and freezing of muscle should be carried out by Biomedical Scientists authorised to perform these procedures.

#### Risk Assessment:

You should perform a risk assessment prior to carrying out procedure.

#### COSHH:

Specify if procedure involves reagents subject to COSHH regulations.

#### PPE:

All personnel should wear a Howie laboratory coat and protective gloves.

#### 1. Muscle sampling for neuropathological investigations.

**NB:** DNA studies, respiratory chain studies along with metabolic investigations require the muscle to be frozen rapidly within 20 minutes of it being taken, Rapid freezing is essential due to the breakdown of enzymes once the tissue has been removed.

The quantity and type of samples required will depend on the investigations requested by the clinician. Always check with the clinician what they are requesting.

Refer to Table 1 which lists the type of samples, amount of tissue required and what investigations they are used for as an aid in sampling the biopsy.

**Table 1: Sampling required for neuropathological investigations**

Sample preparation	Sample size	No of Samples	Purpose
Cryostat	Ideally 0.5 x 0.5 x 0.4cm (depends on size of sample taken)	X2 (if large sample of muscle taken)	Histochemical & Immunocytochemical investigations

<b>Sample preparation</b>	<b>Sample size</b>	<b>No of Samples</b>	<b>Purpose</b>
Snap Frozen	Approx 0.5 x 0.4 x 0.4cm diameter	X1	Western blotting
Snap Frozen	Approx 0.5 x 0.4 x 0.4cm diameter	X1	DNA investigations
Snap Frozen	Approx 1.5 x 0.4 x 0.4cm (250ug)	X1	Respiratory chain investigations
Snap Frozen	Approx 0.5 x 0.4 x 0.4cm diameter	X1	Biochemical investigations
Electron Microscopy (EM)	0.1 mm <sup>3</sup>	X4	Ultra-Structural investigations
Paraffin	Whatever muscle is left over (there may be none)	X1	Histological Analysis

## 2. Preparation of EM and paraffin samples.

- a) EM sample – place x 4 1mm<sup>3</sup> pieces of muscle into a labelled tube containing enough EM fixative to cover tissue.
- b) Paraffin sample – if any tissue is left place into a labelled universal container with enough formalin to cover tissue.

## 3. Preparation and technique for cryostat samples.

The muscle biopsy must be frozen in iso-pentane which has been pre-cooled in liquid nitrogen.

On no account must the sample be placed directly into liquid nitrogen as the fibres will be blighted with ice crystal artefact rendering the sample inadequate for diagnosis.

### a) Equipment / reagents required:

- i. Dewar for liquid nitrogen.
- ii. 50ml plastic container, 250ml plastic container.
- iii. Cork disc, OCT mounting media.
- iv. Labelled EM tube plus EM fixative, foil for snap samples.
- v. Liquid nitrogen (BOC)
- vi. Iso-pentane (VWR)

**b) Preparation of iso-pentane (pre-cooled in liquid nitrogen):**

- i. Fill a 250ml plastic container with liquid nitrogen. Fill to just over half way.
- ii. Fill a 50ml plastic container to the top with iso-pentane.
- iii. Lower the iso-pentane container gently into the liquid nitrogen container. White clouds of vapour will be given off as evaporation of nitrogen occurs.
- iv. Top up the liquid nitrogen container when level becomes low. The optimum freezing temperature occurs when a white solid layer has formed over the bottom and up the sides of the iso-pentane container. (Usually takes about 4-5 minutes)

**c) Freezing technique for cryostat sampling:**

- i. Write the patients name on the cork disc.
- ii. Place a small amount of OCT mountant in the centre of the disc.
- iii. Orientation of the muscle is very important. Place a transverse sample of muscle on the OCT / cork disc. (Transverse orientation reveals the fibre mosaic pattern).
- iv. Make sure the iso-pentane has reached its optimum freezing point.
- v. Plunge the cork disc / tissue into the iso-pentane for approximately 30 – 40 seconds.
- vi. Once the tissue is frozen it can be dropped into liquid nitrogen for transportation. Wrap the sample in aluminium foil and place in a -80°C freezer for storage until ready to arrange transport. Place samples into a sealed plastic bag to prevent dehydration.

**d) Freezing technique for snap frozen samples.**

- i. Wrap each sample individually in a piece of foil (labelled with patient's name) and drop into liquid nitrogen.
- ii. The samples can then be transferred to a -80 freezer for storage until ready to arrange transport. Place samples into a sealed plastic bag to prevent dehydration.

**4. Sending frozen samples prepared by your cellular pathology department.**

**a) Letting us know**

- i. Contact the Neuropathology laboratory (01865 234417) at least 24 hours prior to sending the sample to ensure someone is available to take receipt of the samples.
- ii. Pass on the patient's name and the date the sample will be sent to the person taking the call.

**b) Request cards.**

A request card must accompany the sample and must contain all the relevant information. Refer to section 4c for requirements.

**c) Time of delivery.**

It is important for biopsies to arrive no later than 4pm, Monday to Friday only (no Bank Holidays).

**d) Packaging samples.**

- i. Fill a polystyrene container (measuring approximately 30 x 30 x 30cm) halfway with dry ice pellets.
- ii. Place plastic bags containing snap frozen and cryostat samples into container and fill to the top with the remaining dry ice, ensuring the samples are completely covered.
- iii. DO NOT pack the dry ice with polystyrene chips – the chips mix with the ice during transit, effectively insulating the sample from the dry ice and causing it to thaw.
- iv. Place relevant paperwork / request card along with the paraffin and EM samples in to an envelope and tape to the top of the box.
- v. Ensure the correct address label (see appendix 2) is placed on to the top or side of the container and clearly visible. Also attach a label showing the UN safety sign for dry ice on the same side.
- vi. The parcel is now ready for sending.
- vii. Please ensure your chosen transport delivers directly to our department at the John Radcliffe Hospital.

**e) Day of sending samples.**

Call the laboratory contacts (Louise Young or Nicky Sullivan) on the day the samples are being sent with an approximate time of arrival. Contact number: 01865 234417.

**5. Training.**

We are happy to offer training within our department for experienced BMS staff who would like to learn how to sample and freeze muscle biopsies correctly. If you are interested please call Louise Young on 01865 234417 or e-mail [louise.young@orh.nhs.uk](mailto:louise.young@orh.nhs.uk) to make arrangements.

## Appendix 2

### a) Address Label:

PLEASE HAND DELIVER TO:

Louise Young / Nicky Sullivan

Neuropathology Department  
Level 1, West Wing  
(Via Children's Hospital Entrance)  
John Radcliffe Hospital  
Headley Way  
Headington  
Oxford, OX3 9DU

Tel: 01865 234417