Method for preserving muscle and nerve biopsies (Procedures 1 and 2)

From each biopsy, pieces of tissue should be taken for the following:


More details for carrying out the above procedures are outlined overleaf. For further information please contact:

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Procedure 1: MUSCLE BIOPSY PROCEDURE

SURGICAL PROCEDURE:

In order for the biopsy to be maximally useful for diagnosis, four criteria must be fulfilled:

1. The tissue should be removed as gently as possible to avoid causing contraction of muscle fibres.

2. The tissue should be taken so that precisely oriented transverse sections can be cut.

3. The specimen should be removed from a clinically affected muscle (not weaker than MRC -4). In chronic wasting disease, a less severely involved muscle may be better, as fatty connective tissue replacement is not diagnostic. Muscle sample should look like muscle.

4. Avoid the site of the recorded EMG or other needle insertion, into muscle. Transporting tissue must be done quickly so as to avoid drying or warming of the tissue. Freezing must be carried out extremely rapidly to avoid formation of ice crystals in the muscle fibres.

PRESERVATION OF MUSCLE BIOPSY

The optimal size of the gross specimen removed from the patient is 1cm x 1cm x 2cm. The specimen should be bisected along its longitudinal axis so that you obtain several pieces about 0.3 x 0.5cm (each piece should be as round as a pencil).

These pieces are preserved for histochemistry and immunohistochemistry, for electron microscopy and for histology (see attached instructions ie Appendices A, B, C).

Note that most of the muscle tissue is frozen for histochemistry.
**Procedure 2: SURAL NERVE BIOPSY PROCEDURE**

**SURGICAL PROCEDURE**

1. Make a vertical incision about 5cm long on the lateral aspect of the leg, just posterior to the lateral malleolus, between it and the achilles tendon. Locate the sural nerve in the subcutaneous adipose tissue.

2. Loosely attach 2 sutures, 2cm apart along the nerve.

3. Cut the nerve about 0.5cm outside each end of the 2cm sutured area, and then tighten both sutures. Cut the proximal end first and then transect the distal part. Remove the specimen (total length about 3.0cm) and examine the cut section to make sure that it is nerve rather than the vein which is nearby.

**PRESERVATION OF THE NERVE BIOPSY (see also diagram below)**

1. Tissue for FREEZING
   One 0.5cm piece from the proximal end of the specimen should be frozen immediately with liquid nitrogen and stored at -70°C.

2. Tissue for HISTOLOGY
   The other 0.5cm specimen from the distal end, of the specimen should be fixed in 10% formalin for routine sections. This specimen should be sent to us at room temperature.

3. Tissue for ELECTRON MICROSCOPY AND TEASING
   Take the remainder 2.0cm specimen and pin either end on to a piece of cork using ordinary pins. This is immersed in 3.0% glutaraldehyde.

4. After a 2 hour fixation the specimen should be removed from the fixative and transected into two equal pieces, about 1.0cm each. The edges are also cut free from the pins. The first 1.0cm specimen is placed in phosphate buffer for preparation of teased myelinated nerve fibers. The other 1.0cm piece is returned to the fixative for another 2 hours and then placed in phosphate buffer. This specimen is used for preparation of semithin sections for light and electron microscopy. Both specimens should be stored at 4°C and transported at room temperature.

![Gross Nerve Biopsy Diagram](image-url)
Appendix A - Tissue for Histochemistry and Immunohistochemistry

Introduction: Freezing in Isopentane cooled by Liquid Nitrogen

Liquid isopentane is cooled in liquid nitrogen until ice crystals begin to form on surface of the isopentane or wall of the isopentane container (the isopentane becomes syrupy). At this point, the specimen is quickly immersed, well under the surface of the isopentane for 20 seconds. The specimen turns orange-white on freezing. Upon removal from the isopentane the specimen can be thrown straight into liquid nitrogen and subsequently stored in a plastic capsule (nunc vial) which has been previously immersed in liquid nitrogen so both specimen and capsule are at the same temperature.

- Before cutting into smaller pieces, the muscle biopsy should be well oriented. The direction in which the muscle fibres run (usually parallel) should be evident in each piece.
- Each piece should be about 0.3cm thick and 0.5cm long (i.e. consist of 4-5 fascicles).
- 1-3 blocks may be placed on one piece of card, and fully immersed in cooled isopentane for 20 seconds (the base of the isopentane should be frozen).

The frozen tissue may then be stored in vials (previously cooled in liquid nitrogen) in liquid nitrogen. Each vial may contain several pieces of muscle from the same patient.

Frozen tissues should be stored in liquid nitrogen or in a -80°C freezer.

Transport overseas: to the Cyprus Institute of Neurology and Genetics

The specimen can be transported to Cyprus within a maximum of 48 hours placed in a thick polystyrene box (10cm thick walls) and packed with at least 3kg of dry ice.

Pack biopsies in polystyrene box filled with dry ice. Tape the box with masking tape really tight. Label outside for cold storage/urgent. For transport overseas use only fast courier service like DHL, TNT, or UPS with guaranteed delivery within 2-3 days.

Arrangements should be made in advance of sending the specimen since any delays, resulting in the increase of specimen temperature, will not allow any histochemical or immunochemical testing at all. So please inform me when shipment is arranged so that I know when to expect it in case of a delay.

Additional information on freezing

I. Freezing - For freezing muscle biopsies the following are needed:

1. Liquid nitrogen - Volume ~10-20 litres
2. Isopentane 1-2 liters
3. Dewar flasks
4. Nunc vials

II. Storing - Either in a liquid nitrogen cylinder or in a -80°C freezer.

III. Transporting - Need 3kg of dry ice (solid carbon dioxide).
   - Polystyrene box.
Appendix B - Tissue for electron microscopy (EM)

- Small rectangular pieces (2 mm X 3 mm) should be cut, placed on card and immersed in 3% glutaraldehyde in 0.1M Phosphate buffer pH 7.3 (see Appendix D).

- The tissue is kept in fixative for at least 4hrs or overnight at 4°C. Then washed in 0.1M phosphate buffer. Biopsies may be sent to us in phosphate buffer, at room temperature.

- The above procedure also applies to the fixation of nerve biopsies, for EM.

Appendix C - Tissue for Histology

One piece of tissue is placed on a card and immersed in formalin fixative for histology.

Appendix D - Preparation of glutaraldehyde fixative

After tissues are placed in the glutaraldehyde fixative solution, they are kept in a refrigerator (4°C) for a minimum of 4 hours but may be left overnight. The tissues are then given a phosphate buffer rinse (0.1M phosphate pH 7.3).

Preparation of glutaraldehyde fixative:

3% glutaraldehyde in 0.1M phosphate buffer (pH 7.3) for routine use.

0.1M phosphate buffer solution, is prepared as follows:

Solution A: 0.2M Sodium phosphate monobasic
Sodium phosphate Monobasic (NaH$_2$(PO$_4$)$_3$) ......................... 13.8gm
Distilled water ................................................................. 500ml

Solution B: 0.2M Sodium phosphate dibasic
Sodium phosphate dibasic (Na$_2$H(PO$_4$)$_3$) ......................... 14.2g
Distilled water ................................................................. 500ml

For making 400mls of 0.1M phosphate buffer add the following:

\[
\begin{align*}
pH \, 7.3 & \\
\text{Solution A}^* & : 47\text{ml} \\
\text{Solution B}^* & : 153\text{ml} \\
\text{Distilled Water} & : 200\text{ml}
\end{align*}
\]

*First mix A and B stir well and check the pH; if necessary adjust pH with either solution A or B. Measure volume and add an equal amount of distilled water.