Department of Pathology
Diagnostic Unit

State Neuropathology Service

Procedures Manual

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1. SIGNATURE LOG

Staff Member	Position	Signature	Initial
Paul Kennedy	Senior Scientific Officer		
Veronika Gazdik	Scientific Officer		
Pauline Middelveld	Administrative Assistant		
Matt Reardon	Technical Assistant		

2. **POLICY STATEMENT & INTRODUCTION**

The State Neuropathology Service (SNS) has been in operation for approximately forty years under the direction of Associate Professor Xenia Dennett who retired in 2003. The laboratory is situated in the Department of Pathology at the University of Melbourne and provides a muscle and nerve diagnostic service for hospitals and medical clinics throughout Victoria, Tasmania, the Northern Territory and parts of Southern New South Wales.

Tests are performed to evaluate changes in morphology and enzyme activity of muscle and morphological changes in peripheral nerve specimens.

Muscle biopsies play an integral role in evaluation of a patient with neuromuscular disease. With rare exceptions, it is an essential element in the assessment of a patient with suspected myopathy. In addition to being indispensable for the evaluation of muscle diseases, muscle biopsy also is involved in the evaluation of suspected neuropathic disease, particularly in the distinction of an atypical neurogenic disorder from a primary myopathic one, and for diagnosis of a variety of systemic disorders.

Abnormalities of muscle and nerve tissue (neuromuscular disorders) are diverse and numerous. Neuromuscular disorders may be genetically determined or acquired and whilst some principally affect proximal muscles, others mainly affect distal muscles. Some disorders manifest themselves quickly while others progress insidiously or resolve spontaneously. Moreover, some disorders affect the architecture grossly while others produce minimal change.

A number of neuromuscular disorders encompass defects in the muscle fibre membrane, structural proteins, contractile proteins, neuromuscular junction and the nerves that supply the muscle. On the other hand there are neuromuscular disorders which do not have an architectural component but are caused by a metabolic energy crisis affecting glycolysis, glycogenolysis, lipid, purine or mitochondrial biochemistry. In addition there are some neuromuscular disorders that are drug induced. Classification of neuromuscular disorders traditionally is based on the location of greatest functional or structural abnormality. Clinical symptoms may include muscle weakness and wasting, atrophy, fasciculations, pseudohypertrophy, myotonia, rhabdomyolysis and/or elevated creatine kinase level.

From the range of possible defects it can be seen that the investigation of neuromuscular disorders is a complex process. At the SNS muscle biopsy specimens are stained for a range of muscle enzymes and structural proteins. Based on the results of the staining techniques and an assessment of the muscle morphology a diagnosis is made by a neuropathologist which leads to precise, accurate, specific information useful for the management of neuromuscular disorders. The neuropathologist is advised by the Senior Scientific Officer verbally & via a non-

conformance report form of any changes which could have a significant effect on the interpretation of the result

3. SPECIMEN COLLECTION AND RECEPTION

3.1 The Muscle Biopsy Kit

The muscle biopsy kit consists of the following:

- A plastic bucket, containing the following:
- Three 70mL plastic screw top specimen containers labeled as follows:

1. **"Dry"**
2. **"NBF"** – Into a 70mL specimen container add 25mL of 10% neutral buffered formalin.
3. **"2.5% Glut"** – Into a 70mL specimen container place 15mL of 2.5% glutaraldehyde at the time of sending to the hospital or surgery, and marked with the date of issue.

For interstate clients or clients that store muscle biopsy kits for future use the following procedure applies:

The specimen container labeled "Glut" will contain 18mL of 0.133M cacodylate buffer pH7.2 and a small glass vial which will contain 2mL of a 25% glutaraldehyde solution. Prior to use a qualified laboratory technician will add 2mL of 25% glutaraldehyde to the 18mL of cacodylate.

Please note glutaraldehyde and cacodylate are both toxic. Use gloves, gowns and appropriate fume hood when mixing.

- **Two** biopsy clamps (refer to construction procedure below).
- A plastic specimen bag.
- A specimen request form.
- A muscle biopsy collection instruction pamphlet.
- An address label for return of specimen to SNS.
- Place completed muscle biopsy kit(s) into an esky and label with appropriate address, including for whom the kit is for and a contact phone number of the person ordering the kit. Templates for this are available from the computer (on the desktop).

Muscle biopsy clamps are constructed in the following manner:

Two utilux alligator clips (Middendorp Eletrical P/L, Melbourne, Victoria) are screwed onto a piece of 1.5mm NEMA GRADE GPO-3 EPOXY GLASS 18 x 27mm (Insulcut Services P/L Thomastown, Victoria) backing board with two predrilled holes 20mm apart with a flat head screw driver.

3.2 The Nerve Biopsy Kit

The nerve biopsy kit consists of the following:

- A plastic drinking cup, containing the following:
- A glass tube containing **2.5% glut** and labeled with the date of issue and “2.5% glut”.

For interstate clients or clients that store nerve biopsy kits for future use the following procedure applies:

A glass tube containing 12.6mL of 0.133M cacodylate buffer pH7.2 and a small glass vial which will contain 1.4mL of a 25% glutaraldehyde solution. Prior to use a qualified laboratory technician will add 1.4mL of 25% glutaraldehyde to the 12.6mL of cacodylate.

Please note glutaraldehyde and cacodylate are both toxic. Use gloves, gowns and appropriate fume hood when mixing.

- A plastic tube containing one **nerve hook** (refer to construction procedure below).
- 1 plastic specimen bag.
- A 10mm x 60mm piece of dental wax.
- A specimen request form.
- A nerve biopsy collection instruction pamphlet.
- An address label for return of specimen to SNS.

Construction of nerve hook is as follows:

One steel sewing pin (25mm in length) is threaded through a ball type sinker (size 000, weighing approximately 1gram) (Complete Angler, Melbourne, Victoria). With a pair of pliers crush the sinker around the head of the pin, apply a pair of surgical forceps to the pin immediately above the sinker and bend the pin to a 90 degree angle thus forming a hook which will allow the sinker to be suspended from the nerve biopsy.

3.3 Procedure for Mailing Muscle or Nerve Biopsy Kits Regionally or Interstate

Required

- Prepaid express post bag.
- Complete muscle/nerve biopsy kit (remember to add 2.5% glutaraldehyde and date container).
- One cardboard box (130mm x 130mm)

Procedure

- Place a completed muscle/nerve biopsy kit in a cardboard box and seal with masking tape.
- Place the kit into the prepaid express post bag and address the bag appropriately.
- Peal off sender's barcode sticker from the front of the prepaid post bag and adhere this to the recorded request for the kit in the day to day phone records book. The sender's barcode enables tracking of the kit if anything should go wrong.

3.4 Specimen Reception & Distribution

- The biopsy is received at the State Neuropathology Service on Level 5
- Each biopsy, whether muscle or nerve, is given a SNS Laboratory Biopsy number (M . . .) and entered in the specimen book.
- Fresh biopsy material is snap frozen by SNS staff and is stored in the -70°C freezer.
- Clamped muscle specimens fixed in 10% neutral buffered formalin are given to Histopathology Laboratory staff for routine paraffin processing and sectioning.
- Clamped muscle biopsy specimens fixed in 2.5% glutaraldehyde are placed in the 4oC refrigerator and are collected on a regular basis by the EM staff.
- Nerve biopsies received by the SNS are allowed to fix for a minimum of 4 hours before being cut up and distributed. The length and diameter of each nerve biopsy is recorded by SNS staff.
- Distribution of nerve biopsies is as follows:
- 2 mm portion from sutured end – processed for paraffin sectioning
- 2 mm portion from sutured end – osmicated and processed for paraffin sectioning
- Remainder – to EM

3.5 Specimen acceptance criteria

The following details must accompany every specimen that is received at the SNS:

1. Correctly filled in request slip featuring patient identifiers, date of biopsy along with the biopsy site.
2. Correctly labeled individual specimen pots or nerve biopsy tube.

All specimens will be accepted unless there is no way of identifying the patient from whom it came. For example two specimens from two separate patients are received together in one esky, but the individual pots are entirely unlabeled.

3.6 Corrective action for non-conformance of specimen acceptance criteria, quality control & quality assurance

In all cases were inadequate or unacceptable specimens are received a telephone call is made to the Hospital's operating theatre from which the specimen originated and the surgeon and/or clinician are made aware of the problem. The details of this phone call and any actions taken are recorded on an incident report form.

If specimens are received and appear to be leaking a non conformance report is documented and remedial action is instigated. The use of personal protective equipment (PPE's) is mandatory within the Department. A specimen container or esky is not opened until all PPE and plant use has been undertaken. If a specimen container is leaking the specimen is transferred to another fresh container and new fixative solution is added. The remaining fixative is collected into an appropriate waste container and collected monthly by mediwaste. Remaining fixative is treated as a spill and is mopped up with paper towel and discarded in a biological waste bag.

Where possible tissue controls are stained in parallel with patient tissue sections. For some antibodies used in immunohistochemistry a negative & positive control section is placed on the same coverslip to ensure quality control. If a problem is encountered a non conformance report is raised and corrective actions are followed up in a timely manner. Where any other QC or QA is unsatisfactory an NCR is reported to ensure due process is followed.

For more detail of the process see: Non-Conformances, Complaints and Preventive Actions reporting procedure QMS-PR-003.

4. NERVE BIOPSY PROCESSING

The following are procedures for the processing of a nerve biopsy which is performed by the staff in the Histopathology Laboratory located on level 3 of the Department of Pathology at The University of Melbourne.

4.1 Documentation

1. Details entered into the SNS daybook include:
   - M number
   - Patient's surname and given name
   - Source (i.e. name of Hospital or Clinic)
   - Date of receipt
   - Time of receipt
   - Details of the tissue received.

2. Six photocopies are made of the original paperwork accompanying the specimen. These copies are distributed as follows:

   a) One copy of all paperwork filed in the report folder.

   b) One copy of all paperwork sent to the reporting pathologist together with the slides.

   c) Two copies of the request slip given to Histopathology Laboratory to accompany nerve for processing.

   d) One copy of request slip given to EM unit staff to accompany specimen for EM processing.

   e) One copy of request slip and any additional information is placed into the patient record files. All of this information is entered into the SNS database.

4.2 Nerve Biospy Procedure

1. A nerve specimen is delivered to the staff in the Histopathology Laboratory and placed in the 4^{o}C fridge in W307.
2. The Histopathology Laboratory issue the nerve biopsy with a secondary accession number (UM-number).
3. All patient information and accession numbers are entered into the Histopathology Laboratory Day Book and into an electronic database found on the Pathology server.

This database is backed up weekly, on tape, by the Information Technology Administrator and on zip disc on a monthly basis by the laboratory senior scientist. There is restricted access to the database. Copies of the request slips are kept on file in the laboratory for five years. The State Neuropathology Service keeps the original request slips.

4. A work card is issued for each nerve biopsy that clearly identifies the patient, the sort of tissue available, the amount of tissue processed and blocked and the stains to be performed.
5. Once the tissue is fixed, it is transferred into Sodium Cacodylate Buffer and divided into several pieces. Starting from the proximal end, two pieces are cut which will not receive any treatment with Osmium Tetroxide and one piece that is treated for two hours with Osmium Tetroxide. The remaining piece is issued to the Electron Microscopy Service for further processing. Once treated with Osmium Tetroxide the nerve biopsies are processed overnight on a Sakura VIP processor and then are blocked in paraffin, sectioned at 4 microns on a microtome and stained with Haematoxylin and Eosin, Masson Trichrome and Congo Red. An unstained section is also provided.
6. The completed set is issued to the SNS where patient details and stains performed are logged into a database. The set is then forwarded onto the appropriate Pathologist to issue a report.

4.3 Distribution of Slides

1. Histopathology Laboratory staff members prepare paraffin sections and pass them to SNS staff for distribution to the reporting pathologist.
2. EM staff members produce thick plastic sections for light microscopic examination. These are passed to the SNS staff for distribution to the reporting pathologist.
3. Details of all material sent to the reporting pathologists is recorded in the SNS database. The information recorded includes:
   - M number
   - Date sent
   - Name of reporting pathologist
   - Type of material sent (eg Histochem, Paraffins).
   - Yes/No check box for return of material.
   - Yes/No check box for return of report.
   - Yes/No check box for case complete.
   - (Note: the M number forms a link field to provide patient surname and given name.)

4.4 Reporting

1. All reports on nerve biopsies include assessment of paraffin sections and thick plastic sections from EM blocks.
2. Any further special techniques must be requested by the reporting pathologist and the results of these tests must be reported either as a specific part of the original report or as a supplementary report. These tests include, but are not limited to:
    - Immunohistochemical staining.
    - EM Teased Nerves and Nerve Montage or photomicrographs

    The Pathologist may request further Immunohistochemistry work and these requests are documented and dated on the work card and on the database.
3. The set and a copy of the completed report is sent back to the SNS where they are filed.

4.5 Filing

- Paraffin sections and EM teased nerves and montages are filled numerically in room W304.
- Paraffin blocks are filed in numerical order in room W301 and the work card is filed in alphabetical order in room W306.
- EM thicks are returned to EM staff to be filed.

4.6 Billing

- The financial manager on a monthly basis invoices for services provided by the Histopathology Laboratory and Electron microscopy unit.

5. MUSCLE BIOPSY PROCESSING

5.1 Required Specimens

The laboratory requires three specimens in order to undertake a complete range of tests on each biopsy.

1. A clamped specimen received in neutral buffered formalin to be processed for paraffin sections.
2. A clamped specimen received in 2.5% glutaraldehyde to be processed for electron microscopic examination.
3. A fresh specimen received in a dry sterile container to be snap frozen for cryostat sectioning.

These specimens are received packed in ice and must be dealt with as soon as possible after receipt.

5.2 Treatment of specimens on arrival at the laboratory

1. Fresh specimen must be promptly frozen as detailed below.
2. The specimen fixed in glutaraldehyde is placed in the refrigerator for later collection by the EM staff for processing, sectioning, staining and examination. If the glutaraldehyde is more than 10 days old it should be replaced with fresh fixative. The EM unit staff members check the refrigerator regularly.
3. The final specimen fixed in neutral buffered formalin is delivered to the Histopathology Laboratory for processing, sectioning and staining.

5.3 Documentation

1. Details entered into the SNS daybook include:
    - M number
    - Patient's surname and given name
    - Source (i.e. name of Hospital or Clinic)
    - Date of receipt
    - Time of receipt
    - Details of the tissue received

2. Five photocopies are made of the original paperwork accompanying the specimen. These copies are distributed as follows:

   a) One copy of all paperwork filed in the report folder.
   b) One copy of all paperwork sent to the reporting pathologist together with the slides.
   c) One copy of request slip only given to Histopathology Laboratory to accompany specimen for paraffin processing.
   d) One copy of request slip only given to EM unit staff to accompany specimen for EM processing.
   e) One copy of request slip and any additional information is placed into the patient record files. All of this information is entered into the SNS database.

5.4 Distribution of Slides

1. A complete histochemical set is prepared as soon as possible according to the urgency of individual cases and is dispatched with a copy of all paperwork to the reporting pathologist (see roster of pathologists below). The director determines the degree of urgency.

Neuropathologist	Original of specimen
Associate Professor Penny McKelvie	Biopsies from St Vincent's Hospital and associated hospitals
Dr Michael Gonzales	Biopsies from Royal Melbourne Hospital and Western hospital
Dr Renate Kalnins	Biopsies from Austin Health
Professor Catriona McLean	All other Biopsies

2. Histopathology laboratory staff prepare paraffin sections and pass them to SNS staff for distribution to the reporting pathologist.
3. EM staff members produce thick plastic sections for light microscopic examination. These are passed to the SNS staff for distribution to the reporting pathologist.
4. Details of all material sent to the reporting pathologist is recorded in the SNS database. The information recorded includes:

   - M number
   - Date sent
   - Name of reporting pathologist

- Type of material sent (eg Histochem, Paraffins).
- Yes/No check box for return of material.
- Yes/No check box for return of report.
- Yes/No check box for case complete.

(Note: the M number forms a link field to provide patient surname and given name.)

5.5 Reporting

1. All reports on muscle biopsies include assessment of enzyme histochemical staining set, paraffin sections and thick plastic sections from EM blocks.
2. Cytochrome oxidase/Succinate dehydrogenase (COX/SDH) stains will be performed on all cases. These results may or may not be specifically reported.
3. Any further special techniques must be requested by the reporting pathologist. SNS staff must fill out a *Request for Special Procedures* form (SNS-FO-010). This form will be filed with all other patient documentation in the report folder. The results of these tests must be reported either as a specific part of the original report or as a supplementary report. These tests include, but are not limited to:
   - Immunohistochemical staining.
   - Electron microscopic examination, either direct examination by the pathologist or examination of photomicrographs prepared by the EM staff and sent to the pathologist for assessment.

5.6 Verbal Reporting

The need to verbally report on a case rarely arises however in some muscle myositis cases the need for a verbal report is paramount to the patient's treatment plan. Verbal reporting of such cases is done by the reporting Pathologists only in liaison with the clinicians caring for the patient. All details of the conversation are recorded in the telephone dairy.

5.7 Outstanding Reports

The File Maker Pro data base is equipped to generate an outstanding report list for each Pathologist. A list is generated each fortnight & e-mailed to the Pathologists. Upon receipt of the outstanding reports they are date stamped & signed by the SNS staff. The data base is up-dated & the report is scanned & filed in the appropriate folder.

5.8 Filing

- Histochemistry, Paraffin sections and EM teased nerves and montages are filled numerically in room W304.
- Paraffin blocks are filed in numerical order in room W301 and the work card is filed in alphabetical order in room W306.
- EM thicks are returned to EM staff to be filed.

5.9 Billing

The financial manager on a monthly basis invoices for services provided by the State Neuropathology Service.

6. FREEZING MUSCLE SPECIMENS

6.1 Freezing Open Muscle Biopsy Specimens

Equipment

- 2 scalpel blades: No. 22 and No. 11
- Dental wax
- Wooden applicator
- 2 pairs of fine point forceps
- 1 coarse pair of forceps
- 3 plastic drinking cup
- 1 50 ml plastic beaker
- 50 mL isopentane
- 1 or more NUNC tubes (1 ml or 1.8 ml)
- Cardboard labels
- Lead pencil
- Pen
- Liquid nitrogen
- Vacuum flask contining dry ice
- Dry muscle specimen

Method

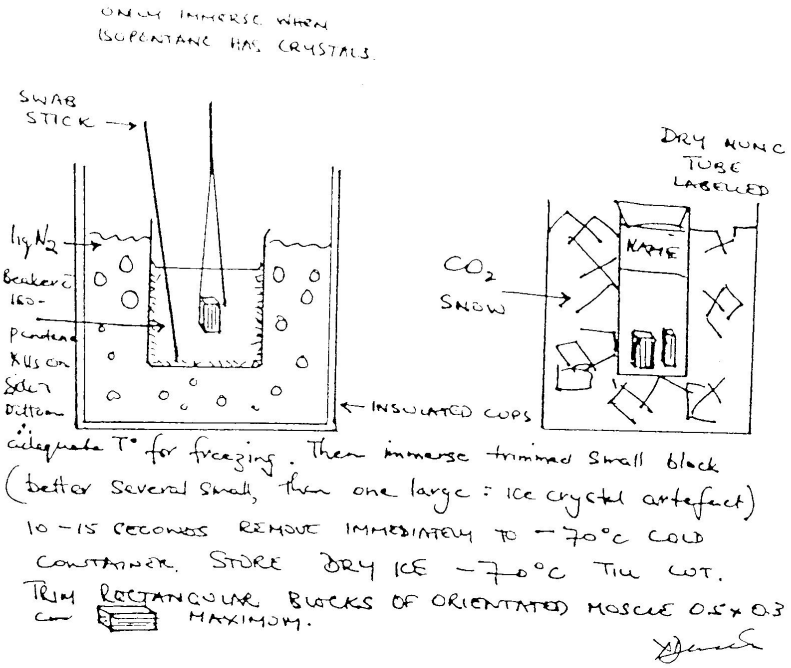
1. Remove needle point forceps from the 70% alcohol solution (this is changed weekly) and dry them completely by wiping inside forceps.
2. Label a Nunc tube with the M-number, the patient's surname and given name.
3. Label a cardboard ticket with the M-number and the patient's surname and place in the Nunc tube.
4. Place the Nunc tube in the dry ice to pre-cool it.
5. Fill plastic cup carefully with liquid nitrogen.
6. Add 50mL of isopentane to a plastic beaker.
7. Carefully place beaker into the liquid nitrogen.
   N.B. If the liquid nitrogen comes into direct contact with the isopentane it will solidify and the procedure must be started again.
8. Allow the isopentane to cool. View the three stages of the isopentane cooling process:
   - ⇒ The first stage - growth of a few crystals of isopentane on the bottom of the beaker (approx -120°C)
   - ⇒ The second stage - the bottom and portions of the sides of the beaker contain crystals the tissue can be frozen without forming microscopic ice crystals (approx -132°C).

⇒ The third stage - a solid mass of isopentane is obtained on the sides and bottom of the beaker together with much opacity and free crystal formation in the remainder of the liquid (minimal temperature of -154.5^{o}C)

⇒ Figure 14 (over the page) represents the cooling curve for isopentane-liquid nitrogen.

9. Remove the fresh specimen carefully with the tip of the needle point forceps and place on to a clean piece of dental wax. Dab specimen on filter paper if excess moisture (Ringer's saline or PBS) remains from specimen pot.
10. Measure the dimensions of the biopsy and record the size and any unusual observations (e.g. pale, fatty etc.) in the muscle specimen book.
11. The pieces to be frozen should be no larger than 5x3x3 mm^3. If they are larger then the specimen needs to be cut to size.
12. This is achieved by using the *two scalpel blade* technique. If the orientation is not apparent from the specimen it can be viewed under a low power stereo dissection microscope.
13. Place a scalpel blade in each hand and cross them over the specimen.
14. Draw the blades across each other evenly.
15. Place the cut-down specimen into the cooled isopentane for approximately 15 - 25 seconds. Depending on the size of the muscle biopsy. For larger biopsies a greater freezing time can be used.
16. The specimen is then placed into the pre-cooled Nunc tube which is in the container of dry ice.
17. The Nunc tube is then placed in the -70°C freezer where it is stored indefinitely.

NOTE: If **NO** glutaraldehyde-fixed specimen is received, a very small (2x2x2 mm) piece of tissue should be placed in 2.5% glutaraldehyde for EM processing.



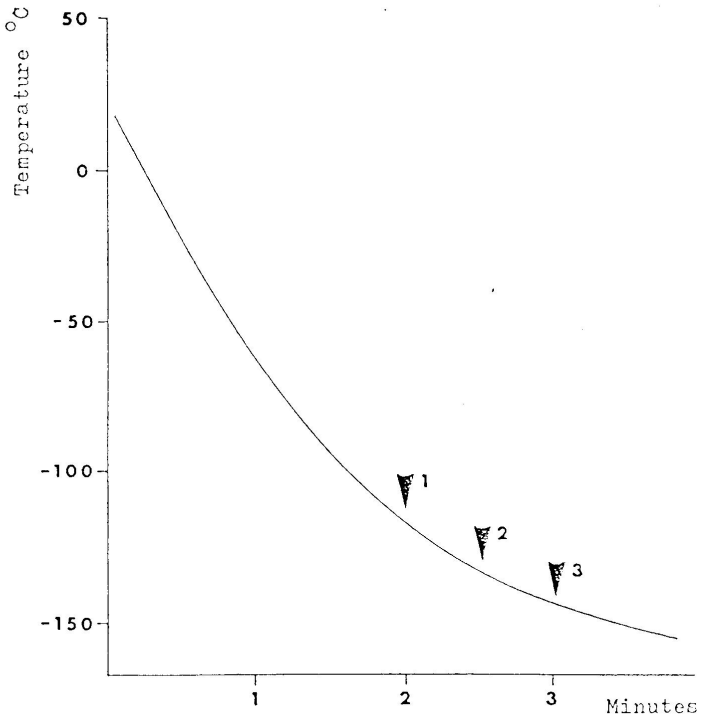


FIGURE 14

Cooling Curve for Iso-pentane in Liquid Nitrogen

Standard curve for pre-cooling 35 mls. isopentane in plastic beaker by liquid nitrogen. Arrow 1 indicates the initial detectable crystal formation (approx.-120°C.). Arrow 2 indicates satisfactory temperature for snap freezing specimens (approx.-130°C.). Arrow 3 indicates dense peripheral freezing and opaque fluid change (-145°C.).

6.2 Freezing Needle Biopsies of Muscle

Equipment

The equipment needed for this procedure is as per the open freezing equipment list. The only addition is the foil scalpel blade rappers which are fashioned into small cylinders approximately (5x3x3 mm) see method for details.

Method

1. Carefully remove the needle biopsies from the specimen container and place them on dental wax.
2. Record the approximate total length of the needle biopsies.
3. Cut the tissue into pieces approximately 5mm in length.
4. If no glutaraldehyde-fixed specimen is received, place a piece of tissue in 2.5% glutaraldehyde for EM processing.
5. Cut several pieces of aluminium foil into pieces of about 1x1 cm^2, and fold them into trough shapes using the end of the white pen stored in the fume hood.
6. Place a number of pieces of tissue into each aluminium trough to make a composite block of approximately 5x3x3 mm.
7. Pick up the tissue in the trough with forceps and snap freeze as in the above method.

6.3 Cutting and Staining Cryostat Sections

Equipment

Micron HM 550 (Ziess), cover slips (22 x 22mm), cutting rack, cover slip holder, gauze, acetone and glass beaker with 25 mL of tap water.

Method

1. Label 20 cover slips (22 x 22mm) with the patients “M” number, with the third and last cover slips label the patient surname (with a solvent proof pen - Grale Scientific).
2. Place the labeled cover slips into the plastic cover slip container with the label facing up.
3. Transport frozen muscle specimens from the freezer in small bucket of dry ice.
4. Remove tissue from the nunc tube and decide on which block of tissue is to be cut.
5. Mount the tissue block in a transverse orientation on the chuck with a small amount of water.
6. Place the chuck in the microtome chuck holder ensuring that it is secure by tightening the chuck screw.
7. Begin trimming the block until there is a full face of tissue.
8. Cut the first three sections and stain the trichrome section (3) immediately. This serves two purposes (a) it identifies and enables the scientist to trace the patient & (b) reduces the risk of staining artifact.

9. Cut the remainder of the sections and store them in the 4^{o}C fridge until ready to stain.
10. Make up staining solutions.
11. For each of the seven 1 hour stains place (sections 6 – 14 except section twelve) one cover slip/patient/Petri dish.
12. Apply stain solutions with a plastic pipette, cover and place into 37^{o}C incubator for 1 hour.
13. At the completion of the incubation take sections from one Petri disk at a time and place them into a staining rack, wash in tap water, post fix in formal saline for 1 minute and wash in tap water then mount with glycerin jelly.
14. All other sections have specific staining methods; refer to technical methods manual for details.

7. LABELING & RECORD-KEEPING

7.1 Routine Histochemistry Labeling

- Slides for normal routine histochemistry set must have the following label information:
    1. Patient M number.
    2. Patient last name.
    3. Patient first name.
    4. Type of stain.
    5. Date and initial of staff member completing the work.
    6. If control tissue is used the name and M number must also appear on the slide label.
    7. The date and the scientist completing the work is entered into the muscle book.

7.2 Immunohistochemistry Labeling

- Slides for immonuhistochemistry should be labeled with the following information:
    1. Control name and M number.
    2. Patient's name and M number.
    3. Date and initials of scientist.
    4. Sequence number.
    5. The product code of the antibody.
    6. The name of the antibody.

7.3 Imunohistochemistry Record-Keeping

- All immunohistochemistry stains should be recorded in the red immunohistochemistry Record Book, including the following information:
    1. Antibody
    2. Batch number of the antibody
    3. Dilution and details of the procedure used
    4. Cross-reference to page number in black workbook
    5. Name of patient and control.

8. STAFFING ARRANGEMENTS & TRAINING

8.1 Staff duties & Responsibilities

The duties and responsibilities for each staff member are documented in a position description which is held by the personal assistance to the Department Head.

8.2 Staff Supervision & Backup

In the event that the Senior Scientific Office (SSO) is away it is the responsibility of the Scientific Officer (SO) to perform the duties normally undertaken by the SSO. In addition the Department Manager is advised of the absence and can be called upon to assist staff.

Authorities and responsibilities of all staff are detailed in Position Descriptions

Table 1: List of Senior Positions, the Incumbent and Deputies

Role	Personnel	Deputy
Approved Pathology Provider	Professor Catriona McLean	Professor Paul Monagle
Senior Scientific Officer	Mr Paul Kennedy	Ms Veronika Gazdik
Scientific Officer	Ms Veronika Gazdik	Mr Paul Kennedy
Administrative Assistant	Mrs Pauline Middelveld	Mr Paul Kennedy & Mr Matt Reardon
Technical Assistant	Mr Matt Reardon	Ms Veronika Gazdik

8.3 Continuing Education

Monthly laboratory meetings are held and minutes are kept of these meetings. A regular agenda is followed with the following items (Quality Management issues, QPulse, Technical concerns, other business). Staff are encouraged to attend national & international conferences.

8.4 Staff Training

At the completion of the Departmental induction process new staff in the State Neuropathology Service are trained in the specific tasks of the service within the scope of their job description. Records of this training are kept within the laboratory & in QPulse. The following items are covered in the training.

Table 2: List of training needs & topics to be covered.

Training Need	Topics to be covered
Introduction to quality management system "policies & procedures"	♦ Writing quality systems documents ♦ Document control ♦ Internal audits ♦ Records & data management ♦ Purchasing goods and services ♦ Non-conformance reporting ♦ Staff training & competency ♦ Management review ♦ Review of contracts ♦ Measurement uncertainty ♦ Protection of privacy
Specimen reception and processing	♦ Safety & Infection Control. ♦ Specimen Reception ♦ Patient data entry ♦ Procedures for unsuitable or inadequately labelled specimens ♦ Specimen labelling ♦ Specimen cut-down ♦ Snap freezing of muscle biopsies ♦ Freezing and thawing artefacts, implications and prevention ♦ Storage and recording of frozen tissues, current and archival
Cryomicrotomy	♦ Care and use of cryostats ♦ Microtomy and cryomicrotomy ♦ Commonly encountered artefacts ♦ Quality control ♦ Coverslip and slide treatment
Routine staining	♦ Routine staining with haematoxylin and eosin, sudan black B and Gomori trichrome

	♦ Additional routine histochemical stains
	♦ Routine enzymatic stains
	♦ Positive and negative controls
	♦ Mounting media and coverslipping
	♦ Labelling individual slides
	♦ Quality control, common artefacts and their prevention and correction
Issuing completed work	♦ Quality control
	♦ Patient data entry
	♦ Signing off process
	♦ Issuing completed work
Immunocytochemistry	♦ Materials and methods
	♦ Ordering and storage of reagents
	♦ Quality control, positive and negative controls
Specialised procedures for processing infected material	♦ Storage and labeling of presumptive infected material
	♦ Glove and masking procedures during Cryotomy
	♦ Shut down and cleaning of cryostat
	♦ Staining procedures

8.5 On-going competency assessment

As part of ongoing training within the unit staff are periodically assessed for competency. The assessment may take the form of parallel testing with the trainer, observation by the trainer and/or testing of QC material (obtain expected results). For example, at the completion of each staining set the Senior Scientific Officer (SSO) views the slides from the days work with scientist who completed the work. The work is assessed for correct labeling, section thickness and artifacts, stain intensity and other significant pathology. Records are kept of ongoing competency training refer to QMS-FO-035.

Staff are informed of their training status and information gathered forms part of the performance appraisal. For further details on assessing competency refer to document number QMS-PR-010.

9. LABORATORY MAINTENANCE

9.1 Instructions for Daily Calibration and Use of the pH Meter

Calibration should be carried out once a day before use.

1. Turn on the pH meter if not already on.
2. Check the level of the saturated KCl solution. It should be no more than 5 mm below the level of the filling hole.
3. Check the level of the KCl crystals. It should be about 5 mm.
4. Check that the crystals are not compacted together. This is done by inverting the electrode several times. The crystals should move freely in the solution. If they do not, then repeated inversion should loosen them.
5. Check for air bubbles. If present, gently tapping the electrode with the finger should release them.
6. Uncover the filling hole.
7. Rinse the electrode with distilled water from a wash bottle.
8. Fill a plastic beaker with the recommended buffer (pH 7.0) and immerse the electrode in the buffer, agitating the solution gently.
9. Press the CAL key once ("1. CAL READY" appears on the display together with the measured pH value).
10. Wait for a stable pH value (i.e. when the decimal point stops blinking).
11. Press the CAL key again ("CALIBRATED" appears on the display for 2 seconds after which "2. CAL READY" is displayed).
12. Rinse the electrode with distilled water.
13. Fill a plastic beaker with the second buffer (pH 4.0) and immerse the clean electrode in the buffer, agitating the solution gently.
14. Wait for a stable pH value (i.e. when the decimal point stops blinking).
15. Press the CAL key ("CALIBRATED" appears on the display along with the pH value).
16. The calculated sensitivity appears on the display for 2 seconds after which the pH meter is ready for measurements.
17. Wash electrode with distilled water and wipe with a tissue.
18. Immerse electrode in the solution to be pH'ed.
19. When reading is completed rinse the electrode again, wipe, and immerse in a the pH 4.0 buffer.
20. The filling hole should be covered when not in use.

(If only a single-buffer calibration is desired, press the pH key after step 10 and the calibration is finished.)

9.2 Routine Maintenance of the pH Meter Electrode

Routine maintenance should be carried out every fortnight.

MODEL: pHC2401 Combined Electrode. Radiometer, Copenhagen.

AUSTRALIAN DISTRIBUTOR: Foss Electric P/L

1. Check levels of saturated KCL solution and KCL crystals. At the measuring temperature a 5 mm layer of KCL crystals will be sufficient. If necessary, refill the KCL reservoir with saturated KCl solution and with fine grained KCl crystals. Make sure that the top of the saturated KCl solution is less than 5 mm below the filling hole.
2. Check for air bubbles. Make sure that no air bubbles are trapped in the KCl crystals, and that no bubbles are present in the glass. If air bubbles are present in the glass bulb, unplug the electrode from the pH meter and release the bubbles by tapping the electrode with a finger or by swinging it in circles.
3. If air bubbles are trapped in the KCl crystals or the crystals do not move freely, warm the electrode in a waterbath at 50-60°C until sufficient KCl has dissolved to release the bubbles or dislodge the crystals. If this is not sufficient replace the KCl with distilled water and repeat the process. Remove the inner solution immediately the crystals have dissolved, otherwise the electrode may be harmed.
4. If replacing the KCl solution and crystals or cleaning the electrode with the proprietary cleaners, follow the reference cards supplied with the electrode.
5. Check for protein contamination. If the porous pin at the end of the reference portion of the electrode appears to be a black colour immerse the electrode in a 5% hypochloride solution for a few minutes and then rinse with distilled water.

9.3 Weekly Cleaning Schedule

1. Change solutions in routine H&E set, wash glassware in RBS solution and dry in oven or by hand.
2. Change isopentane in Biohazard fume hood.
3. Wipe down benches, sinks and incubator with 70% ethanol and then clean with Ajax.
4. Make up solutions for the following week if required:
    - Baker's formal calcium
    - Periodic acid
    - 1% $CaCl_2$
    - 2.5% $CoCl_2$
5. Check stores and order any supplies that may be required.
6. Wipe down inside of Biohazard fume hood with 70% ethanol.
7. Make up Petri dish set for the following week.
8. Send dirty glassware in the acid bath to be washed.

9. Make up any stock solutions that may be required.
10. Make up biopsy kits if required.
11. Perform routine maintenance procedures on the pH meter electrode.

10. SAFETY

10.1 Protocol For Handling Infectious Specimens

Specimens

1. All fresh tissue specimens are to be handled as infectious.
2. A warning label MUST accompany all specimens known to be infected with HIV or Hepatitis virus.
3. Each HIV or HBV specimen is processed individually.

Gloves

1. For handling HIV or HBV double gloves should be worn at all times.
   This includes:
   i) freezing the specimen
   ii) sectioning in the cryostat
   iii) staining of coverslips
   iv) decontamination of:
   (1) instruments
   (2) benches
   (3) cryostat (rubber gloves should be worn over a surgical pair)
   v) handling of infectious waste
2. Gloves should be placed in an infectious waste bag which is sealed and double-bagged prior to incineration.

Gowns

1. Long-sleeved gowns MUST be worn at all times when dealing with HIV or HBV.
2. Material protective gowns are placed in a yellow alginate linen bag, double-bagged into a yellow nylon drawstring bag and then sent for laundering.

Masks & Eyewear

1. Disposable masks and disposable protective eyewear should be worn during procedures that are likely to generate droplets of blood or the spread of tissue debris, thus preventing exposure of the mucous membranes of the mouth, nose and eyes.

Therefore they should be worn when:

i) the fresh specimen is being frozen

ii) the cryostat is being decontaminated after sectioning

Hand-washing

1. Hands should be washed using Chlorhexidine Gluconate 2.5% w/v
2. This is done:
    i) immediately after removal of gloves
    ii) immediately after completion of lab procedures

Sharps

1. Scalpel blades and broken glass should be disinfected with 5000ppm Sodium Hypochlorite for 30 minutes before being placed into a puncture resistant container.
2. The container is then sealed and sent for commercial incineration at 1000°C under E.P.A. regulations by a private waste contractor.

Disinfection / Decontamination

1. Work surfaces:
    i) Should be decontaminated with 5000ppm Sodium Hypochlorite solution at a minimum exposure time of 10 minutes.
    ii) Surfaces can then be washed down with detergent and water.
2. Specimen pots and disposable staining dishes should be treated with 5000ppm sodium hypochlorite for 30 minutes before being placed in an infectious waste bag and sent away for incineration at 1000°C under E.P.A. regulations by a private waste contractor.
3. Instruments should be treated with 5000ppm sodium hypochlorite for 20 minutes, then washed with detergent and water. The instruments are then washed with 70% ethanol and air-dried in a hot oven.

NOTE:

    i) Longer exposure of metal instruments to sodium hypochlorite solution results in corrosion of the instruments.
    ii) Gloves should be worn when using sodium hypochlorite solution as it is a skin irritant.

4. Cryostat:
    i) Inside the cryostat all tissue debris is covered with a cotton wool swab which has been soaked through with 5000ppm sodium hypochlorite, and allowed to stand for a minimum of 10 minutes.
    ii) The cotton wool swab is then discarded into an infectious waste bag for incineration.
    iii) The microtome is dismantled as much as possible inside the cryostat chamber and is washed down with 5000ppm sodium hypochlorite solution.
    iv) The inside of the cryostat chamber is washed down with the 5000ppm sodium hypochlorite and both are allowed to stand for a minimum of 10 minutes.
    v) The microtome and chamber are then washed down thoroughly with detergent and water.

vi) The microtome can then be removed from the cryostat chamber and washed down with 70% ethanol, air-dried, re-oiled and returned to the chamber.

Spills

As fresh tissue is being dealt with the likelihood of a spillage is lessened. However, any contamination resulting from blood droplets being scattered should be treated with a disposable cloth soaked with 5000ppm sodium hypochlorite and left to stand for a minimum of 10 minutes. The residue is washed away with detergent and water and the area is wiped down with a paper towel. Gown and gloves must be worn for blood spill decontamination procedures.

11. **APPENDIX A HISTORICAL EVOLUTION OF THE "MUSCLE BIOPSY CLAMP"**

The following text details the historical evolution of the "Muscle Biopsy Clamp" and general information of muscle biopsy procedures

11.1 The Muscle Clamp

The muscle clamp (R. McDonald Anderson, personal **comm.) consisted of two** chrome plated radio clips (Utilux) soldered or preferably screwed to a base plate of stainless steel. A number of commercially available clamps were tried and/or modified including:

Lung forceps (Price et al. 1965) **Muscle clamp (Carter, 1969) Isometric** biopsy clamp (Rayport, 1969)

Pennington hemostatic forceps (Vick & Olson, 1970) However, all **proved unsatisfactory for the surgeon** who required independent control of each clip during the clamping of the muscle.

Chrome-plated radio clips were easily obtainable, relatively cheap and also had a strong spring enabling the serrated teeth to provide a very firm hold on the **muscle, thus preventing retraction once the muscle was divided.** The **clips were screwed** onto a stainless steel backing plate approximately 3/8" thick, **three centimetres long** and two centimetres wide. The two clips were set about 1.5 and 2.5 centimetres apart for paediatric and adult biopsies respectively. Final blocks of fixed muscle approximately one and two centimetres in length were obtained free of stretch or clamp artefacts. The clamps were relatively rustproof, easily cleaned, readily autoclaved and could be used many times. Eventually the spring weakened and the clip **was no longer effective, but a routine check** each time the **clamp was** cleaned **detected** this. A new clip was easily replaced.

HUMAN BIOPSY MATERIAL

Diagnostic muscle biopsies were most commonly taken under general anaesthetic, and the quadriceps muscle, particularly the vastus lateralis, was the most frequently biopsied. However the distribution and severity of the disease process determined which muscle(s) could provide the most information, and consequently determined which muscle was biopsied.

Portions of each biopsy were processed for electron microscopy, routine paraffin sections and for histochemisty, using the muscle biospy kit previously described.

Thus the first portion of muscle was for electron microscopy, because of the need for minimal trauma and handling. This thin piece of muscle approximately one millimetre thick and two centimetre long was clamped in situ, then removed and immediately placed in ice cold two per cent cacodylate buffered glutaraldehyde. Figure 13 shows the sequence of events for obtaining the initial biopsy specimen.

The snap frozen muscle was then rapidly transferred from the isopentane to a small and dry glass container stored in the dry ice flask. The muscle, together with a label was placed inside the container and a plastic lid provided an air-tight seal. The specimen could be transported or stored at -70°C deep freeze. On occasions, specimens at least two years old had been

subsequently recut and stained, the only deterioration being some peripheral drying due to the "chamber effect".