

Clinician's Guide to Histopathology Sample Collection

CLINICIAN'S HANDBOOK



**Department of Neuropathology
NIMHANS, Bangalore**

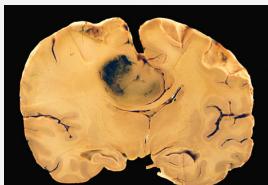
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Our Services

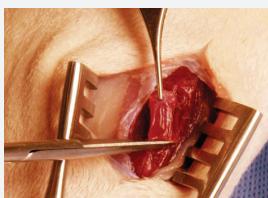
NEUROONCOLOGY (BRAIN TUMOR DIAGNOSIS)



- Intraoperative frozen section*
- Brain tumor diagnosis: routine histology *
- Immunohistochemistry*

FISH for 1p19q co-deletion and MS-PCR for MGMT methylation (in Neuro-oncology Lab)

MUSCLE DISEASES



- Routine histology *
- Enzyme histochemistry*
- Immunohistochemistry*
- Electron microscopy*

Recently introduced: Western blot,
Mitochondrial enzyme assays
(in Neuromuscular lab)

PERIPHERAL NEUROPATHIES



- Routine histology
- Semithin / electron microscopy (where indicated)

Skin punch biopsy for small fibre neuropathy
(only on request)

Note: teased fibre preparation is NOT performed

SKIN BIOPSIES



- Routine histology for Lafora bodies, vasculitis*
- Electron microscopy for NCL/storage disorders (where indicated)

Skin punch biopsy for small fibre neuropathy
(only on request)

Getting Started

LOCATION OF THE DEPARTMENT & ITS SECTIONS

Main Histopathology Laboratory is located on the first floor of Administrative Block.

Electron Microscopy section is also located in the same building in the basement

Transfusion Medicine Centre is housed in the Neurocentre Building, first floor, rear wing.

Clinical Pathology & hematology Section is located in the Casualty Block on the first floor

Mortuary is housed in a separate building behind the Casualty and New Psychiatry buildings

Human Brain Tissue Repository (Brain Bank) is housed in the Neurobiology Research Centre, ground floor, Room No. 004

DEPARTMENT OF NEUROPATHOLOGY & ITS SECTIONS

Department of Neuropathology & its Sections



HISTOPATHOLOGY SECTION



CLINICAL PATHOLOGY
& HEMATOLOGY



ELECTRON MICROSCOPE
LABORATORY



BLOOD BANK



HUMAN BRAIN TISSUE
REPOSITORY (BRAIN BANK)



MORTUARY

DEPARTMENTAL WORKING HOURS

DEPARTMENTAL WORKING HOURS

SECTION	Opening hours	Closing time
Histopathology		
Weekdays (Mon-Sat)	9.00 am	4.30 pm
Second Saturday, & all closed Govt holidays declared by Institute (Sundays holidays)	9.30 am	3.30 pm
Electron Microscopy Weekdays (Mon-Sat) only	9.00 am	4.30 pm
Clinical Pathology	Open round the clock on all days	
Mortuary Staff and faculty are on call (in campus) and functions 24x7	9.00 am (all days including Sundays, & holidays)	4.30 pm



DEPARTMENTAL FACULTY & CONTACT NUMBERS

SECTION	Faculty	Conact No.s
Histopathology	Dr. Vani Santosh Dr. Yasha TC (HOD) Dr. N. Gayathri Dr. Anita Mahadevan	(2699)5132 (2699)5133 (2699)5131 (2699)5137
Electron Microscopy	Dr. B.K. Chandrashekhar Sagar Dr. N. Gayathri	(2699)5738 (2699)5131
Transfusion Medicine Centre	Dr. Sundar Periyavan	(2699)5437
Clinical Pathology	Dr. Sangeetha Seshagiri	(2699)5750
Mortuary	Dr. Anita Mahadevan Dr. Yasha TC Dr. Vani Santosh Dr. S.K.Shankar (Emeritus Prof)	(2699)5137 (2699)5133 (2699)5132 (2699)5786
Human brain Tissue Repository	Dr. S.K.Shankar (Emeritus Prof) Dr. Anita Mahadevan	(2699)5786 (2699)5137

Section	Phone number
Office	(2699)5130
Residents Room (Post Doctoral Fellows)	(2699)5134
Discussion Room	(2699)5135
Laboratory	(2699)5136
Electron microscopy	(2699)5138
Clinical Pathology and Hematology	(2699)5516
Mortuary	(2699)5213
Brain Bank (Neurobiology Research Centre)	(2699)5786
Neuropathology Museum (Neurobiology Research Centre)	(2699)5582

LIST OF TESTS & TURN AROUND TIME

LIST OF TESTS & TURN AROUND TIME

SI No	Tissue type	Specific Test	Turn around Time * , #	Frequency of testing
1	Tumor tissue (Formalin fixed)	Routine Histopathology Special stains or Immunohistochemistry	4 days 7 days	All working days
2	Fresh tissue	Intra-operative smear/ squash	20 mins after sample receipt	All working days
3	Epilepsy surgery	Histopathology & immunohistochemistry	15 days	All working days
3	Fresh Muscle	Histopathology & enzyme histochemistry Immunohistochemistry	4-7 days 4 weeks	All working days Once in 4 weeks
6	Nerve biopsy (glutaraldehyde)	Histopathology	4-7 days	All working days
6	Skin (formalin/ glutaraldehyde)	Routine histopathology Electron microscopy	4-7 days 6 weeks	All working days
7	Liver biopsy (formalin/ alcohol)	Histopathology with special stains	4-7 days	All working days
8	Brain biopsy (Fresh)	Histopathology & immunohistochemistry	10 days	All working days
10	Paraffin block	Routine Immunohistochemistry	4 days 7 days	All working days
13	Slides	Histopathology	2 days	All working days

* in working days

General guidelines: Routine HPE - 4 working days
Special stains/Immunohistochemistry: 7 working days



General Instructions for all Samples

All samples and requests are screened for the following:

- (i) All samples must be accompanied by a histopathology request form, completely filled with all relevant details. The request form for inpatient NIMHANS cases and outside Referral cases are different and non exchangeable.
- (ii) Request should be from treating physician. Requests from patient party are not entertained
- (iii) All samples should have at least two identifiers – patient name, and hospital number , hospital name (if from outside) with age and gender.
- (iv) Personal identifiers of the sample and the request form should match
- (v) Appropriate fixative/ container must be used; and transported/ stored in appropriate conditions to ensure stability of sample and optimum results

Sample receipt timings:

Mon-Sat: 9.00am – 4.30pm

Second Saturdays, Institute holidays: 9.00am-3.30pm

Sundays are holidays.

EHOSPITAL REQUEST - NEUROSURGICAL BIOPSIES

Surgical biopsy

For all biopsies from Neurosurgery and NIIR (including CT guided/stereotactic, tumors, lesions, brain biopsies etc), choose “ SURGICAL BIOPSY” from list of investigations

Epilepsy surgery

For all epilepsy surgeries, choose “ EPILEPSY SURGERY” from list of investigations.

Do NOT choose “Surgical Biopsy” for epilepsy surgery

NEUROSURGERY SAMPLES

Surgical Biopsy

The screenshot shows the e-Hospital @NIC software interface for the National Institute of Mental Health and Neuro Sciences. The top navigation bar includes links for 'e-Hospital @NIC', 'Logout', 'Show Left Member', 'Welcome Mr. NIMHANS Admin', 'Last Updated : Feb 21, 2014, Friday At 04:24:08 PM', 'Add New Investigation(s)/Procedure(s)', 'Search New Patient', and 'Logout'.

The main form is titled 'Patient Information' and contains the following details:

- UHID:** 09130036444 **MRD No:** 1652245
- Name:** NATARAJ S II (Male)
- Address:** null
- Patient Status:** Outdoor
- Dept/Context No:** 20123095
- Age:** 49 years 4 months 26 days
- Payment Category:** B.P.L. Card holder(0 to 19999)
- Department:** Neurosurgery
- Unit / Unit Incharge:** NS II / Dr. S. Indra Devi
- Gen. Cond. of Patient:** null

Below this, there are sections for 'Investigation' and 'Procedure'. The 'Investigation' section is selected, and the category is set to 'NEUROPATHOLOGY'. A dropdown menu for 'Filter Observation Table' shows 'SUG' (Optional). To the right, there are buttons for 'Order Entry Template', 'Select Template', 'Select Page Link', and 'Go >>'. Further down, there are fields for 'Department' (Neurosurgery), 'Unit' (NS III), 'Recommended by' (Dr. Indira B. Devi - Professor & Head), and 'Contact No'. A 'Verified' button is also present.

A 'Service Name' field contains 'SURGICAL BIOPSY (NEUROSURGICAL/ST/BCT GUIDED BIOPSES FROM NIR) (Ra. 0)' with a 'Select' button next to it. On the right, there is a 'Verify Observation(s)/Medicine(s)' section and a table titled 'Investigation' with columns for 'Investigation Category', 'Investigation', 'Date', 'Status', and 'Report'. The table shows one entry: 'NEUROPATHOLOGY' under 'Investigation Category' and 'SURGICAL BIOPSY (NEUROSURGICAL/ST/BCT GUIDED BIOPSES FROM NIR)' under 'Investigation'. The status is 'Active' and the date is '24-2-2014'.

*Choose with care. Remember
the patient pays for our
mistakes....*

EHOSPITAL REQUEST - NEUROLOGICAL SAMPLES

Muscle Biopsy

For all Muscle biopsies from Neurology, choose “ MUSCLE BIOPSY” from list of investigations.

Specify the test required-

- Enzyme histochemistry
- Immuno histochemistry (panels 1,2,3,)
- Do not choose “ Electron Microscopy” (decision for this test is taken only by Neuropathology faculty)

Nerve Biopsy

For all Nerve Biopsy from Neurology, choose “ NERVE BIOPSY” from list of investigations.

- Do not choose “ Electron Microscopy” (decision for this test is taken only by Neuropathology faculty)

Skin Biopsy

For all SKIN Biopsy from Neurology, choose “ SKIN BIOPSY” from list of investigations.

- Do not choose “ Electron Microscopy” (decision for this test is taken only by Neuropathology faculty)

Other Biopsies

For lip, liver, Lymph node etc. choose other “ OTHER BIOPSIES” from list of investigations.

Multiple Biopsies

For more than one biopsy (eg. Nerve and Muscle / Nerve, Muscle and Skin etc.) choose “ MULTIPLE BIOPSY” from list of investigations

NEUROLOGY SAMPLES

e-Hospital @NIC **National Institute of Mental Health and Neuro Sciences**

Welcome Mr. NIMHANS Admin Last Updated : Feb 21, 2014, Friday At 04:24:09 PM

Add New Investigation(s)/Procedure(s)/Medicine(s)/Immunization(s)/Other Service(s) Search New Patient:

Patient Information

UIN: 20130036444 MBD No.: H632145
 Name: NATARAJ S H (Male)
 Address: null
 Patient Status: Outdoor

Dept/Context No.: 20123965 Age: 49 years 4 months 26 days
 Payment Category: BPL Card Holders(0 to 19999)
 Department : Neurosurgery
 Unit/Unit Incharge: H6 8 Dr. S. Indira Devi

Gen. Cond. of Patient

Investigation Procedure

Category: NEUROPATHOLOGY

Filter Observation Table: SU (Optional)

Order Entry Template Select Template Select Page Link Go >>

Department: Neurosurgery Unit: NS III
 Recommended by: Dr. Indira B. Devi - Professor & Head Contact No:

Service Name Select

SURGICAL BIOPSY (NEUROSURGICAL/ST/BCT GUIDED BIOPSIES FROM NIR) (Rs. 0)

Investigation

Investigation Category	Investigation	Date	Status	Report
NEUROPATHOLOGY	SURGICAL BIOPSY (NEUROSURGICAL/ST/BCT GUIDED BIOPSIES FROM NIR)	24-2-2014	Active	X

*Choose with care. Remember
the patient pays for our
mistakes....*

Neurosurgical Biopsies

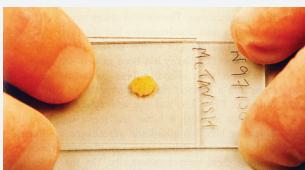
Intraoperative frozen

Caution

1. *Fresh Samples SHOULD reach the Lab within 30min-1hr during lab working hours.*
2. *Intraoperative frozen requests after lab working hours (4.30 pm) or holidays can be performed but should be sent after informing Neuropathology faculty/residents.*
3. *Any sample associated infection risk SHOULD BE CLEARLY STATED ON THE REQUEST FORM (eg., retroviral/HBsAg/ suspected prion disease) and marked BIOHAZARD*

NEUROSURGICAL BIOPSIES

Intraoperative frozen (squash)



Samples for intraoperative frozen (squash) to be sent in FRESH UNFIXED STATE (in normal saline)

- Ensure sample is immersed in saline (should not dry out before it reaches the lab)
- Additional samples collected after frozen section should be sent in 10% formalin (contact OT sister)

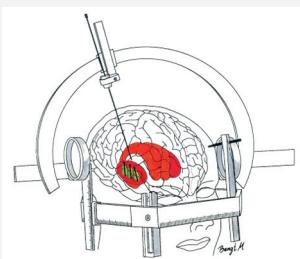
Routine samples (not requiring intraoperative frozen)

Fixative: 10% formalin

Tests Performed :

Routine Histopathology
Special stains
Immunohistochemistry

Small biopsies (stereotactic/CT/MR guided/ endoscopic)



Samples to be sent in 10% FORMALIN:

1. If multiple bottles collected, bottles to be labelled serially clearly INDICATING sites of biopsy

Brain Biopsies and Epilepsy surgeries

Caution

*Diagnostic brain biopsy is a special test and requires extensive co-ordination between clinician and pathologist **BEFORE** biopsy is done for optimal yield. Hence strictly follow the instructions below:*

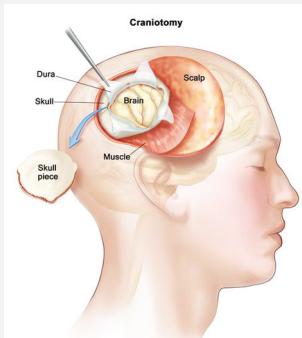
1. Brief the pathologist as to complete clinical details prior to biopsy
2. Confirm date of biopsy previous day
3. Timings: Ensure samples reach within 30 mins DURING working hours
4. If after working hours, inform pathologist prior to sending
5. Indicate clearly if risk of infection (eg., retroviral status/HBsAg/ prion etc) as it requires special precautions from our side. Label biopsy as BIOHAZARD
6. For suspected prion diseases, follow precautions as in pg 22

Tests performed:

- Routine and special stains;
- Immunohistochemistry

NEUROSURGICAL BIOPSIES

Diagnostic brain biopsies: FRESH (no fixative, only saline)



Biopsy Site: dictated by clinical/imaging findings

Biopsy size: 1x1cm cube with overlying meninges (include dura for pachymeningeal disease)

Fixative: Send biopsies in FRESH STATE ONLY (saline)

Epilepsy surgeries: FRESH (no fixative, only saline)



Label each sample clearly as to site
Wrap in saline soaked gauze
Send samples within 30 mins of collection

Fixative: It is ideal to send biopsies in FRESH STATE ONLY (saline)

Aspirates

Caution

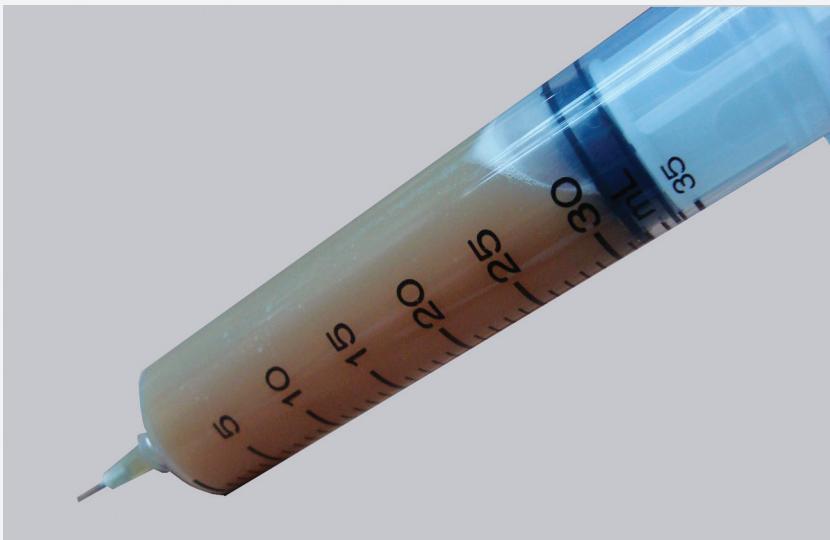
1. *Ensure all samples SHOULD reach the lab within 30 mins.*
2. **If there is delay in sending to lab and it cannot reach on same day, TRANSFER to 10 % formalin. DO NOT STORE in saline and send next day.**

Any sample associated infection risk SHOULD BE CLEARLY STATED ON THE REQUEST FORM (eg., retroviral/HBsAg/ suspected prion disease) and marked BIOHAZARD



NEUROSURGICAL BIOPSIES

Cyst fluid aspirates: FRESH (no fixative)



Samples to be sent in FRESH/UNFIXED state:

1. Fluid (if clear) should be sent to Microbiology department with request for cytospin (use Microbiology request form for cytology)
2. Fluid (turbid/bloody/particulate matter) to be sent to Neuropathology department

Infective Lesions (Eg., Abscess with Pus)

1. Send one sample in 10% formalin to Neuropathology
2. Send one sample (fresh) to Microbiology for culture (esp if fungal infection is suspected) with separate request form of Microbiology department

Muscle Biopsy

Biopsy site:

- Obtain biopsy from affected muscle (clinically/ENMG wise), with MRC Grade 3-4/5. Usual sites – vastus lateralis, biceps
- If involvement is purely distal, chose distal muscles (MRI/USG can help selection)

CAUTION:

- AVOID severely affected muscle with MRC grade <3
- AVOID muscle traumatized by IM injections or by EMG studies.

Collection and Transportation of sample

1. Fresh samples should reach the lab the same day before 12 noon on all working days (Mon-Sat), second Saturdays and Institute holidays.

2. **Biopsies are not accepted on Sundays**

Tests performed

- a. Routine Histopathology
- b. Enzyme histochemistry
- c. Immunohistochemistry for muscular dystrophies (only on FRESH, FROZEN muscle)
- d. Electron microscopy (for diagnosis of congenital myopathy, mitochondrial and storage disorders)

If paraffin blocks sent:

1. Only routine H&E and special stains are possible
2. Enzyme histochemistry/ immunohistochemistry/ electron microscopy **not** possible

Muscle Biopsy

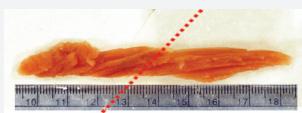
BIOPSY INSTRUCTIONS



Wrap muscle in gauze moistened with few drops of saline to prevent tissue drying



DO NOT USE EXCESS SALINE



Biopsy size – 2x2cms only

Biopsy specifications

- Dissected with minimum trauma along the long axis of the muscle fibers
- Do not use electrocautery or a muscle clamp
- Additional bits if more tests are needed (eg., culture/enzyme studies for mitochondrial or rare genetic disorders)
- Provide site, date and time of biopsy
- State what tests are required

Nerve Biopsies

Biopsy site: dependant on clinical/nerve conductions

- Clinically & electrophysiologically involved nerve
- Sural nerve/superficial peroneal nerve/dorsal cutaneous nerve/superficial radial (commonly biopsied)

Fixative:

- USE ONLY 2.5% gluteraldehyde
- DO NOT USE 10% buffered formalin
- DO NOT SEND FRESH (even for metachromatic stains)

Tests performed

- a. Routine Histopathology
- b. Special stains:
 - Myelin stains (Kulchitsky pal)
 - Masson trichrome for collagen
 - Cresyl violet for metachromatic granules
 - PAS-diastase stain & Lugol's iodine (polyglucosan bodies)
 - Fite Faraco (for lepra bacilli)
 - Congo red (for amyloid) etc
- c. Immunohistochemistry (eg., ubiquitin for polyglucosan bodies, neurofilament for axons etc)
- d. Electron microscopy (USE ONLY 2.5% glutaraldehyde)

Nerve biopsies



CAUTION: BIOPSY SPECIFICATIONS

- Take a minimum length of 2 cms biopsy
- AVOID pinching/crushing nerve during procedure
- DO NOT tie nerve with thread
- AVOID COMMERCIALLY AVAILABLE CIDEX

Skin Biopsies

Biopsy site: dependant on clinical differentials

- For PME diagnosis: axillary skin biopsy (biopsy should be DEEP enough to include Subcutaneous FAT)
- For vasculitis/Hansen's: lesional site (margin)
- For amyloid: abdominal fat aspirate/biopsy
- For CADASIL/Neuroaxonal dystrophy/other suspected storage diseases: axillary skin biopsy

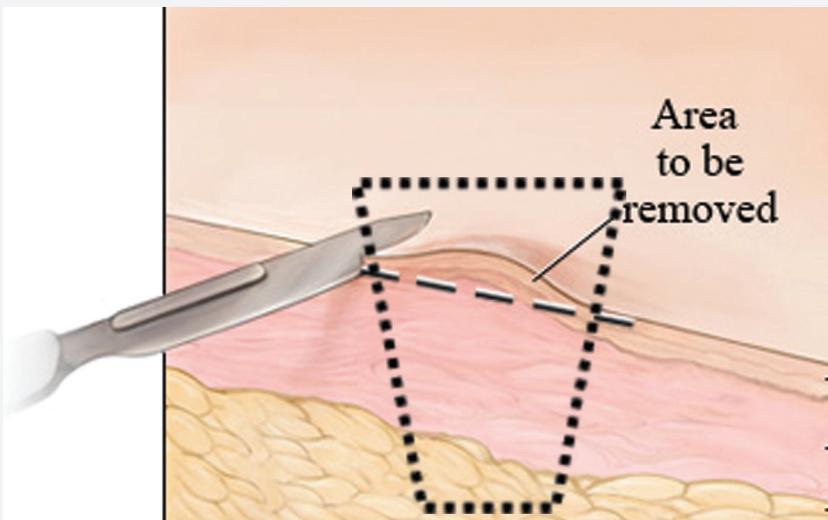
Fixative:

- 2.5% gluteraldehyde for PME/CADASIL/Storage (wherein electron microscopy maybe required)
- 10% buffered formalin for others (vasculitis/amyloid/Hansen's)
- SALINE/FRESH SPECIMEN NOT REQUIRED

Tests performed

- a. **Routine Histopathology**
- b. **Special stains:** PAS-diastase stain & Lugol's iodine (for polyglucosan bodies), Fite Faraco (for lepra bacilli), Congo red (for amyloid)
- c. **Immunohistochemistry** (eg., ubiquitin for polyglucosan bodies, neurofilament & ubiquitin for neuroaxonal dystrophy etc)
- d. **Electron microscopy:** Essential for diagnosis of: NCL, CADASIL, neuroaxonal dystrophy and storage

Skin biopsies



CAUTION

- Ensure biopsy is deep enough to include subcutaneous fat
- DO NOT USE COMMERCIALLY AVAILABLE CIDEX

Note: Skin punch biopsy for small fibre neuropathy IS A DIFFERENT PROCEDURE with specific biopsy sites, fixative (paraformaldehyde-lysine-periodate), and cold chain. This test is not being routinely performed (available for NIMHANS patients ONLY on research mode)

Sample Submission Criteria

Samples should be accompanied by Histopathology request form from TREATING PHYSICIAN, completed in all respects with Priority Status of Request (Urgent intraoperative frozen requirement or Routine) CLEARLY INDICATED ON THE REQUEST FORM

The following information must be documented in a LEGIBLE manner on the request form.

1. *Patient's Full Name (legible, preferably in CAPITALS).
2. *Patient's Neuro Number/UHID No.
3. *Patient's Age/Sex
4. Consultant name / Unit
5. *Specimen Type
6. Date and time of collection
7. Complete Clinical Details with results of relevant investigations and Clinical Diagnoses considered
8. Test requested
9. Signature of resident with Contact No./Pager Number
10. Priority Status of Request (Urgent or Routine)
11. Details of any sample associated Infection Risk

Items marked with an * are minimum identifiers and failure to provide the data required will delay processing of the sample or lead to withholding of results.

ALL Samples should be labeled with the following

1. *Patients' Full name (legible, preferably IN CAPITALS)
2. *Age/Sex
3. *Neuro number
3. *Unit
5. *Specimen Type

Paraffin blocks/slides submitted for review:

Request form should contain block/slide identification number and number of blocks/slides submitted in addition to complete clinical details and diagnoses considered.

Non-Compliance with Submission Criteria

Compliance with submission criteria is mandatory for the referring clinician to ensure accuracy of results.

Patient samples are precious and hence will not be rejected.

However, "non-compliance" with the above requirements will interfere with correctness of results or lead to delay/withholding of results/inability to perform certain tests.

The laboratory management will not bear responsibility for this.

Examples of non compliance:

1. Samples unaccompanied by completed Histopathology request form
2. Mismatch of information on the label and the request
3. Inappropriate transport temperature particularly for muscle biopsies
4. Excessive delay in transportation in case of fresh samples (muscle biopsies after 12.00 noon)
5. Inappropriate transport medium
 - a. specimen received in saline and autolysed at time of receipt
 - b. dried specimen, leakage of fixative due to breakage of container

Safety precautions for suspected "CJD" /prion diseases

1. **Operative procedure:** Follow universal precautions
2. **Send Biopsy Tissue fixed in 10% formalin to our lab, well sealed and double bagged to prevent leakage. DO NOT SEND FRESH**
3. **DECONTAMINATION PROCEDURES (after biopsy)**
 - Disposable clothing /instruments/ gloves/ cotton/gauze etc – double bag and mark for incineration
 - Disposable sharps – immerse in sharps can with concentrate hypochlorite (DO NOT DILUTE) for 30 mins, then mark for incineration
 - Non-disposable instruments - Steam autoclave at 134-136°C for 60mins(gravity displacement) or immerse in 2M NaOH (80gms in 1L)
 - Work surfaces, glassware – 2M NaOH
 - * 2M NaOH can be collected from Neuropathology dept one day before procedure (Ref: CJD Surveillance Unit, Edinburgh)

Release of laboratory reports

Telephonic reports

- Reports will not be given on telephone to patients/ relatives
- Reports over telephone are usually AVOIDED except for intraoperative frozen reports
- Under special circumstances (eg., patient waiting in OPD and reports have not been dispatched/reports not available in file) report maybe provided on telephone only to the treating physician/resident in charge of the case

Reports of intraoperative frozen sections

- Report will be informed telephonically to the Operation theatre by the resident/faculty/trained authorized technicians
- Report will be given after verifying name of patient and operating surgeon
- Entry of the person (resident/surgeon/anesthetist/OT sister/OT technician) receiving the report, date and time will be recorded on the reverse side of the HP request form.
- Inability to reach anyone in OT to give reports will also be recorded.

Release of laboratory reports

- All reports typed in by the office staff will be checked for typographic errors and patient demographic details by residents/faculty.
- Verified reports will be released in ehospital which is accessible to clinicians immediately
- A printed copy of reports will be also be sent by dispatch the next day to neurology/neurosurgery offices for filing in patient records
- COPIES OF REPORTS WILL NOT BE HANDED OVER TO PATIENT PARTY except in exceptional cases (eg., reports not available in case files/lost). In such cases, written request must be sent by the clinician to supply duplicate report if they have no facility for printing out from ehospital files.

Reports of test results considered “Critical Alert”

- In consultation with clinicians, following have been considered as requiring “critical alert” for immediate notification to treating physician
 - diagnosis of fungal infection, viral infections, demyelination
 - lymphoma, germinoma (radio / chemosensitive)
 - CSF cytopsin positive for malignant cells/cryptococci
 - Failed biopsies (muscle and nerve)

Reports are immediately informed (telephonically) to treating physician/resident in charge of the case. The clinician informed, including date and time is documented on the request form. A final written report is also sent subsequently

Retention time of specimens/blocks/reports after reporting

Retention time of specimens/blocks/reports after reporting

1. Tissues remaining after grossing and generation of reports will be reviewed by reporting faculty. Those of research/teaching interest will be retained, and others will be discarded within 6-8 months from the date of reporting
2. All paraffin blocks and slides will be preserved under appropriate conditions, chronologically numbered and will not be discarded.
3. Printed copies of all reports are bound and preserved protecting from any accident/loss due to fire etc. Soft copy in the computers are available in ehospital from Apr 2013.
4. Frozen muscle bits are retained for 2 years. Extra unstained cryosections for immunohistochemistry are retained for 1 year

Additional tests that can be performed on paraffin blocks can be requested at any time. Additional tests on frozen muscle biopsy/sections possible only if requested within the retention period

Review of reports

Review of reports

Review of previously reported slides can be undertaken at any time as paraffin blocks are not discarded.

All requests should be accompanied by request form with clinical details and specifying reasons for requesting review.

Request for duplicate slides

Duplicate slides for referral to oncology centres/ to obtain second opinion can be provided

Requests should be from treating physician only



Summary of tests

Sample	Tests possible (dependant on fixative)	Appropriate fixative, Special instructions, if any	Tests NOT possible due to sample type	Turn around time (average)
Tumor Fresh, unfixed	Frozen section/ squash for intraoperative diagnosis (preliminary)	Saline Should reach lab within 30mins	Routine HPE, immunohistochemistry	15-20 mins
Tumor Formalin fixed	Routine histopathology (HPE) Immunohistochemistry*	10% formalin	Frozen sections	4-7 days [#] 7 days
Paraffin block	Case review	NA	Frozen section	3 days (routine) 7 days (IHC)
Nerve biopsy (fixed)	Routine histopathology, Myelin stain (Kpal) immunohistochemistry*	Use ONLY 2.5% glutaraldehyde	-	7 days [#]
	Semithin*	Use ONLY 2.5% glutaraldehyde NOT CIDEX		21 days
	Electron microscopy*	Use ONLY 2.5 % glutaraldehyde		6 weeks
Paraffin block	Review		Kpal stain not possible (alternative myelin stains – Loyez possible), semithin, EM not possible	4-7 days [#] (routine) 7 days (IHC)
Skin Fixed	Routine HPE Electron microscopy	10% formalin/ 2.5% glutaraldehyde	-	4-7 days [#] EM – 6 weeks

in working days. Routine HPE 4 days, Special stains/immunohistochemistry - 7 days

At a glance...

Sample	Tests possible (dependant on fixative)	Appropriate fixative, Special instructions, if any	Tests NOT possible because of sample type fixative	Turn around time (average)
Muscle Biopsy Fresh	Routine HPE, Enzyme Histochemistry , Immunochemistry Electron microscopy. Western Blot# Enzyme assays#	Saline soaked gauze Should reach lab within 30mins. <u>Do not use too much saline</u>	-	7 days[#] IHC- 4 weeks EM – 6 weeks
Muscle biopsy Fixed	Routine HPE Electron microscopy	10% formalin/ 2.5% glutaraldehyde	Enzyme Histochemistry Immunochemistry Western Blot# Enzyme studies#,	4-7 days[#] EM – 6 weeks
Muscle Paraffin block	Review	Nil	Enzyme histochemistry, semithin, EM not possible	3 days (routine)
Diagnostic Brain biopsy Fresh	Routine HPE, IHC, EM Frozen section for lipid, Culture (for bacterial/ TB/ fungal), PCR for viral / Genetics	Saline (for suspected infective/ neurodegenerative/metabolic etiology)	-	7 days[#] (routine) 7-10 days (IHC) EM – 6 weeks
Brain biopsy fixed	Routine HPE Immunochemistry	10% formalin	Culture, special stains for lipid	7 days[#] (routine) 7-10 days (IHC)
Liver biopsy Fixed	Storage disorder (lipid/glycogen)	10% formalin & 70% alcohol	Note: for lipid/ glycogen demonstration, biopsy in 70% alcohol is MUST	7 days[#]

in working days. Routine HPE-4 days, Special stains/immunohistochemistry - 7 days

Histopathology Request forms

DEPARTMENT OF NEUROPATHOLOGY NATIONAL INSTITUTE OF MENTAL HEALTH & NEURO SCIENCES BANGALORE - 560 029. HISTOPATHOLOGY REQUEST			Date
Name:	Age:	Neuro Path No.:	
Operating Surgeon:	Unit:	Sex:	Neuro No.:
Nature of Specimen & Fixative:			
Clinical History			
Investigations:			
Provisional Diagnosis:			
Operation Notes/ diagnosis:			
Frozen/ Regular:			
Resident Doctor's Name: Signature			

ENSURE ALL FIELDS ARE FILLED UP

Sample transport requirements

PACKAGING SYSTEM FOR TRANSPORT OF PATHOLOGY SPECIMENS TO LAB

All clinical samples, in accordance with UN guidelines are generally classified as Category B and assigned to UN3373 (Biological Substance, Category B) and should be packaged in accordance with UN packaging instructions PI650

(Ref: WHO/CDS/CSRL/LYO/2004.9 entitled Background to the amendments adopted in the 13th revision of the United Nations Model Regulations guiding the transport of infectious substances http://www.who.int/csr/resources/publications/WHO_CDS_CSR_LYO_2004_9/en/).

The following procedures should be adopted for the transport of all specimens to the lab:

Step 1: Primary pack – should be water tight, leak proof, and labeled with patient detail and hospital name.

1. Place specimen with fixative in plastic container with tight screw cap. If there is more than one sample for the same patient, use separate plastic containers for each sample.
2. Label each container with patient name, details and site/biopsy



SAMPLE TRANSPORT REQUIREMENTS

Step 2: Secondary pack – encloses primary pack(s), leak proof and water tight and protects the primary sample

1. Place the primary samples container in a large plastic carrier that can be securely closed with lid.
2. Place large amounts of absorbent material (cotton/filter paper) between primary and secondary receptacle to absorb all spills, if any.
3. Seal securely
4. Box should carry “Biohazard” label
5. Disinfect external surface of container by wiping down with hypochlorite (1000 ppm)
6. Place clinical datasheet/request form securely enclosed within a plastic cover to prevent it from getting soiled with leakage of sample contents.



Packaging for Fresh Muscle Biopsies (without fixative)

Step 1: Primary pack – should be water tight, leak proof, and labeled with patient detail and hospital name.

1. Place muscle biopsy wrapped in gauze moistened with few drops of saline into plastic cover and seal securely.
2. Label container with patient name, details and site/biopsy type.

Step 2: Secondary pack – encloses primary pack(s), leak proof and water tight

1. Place the zip lock cover in Thermocol box/carrier with ice packs to maintain cold temperature in transit.
2. Seal securely
3. Place clinical datasheet/request form enclosed in a separate plastic cover to prevent from getting wet or soiled
4. Biohazard Label on outer container



1. Samples SHOULD reach the lab within 30 mins-1hr of biopsy
2. Samples should reach BEFORE 12 noon on working days (second Saturdays and Govt holidays, lab is open to receive samples)



Department of Neuropathology

CLINICIAN'S HANDBOOK

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Issue No. : 01
Copy No. :
Holder name :
Date of issue : 15.02.14

What is this manual about?

This manual is designed to provide an overview of the services available in the Neuropathology Department and serve as a quick reference guide for all users. Laboratory Management is committed to:

- Ensure stringent adherence to quality in all laboratory procedures that meet requirements of internal and external quality assessment tests.
- Ensure appropriate collection, transport and processing of samples to ensure optimal performance of all the tests required.
- Ensure reports are accurate, timely and clinically useful.
- Ensure use of procedures and methods that are up to date with current practices.
- Ensure continued staff training at all levels to keep them updated with recent advances in the field.
- Periodically assess user satisfaction by feedback forms.

Copies of this manual are available in specimen collection centres, wards, OTs, casualty, short stay wards. Electronic version is available in NIMHANS website.

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