National Guidelines in Histopathology

Collection, Handling and Transport of Surgical Specimens

Second edition
2021

Ministry of Health, Sri Lanka
and College of Pathologists of Sri Lanka

National Guidelines in Histopathology

Collection, Handling and Transport of Surgical Specimens

Second edition, 2021

Content editor of this book: Priyani Amarathunga

Series editors, National Guidelines in Histopathology, second edition:

Dilani Lokuhetty, Isha Prematilleke

Published online by Ministry of Health, Sri Lanka

ISBN 978-624-6147-03-7

First published: 2007

Message by the Director General, Health Services

Ministry of Health, Sri Lanka

Cancer is one of the leading non-communicable diseases in Sri Lanka. With the establishment of national cancer policy on cancer prevention and control, there has been a commendable improvement in the cancer services provided island-wide.

The pathologists play a major role in the diagnosis of cancer and it is of utmost importance to formulate new guidelines as well as to update existing guidelines to improve the quality of diagnosis and to predict the prognosis of the disease in cancer patients.

These guidelines on handling tumours of the gastrointestinal tract, breast and gynaecological region as well as the guidelines on specimen handling and transport appear to be comprehensive guides to the histopathologists practicing in Sri Lanka and hope that these guidelines will help to improve the quality and the consistency of the histopathology reports across the country.

I am very grateful to the College of Pathologists of Sri Lanka for having identified the need and have been able to accomplish this difficult task amidst many hardships faced during the Covid-19 pandemic. I wish to thank the editors, authors and the clinicians who have contributed to these guidelines for their commitment in formulating these guidelines.

As these guidelines will be available in a freely available, easy to use, electronic format and I hope that these will help to improve the quality and delivery of diagnostic services to cancer patients in Sri Lanka.

I wish the College of Pathologists of Sri Lanka all the success in their future endeavors to improve the quality of histopathology services in the country.

Dr. Asela Gunawardena
Director General of Health Services
Ministry of Health
Sri Lanka

Message by the Deputy Director General Laboratory Services

Ministry of Health, Sri Lanka

In the provision of health care services, the laboratory sector plays a vital role by providing timely and accurate test results enabling the clinicians in diagnosis and treatment. Cancer is one of the leading health issues in Sri Lanka which needs effective curative and preventive diagnostic services. In order to achieve this, the contribution of histopathologists is invaluable.

The Laboratory Services Unit, Ministry of Health works with a vision to achieve standards for medical laboratories set by the international organizations for standardization and a mission to provide timely, reliable, high-quality diagnostic services to relevant health care providers. These guidelines published by the College of Pathologists, Sri Lanka have given a valuable contribution to achieving our mission and improving the services provided by the histopathology laboratories to the public.

I am pleased to note that the availability of these guidelines in electronic format hence histopathologists working all over the country will be able to get the maximum use of it.

I wish the College of Histopathologists, Sri Lanka all the success in their future endeavors to provide a tremendous service to uplift the health of the citizens in Sri Lanka.

Dr. Sudath K. Dharmaratne
Deputy Director General-Laboratory Services
Ministry of Health
Sri Lanka

Message by the President

College of Pathologists of Sri Lanka

Since the first series of the National Guidelines in Histopathology were published in 2007, the necessity to revise these guidelines and formulate new guidelines was considered to keep pace with the rapid advancements occurring in the field of histopathology worldwide. The College of Pathologists has been able to complete and publish this new series of guidelines with the objective of improving the diagnostic services in histopathology and histopathology reporting across the country. I am extremely happy that we were able to accomplish this task during the Covid-19 pandemic, utilizing the lockdown periods effectively.

The guidelines have been formulated after extensive discussion by the members of the guideline committees and clinicians in the relevant fields, conforming to the latest, accepted international guidelines in histopathology reporting. These offer a comprehensive guide to the pathologists when handling tumours of the gastrointestinal tract, breast and genaealogical region as well as to specimen handling and transport.

The structure of the guidelines has been made similar to the first series wherever possible with X, Y and Z denoting the mandatory, desirable and optional recommendations respectively. (X; Mandatory; recommendations that can be carried out in most of the institutions in Sri Lanka, Y; Desirable; investigations that can be carried out in selected institutions in Sri Lanka including the private sector and Z; Optional; investigations that are not freely available in Sri Lanka which may be performed in the private sector or abroad).

The guidelines will be in the electronic format to allow maximum visibility to the histopathologists working across the country.

On behalf of the College of pathologists of Sri Lanka, I wish to acknowledge the contributions made by the series editors, content editors, authours, clinicians and all the members of the guideline committees and thank them for their commitment to formulate these guidelines to be on par with international guidelines.

I am also grateful to the Director General of Health Services Dr. Asela Gunawardena and the Deputy Director General Laboratory Services, Dr. Sudath Dharmaratne for facilitating the electronic publication process of these guidelines.

I hope that the histopathologists working across the country will make full use of these guidelines to improve the quality of diagnostic services and reporting in histopathology.

Prof. Dulani Beneragama
President, College of Pathologists of Sri Lanka, 2021.

Technical Committee

Chairperson	Dr. Sriyani Nanayakkara
Convener	Prof. Priyani Amarathunga
Members	Dr. Isha Prematilleke
	Dr. Palitha Ratnayake
	Dr. Cherine Sosai
	Dr. Nayana Ratnayake
	Dr. Chandrani Somaratne

Authors

Chapter 1	**General guidelines for handling of tissue specimens for histopathological examination**	Isha Prematilleke Palitha Ratnayake Cherine Sosai
Chapter 2	**Handling of specimens of specific types**	Palitha Ratnayake Nayana Ratnayake Sriyani Nanayakkara Chandrani Somaratne Cherine Sosai Priyani Amarathunga
Chapter 3	**Intra-operative diagnostic tests and other special situations**	Priyani Amarathunga
Chapter 4	**Handling of specimens for specialized and highly specialized investigations**	Cherine Sosai
Annexure 1	**Preparation of fixatives and transport media**	Palitha Ratnayake Premasiri Mapalagama
Annexure 2	**Preparation of decalcifying solution**	Chandrani Somaratne Cherine Sosai
Annexure 3	**Requisition forms**	Cherine Sosai Priyani Amarathunga Sriyani Nanayakkara Nayana Ratnayake

Design and layout: Isha Prematilleke

Contents

OVERVIEW

These guidelines were first formulated by the College of Pathologists of Sri Lanka, under the auspices of the Health Sector Development Project of the Ministry of Health funded by the World Bank in 2007. This update was considered timely and is meant for the information of surgical theatre and ward staff dispatching surgical pathology specimens, the laboratory staff receiving them, clinicians, clinical postgraduate trainees, pathologists and trainee pathologists who are overseeing the process. It is their collective responsibility to ensure that these guidelines are adhered to in routine practice to offer a high quality and safe histopathology service to the patient.

Histopathology reports not only establish a histopathological diagnosis but also give guidance in clinical management decisions and provide important prognostic data. As histopathology specimens are classified as irretrievable laboratory samples, specimen handling and transport should be done in close liaison between laboratory and clinical staff. There should be shared protocols between clinical and laboratory staff to ensure the above processes.

Mechanical, heat and chemical damage and drying of specimens should be avoided during handling. Prompt fixation should be ensured by immersing the specimen in appropriate fixative in a suitable container. Identification details of the specimen label should correspond with those in the requisition form. Providing relevant clinical and investigation findings will aid the accuracy of the histopathology report.

The recommendations are divided into three levels based on the facilities available in the pathology laboratories in the local setting. They are as follows:
X - Mandatory
Y - Desirable
Z – Optional

To ensure quality in histopathological services and subsequent management, these guidelines should be followed. In addition to the information given in these guidelines, the handling clinicians and/ pathologists may use their discretion in providing additional information or modifying procedures to suit the circumstances.

Committee to formulate National Guidelines in Histopathology on Collection, Handling and Transport of Surgical Specimens, 2nd edition.

CHAPTER 1

General guidelines for handling of tissue specimens for histopathological examination

Introduction

Types of specimens taken at surgery include small biopsies (e.g. endoscopic biopsies) and larger specimens including part of or entire organs (e.g. liver, uterus). Tissues removed at surgery as well as at postmortems will be sent to the histopathology laboratory for histological assessment.

Preparation of these specimens for transport for histopathological examination involves a series of essential steps from the time it is taken from the patient at surgery up to its reception in the laboratory. These include proper collection of specimen, placing the specimen in an appropriate container immersed in an appropriate type and amount of fixative; accurate identification and labelling of the specimen container with corresponding patient details in the requisition form; and completeness of information in the requisition form including relevant clinical details.

It is important to transport specimens for histopathological examination to the laboratory in an appropriate manner in order to optimally preserve tissue integrity for pathological diagnosis. Immediate transport to the laboratory is also important as the pathologist should attend large specimens as early as possible for slicing in order to ensure optimum fixation.

A. Specimen collection: general instructions

The first phase of pre-analytical quality assurance to ensure accurate histopathology reporting is during collection of the specimen from the patient by the surgeon. The following are precautions that should be observed: **[X]**

- Verification of the following at the time of sample collection is necessary:
  - Patient identification, using at least two acceptable patient-specific identifiers (e.g. name and a unique identification number: bed head ticket number/ NIC number/ date of birth).
  - Confirmation of type and site of specimen collection required.
- Human tissue is considered precious and potentially hazardous, and relevant precautions should be followed to protect the specimen as well as the handlers.
- If collecting a limited amount of tissue / small biopsy, the type of test/s required must be kept in mind and adequate and representative tissue should be collected (e.g., medical renal biopsies for multiple tests).
- Areas of necrosis should be avoided when sampling small biopsies to ensure adequacy of diagnostic material. The active edge may be more representative of the lesion than the necrotic center.
- Avoidance of crush artifact - the use of surgical instruments which could crush the tissue, e.g., metal forceps, should be avoided or limited as much as possible when handing the specimen to prevent crushing or damaging the tissue, especially small biopsies, and tissue cores (Refer Chapter 2: Handling of specimens of specific types).
- Air-drying of tissue should be avoided (Refer Section F on fixation).
- Avoidance of cautery artifact - use of surgical instruments driven by heat should be avoided or limited as thermal injury is a common artifact which interferes with diagnosis, especially in small biopsies and when assessing margins.
- Recording of warm and cold ischaemia times (Refer Sections F on fixation, and Chapter 4, Sections A and E on Immunohistochemistry and Molecular tests).

B. Requisition form (request form)

The tissue specimen should be accompanied by a requisition form specially designed for histopathology testing (Annexure 3, Section A). The following information should be included. **[X]**

- Test requested: e.g. Histopathology
- Patient identification data – Name and a unique identification number (at least one, preferably two of these: bed head ticket number/ clinic number, NIC number, date of birth, barcode number)
- Age and gender

- Location from which the specimen was sent - ward/ clinic and hospital
- Date and time of specimen collection
- Site of sampling, and where relevant side, orientation details / diagrams
- Specimens from different anatomical sites should be individually identified
- Relevant history (clinical details and examination findings) including previous biopsy and/or cytology diagnosis/lab reference number if available
- When providing clinical details, please use standard abbreviations only, which are familiar to the clinical and pathological teams.
- Operative findings and details of adjuvant therapy where relevant
- Relevant investigation results (e.g. radiological findings for bone tumours, liver function tests for liver biopsies, renal function tests for renal biopsies, PSA levels for prostatic biopsies, endoscopic findings for gastrointestinal biopsies)
- Copies of X rays, CT scans, ultrasound scans, endoscopy reports should be attached to the requisition form where relevant. Sometimes, pathologist may request the X ray or CT scan films for inspection. **[Z]**
- Time of removal of tissue and the time of immersion of tissue in fixative
- This information is especially important when reporting immunohistochemistry and molecular test results (refer Chapter 1 Section E on Fixation).
- Clinical differential diagnosis where relevant
- Requesting physician's name and designation
- Requesting physician's contact information (phone number) **[Y]**

Only one completed histopathology requisition form is adequate for any number of tissue specimens of the same patient collected during the same procedure submitted at a time. If more tissue is subsequently submitted from the patient (e.g. following a frozen section on the same surgical procedure), send a separate requisition form, indicating that there was tissue previously submitted, with date of such submission. **[X]**

Specimens obtained from more than one procedure (e.g. Thyroidectomy and lumpectomy of breast), should have requisition forms for each procedure, though submitted at the same time. **[X]**

Any special requests should be noted on the requisition form (e.g. "Please call with result ASAP", or "URGENT") **[X]**. If not indicated, the specimen will be processed as routine.

Ideally, it is best to provide a written procedure on how to properly complete a pathology requisition form, prepared by the laboratory, to all medical officers filling the form. **[Z]**

C. Container

All tissue submitted for histopathological examination must be in leak proof, wide mouthed containers with a well-fitting lid which will not be dislodged during transport and handling. **[X]**

The container/s should be kept ready with fixative to assure direct transfer of specimen to the container immediately after removal. **[X]**

The container must be large enough to hold the specimen and an adequate volume of fixative (see section on fixation), with adequate space for the fixative to move freely around the specimen. This is in order for the fixative to be able to penetrate from all sides of the specimen (Figure 1 and 2). **[X]**

Screw-capped containers are recommended, especially for small biopsies (Figure 3). The container should be unbreakable (material approved by relevant national authority) and not glass. **[X]**

Specimens from different anatomical sites taken at the same procedure should be sent in separate containers accompanied by a single requisition form. (e.g. colonoscopy biopsy series)

Figure 1. A suitable container for a large specimen

Figure 2. A large specimen stuffed into an inadequate container. This will result in poor fixation.

Figure 3. A container for a small specimen with a screw-capped lid.

Container

- Leak proof, with a well-fitting lid
- Wide mouthed
- Unbreakable
- Large enough to hold the specimen and an adequate volume of fixative
- Specimens from different sites taken at the same surgical procedure should be sent in separate containers with **one** requisition form

D. Labelling

The following should be clearly and indelibly stated on each container label. **[X]**

- Patient name
- A unique identification number (bed head ticket number/ clinic number, NIC number, date of birth, bar code number)
- Age and gender
- Ward/clinic
- Anatomical site and exact location (laterality, lobes, quadrants etc.)
- Type of surgical procedure
- Date and time of collection
- Test requested

This information should correlate with the information provided in the requisition form.

The label should be firmly attached to the container to prevent removal during transport (Figure 4). Labelling or writing on the lid should be avoided **[X]** (Figure 5).

Never pre label specimen containers. The labelling of specimen containers prior to the sample being taken is a well-established pre analytical error. Once identification and other details are confirmed by surgeon, sample receiving officer (nurse or medical officer) should label the container before putting the specimen into container.

All containers should carry a formalin warning label.

Figure 4. The label on this specimen container has not been firmly attached and may be detached during transport.

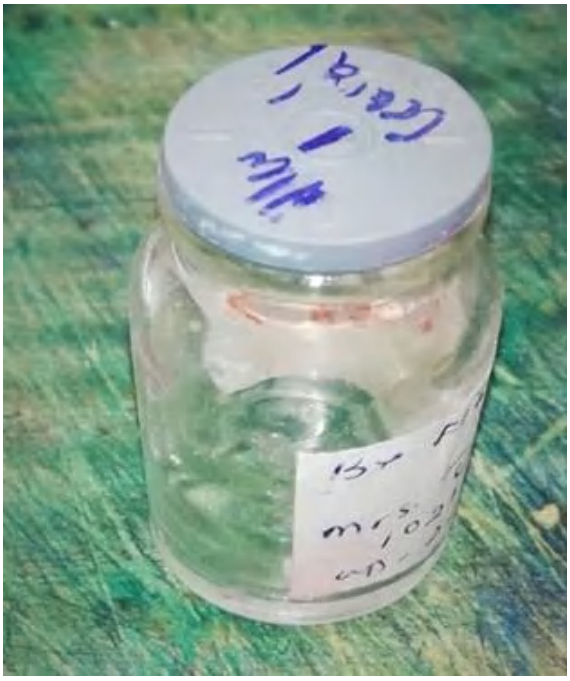


Figure 5. An unsuitable container for a small specimen with a lid that can be easily dislodged. The specimen is labelled on the lid, which should not be done.

E. Specimen orientation

Where required, orientation sutures should be placed on the specimen, and these should be clearly identified in the requisition form. **[X]** An appropriately labeled diagram including orientating sutures should be drawn in the requisition form. The pathologist can then identify the site of the tissue sections related to the anatomic location in the patient using this diagram. **[Y]**

THREE-DIMENSIONAL ORIENTATION

Sutures of variable material, length, or number should be placed to mark surgical margins (e.g., “deep margin”) or margin of concern (e.g., “closest margin”).

A common system is to use two sutures, placed at right angles to each other. A third suture indicating another margin may be placed if necessary.

Two sutures placed at right angles to each other are necessary to identify the remaining four margins. e.g., for wide local excision of breast**:** short superior and long lateral sutures. (Figure 6)

OTHER WAYS OF ORIENTING SPECIMENS

Subdividing areas of a specimen: Different anatomical areas may be submitted as separate specimens or submitted en bloc with orienting sutures. (e.g.,

separating the levels of an axillary dissection for breast carcinoma or compartments of a radical neck dissection)

Suturing a specimen to a surgical drape: The surrounding drape can be used to label areas or to draw the anatomic location.

Small specimens: Small biopsies, such as punch or core needle biopsies, should be placed on a small piece of filter paper prior to submerging in fixative in the container. **[X]** This helps to maintain orientation of skin and mucosal biopsies (Figure 7). This also helps in identification of a tiny piece of tissue which may otherwise be missed. When submerging in the fixative, the filter paper should be placed with the specimen facing downwards (Figure 8). This will prevent detachment of the specimen from the filter paper.

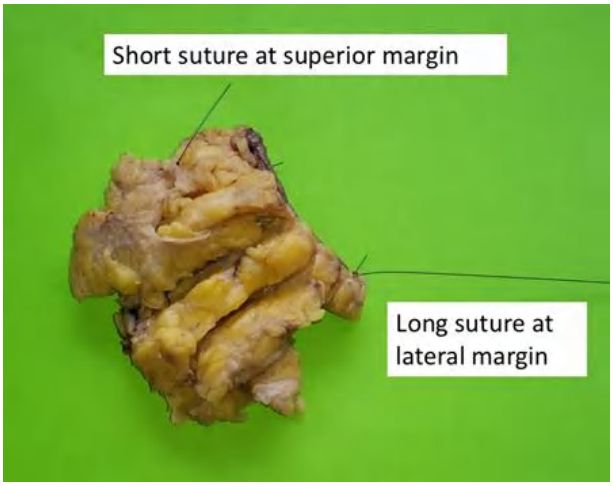


Figure 6. Breast wide local excision specimen with orientation sutures.

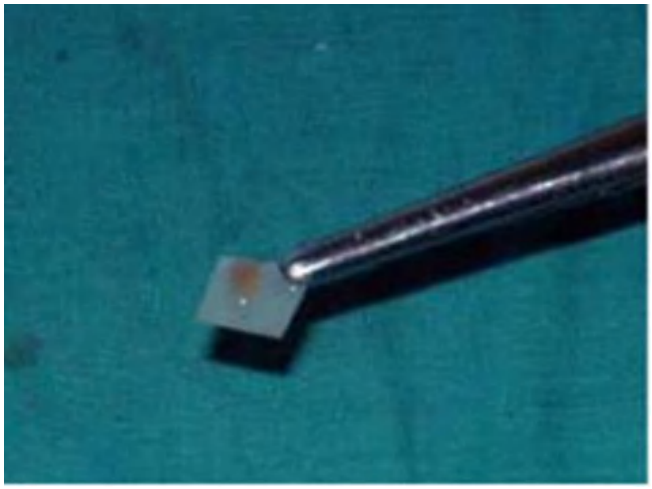


Figure 7. An endoscopic biopsy specimen placed on a piece of filter paper.

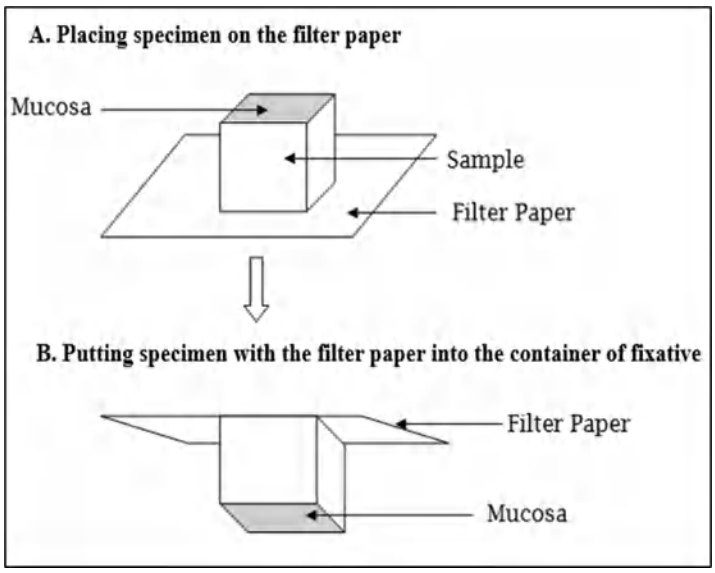


Figure 8. Orientation of a mucosal biopsy on filter paper (A) and placing in container with fixative (B).

- If orientation is not clear to the pathologist, the pathologist may need to discuss with the responsible surgeon to establish correct orientation prior to gross dissection in the laboratory. **[Z]**
- Special orientation methods may be required for the following types of specimens, which are dealt with under the relevant sections.
  - Endoscopic mucosal resection (EMR), Endoscopic submucosal dissection (ESD) and Trans-anal endoscopic microsurgery (TEM), specimens of GI tract: Refer Chapter 2, Section A
  - Wide local excisions of skin: Refer Chapter 2 Section D
  - Block dissection of neck: Refer Chapter 2, Section G
  - Eyeball specimens: Refer Chapter 2, Section H
  - Cone and LLETZ biopsies of cervix: Refer Chapter 2 - Section L
- A photograph of the intact specimen just after removal at surgery may be helpful. **[Z]**

F. Fixation

All tissues, except for specimens requiring special procedures (e.g., frozen sections) or special fixatives, should be put in suitable containers and covered with 10% neutral buffered formalin (NBF) immediately after removal to prevent drying and autolysis.

The time duration from excision of the specimen to immersion of tissue in fixative (cold ischaemia time) should be as minimal as possible and ideally should be less than 1 hour as it affects the results of immunohistochemistry and molecular testing. The time duration from surgical interruption of blood supply to surgical excision of specimen (warm ischaemia time) also matters for molecular testing and is considered when interpreting results.

10% NBF is prepared by the histopathology laboratories and distributed to the operation theatres and other specimen collection rooms (e.g., endoscopy and radiology). Laboratories can refer Annexure 1 on preparation of fixative in this book for preparation instructions.

The ideal volume of 10% NBF should be ten (10) times the volume of the specimen. It is mandatory that the minimum volume of 10% NBF should at least be adequate to completely submerge the specimen. If the volume of fixative is inadequate to cover the whole specimen, this will result in autolysis of part of the specimen (Figure 9). Certain large specimens require to be wrapped in gauze before submerging in 10% NBF to ensure satisfactory fixation, e.g., lung and mastectomy specimens. **[X]**

The usual rate of penetration of tissue by 10% NBF is 1 mm per hour. Hence, the pathologist should attend to large specimens as early as possible and open / slice it to facilitate fixation of tissue inside. However, opening / slicing a large specimen to accelerate fixation before being seen by the pathologist is not recommended in general. **[X]** This is because the opened / sliced specimen distorts it's shape on fixation, and accurate measurement of the distance from the lesion (especially a tumour) to excision margins etc, may be difficult to assess once the specimen is sliced.

Fixative

- All routine specimens are sent in 10% neutral buffered formalin (NBF)
- The minimum volume of NBF should be adequate to completely submerge the specimen
- Slicing a specimen before being seen by the pathologist is not generally recommended

Figure 9. Although the container is adequately large, the fixative volume is inadequate to cover the whole specimen. This will result in autolysis of part of the specimen that is not submerged in the fixative.

Slicing by the surgeon may be specifically allowed in certain situations by prior agreement with the pathologist, on a case-by-case basis. e.g. A single controlled incision is made into the tumour using a sharp long knife (not a scalpel). It needs to be ensured that the anatomical landmarks are not disturbed and the orientation marks (if applicable) are kept in situ. Over-slicing or mutilation of

specimens should be avoided. Slicing should only be done by a person experienced in handling surgical specimens.

- A mastectomy specimen can be sliced by placing a single incision, from the deep margin through the tumour up to the skin, without complete separation of the slice through the skin. A thin gauze pad inserted into this incision will help optimal fixation of the tumour. (refer National Guidelines in Histopathology – Handling and Reporting of Carcinoma of the Breast, second edition 2021 for additional options)
- Colectomy specimens should typically be placed unopened in an adequate volume of fixative. The specimen may be opened along the peritonealized antimesenteric border, leaving the tumour and a 1-2 cm segment proximal and distal to the tumour. This segment is left intact to avoid any subsequent confusion over whether the peritoneal surface or circumferential resection margin is involved. An absorbent (filter) paper or gauze 'wick' can be passed up to the point of obstruction by the tumour, without forcing it through. Bowel specimens should not be cut opened through the non-peritonealized margin (anterior or/and posterior) e.g. in APR specimens.
- Hysterectomy specimens – Opening hysterectomy specimens is best avoided. Injecting 10% NBF into the lumen of the uterus is recommended using a syringe (without a needle) via the os, to facilitate fixation of the tumour in the endometrium (recommended).
- Slicing may facilitate adequate fixation, but this should only be done after careful inspection of the serosal surface, ideally supervised by a pathologist or by a person trained by a pathologist (not routinely recommended).

Special procedures are recommended in the following instances to ensure proper fixation.

- EMR, ESD TEM specimens of GI tract: Refer Chapter 2 Section A.
- Pneumonectomy/ lobectomy specimens:
  - The lung should be removed as intact as possible together with a long bronchus.
  - Eventual tears should be tied or sutured. The blood vessels should be ligated, the bronchi suctioned free of mucus and the lung weighed. **[X]**
  - Lung tissue should be injected with 10% NBF immediately after reception at the laboratory. Therefore, the specimen should be sent without delay as merely submerging the specimen in 10% NBF is inadequate and prior slicing of the lung is not recommended.
  - Injection of 10% NBF is done via the bronchus, thus expanding the lung with the fixative. **[X]** This can be done using a large-volume syringe with a wide nozzle ('bladder syringe'). Alternatively, this can be performed through the supplying bronchus using a reservoir attached to flexible

tubing and nozzle using the pressure gradient generated by elevating the fixative containing container approximately 55–65 cm above the specimen.

- Lung segmentectomy or wedge resection specimens with stapled margins may be inflated with 10% NBF through the pleural surface using a needle and syringe. The specimen is distended until the pleural surface is smooth and it is then placed in an adequate volume of fixative and allowed to fix for approximately 24 hours.
  Inflation must be carried out before or shortly after the specimen has been placed in fixative otherwise fixation of the outer lung may prevent expansion. **[X]**

In justifiable instances when fixative cannot be added immediately, (e.g. surgeon needs to orientate the specimen in fresh state after surgery) the specimen should be placed in a sterile tray and kept moist with sterile saline or be wrapped in saline-dampened gauze and refrigerated until the specimen can be properly placed in fixative.

G. Packaging specimens for transport

Specimens should be transported to the laboratory as soon as possible **[X]** for measurements and inking, followed by slicing for proper fixation procedures to be carried out.

Packaging

The purpose of packaging is to protect specimens during transport and to prevent contact with the handling personnel and the environment.

- **Three-part packaging system**
  - A three-part packaging system (Figure 10) is recommended when transporting surgical specimens to a distant unit via courier or a transport system. **[X]**
  - A three-part packaging system is mandatory for specimens of patients diagnosed or suspected to have WHO risk category 3 and 4 infections. (Ref. https://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf)
  - Three-part packaging consists of
    - A primary container (specimen collection container) as specified in Section C.
    - A secondary container (re-sealable plastic bag, preferably a two-pocket specimen bag - outer pocket for copy of requisition form, inner pocket to contain the specimen and any leakage in the event of damage.

– A rigid outer packaging (specimen transport box- STB) should be used to protect specimen containers from damage if the package is dropped (Figure 11).

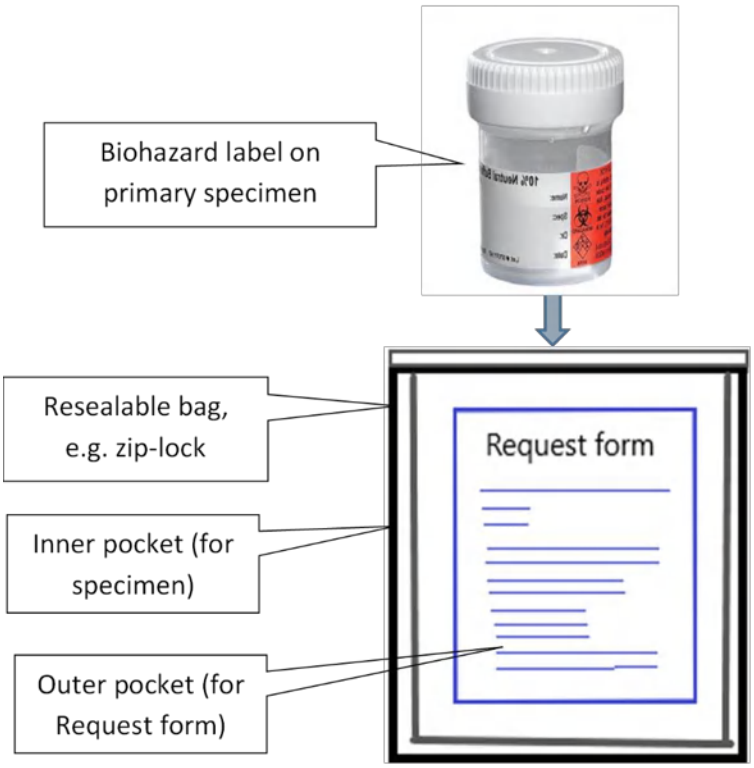


Figure 10A. Specimen container is put into a two-pocket resealable bag.

Figure 10B. Secondary container - a two-pocket resealable bag

Figure 11. A rigid specimen transport box

- **Two-part packaging system**

  If the specimen is being transported within the same building or compound, two-part packaging is adequate (primary and secondary container). **[X]**

Instructions for packaging specimens

- Ensure all container lids are tightly secured before packaging. **[X]**
- All specimens must be packaged carefully to avoid breakage of the container or leakage of fixative. **[X]** The rigid outer packaging should contain absorbent packing material to absorb any accidental leakage.
- Wrapping the specimen container with the requisition form is not recommended.
- It is ideal that the courier be provided with spare STBs, in case of damage to original STB. **[Z]** Clear instruction should be given to the courier on handling of specimen in case of damage during transport, as histopathology specimens are irretrievable.

H. Specimen accession and acceptance criteria

When accepting a specimen to the laboratory for processing and reporting, the following should be checked during accession:

- Patient identification details **[X]**– Identification details should correlate on the specimen label and corresponding requisition form. The minimum requirement is that the name and the unique identification number (two identifiers) should be identical. This is done to avoid mix up of specimens.
- Specimen identification details **[X]**– The type, site and side (if relevant) of the specimen should be clearly stated on the specimen label and should match with the information in the requisition form.
- The specimen (for routine histopathology) should be submerged in an adequate volume of 10% NBF, in a suitable container. **[X]**

Specimens that do not meet acceptance criteria should not be returned, instead the following remedial actions should be taken, as histopathology specimens are considered irretrievable. In the event that any one of these rejection criteria are met, it should be documented and corrected by communicating with the clinical team through appropriate channels.

Table 1. Specimen rejection criteria and recommended remedial actions **[X]**

SPECIMEN REJECTION CRITERIA	REMEDIAL ACTIONS
Patient identification details Patient's name / unique identification number on the container label and the requisition form do not match.	Clinician responsible for specimen collection should be contacted through appropriate channels and the matter rectified at the counter.
Specimen identification details Incorrect, absent or mismatched specimen identification details (type, site and side if relevant) on label and requisition form.	Same as above.
Fixative and specimen container Fixative: lack of fixative, unsuitable type / quality, contamination with blood, inadequate quantity. Container: inadequate size, ill-fitting lid.	Take the specimen to the grossing area and add, change or top up with recommended fixative. Transfer the specimen to a suitable container and affix the label to the new container.
Requisition form Improperly or inadequately completed requisition form, which is lacking in relevant details: e.g. name of requesting clinician, adequate clinical information, previous test findings, other relevant details.	Clinician responsible for specimen collection should be contacted through appropriate channels and matter rectified at the counter.
Specimen sent to the incorrect division of the laboratory for the test requested, e.g. histopathology division receiving fresh tissue for microbiological investigations.	Unless it is a specimen sent for multiple tests in addition to those done in the histopathology laboratory, the specimen should be directed to the correct division.
Specimen not found in the container.	Document on requisition form and inform the responsible clinician through appropriate channels. Ideally the clinician should witness that the specimen is missing. **[Y]**

SPECIMEN REJECTION CRITERIA	REMEDIAL ACTIONS
Completely or partially autolyzed tissue.	Document on requisition form and inform the responsible clinician through appropriate channels. Even if the specimen is autolysed, it should be submitted for histology after fixation
A specimen transported from a distance is bagged improperly or with requisition form in contact with the primary container	Give feedback to the sample dispatch point to follow recommended guidelines
Nonconformity, correction, and corrective action taken should be documented in all above instances, audited regularly and preventive measures taken.	

Specimen accession includes receiving specimens, sorting, allocating a laboratory reference number and registering in the specimen reception record book in the laboratory.

- Reference numbers **[X]**
  - A standard numbering system should be followed.
  - Omission or duplication of numbers should be avoided to ensure tracebility.
  - The number should be written clearly and indelibly on the specimen reception record book (or entered in a database), specimen container and the requisition form.
  - The reference number should include laboratory accession number, institutional name or code and year.
  - If special numbering or lettering is assigned for histology, cytology, postmortems etc., such specified numbering should be documented.
  - It is ideal to use a bar coding system for accession numbering. **[Z]**
- Specimen and patient identification details should be entered in the specimen reception records, and cross checked to avoid mistakes. **[X]**
- A specimen that needs immediate attention, e.g. a specimen sent fresh for the pathologist to slice, should be brought to the attention of the attending pathologist immediately. **[X]**
- Specimens marked ‘Urgent’ should be prioritized. **[X]**

It is best to avoid accessioning the same type of specimens back-to-back to minimize mix-up. If same type of specimens must be accessioned in sequence it is suggested that they be separate by size (e.g., skin punch biopsy followed by skin excision followed by skin punch biopsy).

I. Retention of tissue [X]

- Remaining (unblocked) tissue specimens should be retained for a minimum of twelve (12) weeks from the date of issue of report.
- Containers with no residual tissue should be retained for a minimum of four (4) weeks from the date of issue of report.
- Religious and cultural practices should be honoured in matters of tissue retention.
- Retention of tissue for research or for teaching purposes ideally requires written consent of the patient or next of kin.

J. Specimens which do not require mandatory submission for histopathology

The decision to submit any of the following specimens for histopathology is at the discretion of the clinician. **[Y]**

- Grossly normal placentae of uncomplicated pregnancies
- Extracted normal teeth
- Bone removed as a part of corrective or reconstructive surgery
- Skin or other clinically normal tissue removed during cosmetic or reconstructive surgery
- Prepuce of neonate removed for circumcision
- Rib segment or other tissue removed only for purpose of gaining surgical access (provided the patient does not have a history of malignancy)
- Eye lens
- For any other instances, where the submission of tissue for histopathology may not be required, contact the histopathologist for advice.

References

1. Lott R, Tunnicliffe J, Sheppard E et al. Practical Guide to Specimen Handling in Surgical Pathology. College of American Pathologists, Version: 9.0, revised: April 2020. Pp 4, 17.
2. Pan J F, Yang P C, Chang D B et al. Needle aspiration biopsy of malignant lung masses with necrotic centers. Improved sensitivity with ultrasonic guidance. Chest. 1993 May;103(5):1452-6. doi: 10.1378/chest.103.5.1452.
3. Prematilleke I, editor. The Importance of Correct Specimen Handling and Transport in Histopathology. College of Pathologists of Sri Lanka & Ministry of Healthcare and Nutrition (LK); 2007.
4. Subcommittee for laboratory accreditation in Histopathology, College of Pathologists of Sri Lanka. National guidelines for collection and transport of specimens for histopathological examination. College of Pathologists of Sri Lanka (LK); 2016.
5. National Pathology Accreditation Advisory Council. Requirements for the packaging and transport of pathology specimens and associated materials. Australian Government Department of Health (AU). 4th edition [Internet]; 2013 [cited 2020 June 30]. Available from: https://www1.health.gov.au/internet/main/publishing.nsf/Content/4F97263708B66C49CA257BF0001E012A/$File/Reqmts%20PackagingTransport%202013.pdf
6. Lott R, Tunnicliffe J, Sheppard E, Santiago J, Hladik C, Nasim M, Zeitner K, Haas T, Kohl S, Movahedi-Lankarani S. Practical Guide to Specimen Handling in Surgical Pathology. College of American Pathologists (US). Version: 9.0 [Internet]; April 2020 [cited 2020 June 30]. Available from: https://cap.objects.frb.io/documents/practical-guide-specimen-handling.pdf
7. Royal College of Pathologists and the Institute of Biomedical Science. The retention and storage of pathological records and specimens. Royal College of Pathologists (GB). 5th edition [Internet]; April 2015 [cited 2020 June 30]. Available from: https://www.rcpath.org/uploads/assets/049ea966-df5c-4a9f-9353ba24a69bb808/The-retention-and-storage-of-pathological-records-and-specimens-5th-edition.pdf

CHAPTER 2

Handling of specimens of specific types

A. Gastrointestinal endoscopic biopsies, endoscopic mucosal resection and endoscopic submucosal dissection specimens

Background

Endoscopic biopsies are commonly taken during endoscopic investigation of gastrointestinal lesions and inflammatory conditions.

Endoscopic mucosal resection (EMR), endoscopic submucosal dissection (ESD) and trans-anal endoscopic microsurgery (TEM) specimens are obtained from endoscopic procedures to remove pre-malignant and malignant lesions of the upper and lower gastrointestinal tract.

EMR is a minimally invasive procedure for treating gastrointestinal malignancies with a low risk of lymph node metastasis, as well as premalignant lesions e.g. colorectal adenomas. Resection of large polyps can be performed en-bloc or piecemeal depending on the size and location.

ESD is done for malignancies invading the superficial submucosa, and for lesions that cannot be removed by EMR due to fibrosis of the submucosa, or post-EMR recurrences.

TEM is an advanced minimally invasive procedure to remove benign lesions and early-stage rectal carcinomas. Endoscopic ultrasound guided fine needle aspiration (EUS-FNA) or biopsy (EUS-biopsy) procedures are also done to obtain specimens.

Specimen handling at the point of collection [X]

- Specimens from different anatomical sites should be sent in separate containers suitable for small biopsies (Refer Chapter 1, Section B).
- Biopsies should be placed on a piece of filter paper with the mucosal surface facing upwards (Refer Chapter 1, Section D, Figure 7, 8).
- Ensure biopsy containers are labelled in sequential order (e.g. for colonoscopic biopsies, arrange them in order from proximal to distal anatomical site).
- EMR, ESD and TEM specimens
  - Label specimen containers according to their anatomical location.
  - Orient the specimen with sutures, labeling the 12 o'clock position or proximal (oral) and 6 o'clock position or distal (anal) margins.
  - Place specimen on a hard surface (e.g. Styrofoam board, cardboard or cork board) with the mucosal surface facing upwards, pinned at the periphery. This is to prevent curling and shrinkage during fixation (Figure 1).
    When pinning on to the board,
    - avoid over stretching.
    - avoid pinning through the lesion.
    - include the entire thickness of the specimen.
    - place the pins close to each other to prevent curling and retraction of the margins in between the pins.
  - Multiple specimens must be pinned on separate boards and placed in separate containers indicated by suitable labels and diagrams on the request form.

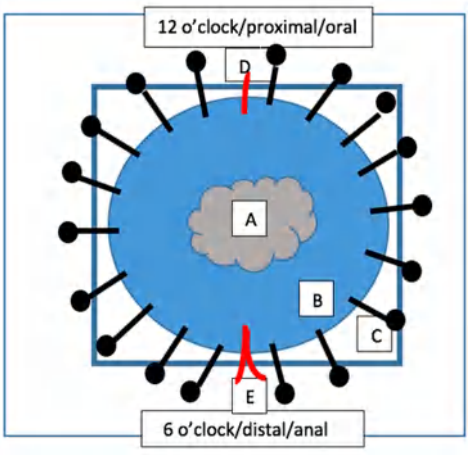


Figure 1. Pin the specimen on a hard surface, placing the pins close to each other.

A - Lesion
B - Surrounding tissue
C - Cork board
D - Orienting suture (12 o'clock / proximal / oral)
E - Orienting sutures 6 o'clock / distal / anal

- EUS-FNA specimens
  Tissue particles obtained during EUS-FNA sampling: particles <2 mm are evaluated using cell block preparation, while tissue particles >2 mm should be placed in 10% NBF for fixation. Instead, with prior discussion with the laboratory, the endoscopist may submit the entire material (including needle washings) immediately to the laboratory, in normal saline, thereby, the laboratory can separate out particles for cell block preparation and routine processing for histopathological examination.

Requisition form

- Attach a copy of the endoscopy report. **[X]**
- Provide following information: **[X]**
- Biopsy indication (diagnosis or follow-up)
  - Oesophagus – presence of Barrett oesophagus
  - Stomach – history of Helicobacter pylori infection, any relevant test results
  - Small intestine – clinical details of malabsorption, suspected infections, any relevant test results
  - Colon and rectum – any relevant history
- Record the type of procedure - mucosal biopsy, polypectomy, EMR, ESD, TEM, EUS-FNA, other procedures (specify). **[X]**
- Record the site of the biopsy. **[X]**
- For EMR, ESD, TEM: record the size of the lesion and the possibility of submucosal invasion. **[Y]**
- State the number of pieces per container. **[Z]**

Specimen transportation

Immerse in an adequate amount of 10% NBF in a suitable container (Refer Chapter 1, Sections B and E). **[X]**

B. Renal core biopsies

Background

Renal biopsies are performed for both medical and surgical conditions. Renal biopsies for surgical conditions are less common and should be handled as per general guidelines for small biopsies.

In this section, guidelines are given for medical renal biopsies, which are taken to identify diseases such as glomerulonephritis and to assess pre- or post-transplant status of renal tissue.

Specimen handling at the point of collection

- A suitably trained person should assess the sample under dissection microscope in a drop of saline for the presence of glomeruli. **[Y]** Glomeruli appear as small red spheres in the cortex (Figure 2). Medulla appears as linear stripes. Small yellow balls of fat will also be present.

Figure 2. Renal biopsy specimen as seen under a dissecting microscope. Note the renal cortex with glomeruli (arrows), recognized as round red areas (wet preparation X 10). (Figure courtesy of S Wijetunge)

  A hand lens should be used if a dissection microscope is not available. **[X]** Blind division of the cores for immunofluorescence and electron microscopy should be avoided.
- Division of the specimen will vary with the type of biopsy and the clinical question being asked
  - Biopsies usually need to be divided into three and submitted for light microscopy, immunofluorescence and electron microscopy.
  - In theory, a minimum of one glomerulus per study is usually adequate for immunofluorescence and electron microscopy.
  - Majority tissue should be submitted for light microscopy. **[X]**
  - If the specimen is limited, division is guided by the clinical question and/or facilities of the individual laboratory.
  - If only minimal tissue is available, it may be possible to retrieve a few more glomeruli for electron microscopy by rinsing out the biopsy needle.

  - Renal transplant biopsies may not require electron microscopy and immunofluorescence unless indicated (e.g Immunofluorescence for C4d).
  - However, it is important not to transfer the entire specimen to 10% NBF until exact requirements are established. **[X]**
- Wooden sticks may be used (in preference to forceps) to gently lift the cores without crushing. Renal biopsies must be carefully handled with clean instruments and separated into different solutions without any cross contamination of fixatives. (Minute amounts of formalin or glutaraldehyde can destroy the antigenicity of the tissue allocated for immunofluorescence). **[X]**

Requisition form

- Indicate the time of collection (to determine minimum time for fixation before processing). **[Y]**
- Mention if urgent, the contact telephone number to inform results. **[X]**
- State the number of cores in the container. **[Y]**
- Include adequate clinical information according to the specified request form (Annexure 3, Section B). **[X]**
- Mention the name (if any) of the reference laboratory to which, parts of the same specimen were sent for immunofluorescence or electron microscopy. **[Y]**

Note: The requisition form should be filled by the clinician attending the patient and not by the radiologist obtaining the biopsy. **[X]**

Specimen transportation [X]

- For light microscopy, immerse in an adequate amount of 10% NBF in a suitable container (Refer Chapter 1, Sections B and E). If alternate fixatives are used, consult the laboratory prior to sample collection.
- Portions of specimens for immunofluorescence and electron microscopy should be sent in relevant fixative/ transport medium as specified in Chapter 4, Section B and C.

C. Liver core biopsies

Background

Needle biopsies are commonly used to evaluate liver diseases which encompass non-neoplastic and neoplastic conditions.

Specimen handling at the point of collection

- Handling of the biopsy with forceps should be minimized in order to prevent crush artefacts and fragmentation of the core. **[X]**
- Fresh tissue without a fixative is required in the following situations.
  - Lipid tests for storage diseases
  - Cytogenetics
  - Flow cytometry
  - Microbiological tests

Requisition form [X]

- Mention the number of cores in the container.
- Provide relevant clinical information and indication for biopsy.
- Provide radiology investigation findings.
- Provide reports of serology and tumour markers where relevant.
- Request any special studies required (e.g. Iron studies, copper studies etc).
- If intraoperative tests were performed, describe the type and results (e.g. frozen section in acute fatty liver of pregnancy/ Reye syndrome, imprints in lymphoma).

Note: The requisition form should be filled by the clinician attending the patient and not by the radiologist obtaining the biopsy. **[X]**

Specimen transportation [X]

- Immerse in an adequate amount of 10% NBF in a suitable container (Refer Chapter 1, Sections B and E).
- Fresh tissue without a fixative is required for certain assessments (See above under specimen handling at the point of collection.

D. Skin biopsies

Background

Skin biopsies are performed for diagnosis of inflammatory and neoplastic dermatological conditions.

Specimen handling at the point of collection [X]

- It is essential that all layers of the skin are visualized in a properly oriented specimen on histopathological examination. Therefore, vertical orientation should be maintained during sampling.
- Punch biopsies are taken parallel to the hair follicles, thus minimizing tangential sectioning, and should include epidermis, dermis and a generous amount of subcutaneous fat.
- Any biopsy including a lesion should include the adjacent normal skin as well.
- Biopsy should include active lesions. Scarred areas should be avoided.
- For shave biopsies, orient the specimen on a piece of filter paper with the epidermal side upwards (As for mucosal biopsies, refer Chapter 1, Section D, Figure 7 and 8).
- For bullous disorders, provide the skin biopsy with intact bulla.
- For alopecia, specify whether vertical and/or horizontal sections are required. If both sections are required, a minimum of two specimens should be sent.
  When selecting the sites, adhere to the following.
  - Non-scarring alopecia - the most thinned area
  - Scarring alopecia - the edge of the lesion including adjacent normal skin
- When hair shaft and nail examination are required, it should be communicated to the reporting pathologist prior to obtaining the specimen.
- Skin and nail scrapings in fresh state, wrapped in a paper may be sent for fungal studies.
- Excision biopsies for suspected skin neoplasms should be orientated using suture/s at least at 12 o'clock position (Figure 3).
-

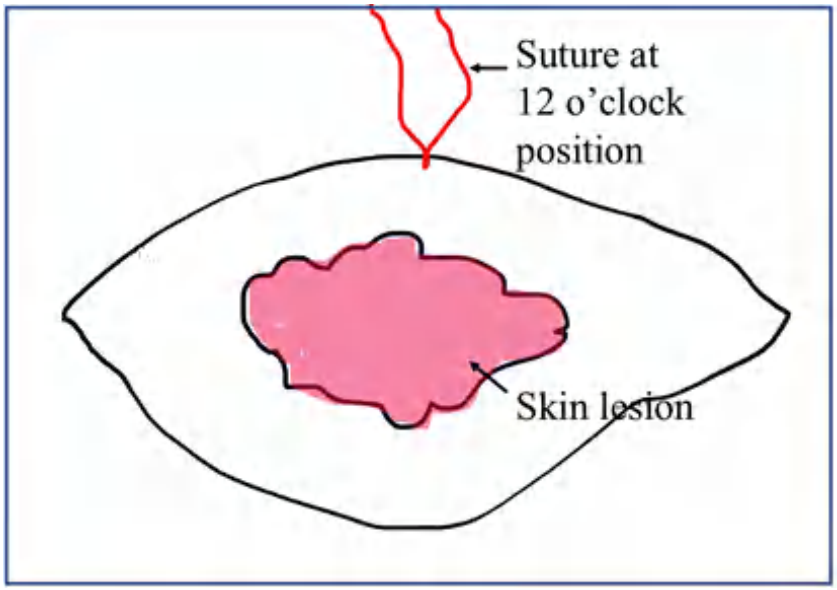


Figure 3. Skin excision for a suspected tumour is oriented using a suture at 12 o'clock position.

Requisition form

- Provide clinical information relevant to disease condition. **[X]**
  - Site, duration and appearance of the lesion
  - Systemic diseases that affect the skin
  - Clinical differential diagnosis
  - Family history
  - Drug history including local applications
  - Previous similar lesions
- Mention simultaneous sampling for slit skin smears, immunofluorescence, flow cytometry, microbiology studies or any other investigations where relevant. **[Y]**
- Mention the type of biopsy (excision, incision, punch etc.). **[X]**

Specimen transportation [X]

- For light microscopy, immerse in an adequate amount of 10% NBF in a suitable container (Refer Chapter 1, Sections B and E).
- For immunofluorescence, refer Chapter 4, Section B.

E. Nerve biopsies

Background

Peripheral nerve biopsies are undertaken to investigate neuropathies and vasculitides.

Specimen handling at the point of collection

- It is recommended to biopsy the most affected peripheral nerve. **[X]**
  - Sural nerve is commonly biopsied.
  - Other biopsy sites include superficial peroneal nerve, superficial radial nerve and obturator nerve.
- Avoid squeezing or stretching the nerve.
- Avoid excessive removal of fat or connective tissue.
- The proximal end of the nerve should be cut first, as cutting the nerve often causes acute pain. If no pain is felt on transection, a blood vessel may have been mistakenly cut.

Requisition form [X]

- Include adequate clinical information according to the specified request form (Annexure 3, Section C)
- Include the following where relevant.
  - Biopsy site and laterality
  - Age
  - Details of onset, distribution and progression of the disorder
  - Involvement of other organs
  - Results of nerve conduction studies, electromyography, MRI of brain, spinal cord and adjacent nerve roots or muscle
  - Drug history
  - Occupational exposure/s
  - Past and current personal and family medical history
  - Plasma creatinine kinase (CK) levels
  - Plasma and CSF lactate levels
  - Other biochemical investigations.

Specimen transportation [X]

- For light microscopy, immerse in an adequate amount of 10% NBF in a suitable container. (Refer Chapter 1, Section B and E)
- Fresh tissue can be sent for immunofluorescence (Refer Chapter 4, Section B)
- If special tests are required for particular diseases, prior communication with the relevant laboratory is recommended.

F. Muscle biopsies

Background

Muscle biopsies are undertaken to investigate myopathies, other muscle disorders and neurogenic atrophy using a range of diagnostic techniques.

Specimen handling at the point of collection [X]

- The laboratory should be notified in advance.
- The biopsy preferably be performed during morning hours, in order to reach the laboratory early in the day.
- The tests required should be decided prior to obtaining the biopsy. e.g. enzyme histochemistry, biochemical analysis, electron microscopy, routine histopathology or any combination of these.

- Select the muscle which is clinically involved. A moderately involved muscle is better than a severely affected muscle.
- Vastus lateralis is a commonly chosen muscle for biopsy. Other muscles include gastrocnemius and biceps.
- Biopsy should be from the belly of the muscle, avoiding the tendon.
- Avoid severely fibrotic / atrophic muscles or previously traumatized area of the muscle e.g. injection/ biopsy/ trauma and electromyography sites.
- A strip of muscle approximately 3 x 0.5 cm in size, parellel to the direction of the muscle fibres should be obtained.
- The muscle strip should be divided into three portions by trained medical personnel as follows:
  - 1.5 cm length on a saline-moistened gauze for enzyme assay
  - 1.0 cm length in 10% NBF for histology
  - 0.5 cm length in glutaraldehyde for electron microscopy
- Specimens should be stretched just beyond its in-situ resting length and mounted by pinning the two ends to a flat surface.
- The biopsy specimen should not be clamped, cauterized, previously dissected, or chopped.
- Muscle biopsies should not be placed at or near any area containing liquid or fumes of formalin, glutaraldehyde or alcohol as these will alter the enzymes.

Requisition form [X]

- Include adequate clinical information according to the specified request form (Annexure 3, Section C)
- Following clinical information should be provided where relevant.
  - Information about the biopsy site and laterality
  - Age at biopsy (essential)
  - Details of onset, distribution and progression of the disorder
  - Involvement of other organs
  - Results of nerve conduction studies, electromyography or MRI of brain, spinal cord and adjacent nerve roots or muscle (if performed)
  - Drug history
  - Occupational exposure/s
  - Past and current personal and family medical history
  - Plasma creatinine kinase (CK) levels (essential)
  - Plasma and CSF lactate levels, other biochemical investigations
  - Specimen transportation:
- Method of transportation will depend on the transit time. Muscle biopsies must ideally be transported immediately to the laboratory, fresh at room

temperature in a manner that prevents the specimen drying out (wrapped in a piece of gauze slightly dampened with saline).
  - If total transit time is less than 2 hours from taking muscle biopsy to receipt at a laboratory, put the specimen into a sterile container without any liquid. Do not immerse biopsy specimens in saline. Some centers place the specimen into moistened gauze or wrap in cling film. Place the specimen container in a Styrofoam container with ice and send immediately. **[X]**
  - If total transit time is more than 2 hours from taking muscle biopsy to receipt at a laboratory, the specimens must be frozen on site and transported on dry ice* (See text box below for reference). **[Z]**
- Electron microscopy is rarely indicated and if so, the specimen should be fixed in gluteraldehyde (Refer Chapter 4, Section C). **[X]**

Note:
- Muscle biopsies for electron microscopy should be immersed in 2.5% gluteraldehyde within 15 minutes of obtaining the biopsy.
- If freezing is required refer* https://www.hopkinsmedicine.org/neurology_neurosurgery/centers_clinics/neuromuscular_pathology/muscle_biopsies/protocol.html

G. Lymph node biopsies

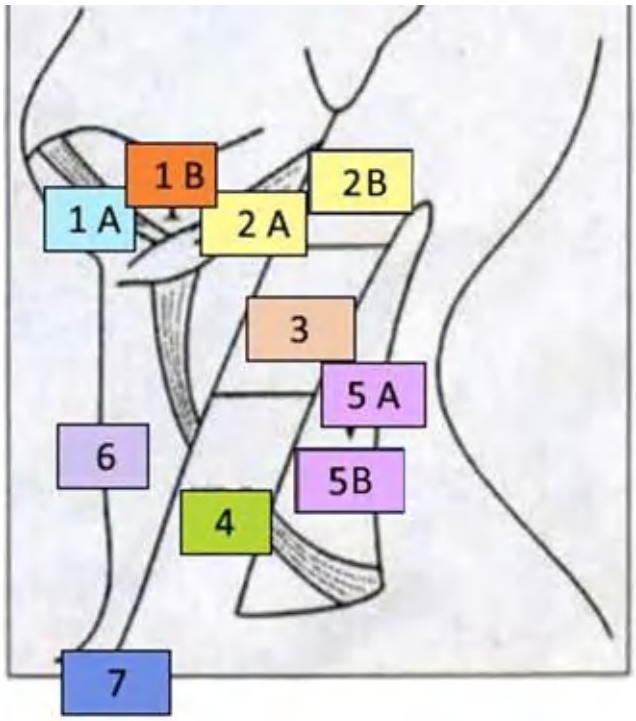
Background

Histopathological assessment of lymph nodes is required for diagnosis of a wide spectrum of diseases including infections and primary or secondary malignancies. Lymph node excisions include regional lymph node biopsies, sentinel lymph node biopsies, block dissections and core biopsies.

Specimen handling at the point of collection [X]

- Lymph node sampling should be done from the most appropriate anatomical site according to the clinical suspicion of the disease.
- It is recommended to sample a larger, preferably a central, deep-seated node.
- When possible inguinal nodes should be avoided, in cases of lymphomas.
- Send the node intact with surrounding fat. Do not slice the node without consulting the laboratory.
- Handling should be gentle and minimal to avoid crush artefacts.
- Block dissections should be orientated by the surgeon and pinned or sutured to cork or polystyrene boards. The general territories of node groups (Figure 4)

should be identified by placing markers such as sutures at the center of the anatomical group. A practical alternative for sending intact block dissections is, for the surgeon to separate the node groups, mark the superior margin of each group with a suture, and place each group in a separately labelled container.
  - A diagram should accompany all block dissections.
  - The surgeon should indicate surgically critical margins.
- For sentinel lymph nodes: If radio labelled substances have been used to identify sentinel node, universal safety procedures should be followed. Container should be labelled as containing radioactive material.
-



1 A	Submental
1 B	Submandibular
2 A and 2 B	Upper jugular
3	Middle jugular
4	Lower jugular
5 A and 5 B	Posterior triangle
6	Pre-laryngeal and paratracheal
7	Upper mediastinal

Figure 4. Anatomical groups of lymph nodes included in block dissection of neck

- Tissues for bacteriological cultures or investigations like flow cytometry require fresh tissue, without a fixative. If such investigations are required, consultation with the laboratory should be done prior to specimen collection.

Requisition form [X]

- Indicate the details of previous pathological investigations (e.g. FNAC, biopsy, blood counts, bone marrow and other relevant investigations if performed).
- Indicate whether it is from a patient suspected of a high risk infection e.g. Drug resistant tuberculosis, HIV, Hepatitis B as those specimens need a longer duration of fixation prior to processing.

- In case of a lymph node group dissection for malignancy, indicate
  - clinical TNM stage.
  - a history of previous radiotherapy or chemotherapy.
  - sentinel node biopsy if performed previously.
  - whether the surgery is palliative or curative.
- If radioactive substances have been used, record it in the requisition form.

Specimen transportation [X]

- If feasible, send the lymph node immediately without fixative to the laboratory for rapid handling of the specimen by the pathologist (to measure and then slice the node and cassette it in formalin to ensure proper fixation). This is especially important for lymphomas, where the nodes are usually large.
- If it is not practical to send fresh lymph nodes, then immerse in an adequate amount of 10% NBF in a suitable container (Refer Chapter 1, Section B and E).
- Fresh tissue without fixative is mandatory, if following investigations are required from the same specimen.
  - Cytogenetics
  - Microbiological cultures
  - Flow cytometry
- If a lymph node is submitted fresh to the laboratory, the following are required.
  - Close liaison with the laboratory staff.
  - The specimen should reach the laboratory and be dealt with, within 60 minutes of its removal.
  - The nodes and needle biopsy specimens should be moistened with a piece of gauze soaked in saline to avoid drying.
  - Specimens should not be sent at the end of the working day.

When sending fresh tissue for cytogenetics, if delay in transport is anticipated, an appropriate transport or tissue culture medium should be used to preserve the material. (Refer Chapter 4, Section E)
Sending air dried imprint smears for fluorescence in-situ hybridization (FISH) is a useful alternative.

H. Eyeball specimens

Background

Eyeball specimens come as enucleation or exenteration specimens which encompasses non-neoplastic and neoplastic conditions. Proper handling of

eyeball specimens is critical as macroscopic and microscopic features contribute to the staging of the tumour which determine prognosis and post enucleation therapy.

Specimen handling at the point of collection

Enucleations

- Minimal handling of the specimen should be ensured. **[X]**
- Orient the eyeball by inserting a suture to the superior rectus muscle. **[Y]**
- Mention the anatomical location of the tumour according to pre-operative radiological findings. **[X]** This will ensure correct tumour sampling with minimal tissue handling at the laboratory.
- Inform the laboratory prior to specimen transport, if the specimen is sent fresh without a fixative (see below). **[X]**
- If fresh tumour sampling has been done by trephining, the site of trephine should be marked before placing the specimen in fixative.

Orbital exenterations

- Orient the specimen with sutures and draw a diagram in the accompanying request form. **[X]**
- Placing gauze or tissue paper between the eyelids during fixation confers no particular advantage.

Requisition form [X]

Include the following where relevant. Use the specified requisition form (Annexure 3, Section D) for retinoblastoma specimens.

- Time of surgery, for fresh specimens
- Clinical staging of the tumour
- Laterality of eye that has been enucleated/exenterated
- Previous therapy: intravitreal chemotherapy and intra-arterial chemotherapy
- Status of the other eye (unilateral/bilateral tumour)
- Family history of retinoblastoma
- Extraocular spread noted at surgery
- Any history of extraocular malignancy
- Any other associated eye diseases

Specimen transportation [X]

- Immerse in an adequate amount of 10% NBF in a suitable container (Refer Chapter, Section B and E).
- For orbital exenterations, place the specimen in an adequately sized container as it requires a greater volume of fixative. (100 mL or more to cover the specimen entirely)
- Avoid injecting formalin or making windows in the eyeball.
- Trephined eyeballs tend to float as these may contain entrapped air bubbles, therefore, ensure that the eyeball is wrapped in gauze before placing in fixative.

Molecular analysis is sometimes performed in specialized ocular pathology centres for retinoblastomas, to determine whether the tumour is of hereditary or sporadic type. In that case, the eyeball should be sent fresh to the laboratory (without a fixative). **[Z]**

I. Respiratory small biopsies

Background

Pulmonary needle biopsies (transbronchial/ endobronchial/ transthoracic), video-assisted thoracoscopic (VATS) and open pulmonary (surgical lung) biopsies and endobronchial ultrasound guided transbronchial needle aspirations (EBUS TBNA) are all methods for investigating pulmonary diseases such as pneumonia, lung neoplasms and mediastinal lesions. Pleural biopsies are also performed for the diagnosis of different respiratory diseases.

Specimen handling at the point of collection [X]

- When performing transthoracic core needle biopsies, a minimum of 3 cores is recommended for optimal diagnostic yield. Additional specimens may be required for ancillary studies.
- All small biopsies should be placed on a piece of filter paper.
- For mucosal surface biopsies, it should be placed on a piece of filter paper with the mucosal surface facing upwards (Refer Chapter 1, Section D, Figure 7, 8).
- Multiple biopsies from different sites should be sent in separate containers which are labelled separately.

Requisition form [X]

Provide the following information where relevant.

- Potential infectious hazard for fresh specimens
- Other investigations requested e.g. culture for tuberculosis, fungi
- Biopsy procedure: Video-assisted (VAST) biopsy, open biopsy, transbronchial needle biopsy, transthoracic needle biopsy, endobronchial biopsy
- Biopsy site - Lung, pleura, bronchus, mediastinal compartment or any other
- Simultaneous sampling for cytology (brush/wash), flow cytometry and microbiological studies.

Note: Respiratory small biopsies including EBUS biopsies are often performed with accompanying cytology sampling, as cytology and histopathology are considered complementary diagnostic techniques with specific advantages and limitations. In addition, respiratory specimens are also used for many ancillary investigations namely, microbiological studies, flow cytometry, cytogenetics and molecular assays.

- Rapid onsite evaluation (ROSE) for assessing specimen adequacy and appropriate triage of material for ancillary investigations is recommended for small respiratory biopsies with prior discussion with the pathologist.
- When performing core needle biopsies or transbronchial forceps biopsies without concurrent cytology, touch preparations may be used for adequacy assessment.
- When performing transthoracic needle aspirations without core needle biopsy, multiple passes are recommended, and should collect sufficient material for a tissue block (i.e., cell block, tissue clot).
- Tissue particles obtained during EBUS-FNA sampling:
  Particles <2 mm in size are evaluated using cell block preparation, while tissue particles >2 mm in size should be placed in 10% NBF for fixation. Instead, with prior discussion with the laboratory, the endoscopist may submit the entire material (including needle washings) immediately to the laboratory, in normal saline, thereby, the laboratory can separate out particles for cell block preparation and routine processing for histology.

Specimen transportation [X]

- For light microscopy, immerse in an adequate amount of 10% NBF in a suitable container (Refer Chapter 1, Section B and E).
- Immunohistochemistry, fluorescent in situ hybridization and molecular assays are also performed on tissues fixed in 10% NBF. (Refer Chapter 4, Sections A, B, E).
- Specimens for bacterial, mycobacterial, viral or fungal cultures should be sent fresh to relevant laboratories without a fixative.
- For specimens sent with multiple tests requested: Refer Chapter 3, Section on Multiple tests requested on a single specimen.

J. Genitourinary biopsies

Background

A range of biopsies are taken to investigate benign and malignant lesions of the genitourinary tract including endoscopic biopsies of bladder, urethra, needle biopsies of testis, prostate and penis and transurethral resections of bladder and prostate. Two types of specimens (testicular biopsies and Transrectal ultrasound [TRUS] guided biopsies) which require special procedures in handling are described below.

J.1 Testicular biopsies

Specimen handling at the point of collection [X]

- An open biopsy should be at least 3 mm in minimum dimension. A transcutaneous tru-cut biopsy is an alternative to open biopsies.
- It may be necessary to obtain bilateral specimens as the nature and/or stage of various lesions may differ from one testis to the other.
- The tissue should not be squeezed with forceps which may disrupt testicular tissue architecture and hamper proper evaluation.

Requisition form

For biopsies done for subfertility, seminal fluid analysis report should be included. **[X]**

Specimen transportation

The European Germ Cell Cancer Consensus Group advocates the use of Stieve's or Bouin's solution for fixation. See text box below. **[Z]**

- 10% NBF is a satisfactory, commonly used fixative for testicular biopsies.
- Non-buffered formalin is not ideal as it may cause shrinkage artifacts.
- The use of Stieve's solution might aid in preserving morphology but hampers intranuclear staining methods if the fixation process is prolonged.
- Bouin's solution is also a good fixative for histological evaluation of spermatogenesis but is infrequently used because of its reduced shelf-life and toxicity.
- Both fixatives could give false negative immunohistochemistry results for OCT 3/4 which is used for detection of germ cell neoplasia in-situ.

J.2 Transrectal ultrasound (TRUS) guided biopsies of prostate

Specimen handling at the point of collection [X]

- The cores from different sites of the prostate should be sent in separate containers.
- The specimens should be submitted in a particular order according to sites and not numbered randomly.
- The cores should be placed on pieces of filter paper to avoid tangling and breakage.

When immersing the filter paper with the cores in fixative, the cores should face downwards (towards the fixative). This will avoid the cores detaching from the filter paper.

Requisition form

- Include PSA values if available, and PIRADS score. **[Y]**
- Indicate the site of each specimen ideally with a diagram. **[X]**

Specimen transportation [X]

Immerse in an adequate amount of 10% NBF in a suitable container (Refer Chapter 1, Section B and E).

K. Bone biopsies

Background

Bone biopsies are taken mainly to identify primary and metastatic bone tumours and for histopathological grading of tumours. Histopathological assessment of a bone tumour needs to take into account the clinical background, features of the

lesion, its radiological appearances and the results of relevant laboratory investigations.

Specimen handling at the point of collection

- Multiple tissue cores are preferable. **[Y]**
- A minimum of three cores may be needed if ancillary studies (molecular genetics, microbiological culture or cytogenetics) are requested. **[X]**

Requisition form

- Specify the type of specimen. (e.g. core needle biopsy, open biopsy, curettage, excision or segmental /en-block resection). **[X]**
- Provide the following clinical information where relevant. **[X]**
  - Age of the patient
  - Correct site of the lesion
  - Duration of symptoms
  - History of trauma
  - Pre-existing skeletal conditions
  - Relevant extra skeletal diseases
  - Occupational or treatment history
- Provide the following laboratory investigation results where relevant. **[X]**
  - WBC
  - ESR
  - Serum protein electrophoresis
  - Serum calcium
  - Serum phosphate
  - Serum alkaline phosphatase
  - Serum acid phosphatase
- Providing radiological information is mandatory **[X]** and wherever possible the pathologist should personally view the radiological images **[Y]**. Provide the following radiological information.
  - Anatomical location
  - Affected region of the bone
  - Whether the tumour has originated in bone or extended into it from surrounding soft tissue
  - The interface between the tumour and the surrounding bone
  - Pattern of bone destruction
  - Extension of tumour through the bone cortex
  - Involvement of surrounding soft tissue
  - Presence of multiple lesions

Specimen transportation [X]

- Where ancillary studies are not required, the specimen should be immediately fixed in 10% NBF (Refer Chapter 1, Sections B and E).
- Specimens should not be placed in a freezer as this may result in formation of ice crystal artifacts.

L. Special gynaecological biopsies

Background

Gynaecological biopsies are obtained in special situations (e.g. Cervical biopsy in high grade squamous intraepithelial lesion on pap smear, endometrial sampling in abnormal uterine bleeding) to ascertain the diagnosis.

Types of gynaecological biopsies

- Vulval and vaginal punch biopsies
- Colposcopy directed punch biopsies of the cervix
- Colposcopy guided wedge biopsies
- Colposcopy guided cone biopsies
- Colposcopy guided large loop excisions of the transformation zone (LLETZ)
- Endocervical curettage
- Endometrial curettage/ Endometrial pipelle biopsy or aspirations
- Trans-cervical resection of the endometrium

Specimen handling at the point of collection [X]

- Careful handling of all of these specimens is recommended to prevent surface trauma and disruption or loss of surface epithelium.
- Opening an intact loop/cone biopsy before fixation is not recommended.
- Vulval biopsies should be handled as for skin biopsies in other sites (Refer Section D)
- For wedge biopsies, cone biopsies of the cervix or colposcopy guided large loop excision of the transformation zone (LLETZ) biopsies
    - Small biopsies (2 mm) should be mounted onto a card or a filter paper.
    - Include a marker suture at the 12 o'clock position to allow the loop to be oriented.
- When sending products of conception for karyotyping, it should be sent fresh without a fixative.

Requisition form [X]

- All specimens should be accompanied by a fully completed request form with adequate clinical information.
- In case of a cervical biopsy, cone biopsy or LLETZ biopsy, include the following
  - Previous cervical cytology results
  - Colposcopic findings

Specimen transportation [X]

Transported in adequate volume of 10% NBF using an appropriate container (Refer Chapter 1, Section B and E).

M. Brain biopsies

Background

Brain biopsies are mainly performed to diagnose tumours. Other indications include diagnosis of oportunistic infections in immunocompromized patients and investigation of unexplained neurological disorders.

There are generally two kinds of biopsies: open biopsies and stereotactic needle biopsies. Specimens are sent for routine histopathology, frozen sections and other special tests as needed.

Specimen handling at the point of collection [X]

- Avoid crushing of the tissue while collecting.
- If multiple sites are biopsied, the specimens should be sent seperately in appropriately labeled containers.

Requisition form

- Provide following information. **[X]**
  - Biopsy indication (diagnosis or follow up)
  - CT or MRI findings
    Wherever possible the pathologist should personally view the radiological images.
- Record the type of procedure – Stereotactic biopsy or surgical excision following craniotomy. **[X]**
- Record the site of the biopsy. **[X]**
- Ideally, state the number of pieces per container. **[Z]**

Specimen transportation [X]

- Transport in 10% NBF using an appropriate container (Refer Chapter 1, Section B and E).
- For frozen sections or electron microscopy refer Chapter 3, Sections A and Chapter 4, Section C.

References

Endoscopic biopsies

1. Draganov PV. Endoscopic Mucosal Resection Vs Endoscopic Submucosal Dissection for Colon Polyps. Gastroenterol Hepatol (N Y). 2018 Jan; 14(1): 50–52.
2. The Royal College of Pathologists of Australasia. Oesophageal-endoscopic mucosal resection in macroscopic cut up manuals [internet] [cited 2021 July 1] Available from: www.rcpa.edu.au/Library/Practising-Pathology/Macroscopic-Cut-Up/Specimen/Gastrointestinal/Oesophagus-and-stomach/Oesophageal-endoscopic-mucosal-resection
3. Geramizadeh B, David A. Owen. Handling and Pathology Reporting of Gastrointestinal Endoscopic Mucosal Resection. Middle East J Dig Dis. 2017 Jan; 9(1): 5–11.
4. Dautel SHP, Baumbach DR, Oldhafer KJ, Oldhafer KJ, Stang A, Feyerabend B. et al. Single center experience of endoscopic submucosal dissection (ESD) in early Barrett´s adenocarcinoma. Surg Endosc. 2015; 29: 1591–7.
5. Biermann K, Escario MDL, Hébert-Magee S, Rindi G, Doglioni C. How to prepare, handle, read, and improve EUS-FNA and fine-needle biopsy for solid pancreatic lesions: The pathologist's role. Endosc Ultrasound. 2017 Dec; 6(Suppl 3): S95–S98.

Renal biopsies

1. The Royal College of Pathologists of Australasia. Kidney biopsy in macroscopic cut up manuals [internet] [cited 2021 May 8] Available from: https://www.rcpa.edu.au/Manuals/Macroscopic-Cut-Up-Manual/Genitourinary/Kidney/Kidney-biopsy
2. Lester SC. Manual of Surgical Pathology, 3rd ed. Philadelphia: Saunders Elsevier; 2010. Chapter 20, Genitourinary specimens; p 384-419.

Liver biopsies

1. The Royal College of Pathologists of Australasia. Liver biopsy in macroscopic cut up manuals [internet] [cited 2021 May 5] Available from:

https://www.rcpa.edu.au/Manuals/Macroscopic-Cut-Up-Manual/Gastrointestinal/Liver/Liver-biopsy
2. Peter J. Scheuer, Jay H. Lefkowitch. Liver biopsy interpretation 7th ed. Philadelphia: Elsevier Saunders; 2005. p 1-11.
3. Jacobson BC, Crawford JM, Farraye FA. GI tract endoscopic and tissue processing techniques and normal histology. In: Odze RD, Goldblum JR, editors. Surgical pathology of the GI tract, liver, Biliary tract and pancreas. 2nd ed. Philadelphia: Saunders Elsevier; 2009. p 3-8.

Skin biopsies

1. Rapini RP. Obtaining a skin biopsy specimen and interpreting the results. Dermatol Clin. 1994; 12(1): 83-91.
2. Lester SC. Manual of Surgical Pathology, 3rd ed. Philadelphia: Saunders Elsevier; 2010. Chapter 18, Dermatopathology; p 310-322.
3. The Royal College of Pathologists of Australasia. Skin biopsy in macroscopic cut up manuals. [internet] [cited 2021 May 20] Available from: https://www.rcpa.edu.au/Manuals/Macroscopic-Cut-UpManual/ Skin-soft-tissue-and-bone/Skin

Nerve biopsies

1. Weis J, Brandner S, Lammens M, Sommer C, Vallat J. Processing of nerve biopsies: A practical guide for neuropathologists. Clinical Neuropathology 2012; 31 (1): 7-23.
2. The Royal College of Pathologists of Australasia. Nerve biopsy in macroscopic cut up manuals [internet] [cited 2021 April 20] Available from: https://www.rcpa.edu.au/Manuals/Macroscopic-Cut-Up-Manual/Neuropathology/Nerve-biopsy.
3. Lester SC. Manual of Surgical Pathology, 3rd ed., Philadelphia: Saunders Elsevier; 2010. Chapter 29, Neuropathology specimens; p 533-534.
4. Ducic I, West J A modified approach to sural nerve biopsy: minimizing complications. Ann Plast Surg. 2009; 62: 220–222.

Muscle biopsies

1. Joyce NC, Oskarsson B, Jin L, Muscle Biopsy Evaluation in Neuromuscular Disorders. Phys Med Rehabil Clin N Am. 2012 August; 23(3): 609–631.
2. Cai C, Anthony DC, Pytel P. A pattern-based approach to the interpretation of skeletal muscle biopsies. Mod Pathol 2019; 32: 462–483.
3. Dastgir J, Rutkowski A, Alvarez R et al. Common Data Elements for Muscle Biopsy. Arch Pathol Lab Med 2016; 140: 51–65.
4. Lester SC. Manual of Surgical Pathology, 3rd ed., Philadelphia: Saunders Elsevier; 2010. Chapter 29, Neuropathology specimens; p 533-534.

5. University of Louisville, Protocoal for muscle and nerve biopsies. [internet] [cited 2021 June 10] Avaialable from: https://louisville.edu/medicine/departments/pathology/divisions/anatomic-pathology/muscle-and-nerve-protocol.
6. Gaspar BL, Vasishta RK, Radotra BD. Muscle Biopsy Procedure and Processing. In: Gaspar BL, Vasishta RK, Radotra BD, editors. Myopathology. Singapore: Springer; 2019. p 11-22.

Lymph node biopsies

1. Fitzgibbons PL. LiVolsi VA. Recommendations for Handling Radioactive Specimens Obtained by Sentinel Lymphadenectomy. Am J Surg Pathol 2020; 24(11): 1549–1551, 2000.
2. Ramsay A, Pomplun S, Wilkins B. Tissue pathways for lymph node, spleen and bone marrow trephine biopsy specimens. The Royal College of Pathologists. Version 3 [internet]; London, UK: 2017 [cited 2021 July 3] Available from: https://www.rcpath.org/uploads/assets/a2780c20-edc8-4023-a7e5bd45175dce76/G062-Tissue-pathways-for-lymph-node-spleen-and-bone-marrow-trephine-biopsy-specimens-For-publication.pdf
3. Safe handling and despatch of specimens in the operating theatre, Standard operating procedure, Leeds Teaching Hospitals NHS trust 2018 [internet][cited 2021 July 04] hAvailable form: http://lhp.leedsth.nhs.uk/detail.aspx?id=5622#31
4. Cibull ML. Handling of sentinel lymph node biopsy specimens, A work in progress, Arch Pathol Lab Med 1999 Jul; 123(7): 620-1.

Eyeball specimens

1. Ocular Pathology Laboratory, Department of Ophthalmology, University of Iowa Hospitals and Clinics. Handling, labeling and transportation of specimens and ocular pathology consultation request forms. [internet] January 2013, Revised April 2014 [cited 2021 July 5]; Available from: https://medicine.uiowa.edu/eye/sites/medicine.uiowa.edu.eye/files/wysiwyg_uploads/Blodi%20in-house%20handling.pdf
2. Lott R, Tunnicliffe J, Sheppard E, Santiago J, Hladik C, Nasim M et al. College of American Pathologists. Practical guide for specimen handling in surgical pathology, Version 9 [internet]; Revised April 2020 [cited 2021 July 5]. Available from: https://cap.objects.frb.io/documents/practical-guide-specimen-handling.pdf
3. Ford AL , Mudhar HS, Farr R, Parsons MA. The ophthalmic pathology cut-up—Part 1: The enucleation and exenteration specimen. Current Diagnostic Pathology 2005; 11: 284–90.

Respiratory biopsies

1. Roy-Chowdhuri S, Ghofrani M, Illei PB et al. Collection and handling of thoracic Small biopsy and cytology specimens for ancillary studies. Arch Pathol Lab Med 2020; 144 (8): 933–958.
2. Nicholson AG, Kerr K, Gosney J. Standards and datasets for reporting cancers. Dataset for histopathological reporting of lung cancer. The Royal College of Pathologists. Version 6 [internet]; London, UK: September 2018 [cited 2020 June 27] Available from: https://www.rcpath.org/uploads/assets/265cdf74-3376-40b0-b7d0e3ed8a588398/G048-Dataset-for-histopathological-reporting-of-lung-cancer.pdf

Genitourinary biopsies

1. Suvarna SK, Layton C. The gross room/surgical cut-up. In . In Suvarna SK, Layton C, Bancroft JD (editors). Bancroft's Theory and Practice of Histological Techniques, 7th ed. New York, NY: Churchill Livingston; 2013, 95-103.
2. Carson F, Hladik C. Histotechnology A Self- Instructional Text, 3rd ed. Chicago, IL: ASCP Press; 2009.
3. O'Rourke D, Allen D. Tissue Pathways for Urological Pathology. The Royal College of Pathologists. Version 2 [internet]; London, UK: 2017 [cited 2021 May 5] Available from: https://www.rcpath.org/uploads/assets/88d7ee53-9a54-4b9b-8bb89b03318b4c47/Urological-tissue-pathways.pdf

Bone biopsies

1. Mangham DC, Athanasou NA. Guidelines for histopathological specimen examination and diagnostic reporting of primary bone tumours. Clinical Sarcoma Research 2011, 1:6 [cited 2021 July 2] Available from: https://doi.org/10.1186/2045-3329-1-6
2. Sterchi DL. Bone. In Suvarna SK, Layton C, Bancroft JD (editors). Bancroft's Theory and Practice of Histological Techniques, 7th ed. New York, NY: Churchill Livingston; 2013, 317-52.

Special gynaecological biopsies

1. Lester SC. Manual of Surgical Pathology. Philadelphia: Saunders Elsevier; 2010. Chapter 13, Small Biopsies; p. 243-245.
2. Suvarna SK, Layton C. The gross room/surgical cut-up. In . In Suvarna SK, Layton C, Bancroft JD (editors). Bancroft's Theory and Practice of Histological Techniques, 7th ed. New York, NY: Churchill Livingston; 2013, 95-103.

3. Brown L, Andrew A, Hirschowitz L, Millan D. Tissue pathways for gynaecological pathology. The Royal College of Pathologists. Version 1 [internet]; London, UK: 2008. [cited 2021 May 7] Available from: https://www.uhb.nhs.uk/Downloads/pdf/CancerPbRcpTissuePathways.pdf

Brain biopsies

1. Bai HX, Zou Y, Lee AM, Lancaster E, Yang L. Diagnostic Value and Safety of Brain Biopsy in Patients with Cryptogenic Neurological Disease. Neurosurgery 2015; 77 (2): 283-95.
2. Brandner S, Jaunmuktane Z, Roncaroli F, Hilton DA, Thom M, Ansorge O. Dataset for histopathological reporting of tumours of the central nervous system in adults, including the pituitary gland. Version 5 [internet]; London, UK: The Royal College of Pathologists. March 2020. [cited 2021 February 20] Available from: https://www.rcpath.org/uploads/assets/abc54563-f574-40a0-b6b9a9a2cfbaa89d/g069-dataset-for-histopathological-reporting-of-tumours-of-the-central-nervous-system-in-adults-including-the-pituitary-gland.pdf
3. Brandner S, Hofer M, Jaunmuktane Z, Phadke R. Tissue pathways for non-neoplastic neuropathology specimens. The Royal College of Pathologists. Version 4 [internet]; London, UK: August 2020. [cited 2021 July 1]; Available from: https://www.rcpath.org/uploads/assets/d8dc43af-4f19-4ff3-82d0573b8597292a/5aa079b0-3d36-4396-a491d2d755f70980/G101-Tissue-pathways-for-non-neoplastic-neuropathology-specimens-For-Publication.pdf

CHAPTER 3

Intra-operative diagnostic tests and other special situations

A. Intra-operative diagnostic tests

Intra-operative diagnostic tests are performed when the surgeon needs histopathological information to decide the type and the extent of the ongoing surgical procedure. The report will then be conveyed as soon as possible to the operating surgeon via telephone. Tissue morphology in intra-operative diagnostic tests is examined by using routine stains and does not utilize any additional tests such as special stains or immunohistochemical stains. The commonly used procedure for intraoperative diagnosis is histopathological examination of frozen sections. If frozen section facilities are not available in the laboratory, imprint or crush smears for cytopathological examination would be an option.

Indications for frozen sections:

Frozen sections or other intra-operative diagnostic tests should be done with a clear indication. Some common situations are as follows.

- To confirm a malignant diagnosis, to make a decision regarding further surgery

  e.g., papillary carcinoma of thyroid: to continue with lymph node dissection, sentinel lymph node in breast carcinoma: to proceed with axillary lymph node clearance.

- To confirm a benign diagnosis, so that the extent of surgery can be limited
  e.g., massive ovarian oedema
- To determine the extent of a disease e.g., Hirschsprung disease
- To assess the adequacy of resection e.g., eyelid malignant lesions

Limitations of frozen sections

- Frozen sections should not be used as a replacement for routine diagnostic tests, as it is inferior in quality to the latter due to its limitations. Despite these

limitations, the pathologist needs to arrive at a decision within a short period of time based on experience, judgment and knowledge of the specialty and clinical context.

Limitations of frozen sections

1. Sampling errors
   - Inadequate or non-representative sampling of tissue
   - Extensive tumour degeneration or necrosis
   - Inability to assess capsular or vascular invasion
2. Technical problems
   - Freezing artifacts / Xylene artifacts
   - Bloated cell morphology
   - Poor quality sections
   - Poorly stained sections
3. Interpretative errors, inherent to the lesion concerned
   - Lesions that are difficult to diagnose: e.g., chronic pancreatitis vs pancreatic carcinoma
   - Heterogeneity of the tumour: e.g., bone and soft tissue sarcomas
   - Variable degrees of tumour differentiation: e.g., limited sampling of the malignant component in an ovarian teratoma

Specimen handling at the point of collection [X]

- Frozen sections should be an elective procedure and the case should be discussed by the clinician and the pathologist prior to the procedure, with regard to its purpose and limitations.
- After taking into consideration the purpose for frozen section, the clinical presentation of the case and all relevant investigations done on the patient, the pathologist should finally decide whether the frozen section is indicated.
- Frozen section should be performed only if the results will in any way influence the surgical procedure.
- The time should be fixed in collaboration with the laboratory.
- The biopsy should be a representative sample of the lesion concerned. Multiple biopsies are better than a single biopsy, however processing and interpretation of multiple biopsies will take more time.
- Necrotic tissue and bony tissue should be avoided. Attached fatty tissue or fibrous tissue also will make section cutting difficult, hence should be removed.
- Excision specimens in which resection margins to be assessed, should be oriented and identified regarding the location of surgical margins and other significant anatomic structures.

Requisition form [X]

- Histopathology requisition form should accompany the sample.
- The following details should be included.
  - Patient identification details
  - Type of tissue, location of biopsy, indication for frozen section
  - Identification of orientation marks, if applicable
  - Contactable telephone number of the surgeon to discuss the findings
- Radiological investigation findings may be important to the pathologist especially for brain tumours.

Specimen transportation

- Send the specimen fresh (without adding a fixative) in a labled, closed, clean container immediately to the laboratory, adhering to standard safety precautions. **[X]**
- Specimen may be placed on a piece of sterile gauze which has been soaked in saline in order to avoid drying during transport. **[Y]** Do not suspend the tissues in saline or other fluids.
- Specimens sent in this manner can be used to prepare crush or imprint smears as well.

> **Note**: Frozen section procedure takes at least 20 minutes for the technical procedure and interpretation.

B. High risk infectious specimens

When sending specimens of patients suspected or known to have infections with high risk organisms (e.g. Yersinia pestis - black plague, HIV, SARS virus, Ebola virus, Marburg virus, Lassa virus, drug resistant TB, CJD), the laboratory should be informed prior to sending the sample, as the laboratory may have to process them differently. Interim guidelines issued during any emerging epidemic should also be followed e.g. guidelines issued during COVID 19 pandemic.

Submitting fresh tissue for frozen sections, immunofluorescence or any other investigation should be avoided when possible. If such an investigation procedure is mandatory, the laboratory should be informed prior to sending the specimen, therefore the laboratory can take special measures while handling. All brain biopsies for dementia should be handled as possible CJD cases and frozen sections should not be done. Touch imprints are preferable to frozen sections in suspected cases of tuberculosis.

Specimen handling at the point of collection [X]

- Tissue submitted for routine histopathology should be placed in an adequate volume of 10% NBF in a leak proof container.
- The container should have “High risk - Danger of infection” label.

Requisition form [X]

- Include patient identification details and other relevant information as per Chapter 1 Section A.
- Indicate “HIGH RISK- DANGER OF INFECTION”
- Send the requisition form seperately in a sealed plastic bag. Do not wrap or pack the it with the specimen container.

Specimen transportation [X]

- Triple packaging during transport is mandatory for high-risk specimens. (Refer Chapter 1, Section F)
- The primary container should be watertight, leak-proof and appropriately labeled as to the content.
- The primary container should be wrapped in enough absorbent material to absorb all fluid in case of breakage or leakage.
- A single or several wrapped primary containers may be placed inside a watertight, leak-proof secondary packaging to enclose and protect the primary container/s.
- The secondary package should be placed in a third package to protect it from physical damage while transporting.
- The packaging should not be opened while transporting.
- In the event of damage to packaging and spill while transporting, the specimen should be retrieved as much as possible before clean-up procedure, as histopathology specimens are irretrievable. Contact the laboratory immediately for advice. Utilize the extra package give to the courier, to re-package the specimen.

C. Multiple tests requested on a single specimen

Background

Sometimes, tissue samples are obtained for multiple tests including routine histopathology, microbiology, cytogenetics etc. It is preferable to obtain separate specimens in appropriate containers for different tests. However, at times, a single specimen may have to be used to perform multiple tests. At which point, it is advisable to contact histopathology and other relevant sections (if needed) of the laboratory and follow the given instructions.

Specimen handling at the point of collection

- If a single specimen is to be shared for multiple tests, adequate material should be obtained under strict aseptic conditions with minimal handling, as tissue portion for histopathology requires tissue without disruption of architecture/ morphology. **[X]**
- If there is a delay in trasport (e.g. when the specimen is collected at night and sending to the laboratory, in the following morning) it should be refrigerated. Wrap the specimen in a piece of sterile gauze moistened with sterile saline and place it in a sterile container to avoid drying.

Requisition form [X]

- Separate requisition forms should be sent for each test requested.
- In *each* request form
  - indicate the other laboratory tests requested.
  - indicate contact telephone number of the clinician.
- Transport the specimen immediately to the laboratory, adhering to safety precautions without a fixative.
- If there is a delay in transport, wrap the specimen in a piece of sterile gauze moistened with sterile saline and place it in a sterile container to avoid drying. **[Y]**

References

1. Royal College of pathologists of Australasia guidelines, Frozen Section: minimization of infection hazards associated with cryostats. [internet]; September 2018. [cited 2020 June 20], Available from: https://www.rcpa.edu.au/getattachment/2b50b88c-9aa3-4811-aade-dc26470fb44b/Frozen-Section-Minimisation-of-Infection-Hazards-a.aspx
2. Jaafar H. Intra-operative frozen section consultation: concepts, applications and limitations. Malaysian Journal of Medical Sciences 2006; 13(1); 4-12.
3. Crain BJ. Creutzfeldt-Jakob Disease, Safety tips for anatomic studies of possible CJD. CAP In the News - Reprints of Interest. [cited 2020 April 15], Available from: https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.516.7557&rep= rep1&type=pdf
4. World Health Organization Laboratory biosafety manual. – 4th ed. Geneva: 2004. [cited 2020 June 20], Available from: https://www.who.int/publications/i/item/9789240011311
5. Safe handling and despatch of specimens in the operating theatre, Standard operating procedure, Leeds Teaching Hospitals NHS trust 2018 [internet] [cited 2021 July 04] Available from: http://lhp.leedsth.nhs.uk/detail.aspx?id=5622#31
6. Roy-Chowdhuri S, Ghofrani M, Illei PB et al. Collection and Handling of Thoracic Small Biopsy and Cytology Specimens for Ancillary Studies. Arch Pathol Lab Med 2020; 144 (8): 933–58.

CHAPTER 4

Handling of specimens for specialized and highly specialized investigations

Introduction

Different types of specimens (e.g., paraffin wax tissue blocks, fresh tissue) may be required to be used for performing specialized tests. The specialized / highly specialized tests include immunohistochemistry, immunofluorescence, flow cytometry, electron microscopy, molecular tests and cytogenetics.

The procedures for optimal collection, storage and transport of specimens for these investigations may vary between laboratories. Hence, communication with the relevant laboratory is mandatory, to obtain appropriate instructions prior to collection.

A. Immunohistochemistry

Background

Usually immunohistochemistry (IHC) is requested by the reporting pathologist following examination of the initial H and E-stained sections. However, in the event that a malignancy report is sent to an oncologist which requires immunohistochemistry, the oncologist may then request the pathologist to arrange further tests.

Specimen handling at the point of collection

- The most suitable specimen for performance of immunohistochemistry test is the paraffin wax tissue block.
- The wax tissue block should have been prepared from tissue fixed in 10% NBF and processed using standard / recommended quality chemicals validated for optimum antigen preservation. **[X]**
- Use of any other fixative (e.g., Bouin solution) or processing method (e.g., decalcification) should be mentioned in the test requisition form.
- The wax tissue block should bear the identification numbers written clearly (laboratory accession number and block identification number). **[X]**
- Some reference laboratories may require the relevant H and E stained section to accompany the wax tissue block. **[Y]**
- If any IHC stain has been performed previously, those slides also should ideally be sent. **[Z]**
- In case of small biopsies, it is preferable to send a few unstained sections cut on positively charged glass slide, in order to avoid loosing tissue during re-embedding and trimming. **[Y]**

Requisition form

- The specified test requisition form should accompany the wax tissue block (Annexure 3, Section E). **[X]**
- The following details should be included. **[X]**
  - Patient identification details as per Chapter 1 Section A
  - The requested immune marker/s
  - The site and laterality (e.g. left breast lump) and the type of biopsy tissue in the wax tissue block (e.g. core biopsy, lumpectomy, mastectomy etc.)
  - The morphological diagnosis and/or the differential diagnosis
  - The time duration from excision of the specimen to transfer to fixative (cold ischaemia time) and the duration of fixation (tissue in fixative) as it may affect the test results
  - Name and signature of the requesting/reporting consultant pathologist

Wax tissue block and slide transportation [X]

- The tissue on the block should be covered with paraffin wax or a suitable covering material such as a thin cling wrap or aluminum foil to prevent damage.
- The wax tissue block should be
  - placed in a cardboard box or suitable container to prevent physical or heat damage and to protect from pests during transport.

- kept in a cool dry place prior to and during transport (preferably below 27°C)
- The glass slides should be packed in a box with a slide holder to prevent breakage during transport.

B. Direct immunofluorescence

Specimen handling at the point of collection

- The most suitable specimen for immunofluorescence is fresh tissue.
- For renal biopsies, ideally, assessment should be done for the presence of glomeruli (Refer Chapter 2 Section B on renal biopsies). **[Z]**
- For skin biopsies, the optimal site for taking a biopsy depends on the suspected disease. **[X]**

  Autoimmune bullous diseases: Normal-appearing perilesional skin less than 1 cm from a bulla. Avoid specimens from lower extremities as false-negative results may occur.

  Connective tissue diseases: An established lesion from sun-exposed areas, ideally more than 6 months old, but still active. Take an additional specimen from a lesion in a sun-protected site.

  Vasculitis: A punch biopsy or a deep-shave biopsy of a lesion that is less than 24 hours old.

Requisition form

- The specified test requisition form should accompany the specimen (Annexure 3, Section F). **[X]**
- The following details should be included. **[X]**
  - Patient identification details as per Chapter 1 Section A
  - Test parameters (types of antibodies / complement components)
  - Site and laterality (e.g., left renal biopsy) and the type of biopsy tissue (e.g., core biopsy, punch biopsy)
  - For renal biopsies, whether native or from transplanted kidney
  - Relevant clinical details and laboratory investigations (e.g., skin biopsy-the type of bullous lesions)
  - Morphological diagnosis and/or the differential diagnosis
  - Name, contact telephone number and signature of the requesting clinician / pathologist

Specimen transportation

- The tissue biopsies should be placed/ wrapped in a wet filter paper (soaked in normal saline). **[X]**
- Place it in a leak proof container, labeled with patient identification details. **[X]**
- The specimen container should be surrounded by ice cubes inside a cool box. The ice cubes should be in a leak-proof bag. **[X]**
- If transported by courier, the cool box should be placed in another box to prevent damage during transport (Refer Chapter 1, Section F). **[X]**
- If the specimen is transported in ice, it should reach the laboratory preferably within one hour of collection, and at least within 6 hours if the institution is at a distance. **[X]**
- If a further delay is anticipated, it is preferable to use a standard transport media (Michel transport medium). The transport media should be requested from the relevant laboratory. **[Z]**
    - Extended time in transport medium also will increase the autofluorescence. Keeping tissue in the medium for >5 days is not recommended.
    - During transport or storage in transport medium, maintain cool to ambient temperatures of 4°C to 22°C.

C. Electron microscopy

Specimen handling at the point of collection

- Tissue specimens should be placed directly in 2.5% buffered iso-osmolar glutaraldehyde immediately after removal. **[X]** This will help prevent autolysis and air-drying/ shrinkage artifact.
- Small biopsies should be placed on a wet filter paper and then transferred to the fixative. **[X]**
- The specimen should not be frozen.
- In the event that glutaraldehyde fixed tissue is unavailable, formalin fixed tissue, tissue retrieved from wax tissue block, or haematoxylin and eosin-stained tissue sections may be used but may not yield optimum results. **[Y]**
- Though solid tissue is the ideal, fluids such as blood, bone marrow aspirates, fine needle aspirates, fluid suspensions (e.g. nasal brush biopsies) are also used. If so, the fluids should be centrifuged, and the pellet should be fixed in 2.5% glutaraldehyde. **[Y]**
- For nerve biopsies
    - Obtain at least 4-6 cm long biopsy.
    - Divide the specimen into two pieces; one should be 2-3 cm long for glutaraldehyde fixation. The other piece is placed in 10% NBF.

- Place the specimen with fixative in a refrigerator overnight, ensuring that the biopsy is stretched and in upright position (Figure 1).

Requisition form [X]

- The specified test requisition form should accompany the specimen (Annexure 3, Section G).
- The following details should be included.
  - Patient identification details as per Chapter 1 Section A
  - The specific structure/ feature that requires to be identified by electron microscopy
  - The site and laterality (e.g., liver, right kidney) and the type of tissue biopsy sent (e.g., soft tissue)
  - Relevant clinical details and laboratory investigations
  - The morphological diagnosis and/or the differential diagnosis
  - Name, contact telephone number and the signature of the requesting clinician / pathologist

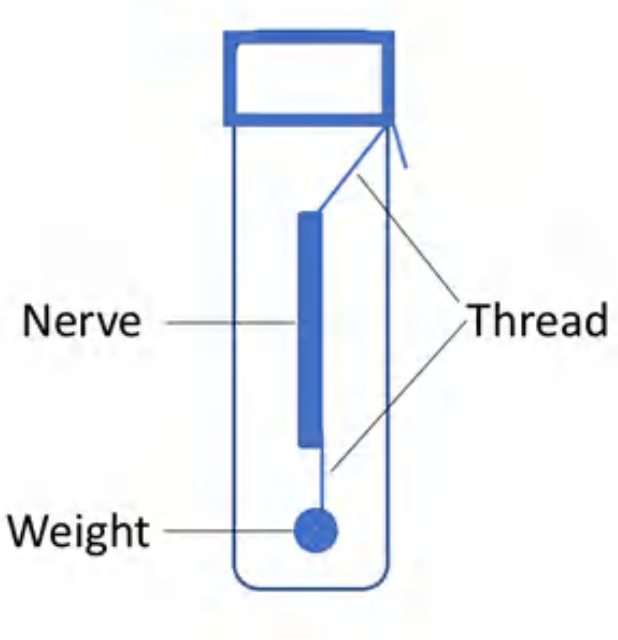


Figure 1. Nerve biopsy is stretched and kept in upright position.

Specimen transportation [X]

Refer general guideless on specimen transport Chapter 1.

D. Flow cytometry

Specimen handling at the point of collection

Suitable specimens for flow cytometry include, peripheral blood, bone marrow aspirate, body fluids, fine needle aspirates, fresh tissue scrapings, solid tissue (see below). It is mandatory to check with your laboratory the type/s of samples they will accept for flow cytometry. **[X]**

- **Fine needle aspirates**
  - Multiple aspirates are better than single aspirates from the site. Single aspirate specimens often contain too few cells for satisfactory analysis.
  - Collect the specimen into a tube containing commercially available preservative (e.g., RPMI tissue culture medium).
  - The specimen may also be placed in saline if it is to be transported to the laboratory within 1 hour.

- **Body fluids**
  - Body fluid specimens containing small numbers of cells may not be adequate for immunophenotyping.
  - 20-50 mL of fluid (minimum 5 mL) should be collected to a sterile container.
  - For CSF, 5-10 mL (minimum 1 mL) is recommended.

- **Solid tissue (e.g., lymph nodes)**
  - A minimum of 0.5 cm^3 of tissue should be placed in a sterile container with 15 mL of commercially available preservative (e.g. RPMI tissue culture medium).
  - Fresh tissue scrapings should be transferred to 15 mL of RPMI.
  - Fresh tissue may also be placed in saline or a saline soaked gauze if it is to be transported to the laboratory within 1 hour.

Requisition form [X]

- The specified test requisition form should accompany the specimen. (Annexure 3, Section H)
- The following details should be included.
  - Patient identification details as per Chapter 1 Section A.
  - The requested panel of cell markers (e.g. lymphoproliferative disorder panel, screening panel).
  - The site and laterality (e.g. left supraclavicular lymph node) and the type of specimen sent (e.g. fine needle aspirate, fresh tissue scrapings).
  - Relevant clinical details and laboratory investigations (e.g. LDH levels).

- The morphological diagnosis and/or the differential diagnosis.
- Name, contact telephone number and the signature of the requesting clinician / pathologist.

Specimen transportation [X]

- Transport the sample to the laboratory within one hour at room temperature Refrigerate at 40°C if a delay is anticipated. Do not freeze.
- Specimens should reach the laboratory in order to be processed within 24 hours of collection.

E. Molecular tests

Specimen handling at the point of collection [X]

- The most suitable specimen for performance of FISH is the wax tissue block.
- Touch preparations (e.g. soft tissue tumours and lymph nodes) and fresh tissue (e.g. chorionic villi) are also used.
- Amniotic fluid and bone marrow aspirates are suitable specimens for karyotyping.
- The wax tissue block should be prepared from tissue fixed in 10% NBF and processed using standard / recommended quality chemicals validated for optimum antigen preservation.
- Tissue fixed in fixatives other than 10% NBF (e.g. Bouins solution) or tissue subjected to decalcification are unsuitable for FISH.
- The wax tissue block should bear clearly readable identification details (lab accession number and block number).
- The wax tissue block should contain more than 10 mm3 area of target tissue required for the test.

Requisition form [X]

- The specified test requisition form should accompany the samples. (Annexure 3, Section I)
- The following details should be included.
  - Patient identification details as per Chapter 1 Section A
  - Specific test and the type of genetic abnormality to be identified (e.g. FISH for X:18 translocation, karyotyping for trisomy 21)
  - The site and laterality (e.g. left breast lump) and the type of sample sent (e.g. whole blood, fresh tissue, paraffin wax block.

- Relevant clinical details and laboratory investigations (e.g. immunohistochemistry test results for Her2 neu in breast carcinoma
- The duration from interruption to blood supply to surgical excision of specimen (warm ischaemia time), the duration from excision of the specimen to transfer to fixative (cold ischaemia time) and the duration of fixation (tissue in fixative) as it may affect the test results
- The morphological diagnosis and/or the differential diagnosis
- Name, contact telephone number and the signature of the requesting clinician/ pathologist

Specimen transportation [X]

- The tissue on the block should be covered with paraffin wax or a suitable covering material such as a thin cling wrap or aluminum foil to prevent damage.
- The wax tissue block should be
  - placed in a cardboard box or suitable container to prevent physical or heat damage and to protect from pests during transport.
  - kept in a cool dry place prior to and during transport. (Preferably below 27 C)
- Fresh tissue or touch preparations should be sent immediately after removal/ preparation placed in a sterile container.

F. Liquid biopsy

Liquid biopsy is a test done on a sample of blood to look for circulating cancer cells from a tumour or for pieces of DNA from tumour cells that are in the blood. Although this test is not currently available in Sri Lanka, it may come into use in the near future, at which point, recommended sources of information should be used for sending samples.

G. Transporting blocks for histochemical stains and referral opinions

The same procedure as for sending wax tissue blocks for immunohistochemistry should be followed for sending wax tissue blocks for histochemical stains and referral opinions. Please refer Section A of this chapter.

References

1. Khoury T. Delay to Formalin Fixation (Cold Ischemia Time) effect on breast cancer molecules. American Journal of Clinical Pathology April 2018; 149 (4): 275–92.
2. Holland NT, Smith MT, Eskenazi B, Bastaki M. Biological sample collection and processing for molecular and epidemiological studies. Mutation Research 2003; 543: 217–34.
3. College of American pathologists. Molecular pathology check list [internet] [cited 2021 July 1]; Available from: https://webapps.cap.org/apps/docs/education/OnlineCourseContent/2012/LAP/Resources/Checklists/MOL09252012.pdf.
4. Suvarna SK, Layton C, Bancroft JD. Bancroft's Theory and Practice of Histological Techniques, 7th ed. New York, NY: Churchill Livingston; 2013.
5. Engel KB, Moore HM. Effects of preanalytical variables on the detection of proteins by immunohistochemistry in formalin-fixed, paraffin-embedded tissue. Arch Pathol Lab Med. 2011; 135: 537–43.
6. Deborah Levenson. A New Era for Liquid Biopsy. Clinical Laboratory News. Available online at https://www.aacc.org/cln/articles/2020/november/a-new-era-for-liquid-biopsy. Published November 1, 2020. Accessed January 5, 2021
7. Kapila SN, Boaz K, Natarajan S. The post-analytical phase of histopathology practice: Storage, retention and use of human tissue specimens. Int J Appl Basic Med Res. 2016 Jan-Mar; 6(1): 3–7.

Annexure I: Preparation of fixatives and transport media

A. Formalin-based fixatives

Ideally surgical pathology samples should be sent to the laboratory in fresh state immediately. If there is a delay in sending or the laboratory is unable to handle the fresh samples, a fixative should be added. Unless otherwise specified, the fixative recommended is 10% neutral buffered formalin (10% NBF) and should be obtained from the laboratory.

A1. Preparation of 10% NBF

The standard stock solution of formalin available is 37-40% formaldehyde (a gas) in aqueous solution with the specific gravity ranging from 1.080 to 1.090 and a pH of 3-4.

- Water is used (preferably distilled water) to dilute stock solution to prepare 10% formalin.
- In order to achieve the correct concentration of fixative, even if the stock solution of formalin is labeled mentioning the concentration, it is recommended that the specific gravity of the solution is checked to obtain the exact concentration.
- If the concentration of formaldehyde in stock solution is less than 37%, then water should be mixed with formalin as given in table 1 to prepare 10% formalin.

Table 1. Preparation of 10% formalin: Calculation of the proportions of formalin and water based on the specific gravity of formaldehyde stock solution.

Density (specific gravity) of stock solution of Formalin	Percentage (%) of Formaldehyde	Milliliters of formalin	Milliliters of distilled water /tap water
1.075	35.15	113.7	886.3
1.070	33.30	120.0	880.0
1.060	29.60	133.5	866.5
1.055	27.75	144.0	856.0
1.050	25.90	154.4	845.6
1.045	24.05	166.2	833.8
1.040	22.20	180.0	820.0
1.035	20.35	196.1	803.9
1.030	18.50	216.5	783.5
1.025	14.80	270.0	730.0
1.020	12.95	309.2	690.8
1.015	11.10	361.0	639.0
1.012	9.25	432.4	567.4
1.010	7.40	540.0	460.0
1.008	5.55	720.7	279.3
1.006	4.00	1000.0	00.0

- Adjusting pH using a buffer: As formaldehyde aqueous solution is acidic, formalin can react with haemoglobin in the tissues to produce dark brown acid formaldehyde haematin precipitates (formalin pigment), which can interfere with histopathological assessment. A buffer is used to neutralize the acidity and to prevent formation of formalin pigment.
- The pH, and presence or absence of buffer also affect the quality of immunostaining in formalin fixed paraffin embedded specimens.

Solutions and reagents for 10% NBF (in phosphate buffer)

If the concentration of formaldehyde in stock solution is 37- 40% the following quantities are used to prepare 1L of 10% NBF. The pH should be within 7.2-7.4.

Formaldehyde (37- 40% stock solution)	100 mL
Distilled water (ideal) / tap water	900 mL
Sodium phosphate monobasic monohydrate NaH_2PO_4	4 g/L
Sodium phosphate Na_2HPO_4 (dibasic / anhydrous)	6.5 g/L

* See table 1 for stock solution concentrations below 37%.

A2. Preparation of other formalin-based fixatives

10% formalin is not isotonic and there is a possibility that erythrocytes may be damaged by lysis. Formal saline is a little more effective than 10% formalin as it is isotonic and there is less likelihood that erythrocytes will be damaged.

Solutions and reagents for Formalin, buffered saline (10% buffered formal saline (10% BFS)

If the concentration of formaldehyde in stock solution is 37- 40% the following quantities are used to prepare 1L of 10% BFS.

Formaldehyde (37- 40% stock solution)	100 mL
Distilled water (ideal) / tap water	900 mL
Sodium chloride (NaCl)	9 g
Sodium phosphate Na_2HPO_4 (dibasic / anhydrous)	12 g

* See table 1 for stock solution concentrations below 37%.

10% formal saline (unbuffered) is the common fixative available in histopathology laboratories in Sri Lanka. However, it is recommended that laboratories work towards ensuring that 10% neutral buffered formalin (10% NBF) which is the ideal fixative is used.

Solutions and reagents for 10% Formalin saline (unbuffered)

Formaldehyde (37- 40% stock solution)	100 mL
Distilled water (ideal) / tap water	900 mL
Sodium chloride (NaCl)	9 g

* See table 1 for stock solution concentrations below 37%.

B. Special fixatives / transport media

Use of any fixative other than those described above for special purposes, should be decided with the reporting pathologist.

B1. Zenker fixative

Zenker is a nuclear fixative and is used as a mordant in some staining procedures.

It gives good nuclear preservation but lyses red blood cells due to the presence of acetic acid.

Zenker fixative has been recommended for congested specimens and gives good results with PTAH and trichrome staining.

Solutions and reagents

Zenker stock solution

To prepare 1L of Zenker stock solution – add following into a suitable container and mix well

Mercuric chloride	50 g
Potassium dichromate	25 g
Sodium sulfate	10 g
Distilled water	1000 mL

Working solution

Mix following thoroughly *just before use*.

Zenker stock solution	95 mL
Glacial acetic acid	5 mL

Instructions for usage of the solutions

- When using Zenker fixative samples should be fixed for 4-24 hours.
- The tissues must be washed well in running water overnight after fixation to remove the excess potassium dichromate, or the dehydrating alcohols will cause a chrome pigment.
- Zenker fixative should be discarded following hazardous waste regulations as it contains mercury.
- The stock solution is stable for 2 years.

B2. Bouin fixative

Bouin fixative is a compound fixative which is composed of picric acid, acetic acid, and formaldehyde in an aqueous solution. It is a good fixative when tissue structure with a soft and delicate texture needs to be preserved. The acetic acid in this fixative lyses red blood cells and dissolves small iron and calcium deposits in tissue. Bouin fixative is commonly used for testicular and gastrointestinal biopsies and also as a mordant for staining procedures

Solutions and reagents

To prepare 1 L of Bouin fixative:

Picric acid (saturated aqueous solution)	75 mL
Formalin (40% w/v formaldehyde)	25 mL
Glacial acetic acid	5 mL

Method of preparation

1. Prepare 75 mL of saturated aqueous solution of picric acid
2. Add 25 mL of formalin (40% w/v formaldehyde)
3. Add 5 mL of glacial acetic acid

Instructions for usage of the solutions

- Small biopsies should be fixed for 2 to 4 hours, large specimens may remain in fixative up to 3 days. Some tissue will become brittle if left in fixative for long.
- Remove the picric acid from the tissue prior to processing with several changes of 70% alcohol until no more yellow exudates from tissue.
- Prepared solution is stable for 1 year.
- Picric acid is explosive in crystal form and if allow to dry.

B3. Michel transport medium for immunofluorescence

Michel transport medium is used for biopsies to maintain tissue-fixed immune-reactants prior to direct immunofluorescence and immunoelectron microscopy. It provides a stable medium for transport of fresh unfixed tissues, such as renal, skin and oral mucosal biopsies, which will undergo subsequent frozen sectioning and immunofluorescence.

Tissue sent in Michel transport medium should be washed with Michel wash buffer solution prior to sectioning.

Solutions and reagents

Michel transport medium

To prepare 100 mL of Michel transport medium:

$(NH_4)_2SO_4$	55 g
1M potassium citrate at pH 7	2.5 mL
0.1M CN–ethylmaleimide	5 mL
0.1M $MgSO_4$	5 mL
Distilled water	87.5 mL
1M KOH	

Method of preparation

1. Add 55 g of $(NH_4)_2SO_4$ to 2.5 mL of 1M potassium citrate, pH 7
2. Add 5 mL of 0.1M CN–ethylmaleimide
3. Add 5 mL of 0.1M $MgSO_4$
4. Add 87.5 mL of distilled water
5. Adjust to pH 7 with 1M KOH

Michel wash buffer

To prepare 100 mL of Michel wash buffer:*

1M potassium citrate	2.5 mL
0.1M CN–ethylmaleimide	5 mL
0.1M $MgSO_4$	5 mL
Distilled water	87.5 mL
1M KOH	

Method of preparation

1. Add 2.5 ml of 1M potassium citrate pH7 to 5 ml of 0.1M $MgSO_4$
2. Add 5 ml of 0.1M CN–ethylmaleimide
3. Add 87.5 ml of distilled water
4. Adjust to pH 7 with 1M KOH

* This is the same as for fixative solution except for lack of $(NH_4)_2SO_4$.

Instructions for usage of the solutions:

- Place fresh tissue in an adequate amount of Michel transport medium as soon as possible after obtaining the specimen.
- Ensure that the specimen is completely covered with Michel transport medium and is free floating.

- Transport tissue to the laboratory in Michel transport medium as early as possible. Extended time in transport medium will increase the autofluorescence. Keeping tissue in the medium for more than 5 days is not recommended.
- During transport or storage, maintain cool to ambient temperatures of 4°C to 22°C.
- Upon receipt, wash tissue with Michel wash buffer solution; three changes, 10 minutes each.
- Freeze tissue sample(s).
- Tissue placed in Michel transport medium may provide adequate results when processed for light microscopy review. Wash tissue 2-3 minutes in tap water and place in appropriate fixative prior to processing.

Note: Michel transport medium is *not a fixative* and does not have any fixative properties. It is not suitable for transporting cells for flow cytometry or tissues used for fluorescent in-situ hybridization (FISH).

B4. Fixative for electron microscopy

To be able to view a biological sample in the electron microscope it must first be stabilized (fixed), preferably in a way that the ultrastructure of the cells or tissue remain as close to the living material as possible.

Glutaraldehyde is the most common fixative used in electron microscopy, which reacts with many nucleophiles in the cell (most commonly amines). It produces irreversible cross-linking networks throughout the cytoplasm in seconds to minutes. Too high concentration of glutaraldehyde can inhibit the formation of rapid cross-links hence preparation of the fixative should be meticulous.

Electron microscopy is not widely used in Sri Lanka at present, hence recommendations for fixative preparation may be included in future editions of this guideline. References available for preparation of this fixative are given below.

References

1. Formalin Fixative. University of Surrey. [internet] [cited 2021 March 12] Available from: https://www.surrey.ac.uk/sites/default/files/Formalin-Fixative.pdf
2. Ackerman LV, Rosai J. Rosai and Ackerman's Surgical Pathology. 10th ed. Edinburgh: Mosby; 2011.
3. Carson FL, Cappellano CH. Histotechnology. A self-instructional text. 4th ed. Chicago: ASCP Press; 2014.
4. Dapson JC, Dapson RW. Hazardous materials in the histopathology laboratory: regulations, risks, handling, and disposal. 3rd ed. Battle Creek, MI: Anatech Ltd; 1995.
5. Hayet M. Fixation for Electron Microscopy. 1st ed. Academic Pres; 1981.
6. Woods AE, Stirling JW. Transmission electron microscopy in Suvarna SK, Layton C, Bancroft JD (editors) Bancroft's Theory and Practice of Histological Techniques, 7th ed. New York, NY: Churchill Livingston; 2013; p. 493-538.

Annexure II: Preparation of decalcifying solutions

Introduction

Inorganic calcium must be removed from the organic collagen matrix, calcified cartilage and surrounding tissues in order to obtain satisfactory paraffin sections. It is carried out by chemical agents.

Satisfactory decalcification must result in complete removal of calcium salts with minimal distortion of cell morphology and produce no interference during staining. Decalcification is carried out by different methods and the most commonly used is by using acid solutions. Various acid solutions may be used alone or in combination with a neutralizer. The neutralizer helps in preventing the swelling of the cells.

The tissue is cut into small pieces of 3 to 5 mm size. This helps in faster decalcification. The tissue is then suspended in decalcifying medium with waxed thread. The covering of wax on thread prevents from the action of acid on thread. The volume of the decalcifying solution should be 50 to 100 times of the volume of tissue. The decalcification should be checked at the regular intervals.

A. Types of decalcifying agents

- Acid decalcifiers (form calcium salts)
  - Strong (inorganic): 5-10%, nitric acid, hydrochloric acid
  - Weak (organic):
- Chelating agents (bind to calcium ions): used when molecular elements must be preserved for IHC, FISH, PCR
- Ethyline diamine tetra-acetic acid (EDTA)

Commonly used strong acids

- Aqueous Nitric acid, 5-10%
- Perenyi's fluid
- Formalin- Nitric acid,
- Hydrochloric acid in distilled water

Advantages and disadvantages of strong acids

- Decalcify rapidly: within 24 hours.
- Cause tissue swelling and damage if used for more than 24 hours.
- Old nitric acid is particularly damaging.
- Used for needle and small biopsy specimens to permit rapid diagnosis within 24 hours.
- Used for large heavily mineralized specimens with careful monitoring by decalcification end point test.

Commonly used weak acids

- Formic acid, 10% in distill water (primary decalcifier only)
- Aqueous formic acid
- Formic acid formalin – for very small bone pieces
- Buffered formic acid
- Acetic acid
- Picric acid

Advantages and disadvantages of weak acids

- Can cause tissue damage, so should be end-point tested.
- Decalcification usually complete in 1-10 days.
- Picric acid can cause tissue swelling and damage (found as components of Carnoy and Bouin fixatives).

B. Solutions and method of preparation

STRONG ACIDS	
Aqueous Nitric acid, 5-10%	
Nitric acid	5-10 mL
Distilled water	Up to 100 mL
Perenyi's fluid	
10% nitric acid	40 mL
Absolute ethanol	30 mL
0.5% chromic acid	30 mL
Mix shortly before use	
Formalin-Nitric acid	
Formaldehyde (37-40%)	10 mL
Distilled water	80 mL
Nitric acid	10 mL
WEAK ACIDS	
Formic acid, 10% in distilled water (aqueous formic acid)	
90% stock formic acid	5-10 mL
Distilled water	Up to 100 mL
Picric acid	
Picric acid (hydrated)	1.6 g
Distilled water	100 mL

References

1. Sterchi DL. Bone. In Suvarna SK, Layton C, Bancroft JD (editors). Bancroft's Theory and Practice of Histological Techniques, 7th ed. New York, NY: Churchill Livingston; 2013, 317-52.
2. Decalcification of Biopsy Tissue. 1st ed. LAP LAMBERT Academic Publishing; 2016: p. 28-34.

Annexure III: Requisition forms

Introduction

Histopathology laboratories use specially designed forms for accepting specimens for routine histopathology and other specialized and highly specialized investigations performed on tissue. Interpretation of histopathology and other specialized and highly specialized tests requires correlation with clinical information and other investigation findings, such as radiological or endoscopic findings and previous biopsy / cytology reports, hence adequate space should be available in the form to provide such details.

Histopathology requisition form includes the following, which need to be completed by the requesting clinician:

- Part I: Header with the name, address, contact information and the logo of the institution/ ministry, the type of test requested and whether urgent diagnosis is required or not.
- Part II: Patient identification information, ward/clinic and hospital name and date and time of specimen collection
- Part III: Specimen details, relevant clinical information, investigation findings, operative findings, therapeutic measures, previous biopsy/ cytology details
- Part IV: Information about the requesting clinician

The following details may be included in the histopathology requisition form, however, they may alternatively be included in a separate laboratory worksheet.

- Acceptance checklist
- Procedural records
- Quality parameters
- Reporting form
  i. Macroscopy
  ii. Microscopy

iii. Conclusion/ summary
iv. Comments and recommendations if any
v. Name and signature of the pathologist
vi. Date of reporting

As these guidelines are for handling of histopathology specimens during collection and transport to the laboratory, only the model requisition form is included at the end of this chapter. The laboratories can design laboratory worksheets and reporting forms as required ensuring that above details are included.

Part I, Part II and Part IV of the model requisition form are the same for, requesting routine histopathology or any other specialized investigation, **except for the type of test requested** (in Part I). However, Part III which contains clinical information, varies for different types of specimens and for different types of tests requested. Hence, Part III relevant to special specimen types and for specialized investigations is included separately.

Note:

- These forms contain minimum information required for testing purposes, however, individual laboratories may require additional information. The clinicians should comply with such requirements.
- For further details regarding information to be included in the requisition form, refer relevant sections in Chapter 1 (Section A), Chapter 2 and Chapter 4 under each specimen type or test category.

A. Histopathology requisition form: Parts I-IV

Part I

Logo of the institution

Department of Histopathology

Name, Adress and contact details of the Instititon

Histopathology requisition form

☐ **Urgent** ☐ **Routine**

FOR LABORATORY USE ONLY
Lab reference No. /Barcode .....
Pathologist .....
Date and time of reception.....

Part II.

Name of patient: ..... Date of birth: .....
Age: ..... NIC No.: .....
Gender: ..... BHT/Clinic No: .....
Ward /Clinic: ..... Date and time of sample collection:
Contact tel. number of patient: ....

Part III.

Type of specimen and site /(side if relevant): Multiple specimens should be identified seperately.
1.....:..... 2. ..... 3. .....
Surgical procedure:
.....
Time of removal of tissue.....Time of immersion of tissue in fixative.....(for breast specimens)
Clinical history and examination findings:
.....:.....
.....
Clinical diagnosis /differential diagnosis:
.....
.....
Relevant radiological findings / endoscopic findings / investigation results:.....
.....
.....
Reports (Radiology/endoscopy) attached: Yes / No /NA
Operative findings with specimen orientation details:
.....
.....
.....
Pre-operative chemotherapy given: Yes/ No/ NA
Radiotherapy given: Yes /No/ NA

Previous diagnostic data:	Lab ref. no.	Institution performed	Diagnosis
Histopathology			
Cytology/ FNA diagnosis			

Part IV.

Referring consultant (name stamp): .....
Name and designation of requesting medical officer: .....
Signature: ..... Contact tel. number:.....

B. Renal biopsy histopathology requisition form: Part III

Note:

- Part I, II and IV should be the same as for histopathology requisition form (Annexure III A)
- Replace header "Histopathology requisition form" with “Renal biopsy histopathology requisition form”

Part III.

Type of specimen and side if relevant:

……:……

Indication for renal biopsy: (please tick)

A. Isolated proteinuria
B. Isolated / microscopic haematuria
C. Proteinuria + haematuria
D. Nephritic syndrome
E. Nephrotic syndrome

Relevant medical history: ……..

……

Clinical diagnosis/ Differential diagnosis: ……..

Investigations:

A. ESR: ……
B. CRP: ……
C. Blood urea: ……
D. Serum electrolytes: ……
E. Serum creatinine: ……
F. UFR: ……
G. Urine dysmorphic cells: ……
H. Urine protein/creatinine ratio: ……

Ultrasound scan: ……

Drug history: ……

……

Family history: ……

Specimen fixed in (A) Formalin

(B) Any other fixative

Native/ transplant: ……

Side of biopsy: …… No. of cores: ……

Hospitals / institutions where biopsy sent for other investigations: (Indicate results if already available)

Immunofluorescence……

Electron microscopy……

Previous biopsy diagnostic data:

Lab ref. No.	Institution performed	Diagnosis and treatment details

C. Muscle / nerve biopsy histopathology requisition form: Part III

Note:

- Part I, II and IV should be the same as for histopathology requisition form (Annexure
- Replace header "Histopathology requisition form" with “Muscle/ nerve biopsy histopathology requisition form”

Part III.

Type of specimen: Muscle/ Nerve Biopsy procedure: Open/ Needle

Site of biopsy: ……

(Should be from moderately affected muscle with at least grade 4 power or moderately affected muscle confirmed by ultrasound scan or MRI)

Clinical details

Muscles affected: ……

Age at onset: …… Current functional level: ……

Ambulant and able to climb stairs: …… Uses wheelchair full time: ……..

Unable to climb stairs: …… Contractures (describe): ……..

Skin changes: …… Any vasculitic syndromes: ……..

Maximal mobility achieved (if different): ……

Describe pattern of weakness and age at onset (Please state what are the muscle groups that are weak or paralyzed accurately……

……

Muscle wasting (Describe):

……

Muscle hypertrophy (Describe):

……

Myopathy facies		Epilepsy	
Scoliosis		Learning difficulties	
Spinal rigidity		Cardiac involvement	
Swallowing difficulty		Eye involvement	
Recurrent chest infections			

Drugs the patients is on: …… Tiredness after exertion: ……

Muscle cramps after exertion: ……

Genetic history

Consanguinity: …….. Other affected family members: ……

Pedigree included: ……

……

(Name and relationship of other affected family members - include clinical and biopsy details if possible).

Investigations

ESR: …… CRP: …… CK levels: …… CK: Normal/ Upper limits of lab: ……..

Result of EMG: ……

Result of NCS: ……

Result of brain imaging: ……

Any other information: ……

Clinical diagnosis or differential diagnosis: ……

D. Retinoblastoma histopathology requisition form: Part III

Note:

- Part I, II and IV should be the same as for histopathology requisition form (Annexure III A)
- Replace header "Histopathology requisition form" with “Retinoblastoma histopathology requisition form”

Part III.
Type of specimen
……
Site / side (if relevant):
……:……
Surgical procedure: Eyeball enucleation/ Orbital exenteration
……
Clinical history and examination findings:
……:……
……
……

Retinoblastoma diagnosis: Unilateral or bilateral ……
Age at diagnosis: ……(in months, if < 3 years)
Treatment prior to surgery:
Chemotherapy/ Radiotherapy/ Chemoradiation: ……

Family history of retinoblastoma: ……
If yes, relationship to the patient: ……

Clinical staging at surgery…… Extra-ocular spread noted at surgery……
Blood sample of the patient is submitted for DNA testing: Yes/ No
Test requested: ……

Has anyone in the family had DNA testing for retinoblastoma?……
If yes, name of the person and relationship ……

Previous diagnostic data: Ocular or extra-ocular

	Lab ref. No.	Institution performed	Diagnosis
Histopathology			

E. Immunohistochemistry requisition form: Part III

Note:

- Part I, II and IV should be the same as for histopathology requisition form (Annexure III A)
- Replace header "Histopathology requisition form" with “Immunohistochemistry requisition form”

Part III.

Type of specimen and site / (side if relevant):

……

Surgical / biopsy procedure: Tru-cut biopsy / excision / resection / other

……

Material submitted for IHC: (Please tick)

Tissue in fixative: ☐

Paraffin block/s: ☐

Number of blocks ……

Block reference number/s……

Unstained slides:

Number of slides……

Slide reference number/s……

Accompanying H & E slide: ☐

Type of fixative used:

☐ 10% Neutral buffered formalin

☐ Other (specify)……

Cold ischaemia time: ……

Duration of tissue in fixative……

Referring hospital……

Types of antigens/ immune markers requested:

1. …… 2. ……

3. …… 4. ……

5. …… 6. ……

Relevant clinical history:

……

……

Relevant radiological findings / investigation results:

……

……

Pre. Op. Chemotherapy given: Yes/ No/ NA Radiotherapy given: Yes /No/ NA

Previous diagnostic data of same surgical/ biopsy specimen

		Lab ref. No.	Institution performed	Diagnosis/ Differential diagnosis/ IHC markers positivity	H & E and IHC slides accompany the wax tissue block (Yes/No) If “No”, the reason
Histopathology					
Immune markers if performed					

Diagnostic data of previous biopsy procedures

	Lab ref. No.	Institution performed	Diagnosis
Histopathology			
Cytology/ FNA diagnosis			
Immunohistochemistry			

F. Immunofluorescence requisition form: Part III

Note:

- Part I, II and IV should be the same as for histopathology requisition form (Annexure III A)
- Replace header "Histopathology requisition form" with “Immunofluorescence requisition form”

Part III.

Type of specimen and site /(side if relevant):
……:……
Surgical / biopsy procedure: ……

Material submitted for immunofluoresence: Fresh tissue/ Snap frozen tissue/ Tissue in transport media/ Other ……

Types of immune markers/ complement components / other markers requested:

1. …… 2. ……
3. …… 4. ……
5. …… 6. ……

Clinical diagnosis/ Differential diagnosis:
……

Referring hospital: ……

Hospitals/ institutions where biopsies sent for other investigations: (Indicate results if already available)

Histopathology ……
Electron microscopy ……

Relevant clinical history:
……
……
Relevant radiological findings / investigation results:
……
……
Diagnostic data of previous biopsy procedures

	Lab ref. No.	Institution performed	Diagnosis
Histopathology			
Immunofluorescence			

G. Electron microscopy requisition form: Part III

Note:

- Part I, II and IV should be the same as for histopathology requisition form (Annexure III A)
- Replace header "Histopathology requisition form" with “Electron microscopy requisition form”

Part III.
Type of specimen and site /(side if relevant):
…….:……
Surgical / biopsy procedure: ……
Material submitted for electrone microscopy: Tissue in fixative/ Paraffin blocks/ slides/ Other
……

Type of fixative used: 2.5% glutaraldehyde / other ……
Specific structures or features to be identified:
1. …… 2. ……
3. …… …… 4. ……

Clinical diagnosis / Differential diagnosis:……

Referring hospital: ……

Hospitals/ institutions where biopsy sent for other investigations: (Indicate results if already available)
Histopathology ……
Immunofluorescence …… ……

Relevant clinical history:
……
……
Relevant radiological findings / investigation results:
……
……
Diagnostic data of previous biopsy procedures:

	Lab ref. No.	Institution performed	Diagnosis
Histopathology			
Other			

H. Flow cytometry requisition form: Part III

Note:

- Part I, II and IV should be the same as for histopathology requisition form (Annexure III A)
- Replace header "Histopathology requisition form" with “Flow cytometry requisition form”

Part III.
Type of specimen and site /(side if relevant):
……
Surgical / biopsy procedure:
……
Material submitted for flow cytometry:
Fresh tissue / Fine needle aspirates / Body fluids: ……/ Other ……

Types of antibody panels requetsed: Screening panel / Lymphoproliferative panel / Immunodeficiency panel/ other
……
……

Clinical diagnosis/ Differential diagnosis. ……

Referring hospital: ……
Hospitals/ institutions where biopsy sent for other investigations: (Indicate results if already available)
Histopathology ……
Immunohistochemistry ……

Relevant clinical history:
……
……
Relevant radiological findings / investigation results:
……
……

Diagnostic data of previous biopsy procedures:

	Lab ref. No.	Institution performed	Diagnosis
Histopathology			
Other			

I. Molecular testing requisition form: Part III

Note:

- Part I, II and IV should be the same as for histopathology requisition form (Annexure III A)
- Replace header "Histopathology requisition form" with “Molecular testing requisition form”

Part III.

Type of specimen and site /(side if relevant):
……..:……

Surgical / biopsy procedure:
……

Types of molecular tests requested: PCR/ RTPCR/ FISH/ CISH/ NGS/ Karyotyping/
Other…… ……

Type of abnormality 1. ……

2. ……

Reason for testing: Diagnosis/ Confirmation/ Carrier/ Familial mutation/
other……

Previously knows molecular abnormalities
……

Clinical diagnosis/ Differential diagnosis.
……

Hospitals/ institutions where biopsy sent for other investigations: (Indicate results if already available)
Histopathology ……
Immunohistochemistry……
Electron microscopy……

Relevant clinical history: ……
……
……

Relevant radiological findings / investigation results:
……
……

Diagnostic data of previous biopsy procedures:

	Lab ref. No.	Institution performed	Diagnosis
Histopathology			
Other			