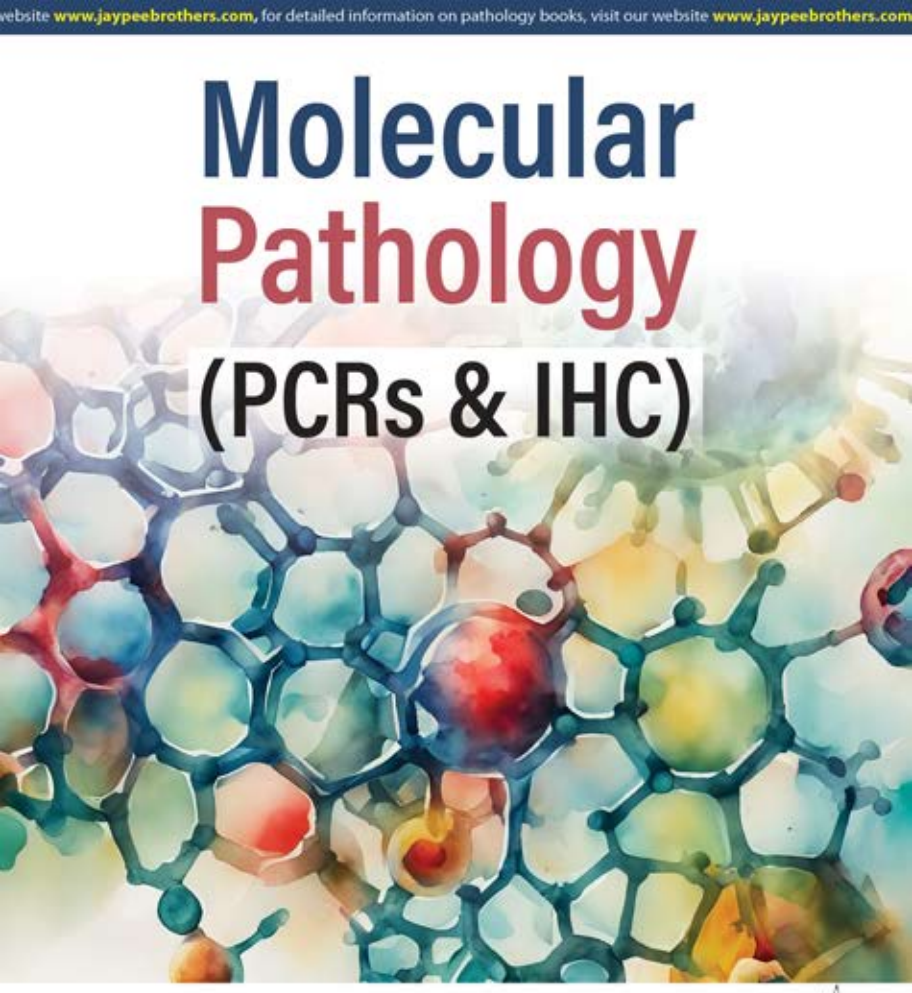
Molecular Pathology

(PCRs & IHC)



Ramnik Sood
Deepak G Tripathi

JAYPEE

Contents

6 CHAPTER Histopathology (Prelude to Immunohistochemistry)

INTRODUCTION

This book comprises of molecular pathology technology, techniques, and protocols mainly PCRS and immunohistochemistry. Imunohistochemistry comprises of technical preparation guide for specimen removed out of body during surgery, aspirates, body fluids. Macroscopic and microscopic examination (*Histopathology-by Greek definition histos–tissue; pathos–suffering; logia–study of*) of the specimen enables understanding of changes occurred in the disease and diagnose, on basis of which the medical treatment starts. Tissue has to undergo various stages of processing for preparation to a microscopic slide for evaluation (*histotechnology*). This involves tissue processing, embedding, microtomy, staining and mounting.

Histotechnician/histotechnologist are trained personnel with excellent mechanical skills, having expertise on equipments and knowledge of anatomy, science of tissue processing and various chemical reaction in staining protocol. They play a very important role in preparation of slide for microscopic evaluation. Preparation of slides for frozen sections, routine H&E stain, special stains like PAS, rectic, masson and many more, Immunohistochemistry. Molecular tests have become important ancillary tests identifying type of tumors for diagnosis, prognosis, and therapeutic aspect.

The sensitivity of the technique has improved over a period of years from immunofluorescence labeled antibody system to labeled polymer-based systems and also overcome the effects of formalin fixation for identifying epitopes. With the introduction of digital pathology, it is essential to monitor quality of slide preparation.

Understanding Basics of Tissue Processing (Fig. 6.1)

Action for removal of water.

Water is found in tissue in two forms, free and bound water. The bound water molecule is an integral part of macromolecules of the cell. Proteins, lipids, carbohydrates, and nucleic acids are major macromolecules of the cell having their own specific function within living cells. Correct dehydration defines removal of free water, leaving bound water intact.

- **Fixation and gross examination:** Fixation is the process of preserving tissue from decomposing and are placed in a solution called fixative. All fixative solutions are aqueous in nature.
- Gross examination is the macroscopic observation and dissection of tissue for purpose of diagnosis.

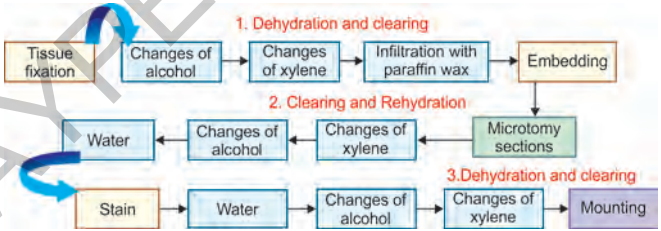


Fig. 6.1: Basics of tissue processing cycle—dehydration-rehydration-dehydration.

- **Processing and embedding—step 1:** Paraffin wax is universally used for embedding tissue section. Most of the fixatives used for tissue preservation are aqueous solutions. Water is removed from aqueous fixed tissue sections using changes of alcohol and alcohol is removed using xylene making place for paraffin wax to infiltrate. This process hardens tissue and helps in section cutting.
- **Microtomy:** Microtomy is an art by means of which tissue is cut into thin sections. A microtome is an instrument used for cutting sections.
- **Staining and mounting—step 2:** Staining procedure imparts color to tissue components which can be visualized under microscope. Paraffin wax is removed using xylene and sections are brought to water treating with alcohol.
- **Mounting:** After staining is complete tissue sections dehydrated, cleared, and are mounted using mounting medium DPX—**step 3**.

TISSUE FIXATION AND GROSS EXAMINATION

Histopathology Specimen

Specimens received for histopathology examination are mainly collected by surgeons in the operation theatre. They are removed out of the body using various techniques at the time of surgery.

Specimens are received for examination in form of:

- **Incision biopsy:** Only portion of the lesion is sampled from suspected area usually sent for confirmation by frozen section.
- **Excision biopsy:** The entire lesion is removed usually with the rim of normal tissue. Therefore, this serves both diagnostic and therapeutic purpose.

- **Punch biopsy:** Small round tissue is removed using punch of desired diameter. Useful in lesions from skin, vulva, cervix.
- **Cone biopsy:** Cone shaped piece of lesion is removed from cervix.
- **Wedge biopsy:** Triangle shaped tissue lesion with small amount of normal tissue around it
- Biopsies are collected using Tru-cut needle, endoscope, CT/MRI, trephine needle.
- **Surgical resection:** Lumpectomy specimens, uterus, lymph nodes, gallbladder, Appendix
- **Complete resection:** Breast, kidney, intestine, larynx
- Amputations/limbs
- Slides and blocks for second opinion or ancillary tests

Accessioning of Specimen

Every specimen must be submitted with a complete requisition form **(Fig. 6.2)**.

Requisition must include:

- **Patient demographic details:** Name/Age/Sex
- OPD/IPD No./Bed. No./Ward
- Specimen site/Nature of operation
- Clinical notes by clinician/surgeon
- **Details of investigations:** Blood reports, CT/MRI/USG findings, X-rays
- Information of previous surgery, biopsy, cytology, PAP smear reports for correlation and documentation
- **Nature of specimen sent:** Tissue/slides/paraffin blocks/smears
- Details of tests billed

Histopathology requisition form								
Patient name:	Date:/___/							
Age:_____ Years_____Sex:_____ Bed no: _____/Ward:- _____OPD/IPD no.					Histopathology previous no.			
To be filled by the doctor								
Nature of operation/procedure done:								
Material sent:								
Brief history:								
Relevant past history:								
Previous surgeries:								
Investigations:								
Treatment resolved [phase a tick]:								
Chemotherapy		Radiotheraphy			Any other			
To be filled by the i ar only								
Nature of material received: Second option: Number of blocks:_____Number of sliders/smears___-								
Blocks/ slides/smears no:-_____								
Forum-section	Small specimen	Medium specimen	Large specimen	Large R complex	FNAC	PAP smears	IHC	FISH/ PCR

Fig. 6.2: Requisition form.

Specimen Identification and Receiving

This step involves receiving of specimen in the department for processing and reporting.

Specimens are received in the department from OT or OPD in the hospital and are also received in standalone/corporate laboratories by walk ins and nursing homes.

On receiving specimen in the department verify patient identification and information on the requisition form and the specimen container is matching with each other along with the tally of number of containers received. Also, observe and document the condition in which the specimen is received, fresh and unfixed, fixed inadequate or adequate formalin or autolyzed.

Labeling of Specimens

This is very important that specimens are properly accessioned and signed with a specific identification number as soon as specimen is received. This number should remain throughout entire processing steps and then in record keeping. This number includes organization identity/serial number/year – ABC/123/22. This number can be defined manually or also can be generated from software. Labeling should be done very carefully. Printed labels will take care of errors of manual labeling. Use lead pencil to label cassettes and slides as this number will remain during processing and staining. Also, use of Tissue Tek/Vogel system can be done which allows labeling of cassettes and slides. These numbers are retained permanently during processing and storage. Laboratory information system (LIS), automated labeling system will increase efficiency of laboratory by reducing labeling errors. Barcode labels are scanned for confirming identity at every step.

Fig. 6.3: Bar code.

Maintain a logbook for specimens received in the department. This register will include: Date received, Department serial number, Patient ID (in case of Hospital patient), Patient demography, Referring Doctor, Specimen details, and Diagnosis.

Histopathology Register

Date	HP no.	Patient ID	Patient name	Age/ sex	Ref. by	Specimen	Diagnosis

Criteria for Specimen Rejection

As per NABL112 criteria *histopathology specimens should not be rejected on the ground of poor specimen integrity.*

- Specimen with no label/incorrect label:
  - Lack of minimum information on requisition
  - Specimen site
  - Date and time of collection
  - Patient's demographic data
  - Referring doctor's name
- Container label if does not match with requisition form
- **Incomplete requisition form:** Missing information of/incorrect specimen site details, patient's demographic details
- Improperly packed specimen/fixative leaked on requisition form
- **Specimen improperly fixed:** On receipt of specimen change fixative; add appropriate quantity of fixative into specimen bottle; document the same on the requisition form.
- **Slides broken beyond repair on receipt:** Inform to the source from where the sample is received; ask for the repeat sample if possible; document in the specimen discrepancy log.
- **Lost specimens:** Rare occasion but needs to be documented in criteria for rejection.

Rejected specimens are to be kept on hold and all possible efforts must be made to get the correct information from source as per NABL criteria.

Details including reason for rejection is entered in a specially maintained rejection logbook.

Gross Examination of Specimen

Gross examination is visual observation/description of specimen/dissection for submitting representative sections for processing. Primary responsibility of grossing is of pathologist. Histotechnician is responsible for fixation of specimens, arranging specimens, assisting pathologist during grossing and processing of tissue. Hence, it is important to understand grossing protocol.

Grossing Room

- **Safe grossing:** Use apparel (protective gear and clothing) which includes apron, closed shoes, mask, safety goggles and good quality snugly fitting gloves.
- Grossing room needs to be well equipped with proper ventilation, exhaust fan, proprietary grossing station, grossing board, weigh balance.
- **Grossing instruments (Figs. 6.3 and 6.4)**:
- Straight and curved scissors (small and large, with pointed and blunt ends)
- Scalpel blades with handles
- Forceps (fine and toothed)
- Metal probe, saw
- A ruler
- Paint brush, ink and stainless-steel bowls of varying sizes
- Knives of various lengths

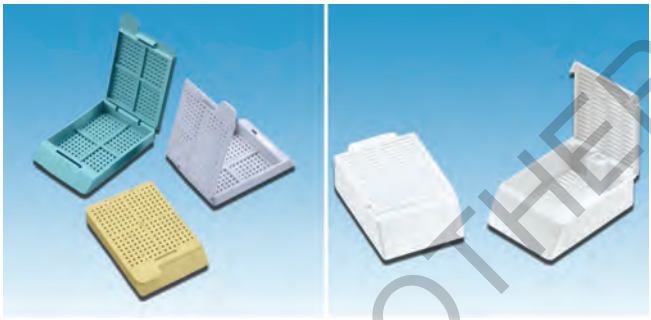


Fig. 6.3: Cassettes for tissue processing.

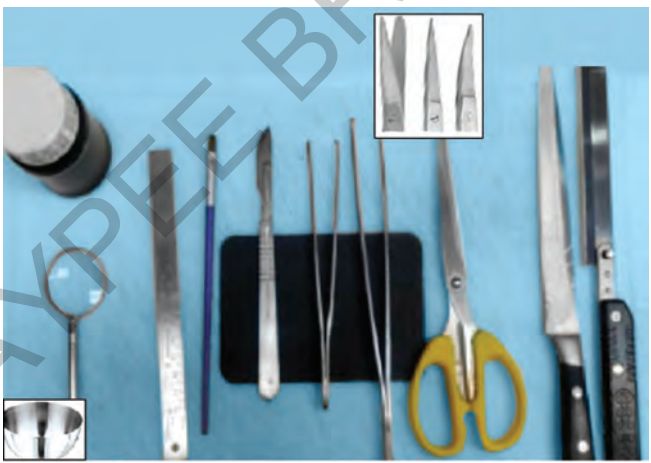


Fig. 6.4: Grossing cutting tools.

- All these instruments are thoroughly cleaned before, during and after grossing and dried to prevent rusting.
- Area for specimen storage

Grossing Station

Commercially available tailor-made modular work station **(Fig. 6.5)** with ergonomic design is made up of steel.

Fig. 6.5: Work station.

Salient Features

- Cutting board
- Rinsing facility on work surface
- While working removal of accumulated formalin and tissue waste below perforated sheet through fully programmed spray system.
- Waste grinder to avoid blockage of drainage system.
- Emergency pullout eye washer
- Backdraft extraction of formalin fumes with maximum efficiency
- Air filtration system to minimize hazardous fumes in the laboratory.

Method

- Arrange all specimens according to the prescriptions. The specimen along with the requisitions are once again to be verified before the grossing begins.
- Open the container of specimen in serial order with gloves in hand.
- Take out specimen, and observe for the gross appearance.
- Take the dimensions with a measuring scale. (a) Weight (b) Size (length, breadth, width) (c) Shape (d) Color (e) Visible abnormality (f) Internal abnormality (if present try to detect by an appropriate cut). Bits are submitted from both pathological and normal looking areas.
- Write the gross description on the receipt.
- Small specimens are fixed after noting all the gross features namely size, shape, color, consistency, hemorrhage, necrosis, etc. In small specimens, inking is of help during embedding.
- Cut specimens and take small sections from representative area. Put smaller bits in appropriately labelled jar containing fixative.

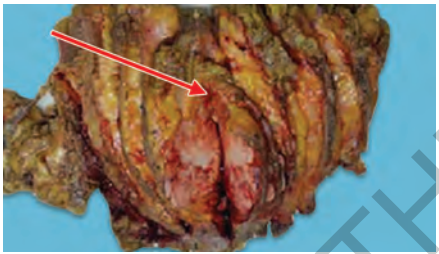


Fig. 6.6: Breast tumor cut in slices for better fixation.

- In the case of biopsy or small specimens submit whole specimen for processing.
- Big resection specimens are preliminarily examined for salient features and the external surface is painted with marking ink. The specimen is then cut opened or bisected or sliced **(Fig. 6.6)** in a regular manner taking care to avoid distortion and facilitate formalin penetration by keeping for overnight fixation. These specimens are grossed the next day by pathologist **(Fig. 6.7)**.
- After grossing tissues are filled in cassettes along with appropriate identification number for further processing.
- Tiny tissue bits are wrapped in filter paper and eosin is added if too tiny to recognize during cutting.
- Entry of tissue bits selected for each specimen are entered in the gross entry book.
- Bony specimens are cut using band saw and are subjected for decalcification.
- Digital images whole specimen is captured before inking and dissecting specimen and also cut surface to demonstrate lesion.

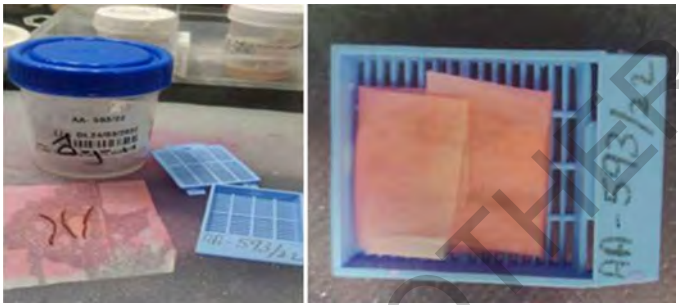


Fig. 6.7: Handling core biopsy.

The date of grossing and the name of the pathologist performing grossing is entered in the requisition. Also, document who's picking sections and number of cassettes.

A key for specimen is summary of noting number of cassettes/blocks for the specimen for, e.g., margins of resection, deepest penetration of tumor, tumor proper breast quadrants, lymph node levels.

The gross description of the specimens is written in detail. Ancillary techniques if identified must be noted, e.g., fungus stain or stains for kidney biopsy, liver biopsy, bone marrow.

Tissue loading work sheet

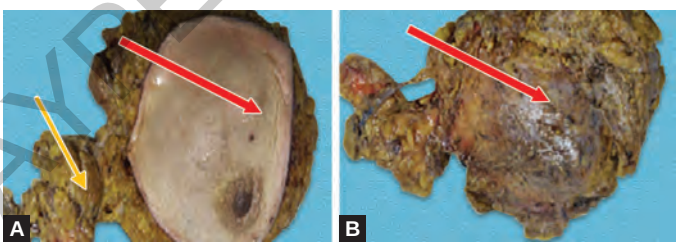
Date:						
Sr. no.	Specimen ID	Key for specimen blocks	Total no. of blocks	More sections	Sign	Remarks

Orientation of Specimen

- **Small specimens:** Endoscopic biopsies are submitted by placing the base of the biopsy on card paper.
- For wide excision specimens margins can be identified with sutures of different length and color. Specimens like breast lumpectomies and wide excisions can be oriented by means of sutures, ink, or clips. Specimens also signify orientation with their anatomical structure; For, e.g., axillary tail in mastectomy specimen **(Figs. 6.8A and B)**.
- Specimens received with staples and suture are neatly resected with scissors as close to the staple line as possible and subsequently the tissue next to it is submitted as margin taking care that the submitted tissue is free of staples or sutures.
- Stents and guide wire are not handled with gloves but are removed with toothed forceps as they have sharp ends.

Gross description involves,

- Type of surgical procedure for, e.g., uterus with bilateral adnexa, radical mastectomy



Figs. 6.8A and B: Breast tissue with axillary tail (Yellow arrow) + Tumor (Red arrow).

Molecular Pathology (PCRs & IHC)

Although Molecular Pathology has taken off since the early 1990s, a compilation of history, standard operating procedures (SOPs), hardware, and consumables associated with the two main branches of Molecular Pathology has been missing terribly. Within the pages of this book, you will find easily digestible basics and details of PCRs, IHCs, IFs, and ISHs.

The book covers:

- Molecular pathology: Introduction, past, present, and future
- PCRs-Technology, techniques, history, and challenges
- Polymerase chain reaction in clinical diagnosis (recapitulation in brief)
- Practical aspects/actual working on systems
- Commercially available—kits of an open system
- Histopathology (prelude to immunohistochemistry)
- Immunohistochemistry
- Annexures

Ramnik Sood MD (Pathology) Gold Medalist is a renowned and eminent medical author, especially when one talks about laboratory medicine and its allied branches. Having authored scores of books, he has written for technologists, undergraduate and postgraduate medical students, and even individuals pursuing PhD programs. Just one click/search on Google will reveal the vastness of his work that he has already accomplished. He has also authored over 150 issues of techno-commercial journals for medical institutions and in vitro diagnostics (IVD) industry. As he has been processing and reporting PCRs and IHCs for many years and is cognizant of the problems inherently faced, he is just the right person to write on the two pillars of molecular pathology.

Deepak G Tripathi is currently the Group President of Tulip Diagnostics (P) Ltd., India, an IVD manufacturer, with its headquarters in Goa, India. He has over 35 years of experience in the IVD industry, R&D, Product Development, and Sales and Marketing of IVD products globally. He was the President of the Association of Diagnostic Manufacturers of India for over 9 years and has represented the Indian IVD industry in many international fora. A chemistry graduate with an MBA, he also has publications in the Indian medical journals as a co-author. His contribution to this book brings about a customer-oriented perspective to the techniques and technologies.

Printed in India

Available at all medical bookstores
or buy online at www.jaypeebrothers.com

JAYPEE BROTHERS
Medical Publishers (P) Ltd.
EMCA House, 23/23-B, Ansari Road,
Daryaganj, New Delhi - 110 002, INDIA
www.jaypeebrothers.com

Join us on facebook.com/JaypeeMedicalPublishers

Shelving Recommendation
PATHOLOGY