



REPUBLIC OF RWANDA
RWANDA MILITARY HOSPITAL



Website: www.rwandamilitaryhospital.rw

P.O. Box: 3377 Kigali, Tel: (+250)252586420, Hotline: 4060

E-mail: info@rwandamilitaryhospital.rw

TISSUE HANDLING IN SURGICAL PATHOLOGY

Presented by Sylvie Umuganwa, Resident-Pathology

Supervisor: Dr. Thierry Zawadi Muvunyi

Outline

- Objectives
- Introduction
- Challenges in the Histopathology lab at RMH
- Tissue specimen collection, fixation, labelling and transport
- Tissue rejection
- TAT
- Consequences of improper handling of specimen
- Recommendations
- References

Objectives

- To understand the importance of proper tissue handling
- To emphasize the importance and usefulness of a complete requisition form

Introduction

- Pathology is a branch of medicine that deals with the study and diagnosis of disease through the examination of surgically removed organs, tissues(biopsies) , bodily fluids and sometimes the whole body (autopsy)
- Two branches: Anatomical Pathology vs Clinical Pathology

Introduction cont'd

- Anatomical Pathology is concerned with the diagnosis of disease based on the gross and microscopic examination of cells and tissues, cytopathology and histopathology respectively.
- **Cytopathology**: main sources of specimen;
 - Exfoliative Cytology (e.g. Effusions, Urine, CSF)
 - Abrasive Cytology: Pap smears
 - Fine Needle Aspiration biopsy: thyroid
 - Imprint/touch: nipple discharge

Introduction cont'd

➤ **Histopathology**

- The main sources of tissue/specimen are biopsy, surgery and autopsy procedures, they include:
 - Excisional biopsy
 - Incisional biopsy
 - Punch biopsy
 - Curettage biopsy
 - Bone: Bone marrow core biopsy
 - Core needle biopsy
 - Autopsy

Introduction cont'd: IHC and special stains

- Immunohistochemistry (IHC) combines histological, immunological and biochemical techniques for the identification of specific tissue components.
- Special stains:
 - Used to stain extracellular products e.g amyloid- congo red
 - Used to demonstrate cellular products for confirmation of the type of malignant cells e.g mucin- PAS,
 - Used to demonstrate infectious organisms e.g Mycobacterial organisms- GMS

Challenges in the Histopathology lab at RMH

- There is delay in sending samples from the operating theatre
- When specimens are sent to the lab, at times they are in inappropriate containers, sometimes the formalin is not enough depending on the specimen size
- The request form is not always well filled
- Once the reports are written in open clinic, the clinicians sometimes do not see them
- The lab often runs out of reagents

Specimen collection, fixation,
labelling, and transport

Preparation for biopsy

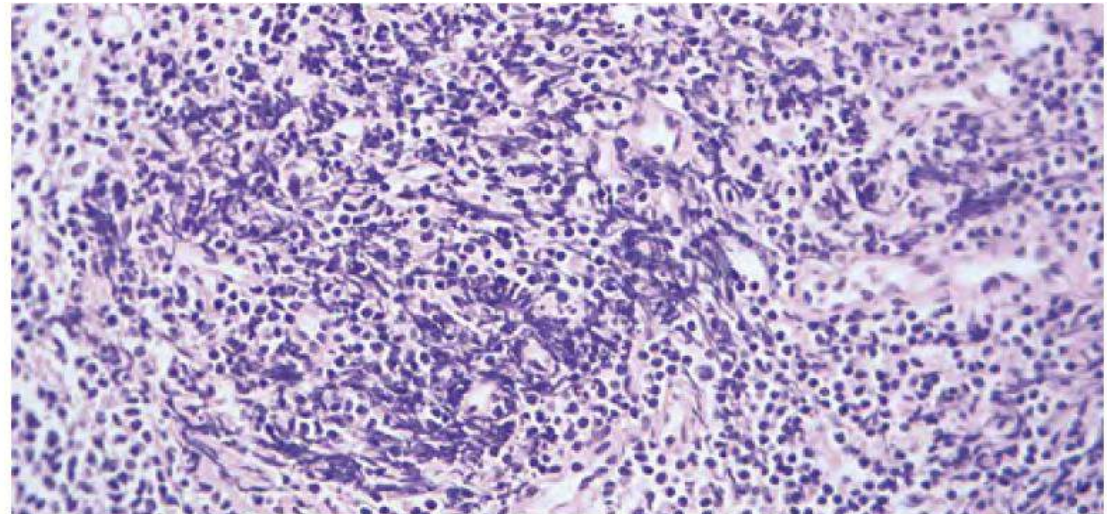
- The surgeon should prepare all the necessities related to the type of biopsy.
 - Is it a small, medium, large biopsy?
 - Is it an incision, total excision, etc...?
 - Is it diagnostic, staging, therapeutic,..?
 - What will I do with the specimen afterwards?
- How will it help in regard to the continuity of patient care?

Specimen collection and transport

- Collection and transport of specimens 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.
- Main steps:
 - Putting the specimen in an appropriate container
 - Specimen is immersed in an appropriate type and amount of fixative
 - Accurate identification and labelling of the specimen
 - A complete request form

Avoid mechanical trauma

- Remove tissue gently (during surgery,...)
- Fragile specimens, make sure they remain intact.
- Avoid tissue crushing/ tearing
- If crushed/torn before fixation, there will be crushing artefact in the sections.



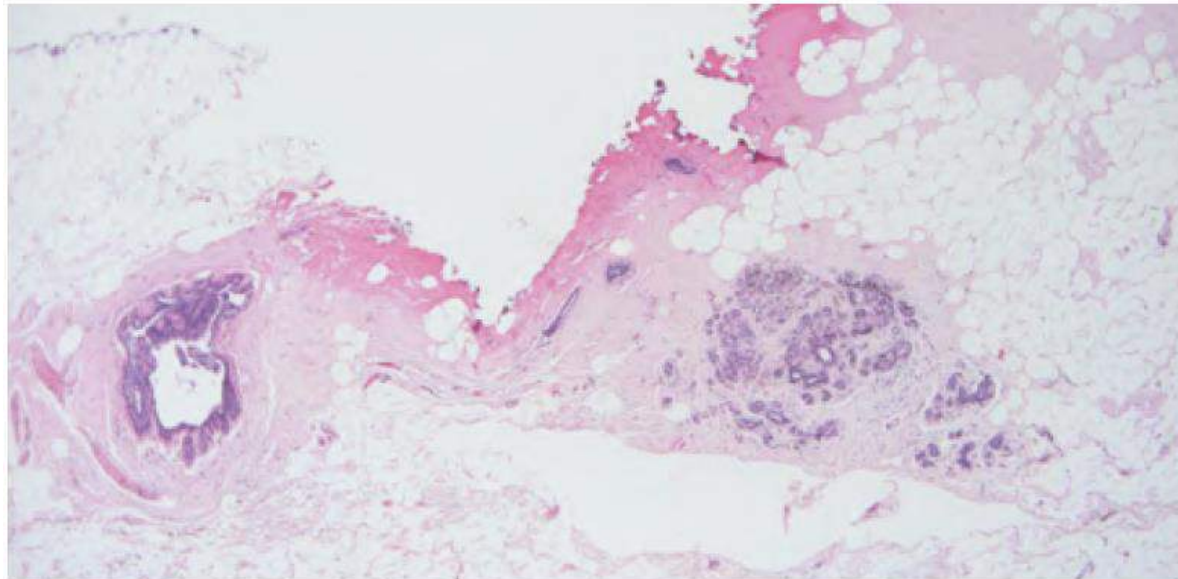
Avoid drying of specimen

- Don't allow specimen to dry out before fixation
- If immediate fixation is not available, the tissue can be wrapped in a gauze moistened with normal saline during the time of waiting



Avoid heat damage

- Avoid any unnecessary local heat damage to the specimen, fresh tissue is susceptible to heat.
- Some damage by cautery is unavoidable

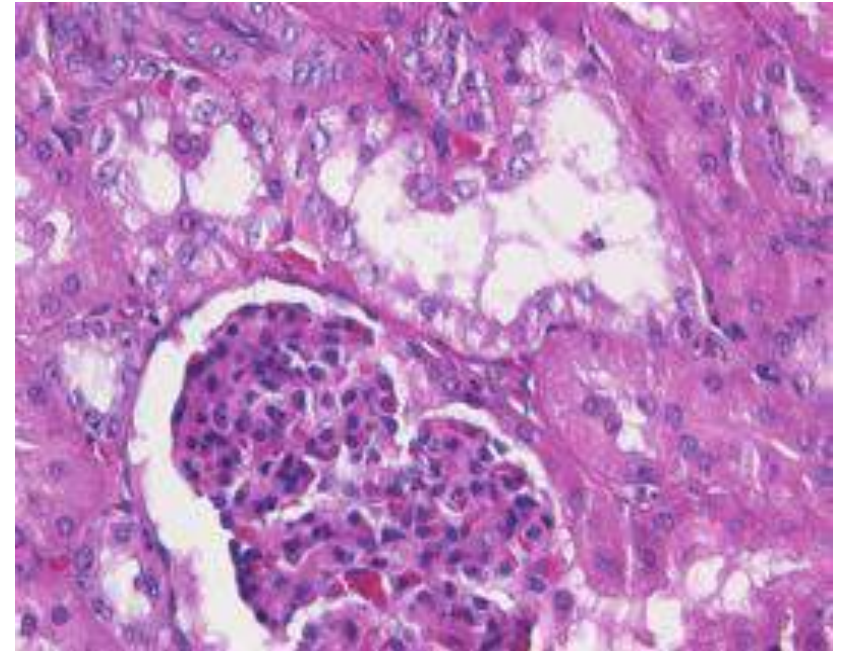
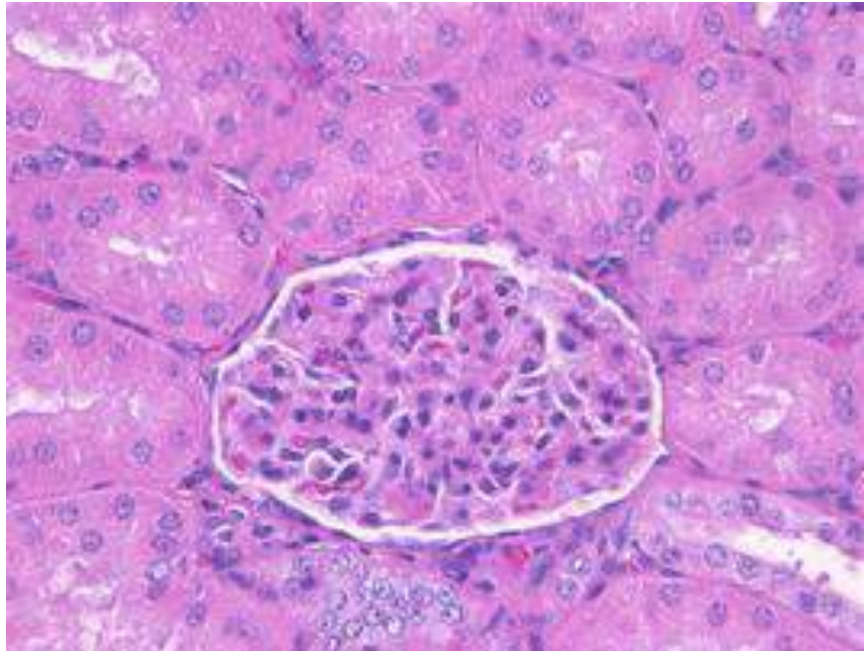


Specimen collection and transport cont'd

- When a tissue/specimen is obtained after a procedure, it should be immediately placed in a fixative medium.
- Formaldehyde is the most common fixative used. (10% formalin is most commonly used)
- Role of fixation:
 - To prevent or arrest the degenerative processes, hence preserve cells and tissue components in a “life-like state”
 - To limit infection from fresh tissue specimen
- Improper fixation may lead to irreversible tissue damage (autolysis)

Ensure prompt fixation

- Fixation is always done immediately after the specimen is removed.
- If it is not going to be remain unfixed for some time, it can be refrigerated at 4°C.



Sufficient amount of fixative and proper container

- An adequate volume of fixative is a ratio of 20:1
- Do not squash a specimen into a small container; an appropriate container should be wide mouthed with a well-fitting lid



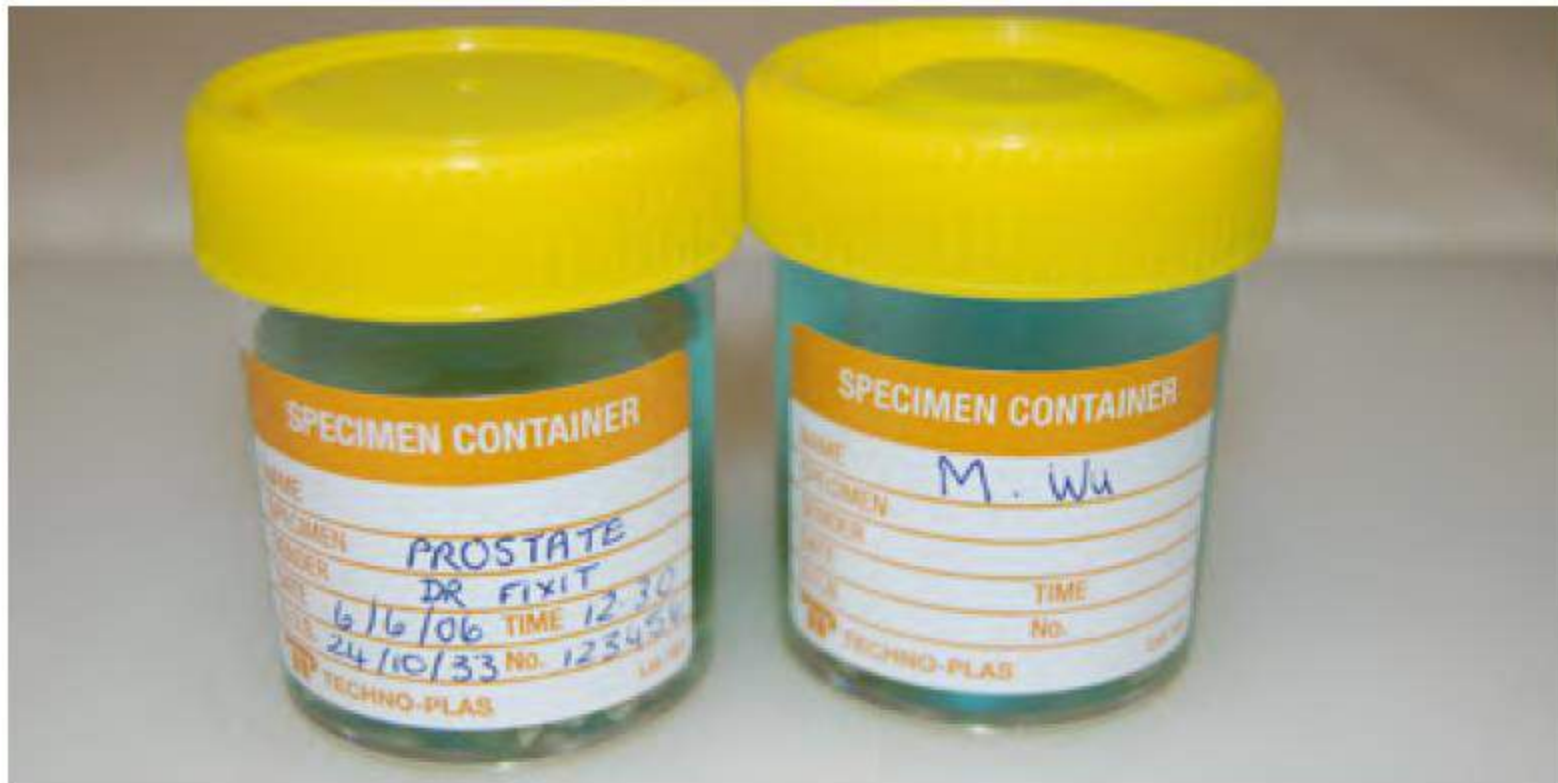
This container is too small for the mass of tissue it contains. There is insufficient fixative present and the specimen may well have been distorted as it was pushed into the container.



- Appropriate container
- Little amount of fixative

Proper labelling of specimen

- Each specimen should be labelled properly and with the correct personal identifiers



Large specimen

- Large specimens should be rapidly transported to the lab to allow grossing/serial slicing for proper fixation.
- When large specimens are left in fixative for an extended time prior to grossing, the center of the specimen may remain unfixed and the tissue can become markedly distorted.



The request form

Request form

- Patient information:

- Name
- Age
- Sex
- Hospital ID number
- Address/ telephone number
- Relevant clinical information

- Specimen information:

- Date of specimen collection/surgery
- Specimen site and laterality if applicable.
- Note if any previous biopsies done at that site.
- Note if there are correlating pap smears, FNA or cultures.
- Identify suture markers if any.
- Document if assessment of margins/adequacy of excision is important.
- Any special requests should be noted on the request form (e.g. "Please urgent").

Health care provider information

- Full name
- Address (name of the clinic or hospital) of the clinician
- His/her telephone number

Rejection of specimen

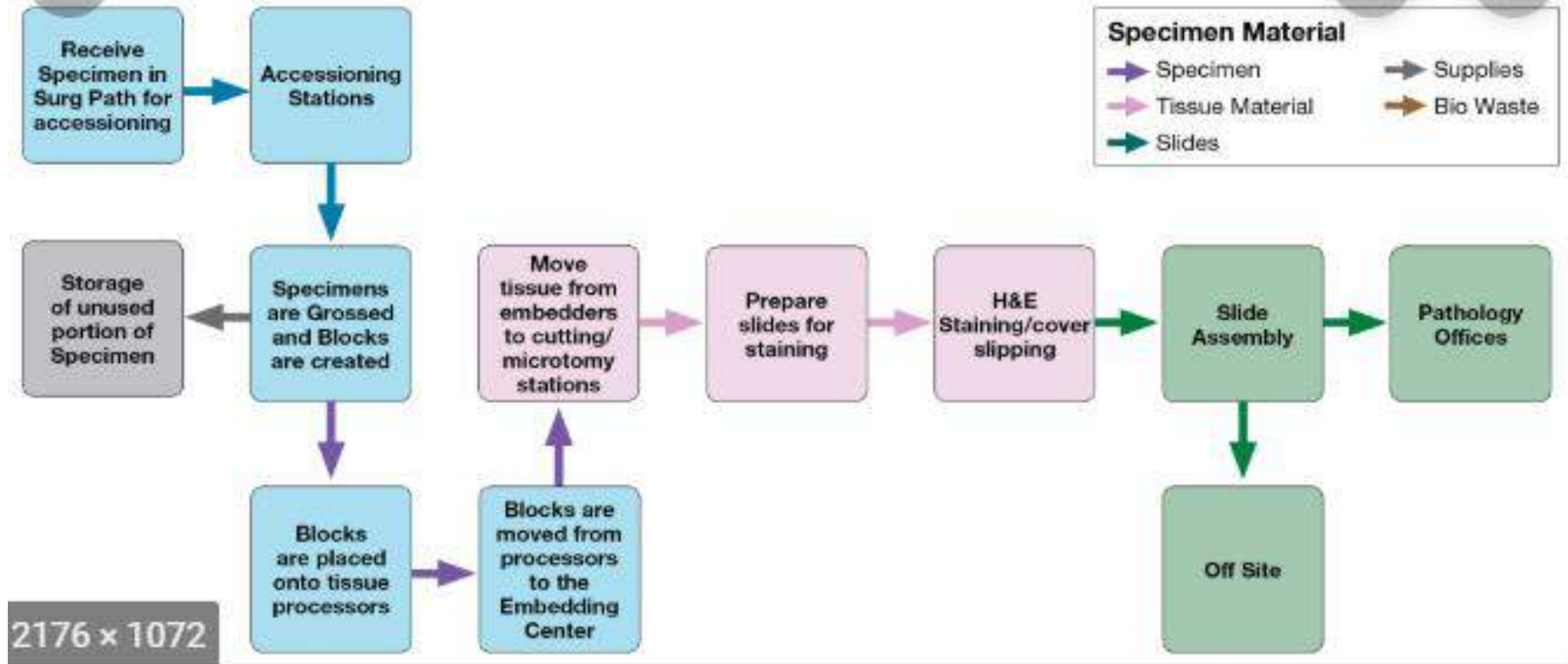
Criteria for rejection of a specimen

- Mismatch of information on the label and the request form
- Unlabelled specimen
- Specimen with no request form/inappropriately filled
- Inappropriate transport medium
- Leakage of container

Turn around time (TAT)

- Turn around time (TAT)

Histology Specimen Workflow Example



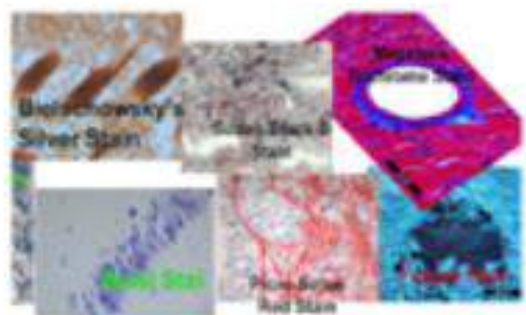
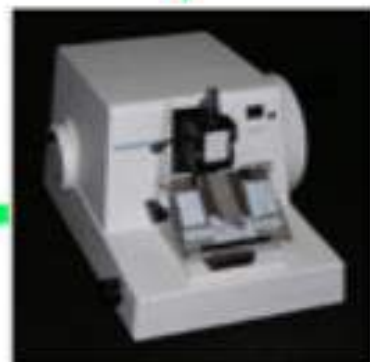
Tissue Fixation



Tissue Processing



Tissue Embedding



Staining Images



Slide Staining

Tissue Sectioning

Consequences of improper handling of specimen

- Long TATs,
- Unsatisfactory or inaccurate reports,
- Inability of the pathologist to render a diagnosis,
- Negative clinical implications for patients,
- Unnecessary treatment with its attendant risk,
- Waste of resources, repeated biopsies,
- And anxiety among the population about the health-care delivery system as a whole.

Recommendations

- Better communication between the surgeon/clinician and the pathologist
- The use of surgical instruments driven by heat should be avoided or limited whenever possible to avoid thermal injury.
- The use of surgical instruments should be avoided or limited as much as possible while handling small specimen to avoid crushing artifacts.
- Request forms should be signed on and stamped by the person who did the surgery
- Specimens should be submitted to the lab immediately after surgery
- Well filled and complete request form

References

- Pathology leaders, 101 steps to better Histology (2016)
- JD Bancroft and L Gamble, Theory and practice of Histological techniques (2009).
- Pranab Dey, Diagnostic Cytology, Jaypee Brothers Medical Publishers, (2014)
- Isha Prematilleke, Dulani Beneragama, 'The Importance of Correct Specimen Handling and Transport in Histopathology', www.slkog.lk

THANK YOU

Any questions?

Any comments?