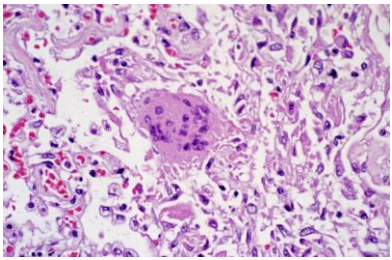
مدينة الملك فهد الطبية
King Fahad Medical City

Histopathology Lab

What is Histopathology?

It is the diagnosis and study of disease of the tissues; which involves examining the tissues under a microscope.



Who is a Histopathology technologist?

Certified technologists have the necessary specialized training to process and stain various tissue samples.

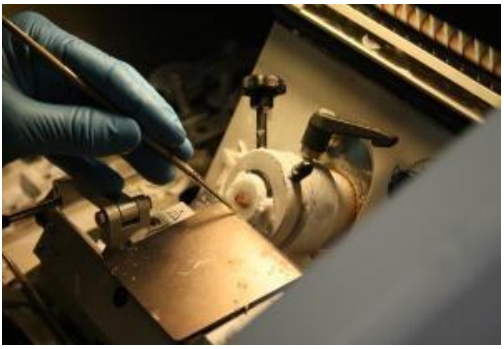
Who is a Histopathologists?

Certified physicians are responsible for interpreting the histological slides and making the diagnosis.

Types of Histopathology Specimens:

- **Fresh specimen for Frozen section:**

- It is a mean for urgent diagnosis to allow the surgeon to make a therapeutic decision during the operation while the patient is under anesthesia.
- Freezing the tissue hardens it so it can be cut on a special refrigerated microtome (cryostat) and stained with a quick procedure.
- The diagnosis should be rendered within twenty minutes from receiving the specimen.

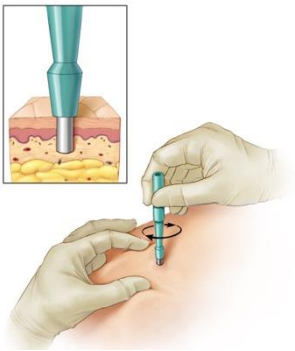


- **Fresh Specimens for special techniques:**

- Sections from the skin and kidney Biopsies are often saved as frozen sections for immunofluorescence studies. The presence (or lack thereof) of certain proteins or protein combinations allows for autoimmune and other disease processes to be diagnosed.
- Freezing the tissue ensures preserving the structures and antigenicity and avoiding exposure to chemical fixatives.
- Muscle biopsies are frozen using liquid nitrogen for studying the enzymes status.
- Specimens from the lymph nodes are also received fresh to take a small part in RPM preservative for flow cytometry study in case of clinical suspicion of lymphoma.

- **Small biopsy:**

This includes endoscopy specimens and core biopsies from solid tumors.



- **large specimens:**

Such as breast, uterus, colon... etc.

- **Outside cases:**

Many cases from other hospitals are also received in the histopathology lab for consultation of the patient's condition.

In addition, and based on the hospital policy, no treatment is offered for a patient based on the outside diagnosis. The department' pathologist should review the referred outside cases prior to the treatment. It consists of a pathology report and blocks or slides.

Steps of preparing slides from the tissue:

Specimen receiving and registration:

- It is the most critical step where trivial error might lead to a disaster. This step should be done with over precautions including; -
- Check both requisition and specimen label for discrepancies.
- Check if the specimen is adequately covered with a fixative.
- Register the specimen and print label.
- Place label on both the specimen's container and the requisition.

Grossing:

- It is describing the status of the received specimen, whether it is fresh or in formalin, the tissue's size, color, consistency, and other characteristics of that tissue.
- The technologist does a gross examination of the small biopsies, placing it inside a filter paper, then in a labeled tissue cassette to be processed.

The large specimens are grossed by pathology resident/ pathologist. and left for overnight fixation.

- The representative sections are submitted in a number of blocks according to the size and type of the specimen.

Fixation:

- It is the most essential step through the process of tissue to arrest autolysis and putrefaction.
- To stabilize the cellular and tissue constituents so that they withstand subsequent processing of tissue.
- It ensures the preservation of tissues as close as possible to a living state.
- The small biopsies should be fixed for 6 hours before processing.

Commonly used fixatives:

- **Formalin:**

It is good general fixative used in 10% natural buffered formalin

Advantages:

- preserves tissue for a long time if the solution is buffered to neutrality.
- preserves proteins and lipids.
- relatively inexpensive.

Disadvantages:

- cause a little shrinkage which is produced when the tissue is subjected to paraffin embedding.
- strong eye, skin and mucous membrane irritant.
- moderately flammable.

- **Glutaraldehyde:**

used for standard electron microscopy.

Advantages:

- less distortion, brittleness, and shrinkage.
- maintains elasticity during manipulation and sectioning.

Disadvantages:

- the comparatively high molecular weight of glutaraldehyde limits its ability to diffuse into thick specimen.
- four carbon chain of glutaraldehyde may mask amine containing peptones, making immunostaining impossible.

Tissue processing:

It is a fully automated process in which the tissue undergoes several stages within a tissue processing machine. This step lasts 16 hours.

- Fixation:

10% neutral buffered formalin.

- Dehydration:

Is the removal of water from tissue, it is usually achieved by replacing the water in the tissue with a dehydrating agent, ethyl alcohol is the re-agent.

- Clearing:

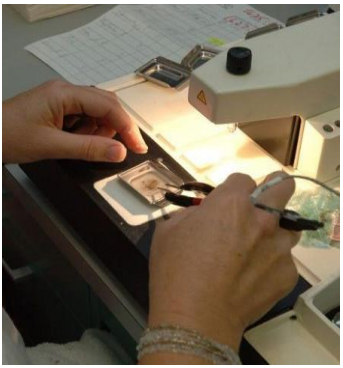
Is a step between dehydration and embedding, it is replacing the dehydrating agent with clearing agent (xylene).

- Wax infiltrating:

Tissue dehydrating and clearing is followed by infiltration with a suitable matrix.

Embedding (blocking out):

Is done by hot liquid media (paraffin wax) which makes tissue solid when cooled down to room temperate. This step is to enable positioning the tissue in the microtome and eases the sectioning.

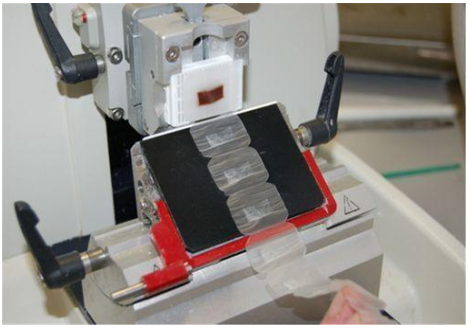


Sectioning:

The tissue must be cut into a section that can be placed on the slide.

Microtome is used for this purpose.

Routine tissue is cut at 3-5 µ, Biopsy tissue is usually cut 2-3 µ.

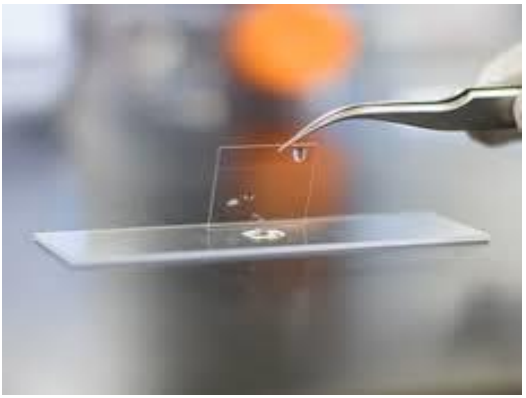


Staining:

The slides are stained with Hematoxylin and eosin to highlights the cells and the nuclear structure.

Cover slipping:

The unstained slide is covered by a thin glass or glass-like material to keep the stained tissue unscratched and save the tissue for a long time.



What is Special Stains?

The study used to identify the presence of organism like bacteria or fungal. To highlight certain constituents like collagen, amyloid, mucin, etc.

SPECIAL STAIN	PATHOGENS
ACID-FAST	Mycobacterium leprae, Mycobacterium tuberculosis
GIEMSA	Helicobacter pylori, Plasmodium vivax, Rickettsia prowazekii, Rickettsia rickettsii, Rickettsia tsutsugamushi
GRAM	Actinomyces israelii, Legionella pneumophila, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides

GROCOTT'S METHENAMINE SILVER (GMS)	Aspergillus fumigatus, Blastomyces dermatitidis, Candida albicans, Coccidioides immitis, Cryptococcus neofarmans, Histoplasma capsulatum, Nocardia asteroids, Pneumocystis carnii, Sporothrix schenskii
MAYER MUCICARMINE	Cryptococcus neofarmans
PERIODIC ACID-SCHIFF (PAS)	Aspergillus fumigatus, Blastomyces dermatitidis, Candida albicans, Coccidioides immitis, Cryptococcus neofarmans, Sporothrix schenskii

Immunohistochemistry:

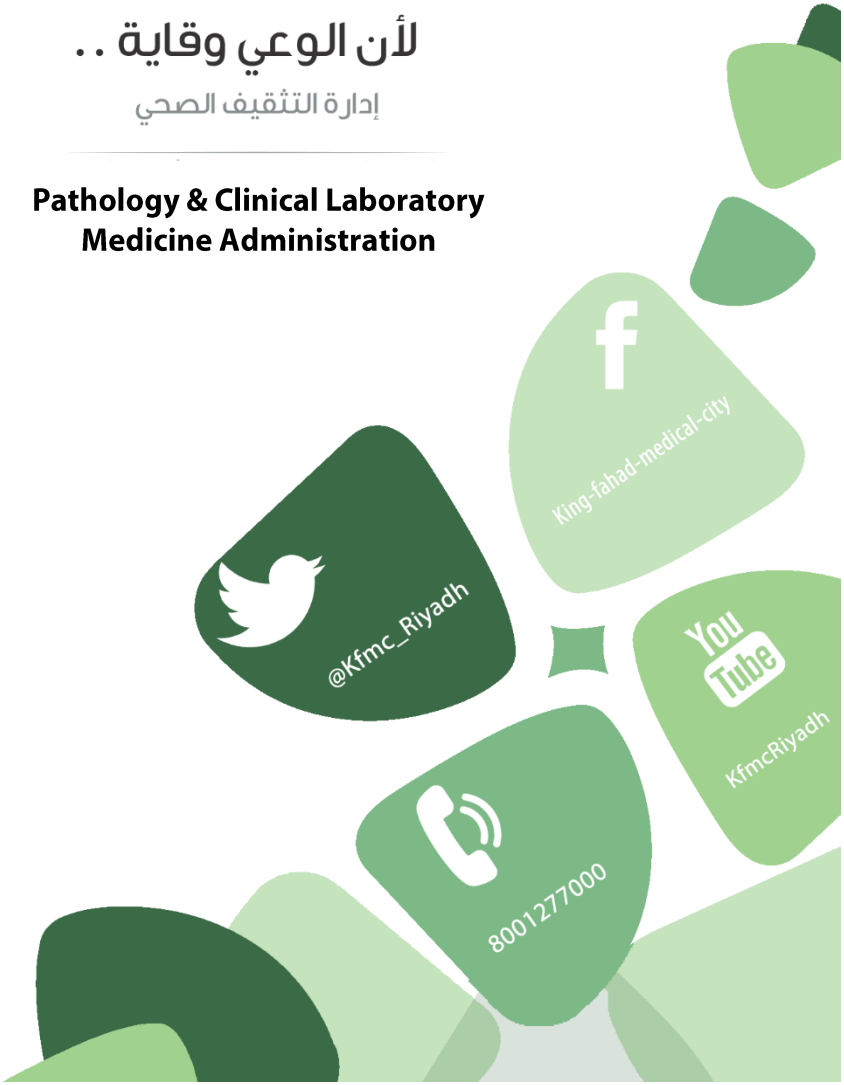
This study is mandatory to identify the origin of cells and the primary of the metastatic tumor.

It is the use of antibodies to detect cell and tissue proteins and provide semi-quantitative data about target protein expression, distribution, and localization. Target expression can be evaluated with the corresponding labeled primary antibody (direct detection) or, more commonly, with the addition of labeled secondary antibodies (indirect detection).

لأن الوعي وقاية ..

إدارة التثقيف الصحي

Pathology & Clinical Laboratory Medicine Administration



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