NOVA

Charting New Horizons in Education

Micro techniques

02

Cellular biology

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Micro techniques For Light microscopy

I
Paraffin
(slow)

II
Freezing
(fast)

Paraffin technique

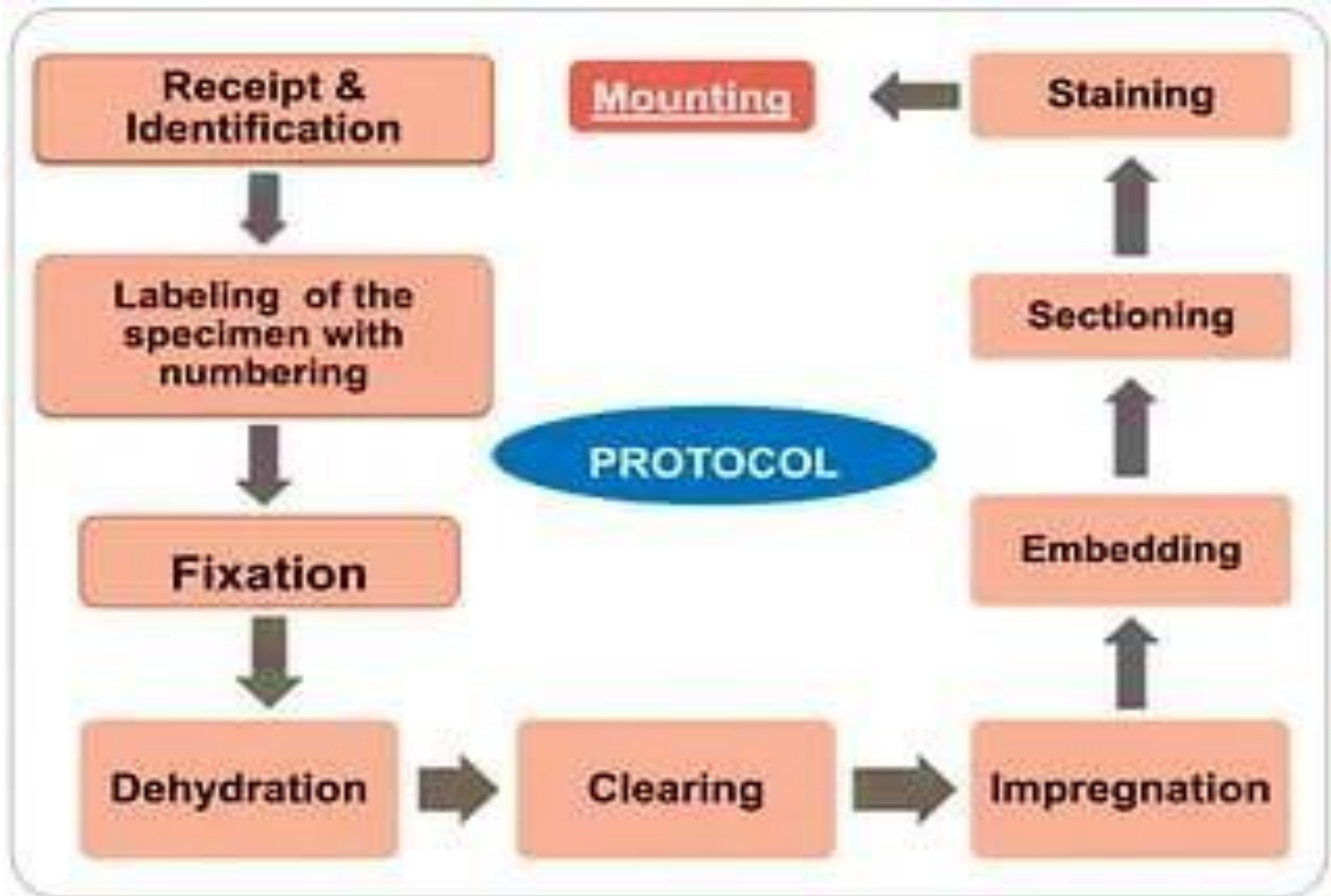
- Technique used to prepare **the tissues** for light microscopy.
- Three ways to obtain a specimen:

1. cadaver
2. Biopsy
3. Animals

- it includes the following steps:

1. **Fixation :** in appropriate solution (formol saline)
2. **Dehydration and clearing:** in alcohol then xylol
3. **impregnation & Embedding:** in paraffin wax (Soft & hard)
4. **Sectioning :** by microtome
5. **Mounting :** on glass slides
6. **Staining of the sections**

Tissue processing



Fixation

- To maintain the structure of the tissue as in the life state
- upon removal from the body the sample Immediately placed in a fixative solution
- Fixation is done as soon as possible to prevent autolysis and to preserve the morphology.

For LM:

- Formol saline

For EM: a mixture of

- Glutaraldehyde
- Osmium tetroxide

ROUTINE FORMALIN FIXATIVES:

10% formal saline: Most commonly used fixative

Water (distilled)	900ml
Sodium chloride	8.5gm
Formalin	100ml

fixation

Advantage of the fixation step :

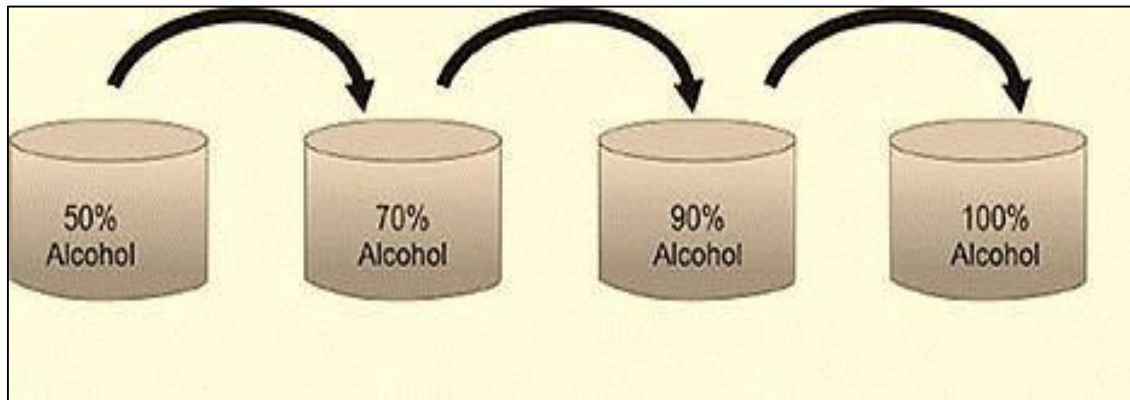
- Hardens the tissue by coagulating its protein → Facilitate the process of cutting & staining & examination

- Prevent putrefaction & stop autolytic changes by killing any bacteria

- Preserves the molecular & morphological structure of the tissue

Dehydration & Clearing

Dehydration : Is done by treating the specimen with ascending concentration of alcohol (50% → 70%→ 100%) ….. Gradual removal of water from the specimen (H2o represent 70% of the body)



Clearing : with this process the tissue become translucent (to allow the light to penetrate the specimen)

the tissue is treated with xylol or benzol …to remove the alcohol

Impregnation & Embedding

Impregnation :

- Tissues are placed in molten soft paraffin wax
- The wax infiltrates the tissue & occupies all the spaces that were originally occupied with water

Embedding:

- Tissue are placed in molten hard paraffin wax
- The tissue is placed in the center of the paraffin, which hardens as it cools → paraffin block

Impregnation

- Complete Infiltration of the tissue with wax
- Essential for production of good sections

Embedding

- Facilitates sectioning
- Prevents tissue damage
- Specimen orientation is very important

Automatic tissue processor

The steps required to take animal or human tissue from fixation to the state where it is completely infiltrated with soft paraffin wax then to be embedded in hard wax for section cutting on the microtome.

Sectioning by Microtome

- A microtome is a mechanical device used to cut extremely thin slices of a fixed tissue block known as **sections.**
- It holds the block of hard paraffin with the tissue in its center against a sharp metal knife that used to cut the block into thin sections (3-10 microns) as it moves up and down.

mounting

Tissue sections are placed on glass slides smeared with egg albumin , then warmed on a hot plate to dry.

The sections are now ready to be stained

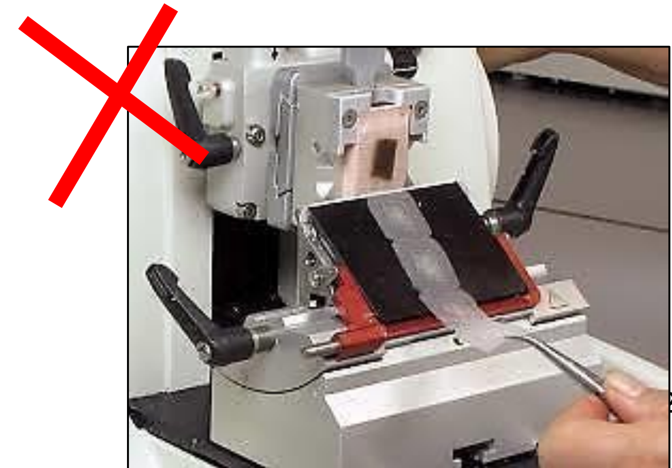
II. Freezing technique

Fresh frozen tissues are cut using **cryostat** (freezing microtome). The sections placed

in cold fluid called Isopentane/liquid nitrogen (-50 C) for fixation then rapidly stained.

Advantages:

- Rapid technique for diagnosis in operation rooms (tumors).
- No fixation , No dehydration & No chemicals are used, so useful for histochemical (enzyme staining) studies.

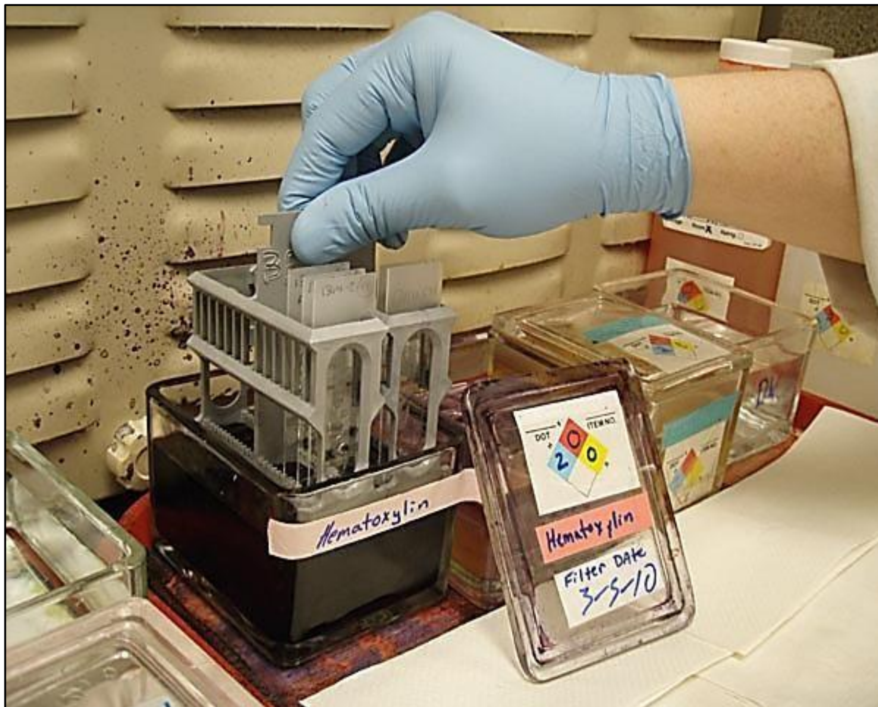


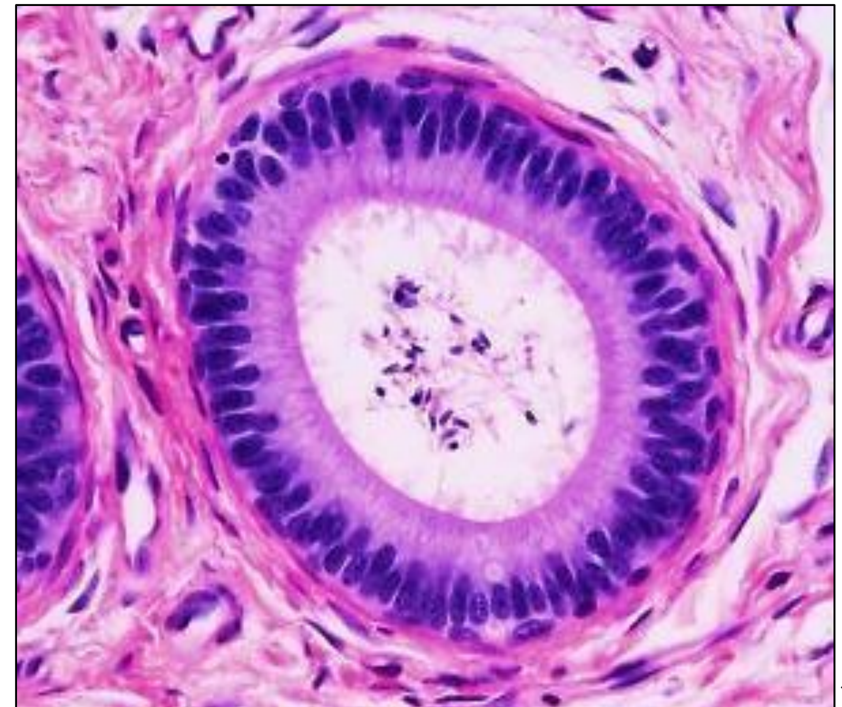
Disadvantages:

- Non serial & Fragmented sections .
- Cannot be preserved for a long time.

Staining

- The tissue sections that will be studied using the light microscope **must be** stained first since most tissues are colorless
- Dyes (stains) used are either **basic** or **acidic**





Staining

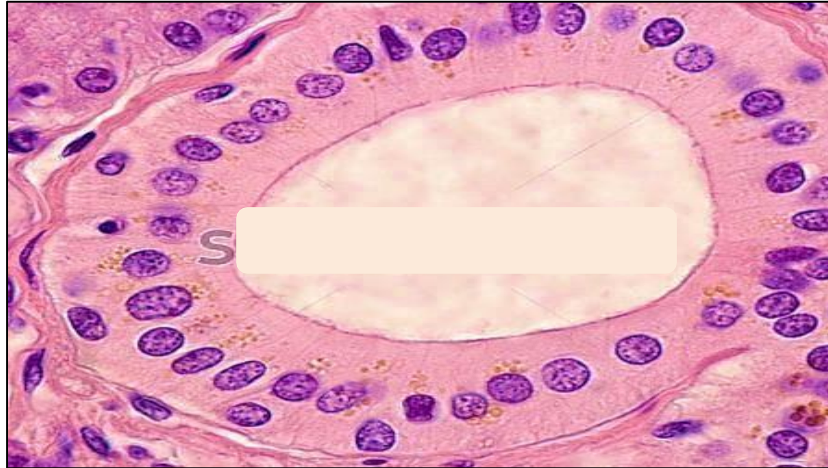
Used to visualize & distinguish the different parts of cells & tissues

Routine stains
H&E

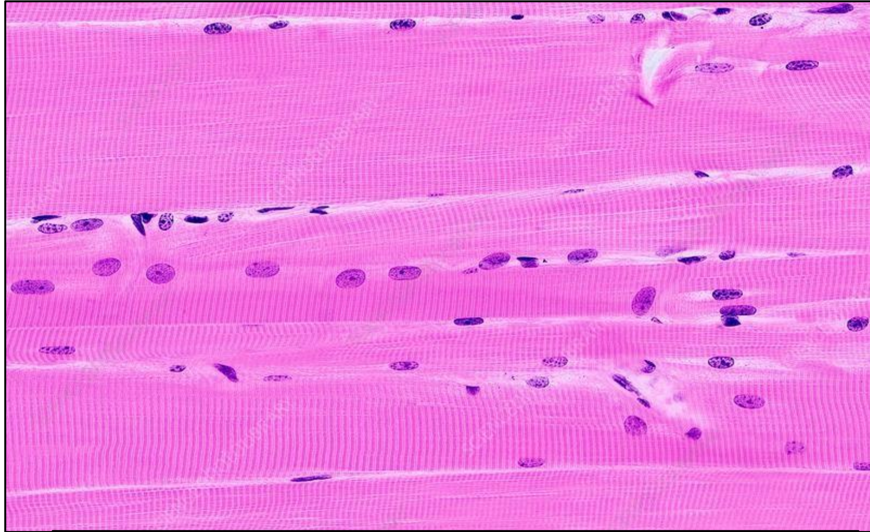
Special stains
e.g. Ag & orcein, trichrome….etc

VA

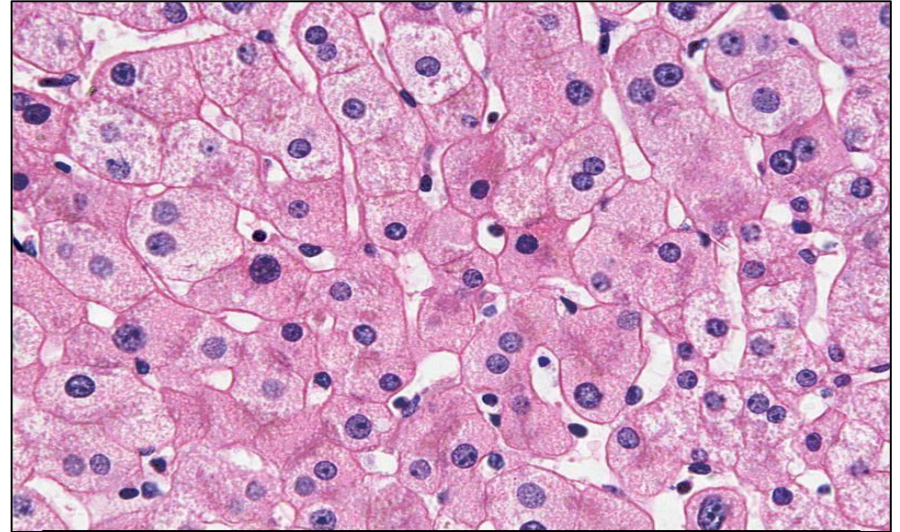
Common (Routine) histological stains H&E



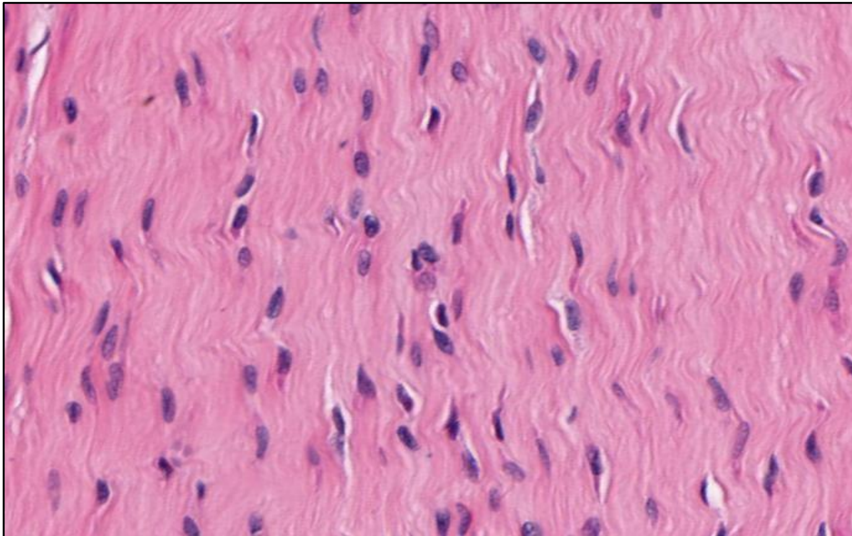
• **Hematoxylin (H) :**	• **Eosin (E):**
blue basic dye (+ve charged)	**red acidic dye (- ve charged)**
• Stains acidic (anionic –ve) components of the cell with a blue color e.g. nucleus, ribosomes (r-RNA)	• Stains basic (cationic +ve) components of the cell with a red color e.g. cytoplasm, mitochondria, muscles !! (it has +ve charged proteins)
Basophilic structure=blue	**Acidophilic structure=red**



L.S skeletal ms. fiber (contains actin & myosin) stained with H &E



Liver cells contains plenty of mitochondria stained with H &E



Collagen fibers stained with H & E

Automatic slides staining machine

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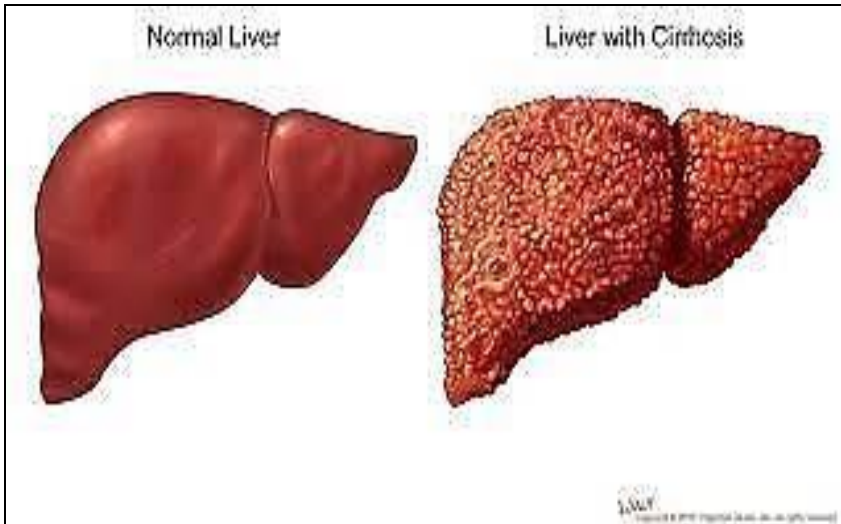
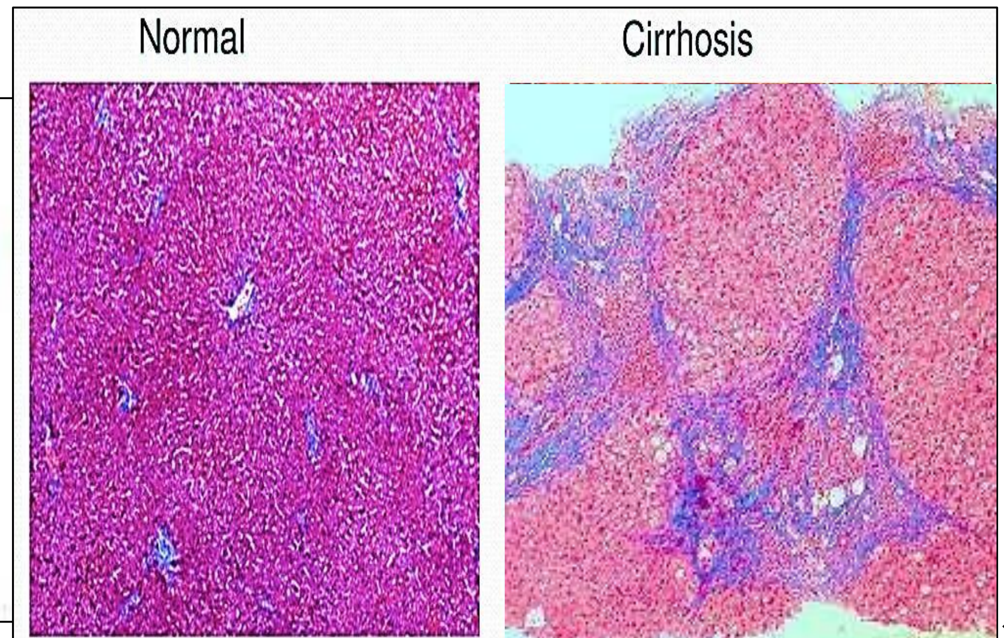
The clinical values of special stains

- Special stains answer specific questions like what type of cells and tissues
- Also Used in the diagnosis of medical diseases like Tichrome stain in case of Liver Cirrhosis





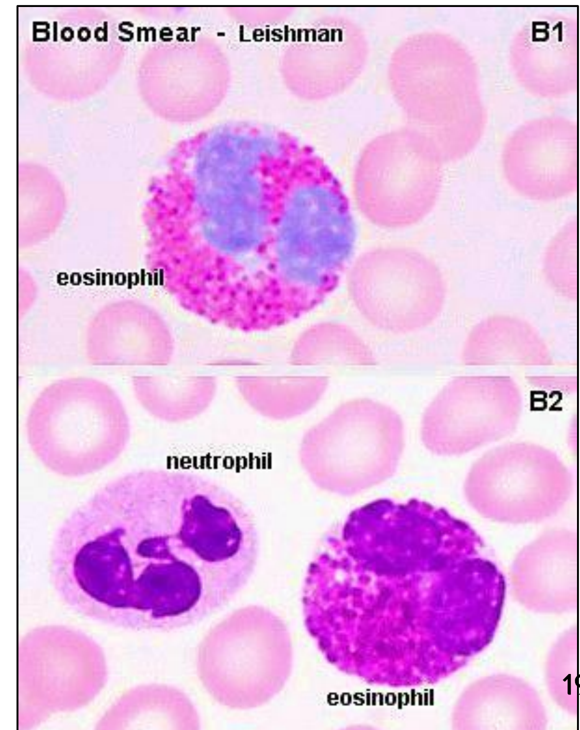
Vital stain: (test the viability of the cell)

Stain the living cells inside the living animal. Done by injecting the dye into living animal prior to examine the tissue .e.g. staining phagocytic cells (macrophages) with Trypan blue & Indian ink

Leishman stain:

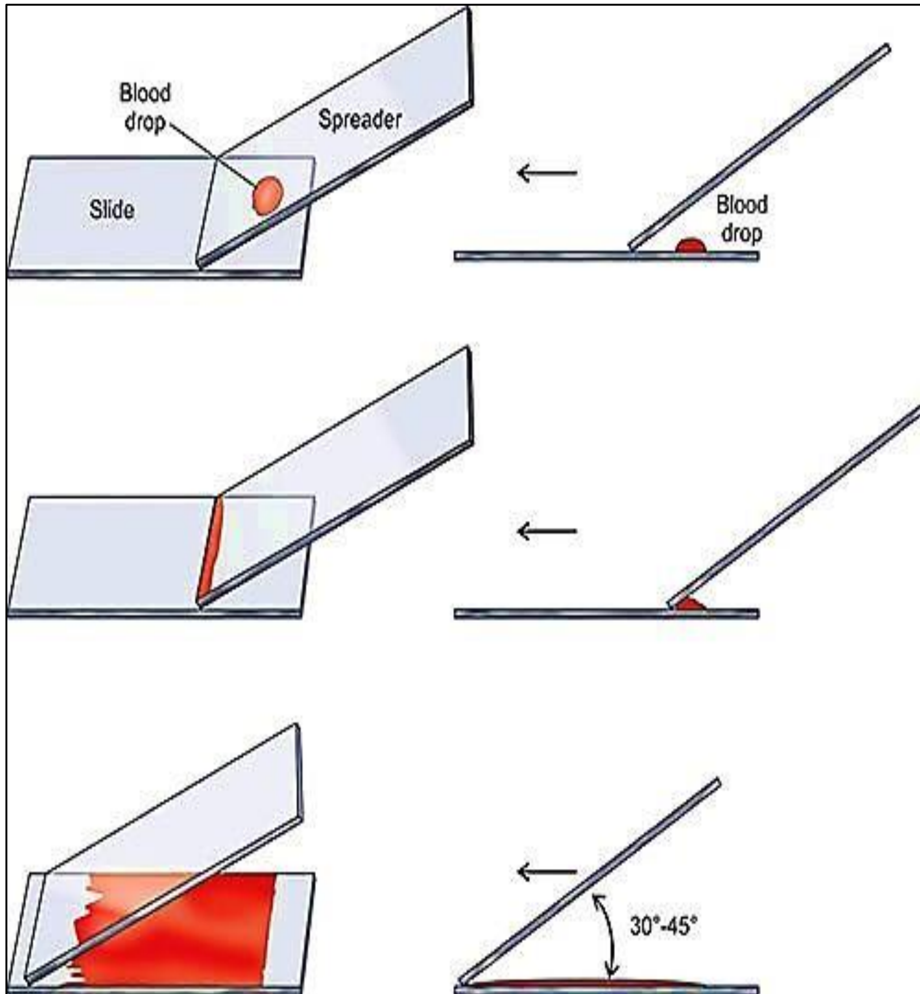
mixture of acidic & basic stains to stain nuclei & cytoplasm

(methylene blue & Eosin)It used to stain blood films to demonstrate white blood Cells e.g malaria parasite

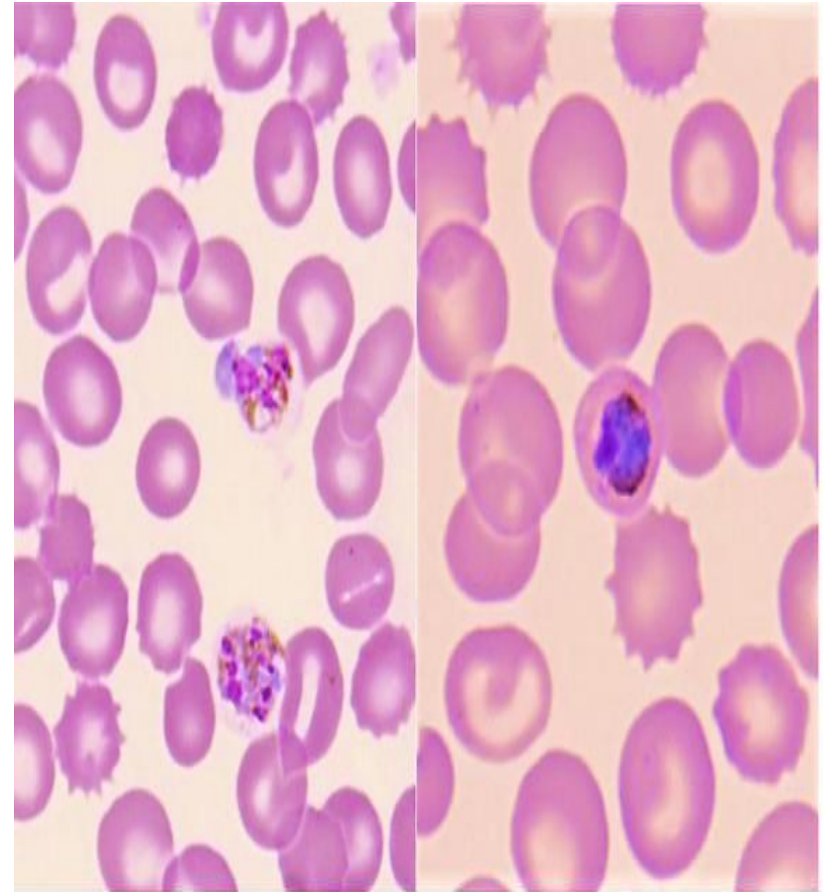


H&E vs. Leishman stain

Feature	H&E (Hematoxylin and Eosin)	Leishman Stain
Primary Use	Histology for tissue sections	Blood smears and cytological prep
Staining Components	Hematoxylin (stains nuclei blue) and Eosin (stains cytoplasm pink)	Leishman stain (stains nucleic acids and cytoplasm)
Cellular Focus	General tissue architecture	Blood cells and parasites
Common Applications	Tumor pathology, organ histology	Malaria detection, white blood cell counts
Visualization	Clear differentiation of tissue structures	Enhanced visibility of blood cells
Color Results	Nuclei: blue, Cytoplasm: pink	Varies, often shades of purple/pink for cells
Preparation Method	Fixed and embedded tissue sections	Fresh blood smears



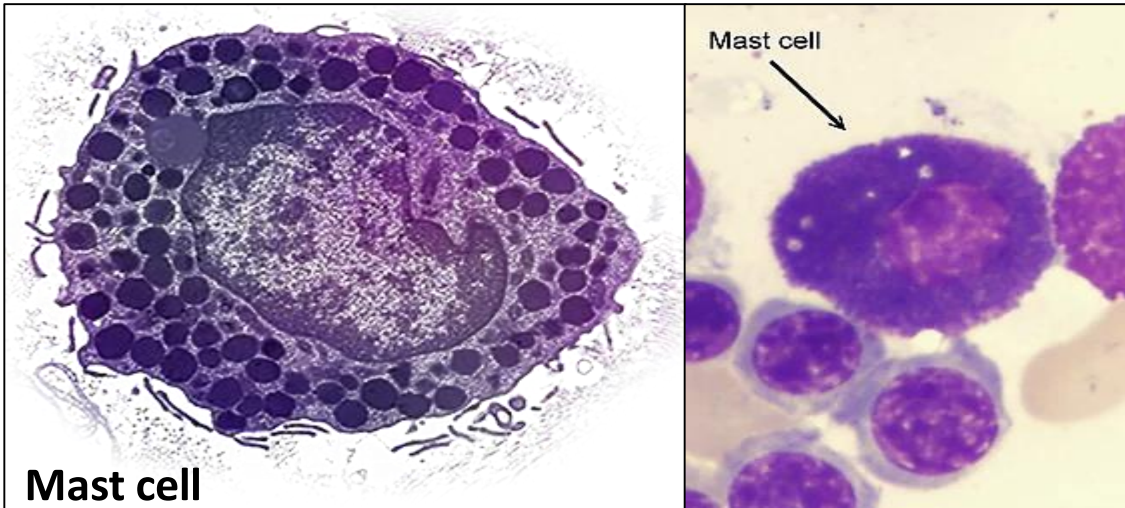
Technique of blood film



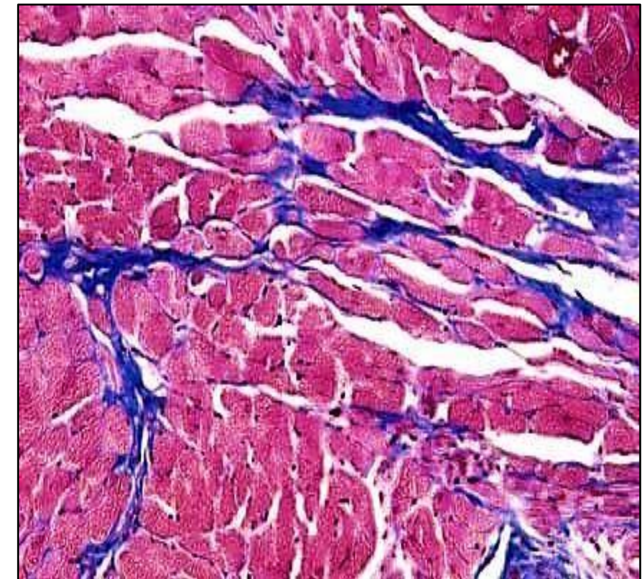
Red blood cells infected with malaria parasite

Metachromatic stain:

Stain gives the tissue new color different from that of the original stain e.g. Toluidine blue when stains Mast cells gives purple color (different from the blue color of the stain). Phenomenon called **metachromasia.**



Trichrome stains: (connective tissue)

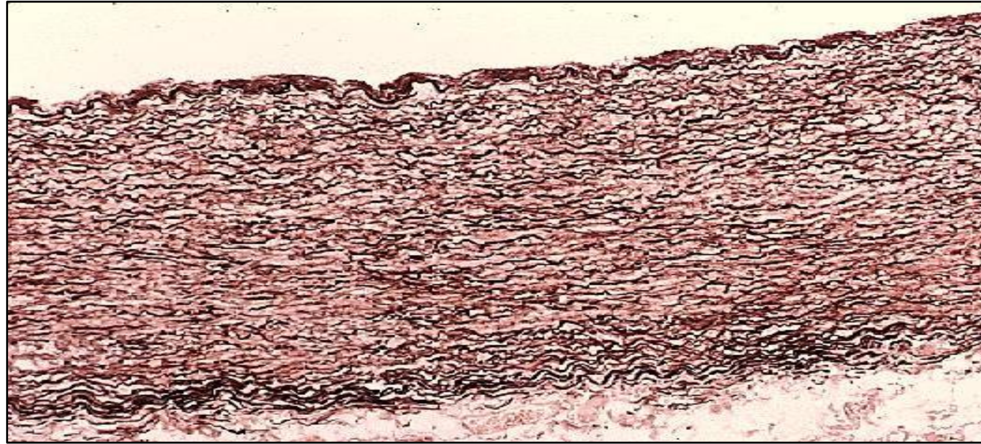


3 stains used in combination to stain different tissues Components e.g. **collagen fibers** stained blue

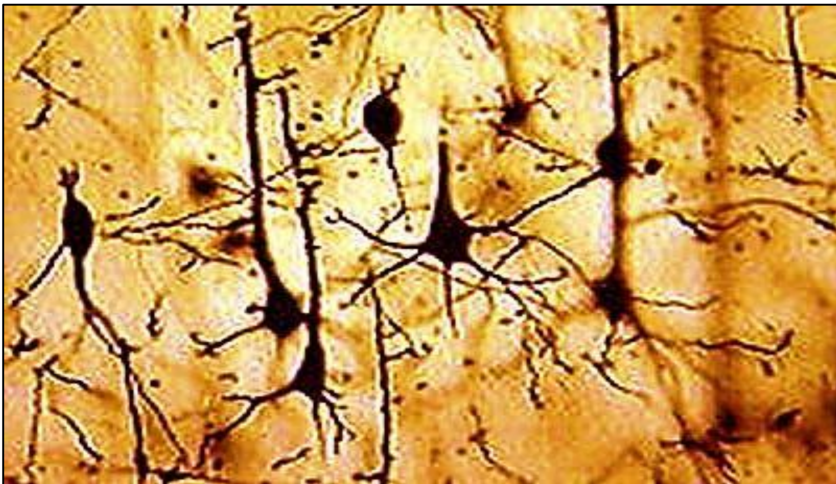
Metachromatic stain:

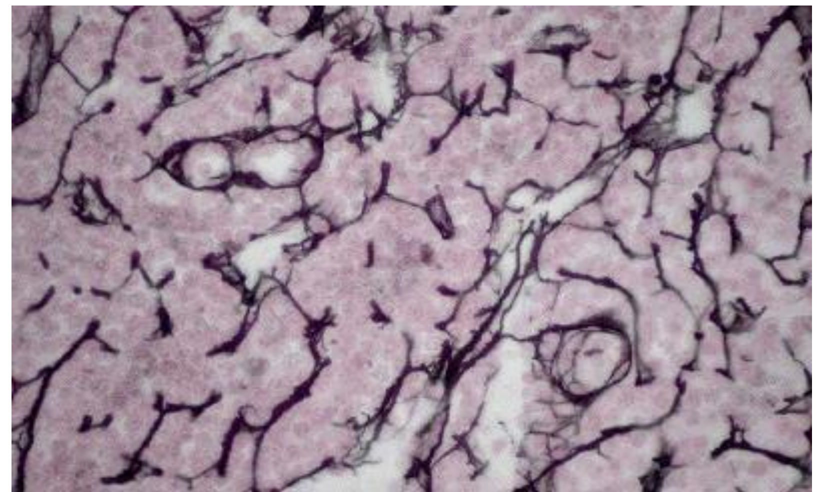
Feature	Metachromatic Stain	Trichrome Stain
Primary Use	Identifying specific cell types and structures, like mast cells	Differentiating tissue components, especially collagen and muscle
Staining Mechanism	Changes color based on the ionic environment; stains certain components in a different color than the dye	Uses multiple dyes to stain different components distinctly
Common Applications	Histology, especially in detecting mucopolysaccharides and mast cells	Tissue histology, especially in connective tissue studies
Color Results	Varies; typically shows a color change (e.g., purple) depending on the target	Often results in green, blue, or red shades for different tissues
Preparation Method	Varies by specific dye used, but generally requires fixation and preparation of sections	Involves multiple steps with different staining solutions

٧٨ **Orcein stain** : stains elastic fibers brown (wall of aorta)



Silver (Ag) stain: nerve cell brown & reticular fibers black





- **Histochemical stains:** (Relate structure to function)

technique used **selectively identify & demonstrate the distribution of chemical compounds or enzymes** within & between the cells e.g. mucine or alkaline phosphatase enzyme

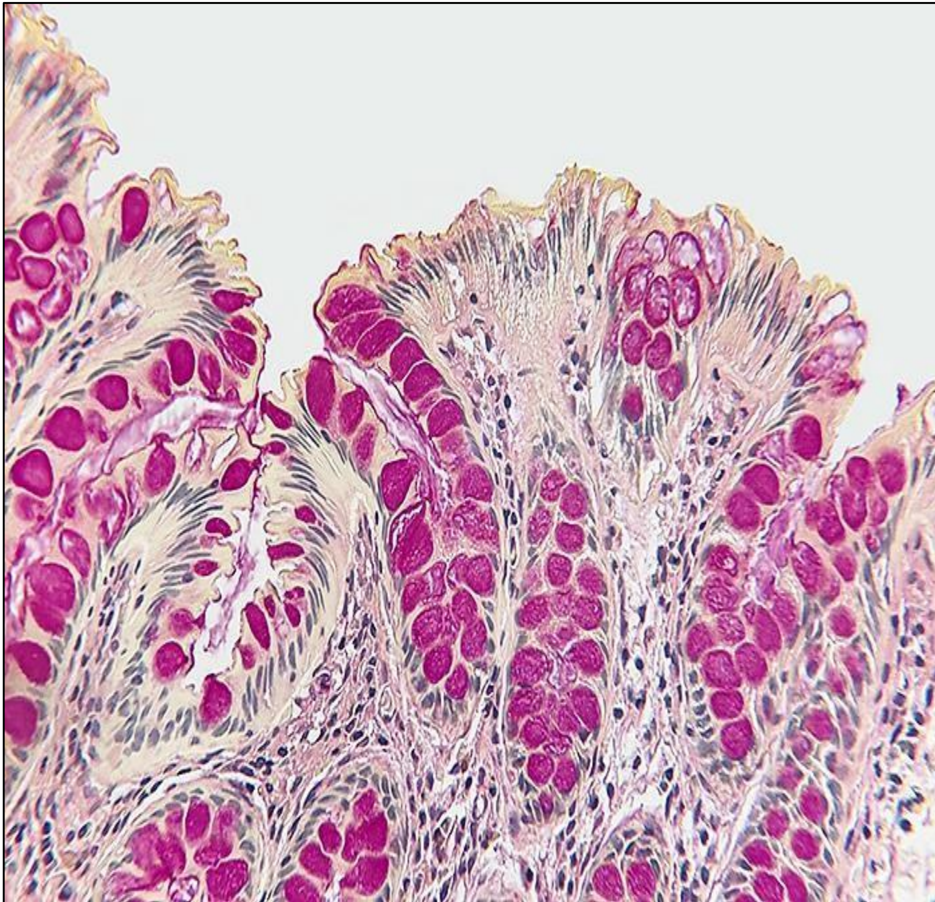
Concept: enzyme of interest in a cell or tissue converts its substrate → colored or florescent product that can be visualized at the site of the activity

cryostat

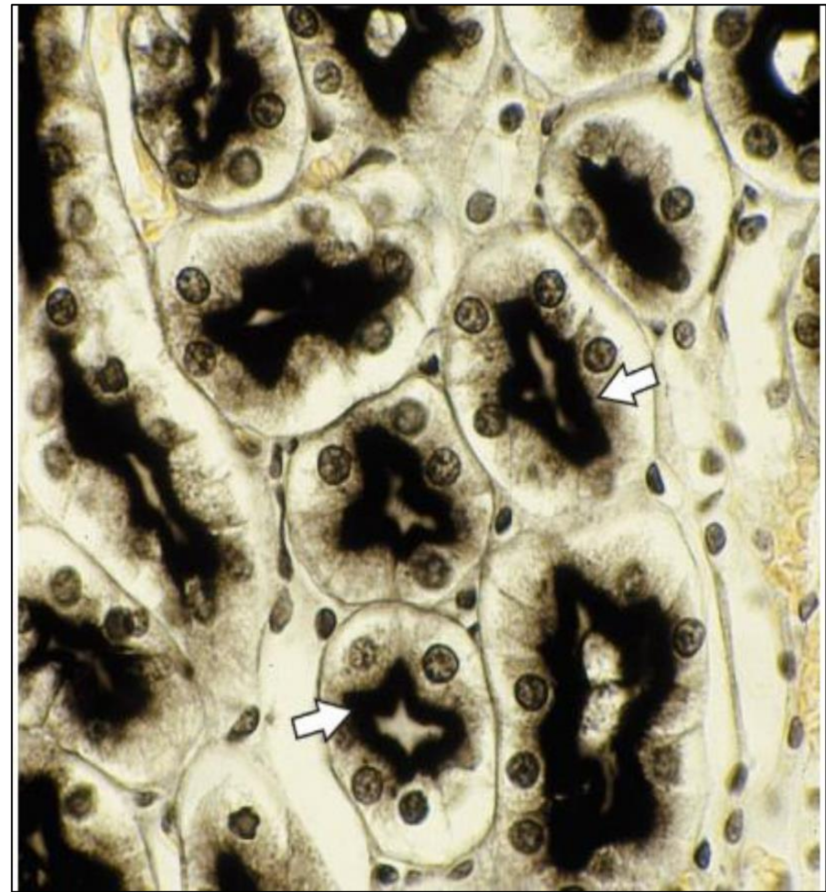
- **Immuno-histochemical (IHC) stains:**

Laboratory method that **selectively identify antigens** using specific antibodies to check for these antigens in a sample of tissue. The antibodies are usually linked to an enzyme or a florescence dye (markers) (Labeled antibodies).

Histochemical stains



for mucin in mucous cells



(Alkaline phosphatase enzyme)
(this enzyme removes phosphate group from protein)

- After the antibodies are linked to the antigen in the tissue sample the enzyme or dye is activated. The localization of the antigen can be seen under the microscope

- <u>This is method is use for visualize both normal & disease status of tissue</u>

used in diagnosis of cancers (markers) and can tell the difference between different types of cancer . Tumor specific

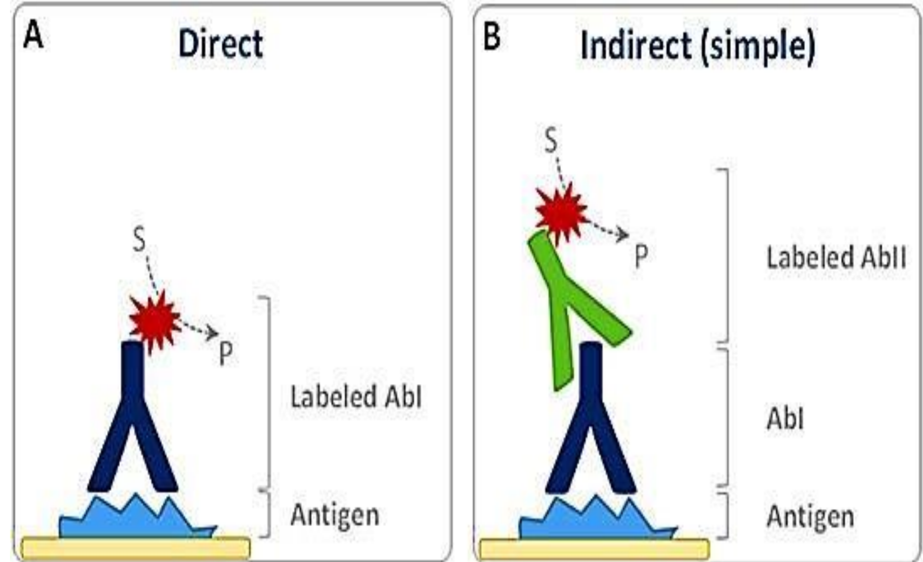
Use of the Immunohistochemistry:

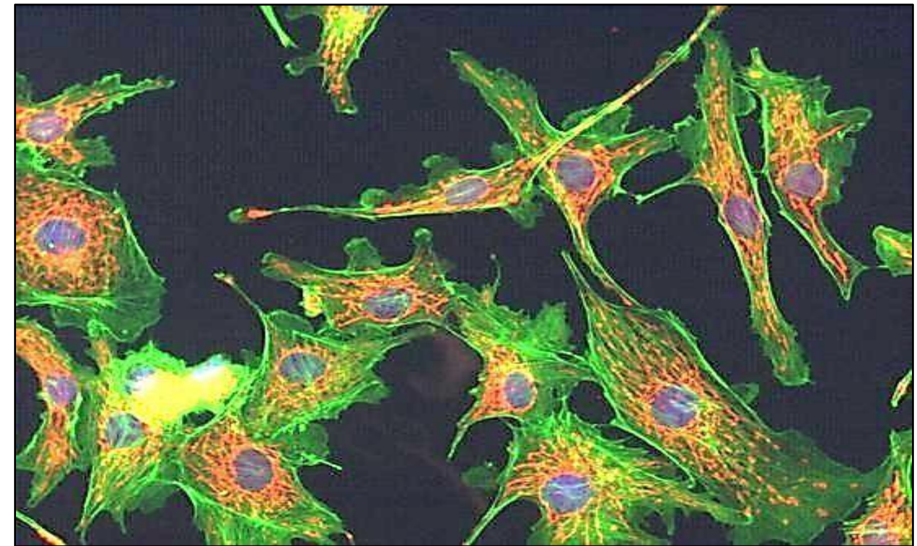
- Intercellular antigens,
- Cell surface antigens,
- Tissue antigen for diagnosing autoimmune diseases
- Protein hormones in histopathological diagnosis
- Soluble antigens of the cell
- Diagnosis of the endocrine tumors
- Small amounts of peptides in endocrine or neuroendocrine cells
- Immunodeposits
- Tumoral markers
- Tumor typing

- Indirect vs direct IHC

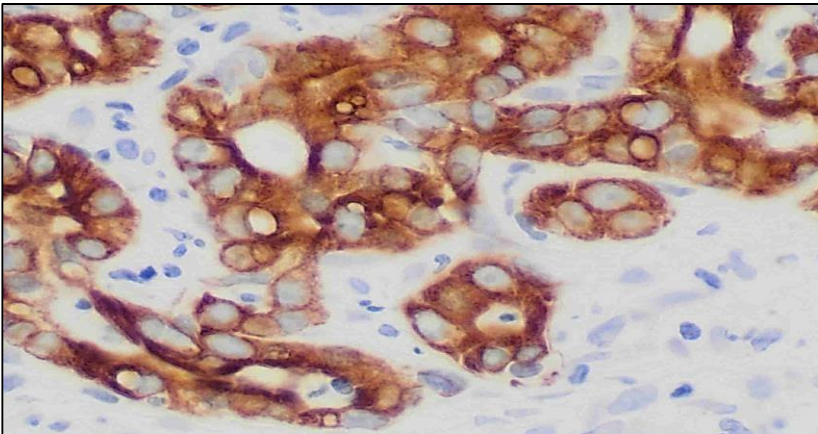
direct is ideal for detecting highly expressed antigens

while Indirect is more suitable for poorly expressed antigens which benefit from signal amplification by the secondary antigen





Immunohistochemistry (IHC)



Molecular analysis

It means **biochemical analysis** of certain components of the cell. It is usually quantitative in nature.

Examples are:

- Protein-electrophoresis
- DNA – electrophoresis
- Fluorescent In situ hybridization (FISH technique)
- Detection of certain ions in the cell e.g. Ca, Fe….etc.

Protein electrophoresis:

Proteins carry a positive or a negative electrical charge, and they move in fluid when placed in an electrical field. Proteins will be separated according to their charge & molecular weight

(e.g. blood cancers:↑ increased gamma globulin protein (M protein) = multiple myloma)

DNA electrophoresis: is technique used to identify & quantify DNA fragments (DNA fragments are -ve charged).

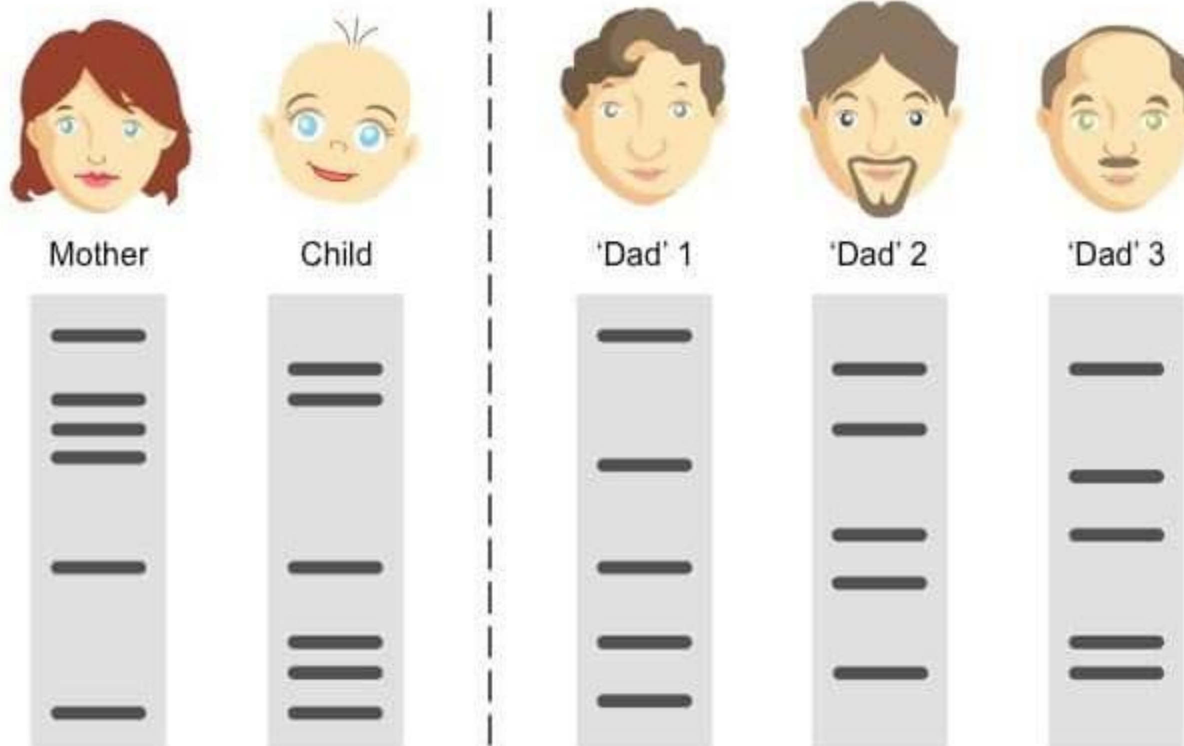
(in this case separation is based on length of the base pair)

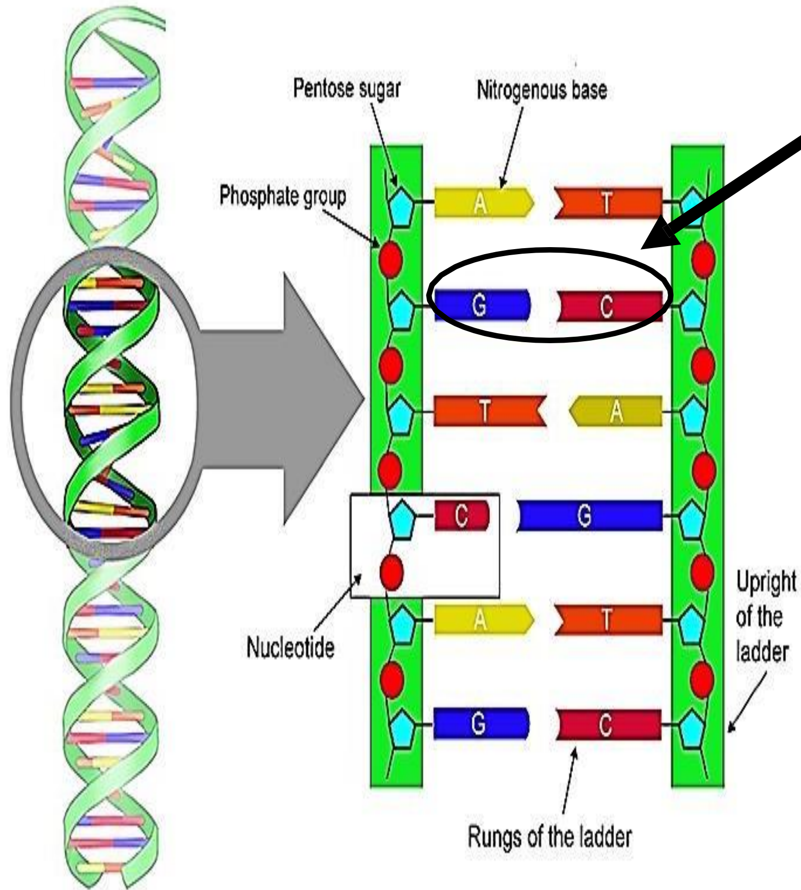
Samples are loaded into wells of an **agarose** or **acrylamide gel** and subjected to an electric field, causing the negatively charged nucleic acids to move toward the positive electrode.

Small fragments will move faster than the large ones

(**DNA fingerprint , gene isolation, disputed paternity**)

DNA Paternity Testing





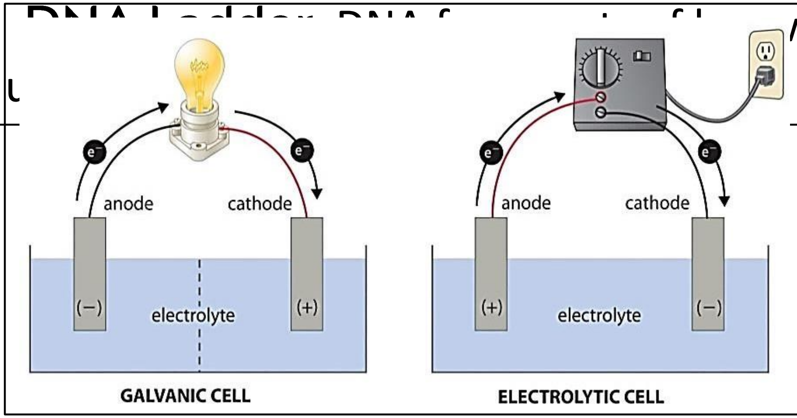
<u>A base pair</u>: is a unit consisting of two nucleobases bound to each other by hydrogen bonds.

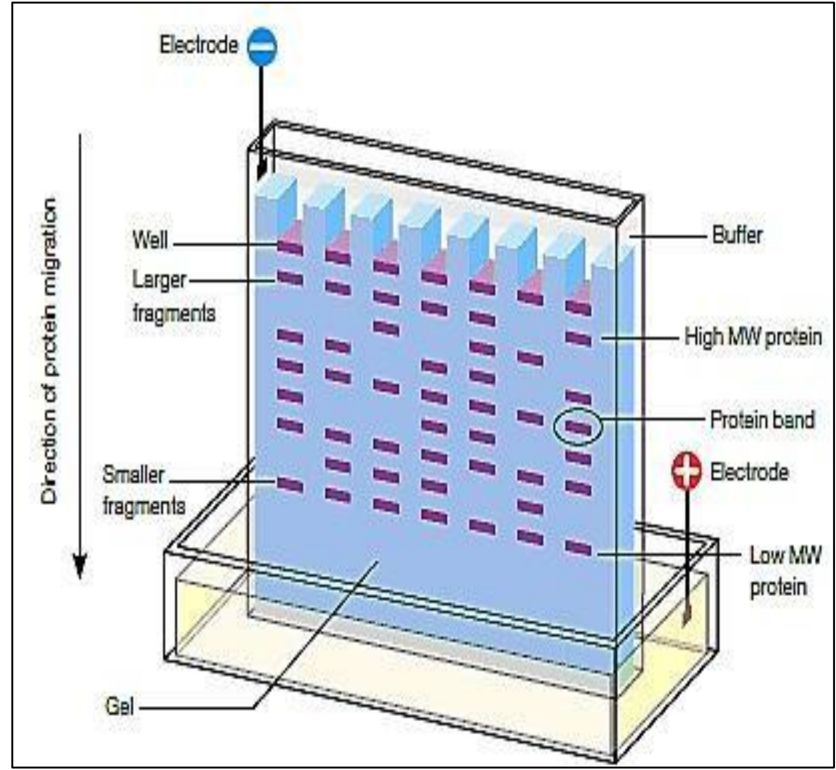
They form the building blocks of the DNA double helix.

Sequence of bases on DNA determine genetic code for a trait

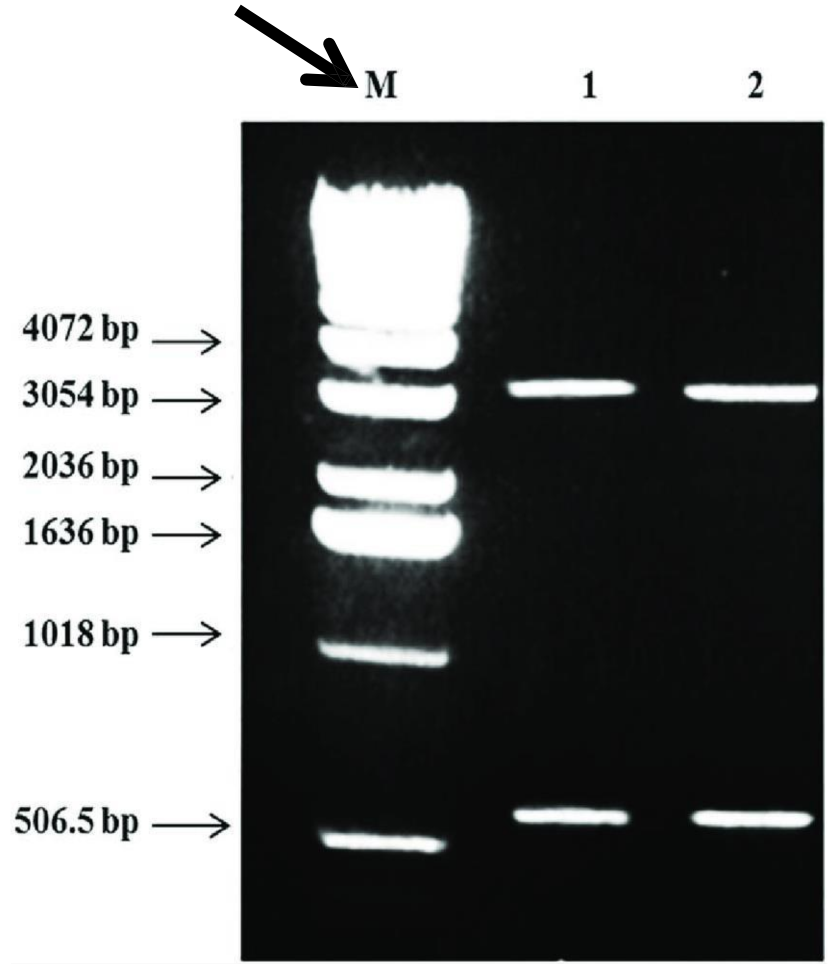
The human genome contains approximately 3 billion of these **base pairs**, which reside in the 23 **pairs** of chromosomes within the nucleus of all our cells

DNA Ladder: DNA fragments of known lengths used to estimate the size of u…





Protein electrophoresis



Fragment size usually referred to as base pair (bp). The shorter fragments travel faster

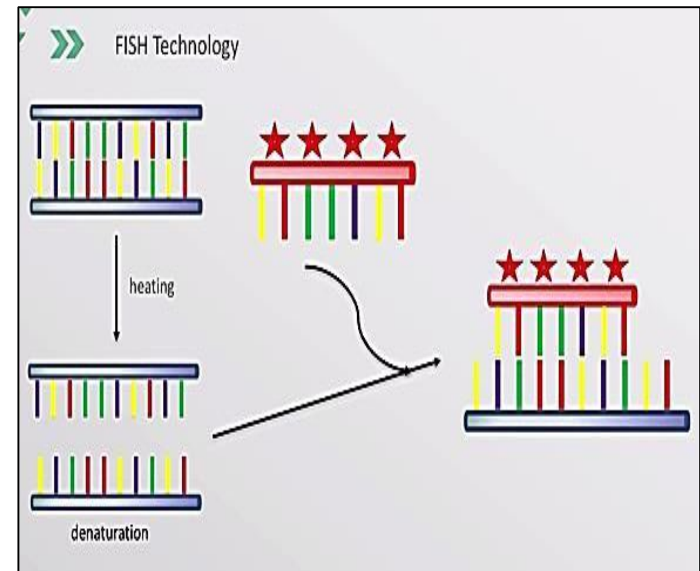
٧٨ Fluorescent In situ hybridization (FISH technique):

Molecular technique used to visualize and map the genetic material. Use to localize the site of the genes on chromosomes using a fluorescent probe

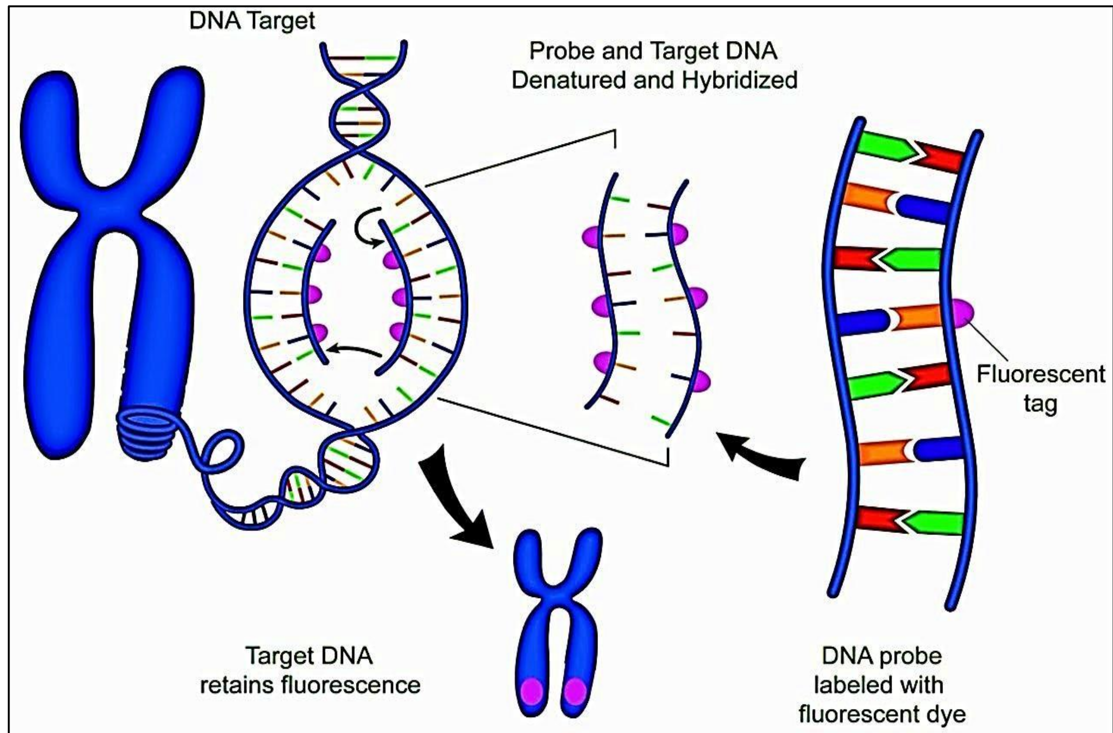
fluorescent probe:

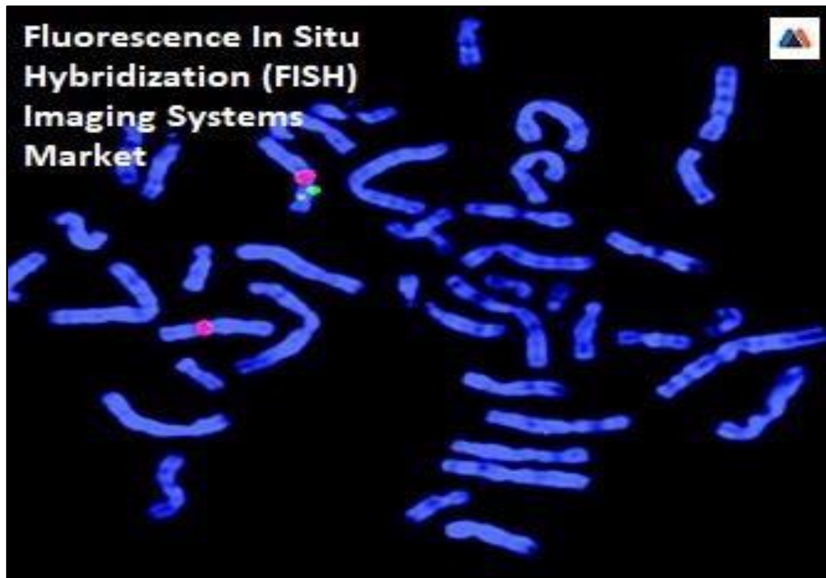
fragment of DNA or RNA of variable length which can be radioactively labeled (probe)

It can be used in DNA or RNA samples to detect the presence or absence of a nucleotide sequence that are complementary to the sequence on the probe .



Useful in detect chromosomal abnormalities


DNA Target
Probe and Target DNA
Denatured and Hybridized
Fluorescent
tag
Target DNA
retains fluorescence
DNA probe
labeled with
fluorescent dye


Fluorescence In Situ
Hybridization (FISH)
Imaging Systems
Market

Methods for study tissues

- **1-In vivo studies: within the living body .** Study of tissues after doing any experiment inside the living body (animal model based testing)

- **2-In vitro studies: outside the body** Study of tissues outside their normal biological context (cell based testing)

Cell and Tissue Culture

- **In vitro** cultivation of tissues & cells at defined temperature(37C) using an incubator & supplemented with a medium containing cell nutrients & growth factors(like animal serum) is collectively known **as tissue culture.**

- Different types of cells can grow in cultures as: white blood cells, fibroblasts, skeletal and cardiac muscle, epithelial tissue (liver, breast, skin, kidney) and many different types of tumor cells.

Medical uses of tissue culture:

1. used in studying chromosomal patterns of individuals, Karyotyping, gene therapy.

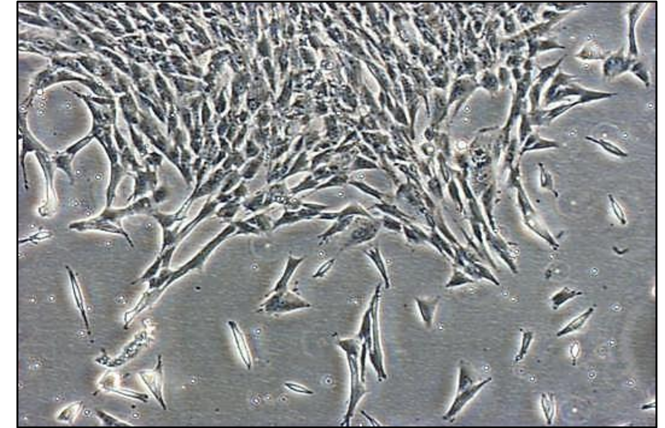
2. Used in researches of cancer.

3. Used in cultivation of bacteria, viruses, in order to prepare different vaccination

4. Study the effects of new drugs

Cell culture

Cells can be isolated from the body for *in vitro* cultures. Cells can be released from tissues by enzymatic digestion Using enzymes such as collagenase and trypsin which break down the extracellular matrix.



Primary cultures:

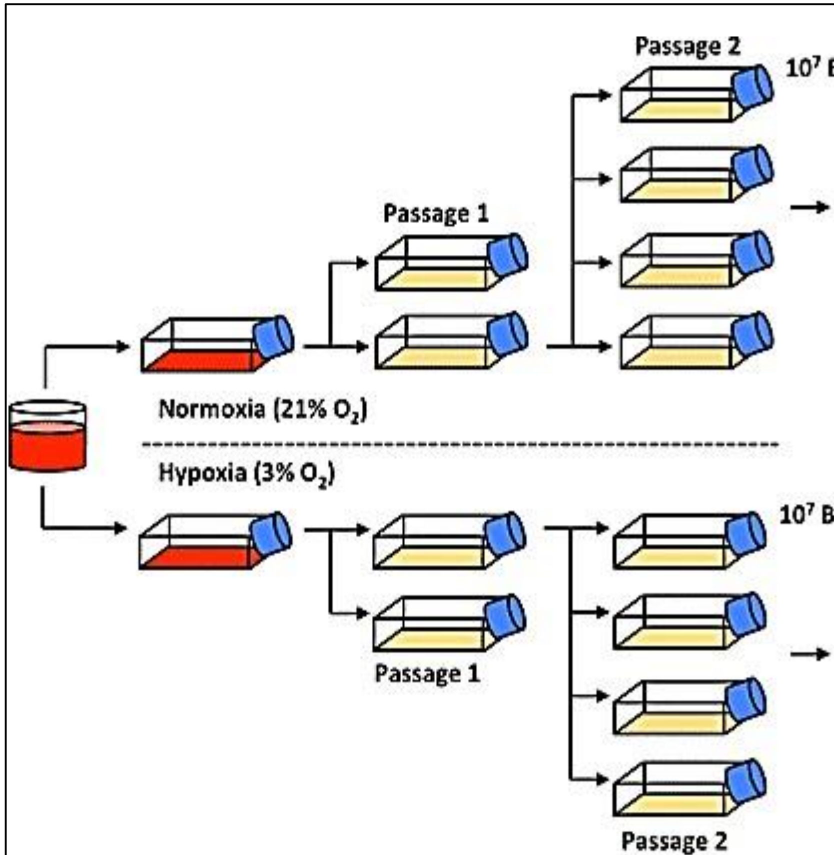
Refer to the cells that are cultured directly from a tissue (parent cells).

Secondary cultures:

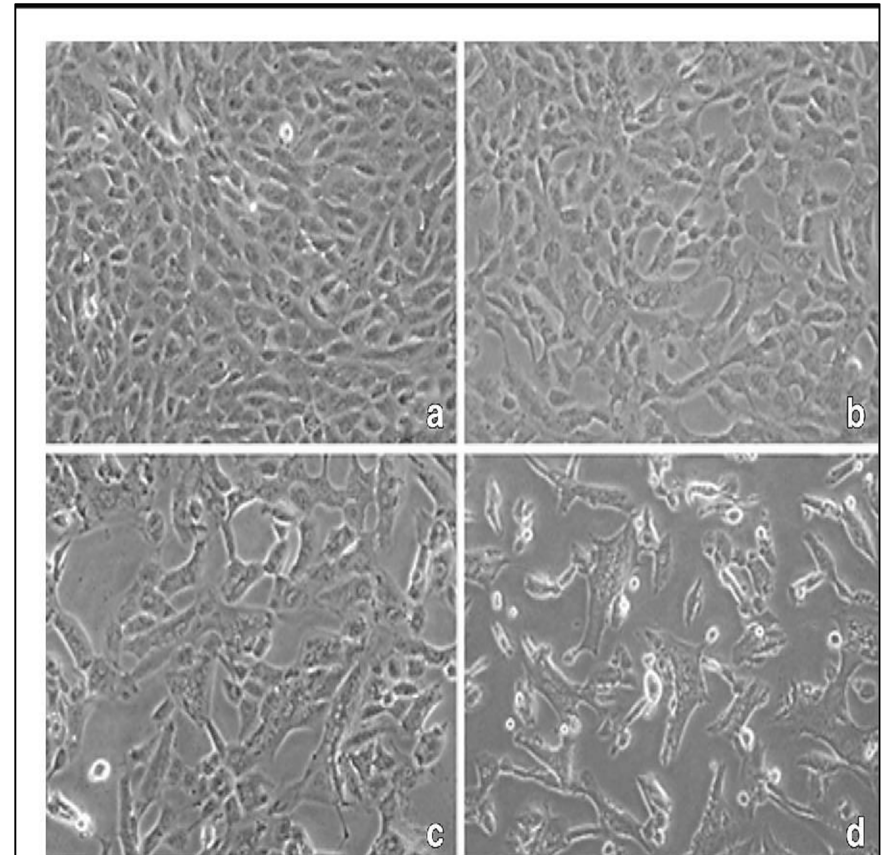
- Once the parent cells reach confluence they have to be **sub-cultured** (i.e. passaged) by transferring them to a new vessel with fresh growth medium to provide more room for continued growth

Confluence:

- stage in which the cells (1ry or 2ry) become adherent to & covering most of the culture surface forming monolayer(e.g. 25%, 50%, 100%)



Cell passage = subculture



Different degrees of confluency

cell line:

- a population of cells (clone)developed from a single cell therefore these cells have a **uniform** genetic make-up (phenotype & function)
- Cell lines have a limited life span, and as they are passaged

immortalized cell line

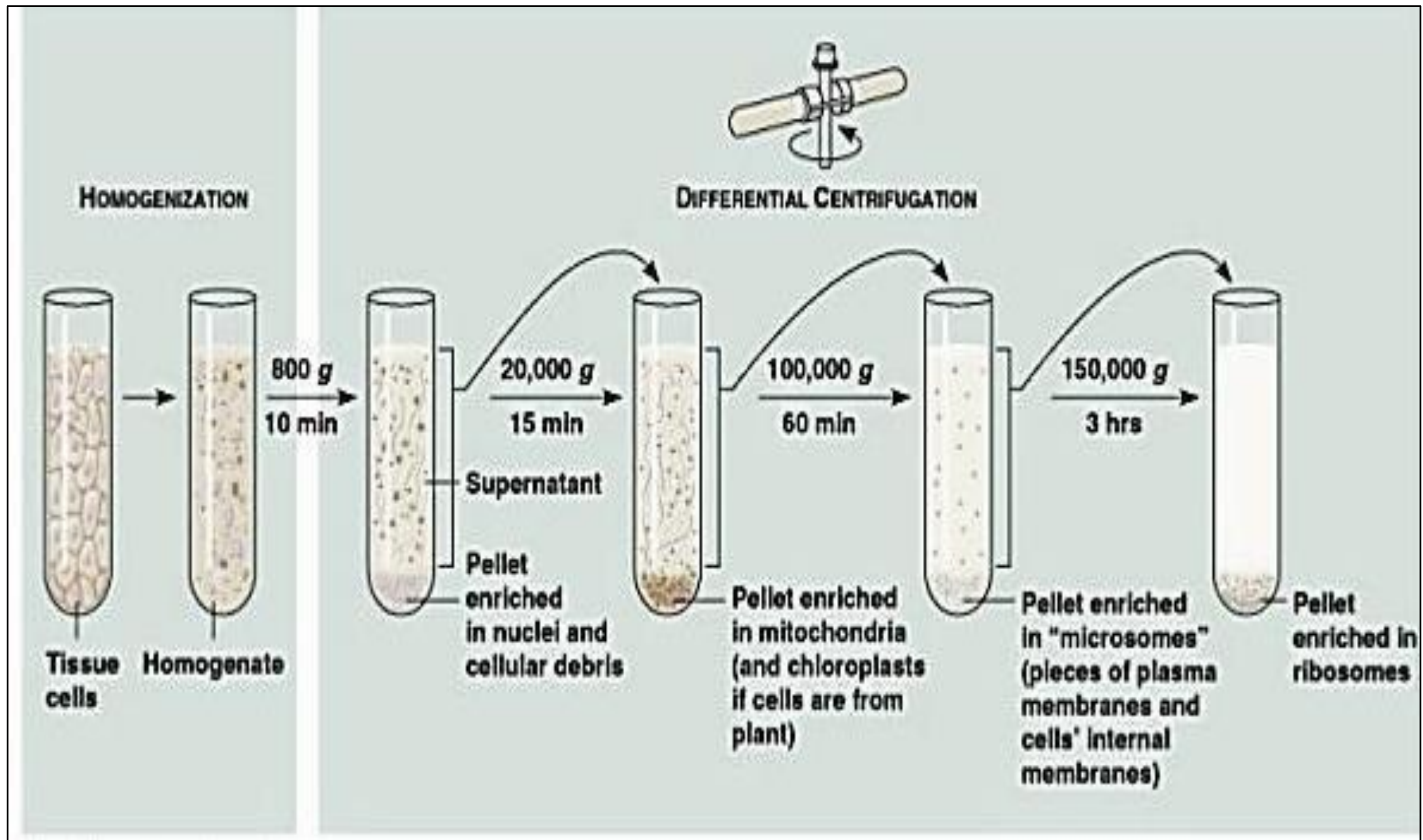
- Has acquired the ability to **proliferate indefinitely.**
- It is obtained from subcultures of the primary culture
- Normal immortalized cell line: stem cells
- Abnormal immortalized cell lines : cancer cells

VA # Cell fractionation

- It means isolation of the cell components (nucleus & organelles) while preserving its individual function to study the features of each.

- This is done by the use of **centrifugation** at different speeds and periods of time. The factor that determine whether a specific cell component ends up in the supernatant or the pellet is size and weight of component

- Nuclei are the first to be separated followed by different cell organelles



The sediment at the bottom of the tube is called **pelle**t, the less dense component at the top is called **supernatant**

«Wherever the art of medicine is loved,

there is also a love of humanity.»

- Hippocrates-

nova

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