

Tissue processing

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Tissue processing

- describes the steps required to take animal or human tissue from fixation to the state where it is completely infiltrated with a suitable histological wax and can be embedded ready for section cutting on the microtome.
- types : manually or automated

Tissue processing steps

1-Fixation.

2-Sectioning

3-Dehydration.

4-Clearing (or de-alcoholisation).

5-Impregnation in wax (Wax infiltration).

6-Embedding in paraffin block.

7-sectioning and slide preparation

8- staining

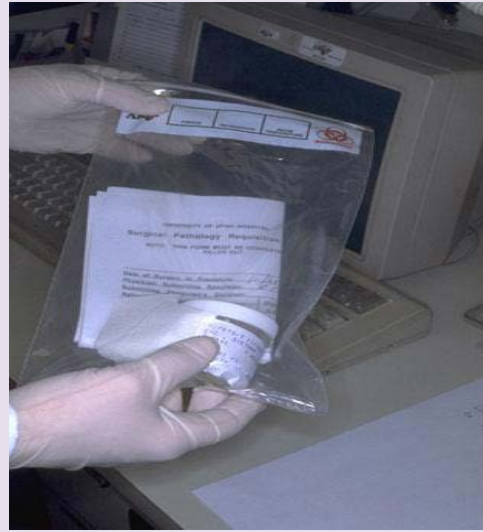
9- mounting

Fixation

- Aiming to preserve a sample of biological materials (tissues or cells) as close to its natural state as possible in the process of preparing tissue for examination.
- Factors affecting fixation:
PH , temperature, volume ,Time interval

Sectioning

Each organ or tissue has a special procedure in sectioning.



Dehydration

- This process must be carried out so that the embedding medium can infiltrate properly.
- Alcohol in various dilutions is usually used.
- water from the tissues should be removed because water is not miscible with wax
- prevent tissue shrinking.

Clearing

- As paraffin wax is insoluble in alcohol, it must be replaced by a fluid that is miscible with or is a solvent of paraffin wax, to get rid of alcohol & make the tissue more translucent.
- **Xylol:** cheap and rapid in action, though tends to harden tissue on prolonged application

Impregnation (Wax infiltration)

- Tissues are impregnated in wax for two reasons:
 1. To surround tissue with some plastic substances to support it on all sides without injury.
 2. To enable natural cavities of tissue to be filled with wax, thus preserving their relationship to each other.
- Paraffin wax used for routine work
- Tissue may be impregnated with paraffin wax using:
 - 56 C° oven or
 - Vacuum embedding.

Paraffin for impregnation must be clean. (i.e. filtered).

Tissues which benefits most from vacuum embedding are:-

Lung, bone, fatty tissue, all tissues from CNS and lymph nodes.

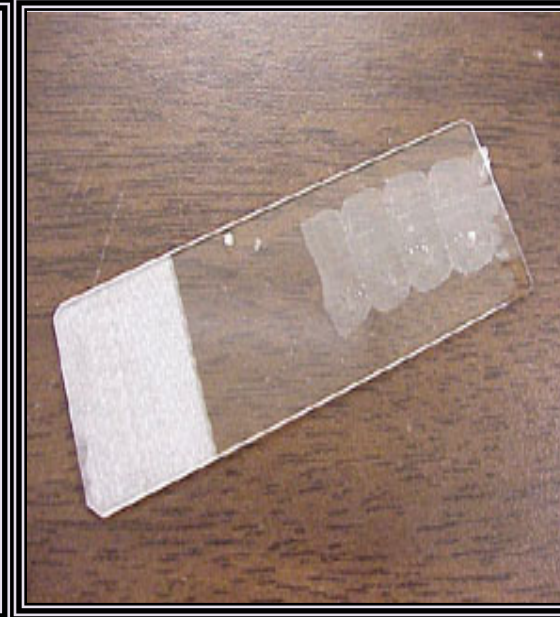
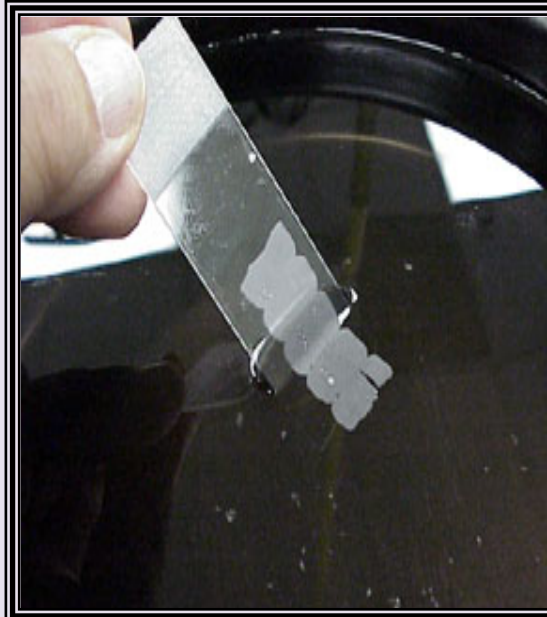
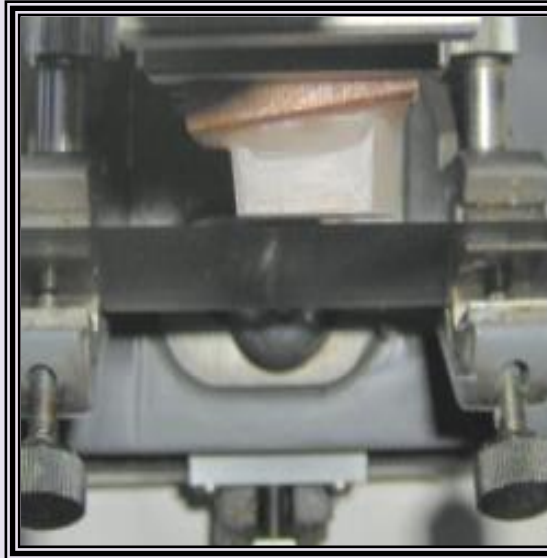
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Embedding

- Process by which tissues are surrounded by a medium wax which when solidifies will provide sufficient external support during sectioning.
- Impregnated tissue transferred from wax bath to a mould filled with molten wax to get a block of wax with the tissue specimen at the center with the cutting surface facing the base of the block
- This may be done using :
 - L-shaped brass boxes.
 - Embedding cassette

Sectioning

- Embedded tissues, to be cut into thin sections of 3- 5 μ with a microtome.
- Cut sections are, floated on a warm water bath
 - Helps to spread the specimen and remove wrinkles.
- Floated sections are picked up on an adhesive coated glass slide.
- Glass slide kept on a slide warmer at 58° temp for 20 min to ensure adhesion
- Egg albumin with additives
 - commonly used adhesive



Staining

- Biochemical technique of adding a class-specific dye to a substrate (DNA, proteins, lipids, carbohydrates) to qualify or quantify the presence of a specific compound
- Commonly used Staining procedures
 - Gram staining
 - Haematoxylin and eosin (H & E) staining
 - Papanicolaou staining (PAP)
 - PAS staining
 - Masson's trichrome staining

H & E stain/ Haematoxylin & Eosin stain

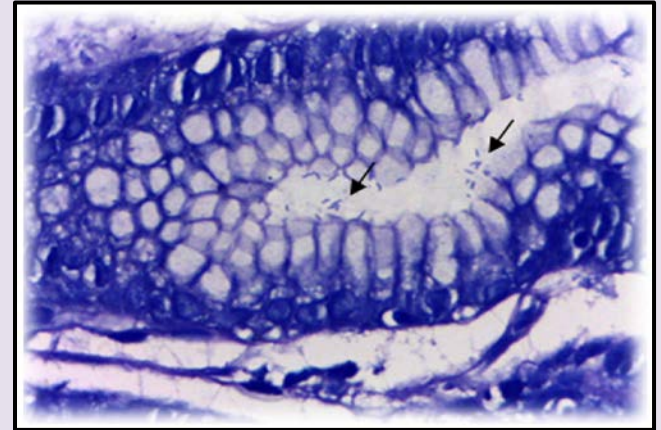
- Most popular staining method in histology.
- Most widely used stain in medical diagnosis.
- The staining method involves application of the basic dye haematoxylin, which colors basophilic structures with blue-purple hue, and alcohol-based acidic eosin Y, which colors eosinophilic structures bright pink.

Mounting

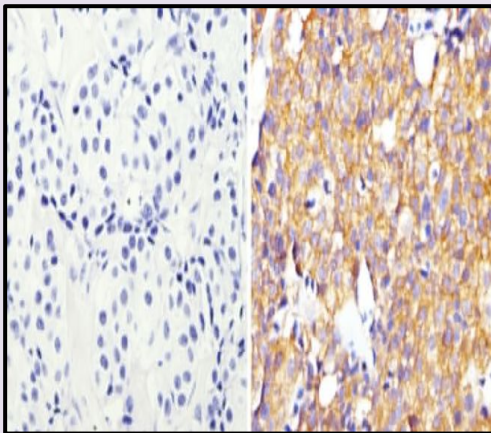
- The stained section on the slide must be covered with a thin glass coverslip to protect the tissue from being scratched, to provide better optical quality for viewing under the microscope, and to preserve the tissue section for years to come
- Mounting medium is used to adhere the coverslip to the slide
- DPX & Canada balsam commonly used

Special techniques

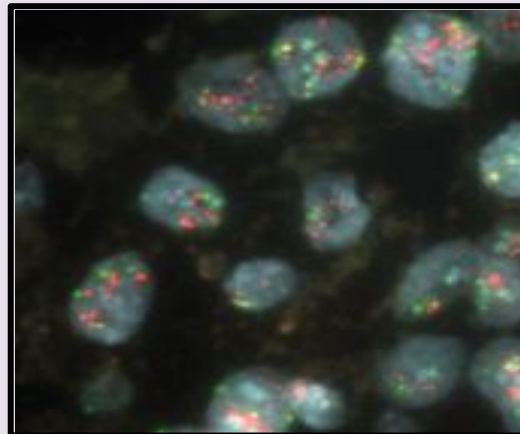
- Immunohistochemistry
- Molecular pathology
- Microbiology



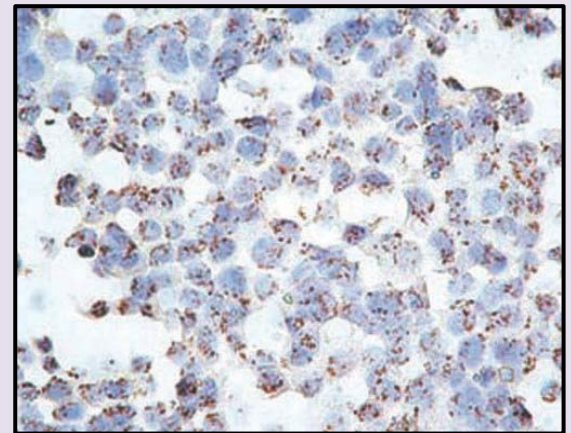
GIEMSA STAIN FOR *H. PYLORI*



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Thank you