



HISTOPATHOLOGY - FIRST COURSE

LAB -11- THE HEMATOXYLINS AND EOSIN

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قسم تقنيات المختبرات الطبية

Third Stage

المرحلة الثالثة

محاضرة رقم 11

Lecture No. 1

الجانب العملي

Practical

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TISSUE PREPARATION AND PREREQUISITES FOR H&E

Before H&E staining can even begin, the tissue must be meticulously prepared.

Errors in these steps are the most common cause of poor staining quality.

A. Fixation : The process of preserving tissue in a life-like state immediately after excision.

Fixative: 10% Neutral Buffered Formalin (NBF) is the standard.

Importance: Stops autolysis (self-digestion) and bacterial decomposition, and

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hardens the tissue. Poor fixation leads to artifactual staining (e.g., washed-out

B. Processing

Dehydration: Removing water by passing tissue through ascending concentrations of alcohol (e.g., 70% $\rightarrow$ 95% $\rightarrow$ 100%).

Clearing: Replacing alcohol with a substance miscible with wax (e.g., Xylene). This step often makes the tissue look translucent (clear).

Infiltration/Embedding: Immersing the cleared tissue in molten Paraffin Wax to provide structural support for sectioning

C. Sectioning :The tissue block is cut using a microtome into

—extremely thin sections, typically 3 to 5 micrometers thick.

These thin sections are then floated onto warm water to flatten them and are picked up onto a glass slide.

6. Technical Variations: Progressive vs. Regressive Staining

The method of applying the Hematoxylin stain significantly affects the final result and is a critical choice in the lab.

A. Regressive Staining :This is the standard, most common method used in diagnostic labs (as detailed previously).

Methodology:

1. **Over-stain** the tissue with Hematoxylin (nuclei, cytoplasm, and background are all dark blue).
2. Use a mild **differentiating agent** (Acid-Alcohol) to *selectively remove* the excess stain from the weakly-bound sites (cytoplasm/background), leaving only the tightly-bound stain in the nuclei.
3. Follow immediately with the **bluing** step.

Principle of H&E Staining: Basophilia and Acidophilia

The mechanism of H&E staining is based on the **chemical attraction between** the dyes and **specific cellular/extracellular components**, determined by their electrical charges.

A. Hematoxylin (The Basic Dye)

- **Staining Component:** Hematoxylin, derived from logwood, is oxidized to **Hematein**. **Hematein**, combined with a mordant (typically aluminum ions), forms a positively charged complex called Hemalum.
- **Chemical Nature:** **Hemalum acts as a basic (cationic) dye.**

- **Target Structures (Basophilic):** It binds to negatively charged **(anionic)** structures, which are referred to as **basophilic** (base-loving). These structures are rich in nucleic acids.

- **Nuclei (DNA):** Stained a **blue-purple** color.
- **Ribosomes and Rough Endoplasmic Reticulum (RNA):**
Stained blue.

Conclusion: The Importance of H&E

H&E staining is more than just a technique; it is the **foundation of histopathological diagnosis**. It provides the fundamental visual information necessary for comparing normal architecture to pathological changes. Its robust nature, reliability, and cost-effectiveness ensure its continued role as the single most important stain in the pathology laboratory.

Would you like me to elaborate on **the chemical formulas** of the dyes, or discuss how **H&E is used as a screening tool** before special stains?

References

- [1] Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. 7th ed. Churchill Livingstone; 2013.
- [2] Rosai J. Rosai and Ackerman's Surgical Pathology.

Any questions?

*Thank
You*