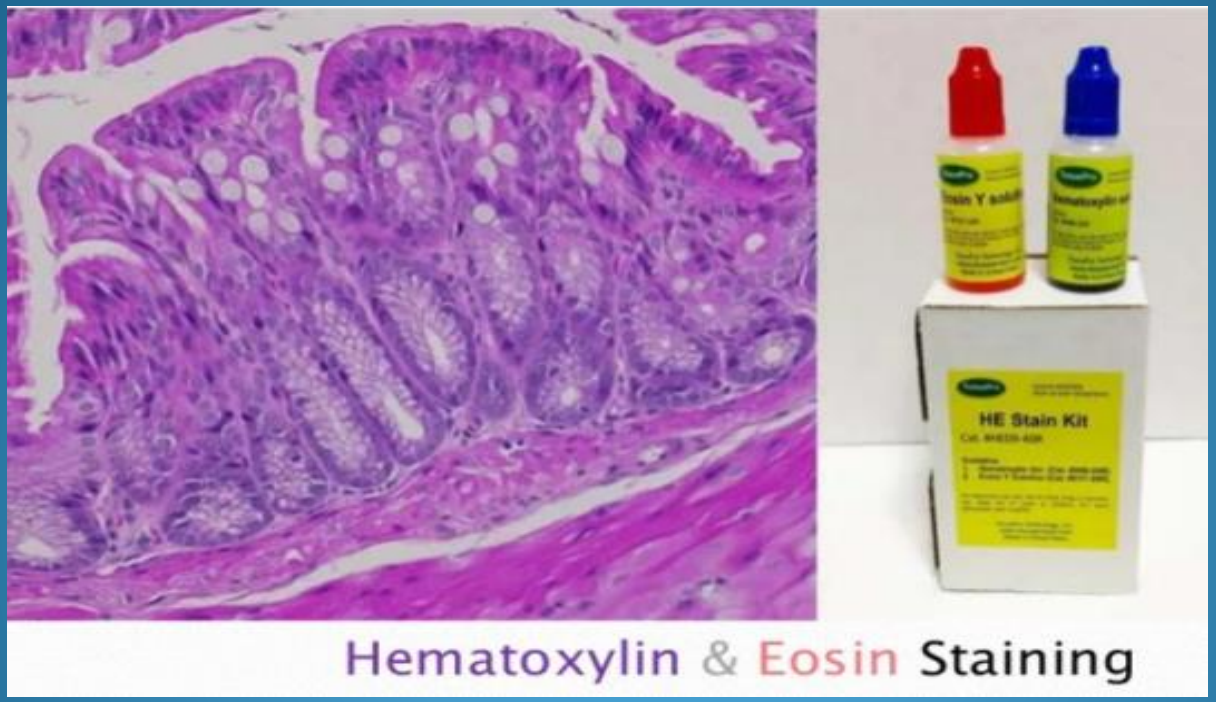

BY-

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MD PATHOLOGY cont.....

EOSIN

- Xanthine dyes which stains connective tissue and cytoplasm in varying intensity and shades (red to pink).
- Available in the following types :
  - Eosin Y (Eosin Yellowish, Eosin water soluble) – most widely available.
  - Ethyl Eosin (Eosin S, eosin alcohol soluble).
  - Eosin B (Eosin Bluish, Erythrosine B).
- Ethyl eosin and eosin B are now rarely used, although occasional old methods specify their use – e.g the Harris stain for Negri bodies.

EOSIN

Eosin Y

- Most commonly used eosin.
- Readily soluble in water.
- Satisfactorily soluble in alcohol.

- Preparation
  - Eosin Y, water soluble 5 gm
  - Distilled water 1000 ml

  - Crystals of Thymol added to inhibit fungal growth.

  - Addition of little acetic acid (0.5 -1000 ml stain) sharpens the staining.

The Hematoxylin and Eosin Staining Technique

Principle

- Hematoxylin and Eosin are principle stains used for demonstration of nucleus and cytoplasm.
- Alum acts as a mordant and the hematoxylin containing alum stains the nucleus light blue which turns red in the presence of acid.
- The cell differentiation is achieved by treating the tissue with acid solution.
- The counterstaining is performed using eosin which imparts pink color to cytoplasm.

The Hematoxylin and Eosin Staining Technique for Paraffin Sections

1. **REMOVAL OF WAX.**
2. **HYDRATION WITH GRADED ALCOHOLS.**
3. **STAINING.**
4. **DIFFERENTIATION**
5. **BLUEING**
6. **COUNTERSTAIN WITH EOSIN**
7. **DEHYDRATION THROUGH GRADED ALCOHOL.**
8. **CLEARING IN XYLENE**
9. **MOUNTING UNDER A COVER SLIP.**

The Hematoxylin and Eosin Staining Technique for Paraffin Sections

REMOVAL OF WAX

- Removal of wax with xylene (impermeable to stains).
- 2-3 minutes of xylene immersion sufficient for sections of 10μ thickness.
- Facilitated by warming the slides at 60 degrees oven to melt the wax.

The Hematoxylin and Eosin Staining Technique for Paraffin Sections

HYDRATION WITH GRADED ALCOHOLS

- Sections are transferred to absolute alcohol for 1-2 minutes - til it becomes opaque.
- Sections rinsed in 2nd bath of alcohol, drained and taken to water.
- Any pigments or deposits should be removed at this stage.

The Hematoxylin and Eosin Staining Technique for Paraffin Sections

STAINING

- Slides immersed in hematoxylin (Mayers / Harris/ Gills)
- If regressive stain is used, longer time is required to overstain the structures

The Hematoxylin and Eosin Staining Technique for Paraffin Sections

DIFFERENTIATION

- Sections are dipped in acid alcohol, agitated and washed in tap water.

- Observed under microscope
- If underdifferentiated – returned to acid alcohol.
- If overdifferentiated – returned to hematoxylin and differentiation repeated.

The Hematoxylin and Eosin Staining Technique for Paraffin Sections

BLUEING

- Slides after draining off hematoxylin is transferred to ammonia water for 2 minutes.
- Section when removed from hematoxylin or acid alcohol are pink in color.
- Washing turns them blue → Blueing.
- Blueing solutions are usually preferred alkaline .

  a)Ammonium hydroxide in 70% alcohol

  b) lithium carbonate stock solution

  c) Scott's tap water
  - ✓ Magnesium sulfate ($MgSO_4$) 30.0 gm
  - ✓ Sodium bicarbonate 2.0 gm
  - ✓ Tap water 3000.0 ml

  d) tap water pH – 7

COUNTERSTAIN WITH EOSIN

- Transfer the slides to 1% aqueous eosin for 2 minutes.
- Wash in running water.

The Hematoxylin and Eosin Staining Technique for Paraffin Sections

DEHYDRATION THROUGH GRADED ALCOHOL.

- After staining the sections are transferred to 90% alcohol and agitated for 10 seconds followed by
- then to absolute alcohol 1for (10-15 sec) followed by
- absolute alcohol 2 for 30 sec.

The Hematoxylin and Eosin Staining Technique for Paraffin Sections

CLEARING

- Slides are transferred to xylene 1 and left until completely clear . It should be tested for clarity.
- Then its transferred to xylene 2 which they be mounted.

The Hematoxylin and Eosin Staining Technique for Paraffin Sections

MOUNTING

- Its required to maintain high refractive index necessary for microscopy and to protect the sections during storage.

- METHOD :
  A drop the mountant is placed on the section , place the convenient sized coverslip into position.
  (Air bubbles may be removed by gentle pressure on the coverslip)

- MOUNTANTS :
- Aqueous mountants
- Resinous mountants

AQUEOUS MOUNTANTS

- USES :
  - To be used with metachromatic dyes.
  - Standard mountant for fat tissues.
- Low refracting index of 1.4 – 1.42.
- Glycerin is added to prevent cracking and splitting of dyes.
- Bacteriostatic agent should be added (thymol).
- Types :
  - Gelatin media
  - Gum arabic media

    Apathy's medium – fluorescent microscopy

    Highman's modified apathy's medium

    Farrant's medium

    Fructose syrup

RESINOUS MOUNTANT

- Its composed of natural or synthetic resins.
- Stained preparations are most transparent when the refractive index is **1.54.**
- Natural resins :
  - Canada balsam dissolved in xylol to 55-70%.
  - Dammar balsam.
  - Colophonium resin - used in alcoholic solutions.
  - Terpene resin.
- Synthetic resins :
  - Plasticizers such as tricreysl phosphate or dibutyl phatalate is added.
  - Commonly used is kirkpatrick and lendrum's DPX

KIRKPATRICK & LENDRUM'S DPX

DIESTRENE PLASTIZISER XYLENE

Distrene 80	10 gms
Dibutylphthalate	5 ml
Xylene	35 ml

- The slides can be cleaned of excess mountant

RINGING MEDIA

- To coat the edges of coverslip , so that no air bubbles develop.
- Commonly used are :
  - Paraffin wax
  - Cement
  - Varnish

The Hematoxylin and Eosin Staining Technique for Paraffin Sections

Results

- Nuclei appear blue/black in color
- Cytoplasm appears in varying shades of pink
- Muscle fibers appear deep pink/red in color
- Red blood cells appear orange/red in color
- Fibrin appears deep pink in color.

The Hematoxylin and Eosin Staining Technique

Cytology smear staining method

1. **HYDRATION WITH GRADED ALCOHOLS.**
2. **STAINING.**
3. **DIFFERENTIATION**
4. **BLUEING**
5. **COUNTERSTAIN WITH EOSIN**
6. **DEHYDRATION THROUGH GRADED ALCOHOL.**
7. **CLEARING IN XYLENE**
8. **MOUNTING UNDER A COVER SLIP.**

The Hematoxylin and Eosin Staining Technique

Papanicolaou staining method

1. Remove polyethylene glycol fixative in 50% alcohol, 2 minutes.
2. Hydrate in 95% alcohol, 2 minutes, and 70% alcohol, minutes.
3. Rinse in water, 1 minute.
4. Stain in Harris's hematoxylin, 5 minutes.
5. Rinse in water, 2 minutes.
6. Differentiate in 0.5% aqueous hydrochloric acid,10 seconds ,approx.
7. Rinse in water, 2 minutes.
8. 'Blue' in Scott's tap water substitute, 2 minutes.

The Hematoxylin and Eosin Staining Technique

Papanicolaou staining method

9. Rinse in water, 2 minutes.
10. Dehydrate, 70% alcohol for 2 minutes.
11. Dehydrate, 95% alcohol, 2 minutes.
12. Dehydrate, 95% alcohol, 2 minutes.
13. Stain in OG 6, 2 minutes.
14. Rinse in 95% alcohol, 2 minutes.
15. Rinse in 95% alcohol, 2 minutes.
16. Stain in EA 50, 3 minutes.
17. Rinse in 95% alcohol, 1 minute.

The Hematoxylin and Eosin Staining Technique

Papanicolaou staining method

Results

- Nuclei appear blue/black in color.
- Cytoplasm (non-keratinizing squamous cells) appear blue/green in color.
- Keratinizing cells appear pink/orange in color.

Rapid Hematoxylin and Eosin Staining Technique for urgent frozen sections

1. Freeze suitable tissue block onto a chuck.
2. Cut cryostat sections at 3-6 µm thickness.
3. Fix section in 10% neutral buffered formalin at room temperature for 20 seconds.
4. Rinse in tap water.
5. Stain in double strength Carazzi's hematoxylin for 1 minute.
6. Wash well in tap water for 10-20 seconds.
7. Stain in 1% aqueous eosin for 10 seconds.
8. Rinse in tap water.
9. Dehydrate, clear, and mount.

The Hematoxylin Staining Technique in PAS Procedure

1. Dewax and hydrate paraffin sections, removing mercury precipitate if indicated.
2. Oxidize for 5 minutes in 0.5% aqueous periodic acid.
3. Rinse in tap and then in distilled water.
4. Place in Schiff's reagent for 15 minutes (10 minutes for frozen sections).
5. Rinse for 2 minutes in each of three changes of freshly made sulfite rinse.
6. Wash 5 to 10 minutes in running tap water.
7. Optional counterstain with Harris' hematoxylin for 1 -3 minutes or in light green (0.1% in 0.1% acetic acid) for 5-20 seconds. Light green is especially useful when searching for fungi.
8. If hematoxylin is used, differentiate by means of 3-5 quick dips in 1% acid alcohol, wash in tap water and blue in Scott's tap water substitute; then wash 5 minutes in running water.
9. Dehydrate, clear and mount.

The Hematoxylin Staining Technique
in
PAS Procedure

Results

- Nuclei appear blue in color.
- PAS +ve materials appear magneta (purple-red) in color.

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