HEMATOXYLIN & EOSIN TROUBLESHOOTING

Prepared by

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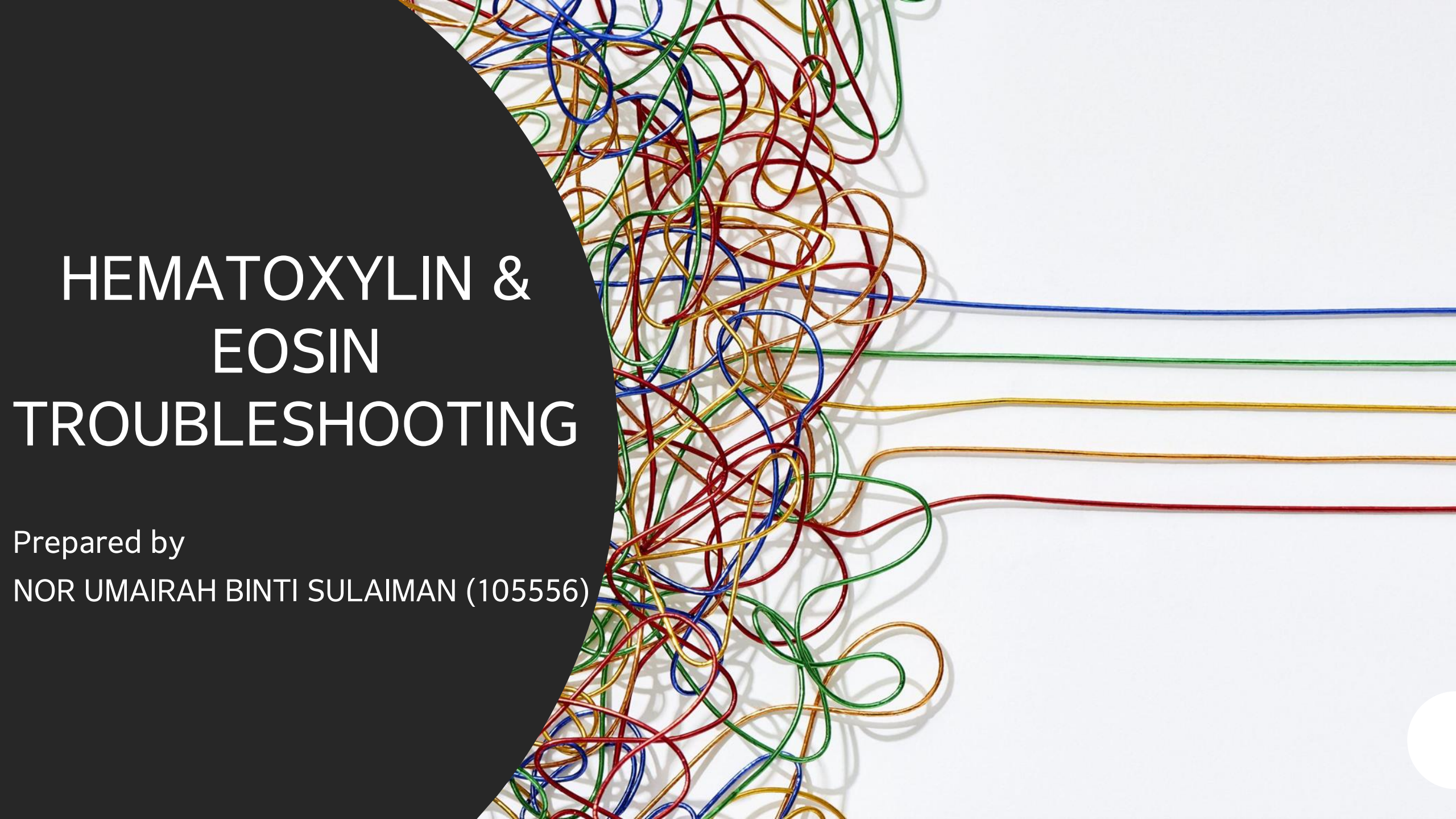


INTRODUCTION TO H&E STAINING

HEMATOXYLIN

- extracted from *Hematoxylon campechianum* tree
- Basic dye, positively charged
- Stain acidic (basophilic) structures of tissues
- Eg; chromatin, ribosomes and cytoplasmic region rich in RNA
- Purplish-blue

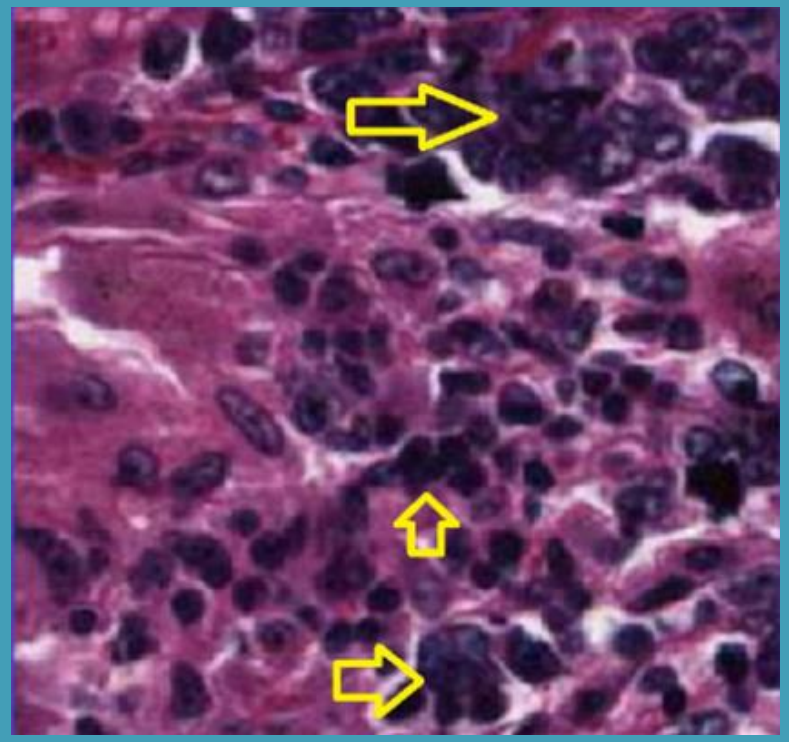
EOSIN

- synthetic dye produced by the action of bromine on fluorescein
- Acidic dye, negatively charged
- Stain basic structures (acidophils)
- Eg; RBCs, cytoplasm, muscle, collagen
- Varying intensities of pink

H&E TROUBLESHOOTING

PROBLEMS

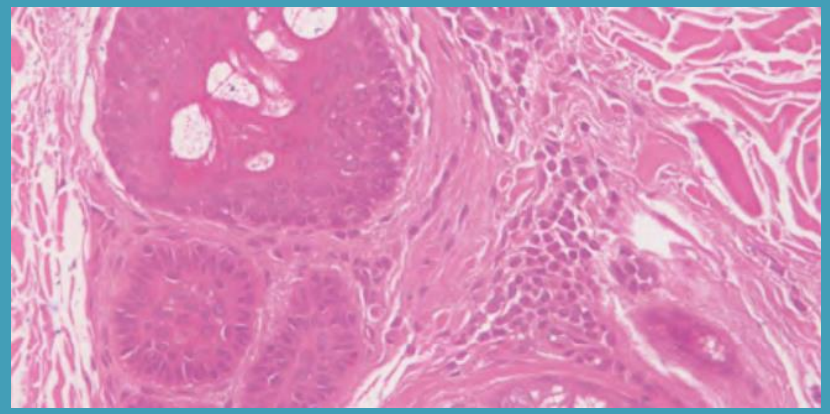
Nuclei not crisp no distinct chromatin pattern seen. There is no variation in the nuclear chromatin patterns among cells, and there is no variation in chromatin staining within one nucleus.



CAUSE	SOLUTION
• Poor or incomplete fixation • Inadequate time of fixation	i. Allow tissues to fix for a longer time. ii. Document time in fixative to assure adequate fixation. iii. Exchange the fixative in the containers at regular intervals throughout the fixation time
• Incomplete water removal during the processing dehydration and clearing	i. Ensure the dehydration and clearing are sufficient by reviewing processing schedule. ii. Ensure that processing solutions are rotated and/or exchanged on a schedule to prevent solution exhaustion.
• Exposure to excessive heat during dying	i. Ensure that tissues are not exposed to high temperatures, and if increased temperature is required that it be as low as possible and exposure time is limited. ii. Assess whether paraffin baths provide radiant heat to the retort. Adjust exposure times to counter increased heat.

PROBLEMS

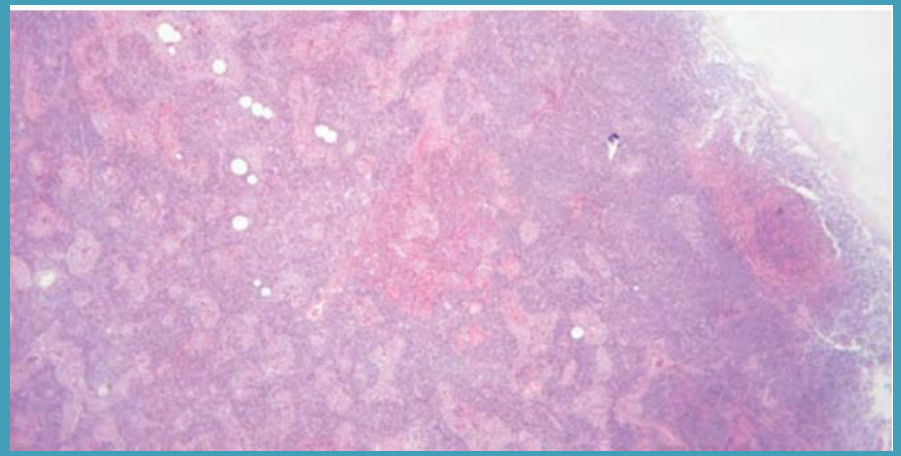
Poor contrast between the nuclear stain and the cytoplasmic stain



CAUSE	SOLUTION
• The nuclear stain is too dark	I. Decrease the intensity of the hematoxylin staining by decreasing the time in the hematoxylin solution II. Increasing the time in the differentiating solution
• Nuclear stain is too pale compared to the cytoplasmic stain.	I. Increase the intensity of the hematoxylin stain by: a) Increasing the time in the hematoxylin stain b) Decreasing the time in the differentiating solution
• The cytoplasmic stain is too dark compared to the nuclear stain	I. Decrease the intensity of the cytoplasmic stain by: • Decreasing the time in the stain • Diluting the eosin stain concentration
• The cytoplasmic stain is too light compared to the nuclear stain	I. Increase the intensity of the cytoplasmic stain by: • Allowing a longer time in the eosin stain • Decreasing the time in the 70% alcohol used for differentiation

PROBLEMS

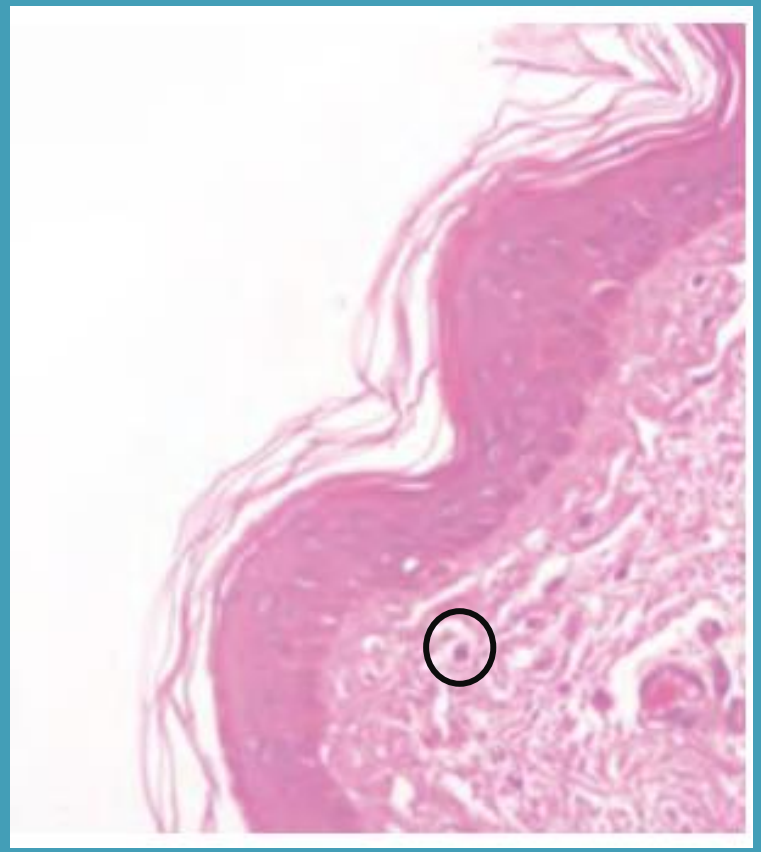
Uneven hematoxylin or eosin staining; the stain varies in intensity in different areas of the section.



CAUSE	SOLUTION
• The section may be thick and/or thin; chatters or venetian blind effect.	I. Recut the section, ensuring that the section is of uniform thickness
• The water rinse was not adequate after hematoxylin staining to remove excess hematoxylin. • The water rinse after acid alcohol was not adequate to stop differentiation of hematoxylin	I. Increase the time and/or fluid levels of water rinses
• The differentiation of eosin in the dehydration alcohol was not adequate	I. Adjust concentration of first alcohol to 70%. II. Increase time in differentiation/dehydration alcohols.

PROBLEMS

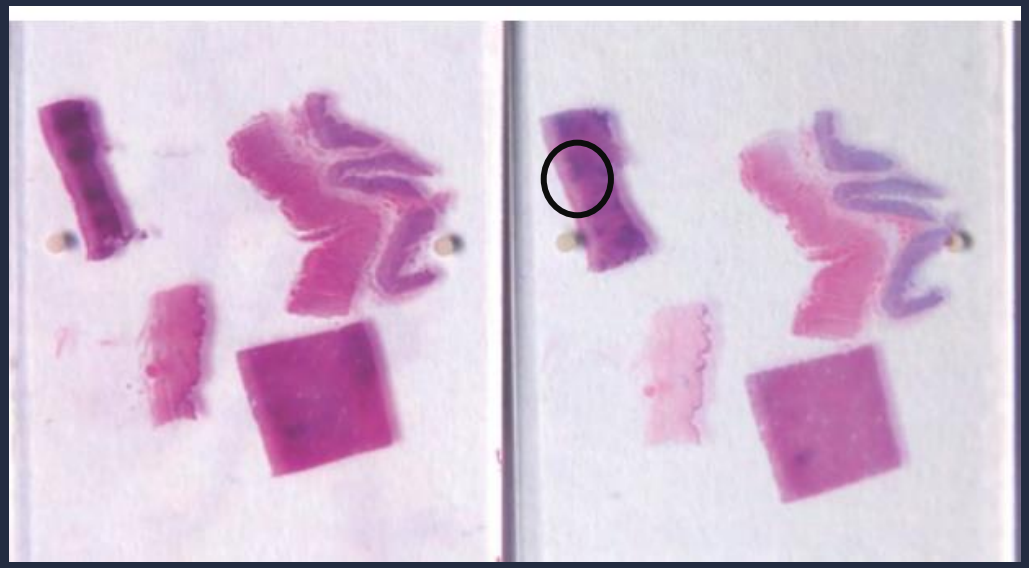
Red brown nuclei. The nuclear stain has a distinct red brown or reddish hue, often seen throughout the entire slide



CAUSE	SOLUTION
• The sections have not been sufficiently blued.	I. Increase the amount of time the sections remain in the bluing solution. II. Ensure the pH of bluing solution is minimally pH 7-8
• The hematoxylin is breaking down due to over oxidation of the hematein	I. Change to a fresh solution of hematoxylin. II. Check expiry date of stock solution.

PROBLEMS

5. Dark precipitate scattered throughout the section; blue-black or purple precipitate is present on parts of the section.



CAUSE	SOLUTION
• Deteriorated hematoxylin. Hematoxylin is used beyond the expiration date or damaged from improper storage conditions.	I. Change to a fresh solution of hematoxylin. II. Ensure proper storage of hematoxylin solutions according to manufacturer's guidelines. III. Check with supplier that hematoxylin was stored correctly during shipping. Excessive heat or freezing can cause solution to decompose.
• Some hematoxylin formulations form a metallic film on the surface of the solution, when exposed to air. This metallic film transfers or adheres to the surface of slide and the tissue section	I. Monitor the hematoxylin solution throughout the day for the appearance of a metallic sheen (hematein). If this is observed, replace or filter the hematoxylin, ensuring that the solution container is clean and free of deposits before reuse.