Alcian Blue

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Introduction:
Barrett’s esophagus is a condition that can lead to adenocarcinoma. Proper diagnosis and follow-up treatment is very important for patients diagnosed with Barrett’s esophagus. It can sometimes be difficult to diagnose. The Alcian Blue – H&E – Metanil Yellow (AB-H&E-MY) stain is very effective in distinguishing between Barrett’s and other gastrointestinal disorders. The stain is simple to perform and the results can easily be duplicated from one staining batch to another. The AB - H&E - MY stain is a very comprehensive stain that can be used for the diagnosis of Barrett’s esophagus.

Discussion:
The GI tract is composed of four distinct layers, the mucosa, submucosa, muscularis propria, and adventia. The epithelium of the GI tract varies distinctly as you transition from one area of the tract to the next, from the esophagus through the stomach and the intestine. The epithelium is very specific to the properties and purpose of each area of the tract. The esophagus is composed of stratified squamous epithelium that provides protection from the contact with the food we swallow. (Figure 3a & 4b) The patient was diagnosed with chronic active inflammation of non-dysplastic Barrett’s esophagus. The patient is recommended to undergo a repeat biopsy in two years.

3. A 65-year-old Caucasian female came to the emergency room presenting symptoms of diarrhea, bloody stool and tenderness in the lower abdominal area; the symptoms have been present for ten days and worsen after she eats. She had a history of dysphagia (difficulty swallowing), esophagitis (inflammation of the esophagus) and gastroesophageal reflux disease (GERD). The patient was scheduled for an endoscopic biopsy to rule out Barrett’s esophagus, and the biopsy was taken from the region of the GE junction. The H&E revealed esophageal mucosa of squamous epithelium that was negative for dysplasia. The gastric mucosa has a large amount of lymphatic infiltrate in the lamina propria. The AB – H&E – MY confirms the presence of focalized intestinal metaplasia. (Figure 5a & 5b) The patient was diagnosed with chronically inflamed, non-dysplastic Barrett’s esophagus.

Materials and Method
Tissue Preparation and Sectioning:
Hollandes fixative is the preferred fixative for gastrointestinal biopsies. Hollandes is a picric acid based fixative that is very effective in preserving the mucin in the tissue. However, since mucin does not break down as quickly as other carbohydrates, neutral buffered formalin can be used. Routinely processed paraffin sections are cut at 4 mm, and sections for the routine H&E and the AB-H&E-MY are cut at the same time.

Solutions:
3% Acetic Acid
Acetic acid 3.0 ml
Distilled water 97.0 ml
Stir together. Stable at room temperature for months.

Alcian Blue, pH 2.5
Alcian blue 1.0 g
3% acetic acid 100.0 ml
Thymol crystals
Dissolve alcian blue in acetic acid. Check pH; adjust the pH as needed using acetic acid to pH 2.5. Add a few crystals of thymol to prevent mold growth. Solution is stable at room temperature for months and may be
reused until weak.

Mayer Hematoxylin: Commercially made

0.25% Hydrochloric Acid
  Hydrochloric acid 2.5 ml
  Distilled water 997.5 ml
  Carefully add hydrochloric acid to the distilled water slowly. Stable at room temperature for months.

0.25% Ammonia Water
  Ammonium hydroxide 1.0 ml
  Distilled water 250.0 ml
  Slowly add ammonium hydroxide to distilled water. Use for one day only.

Eosin: Commercially made

0.25% Metanil Yellow
  Metanil yellow 0.25 g
  Distilled water 100.0 ml
  Glacial acetic acid 0.25 ml
  Mix together well. Stable at room temperature for up to one year

Method:
1. Deparaffinize and bring section to water
2. Stain with Alcian Blue, pH 2.5 solution 15 minutes
3. Wash well with water
4. Stain in Mayer Hematoxylin* 4 minutes
5. Rinse in running water, several changes
6. Differentiate in 0.25% hydrochloric acid 2-3 seconds
7. Rinse in running water, several changes
8. Blue in 0.25% ammonia water 2-3 seconds
9. Rinse well in running water, several changes
10. Place in 70% ethanol 1 minute
11. Stain with Eosin Solution 1 minute
12. Dehydrate in 95% ethanol 30-60 seconds
13. Dehydrate in 100% ethanol, two changes 30 seconds each
14. Place in Metanil Yellow solution** 1 minute
15. Rinse with ethanol, 2 changes 10 dips each
16. Clear with xylene, 3 changes 2 minutes each
17. Mount in a resinous medium

*For automated stainers, run a program on the stainer that takes the slides from water, through your routine H&E and stops at the second change of absolute ethanol.
** Timing of the Metanil Yellow is critical. If stained for too long, increased background staining will occur.

Results:
Nuclei - blue
Cytoplasm - pink-red
Mucin – Turquoise for Barrett’s Esophagus Goblet Cells (some gastric mucin will stain a faint blue)
Collagen - yellow
Smooth muscle - salmon

Conclusion:
When the three stains used in the AB – H&E – MY are combined, the stains yield dark blue nuclei, pinkish-red cytoplasm, turquoise mucin in Barrett’s esophagus vs. non staining/pale blue mucin of herniated tissue, yellow collagen and salmon smooth muscle. This stain gives the pathologist a colorful demonstration of the various components of the gastrointestinal tract with which to make a diagnosis. It is very useful in diagnosing Barrett's esophagus and distinguishing it from other GI conditions.

References:
Blount PL, Cowan DS, Reid BJ, Fred Hutchinson Cancer Research Center and the University of Washington:


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