**Alcian Blue-H&E-Metanil Yellow Stain - Technical Memo**

**APPLICATION:**

Newcomer Supply Alcian Blue-H&E-Metanil Yellow Stain is a useful diagnostic screening method for Barrett’s esophagus and distinguishing from other gastrointestinal conditions/disorders. The use of three separate stains and an eosin counterstain, provides a colorful combination of the gastrointestinal tract various components.

**METHOD:**

**Fixation:** Formalin 10%, Phosphate Buffered (Part 1090)

**Technique:** Paraffin Sections cut at 4 microns

**Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the provided staining procedure.

**STAINING PROCEDURE:**

1. If necessary, heat dry tissue sections/slides in oven.
2. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
   a. See Procedure Notes #1 and #2.
3. Place in Acetic Acid 3%, Aqueous (10017) for 3 minutes.
4. Move directly into Alcian Blue Stain 1%, pH 2.5 Aqueous (1003A) for 15 minutes.
5. Wash in running tap water for 5 minutes; rinse in distilled water.
6. Stain with Hematoxylin Stain, Mayer Modified (1202A) for 2 to 4 minutes.
7. Rinse in running tap water until water runs clear.
8. Blue in Scott Tap Water Substitute (1380); 2-3 dips.
9. Wash well in running tap water; rinse in distilled water.
10. Place in 70% ethyl alcohol for 1 minute.
11. Stain with Eosin Y Working Solution (1072A) for 1 minute.
12. Dehydrate in 95% ethyl alcohol for 30 seconds.
13. Dehydrate in two changes of 100% ethyl alcohol; 30 seconds each.
14. Place in Metanil Yellow Stain, Aqueous (12235B) for 1 minute.
   a. See Procedure Note #3.
15. Rinse/dehydrate in two changes of 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

**RESULTS:**

- Nuclei: Blue
- Cytoplasm: Pink-Red
- Mucin/Barrett’s esophagus goblet cells: Turquoise
- Gastric mucin: Some stain faint blue
- Collagen: Yellow
- Smooth Muscle: Salmon

**PROCEDURE NOTES:**

1. Drain slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during procedure.
3. If over-stained in Metanil Yellow Stain, Aqueous increased background staining will occur.
4. If using a xylene substitute, closely follow the manufacturer’s recommendations for deparaffinization and clearing steps.

**REFERENCES:**

3. Modifications developed by Newcomer Supply Laboratory.