**UW Medicine - Pathology**

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Alcian Blue Method for Mucosubstances pH 2.5 Procedure

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| Adopted Date: 06/20/05  Review Date: 09/03/10  Revision Date: 04/16/13 |

PURPOSE

To identify the method for performing the special stain of Alcian Blue Method for Mucosubstances pH2.5.

PROCEDURE

**Fixation:**

10% buffered neutral formalin.

**Sectioning:**

Paraffin sections at 5 microns.

**Solutions:**

(use Type II de-ionized water for all solutions**)**

**3% Acetic Acid**

Glacial acetic acid 3 ml

Distilled water 97 ml

Stable for six months. Discard after use.

**1% Alcian Blue**

Alcian blue, 8GX (CI# 74240) 1.0 gm

Glacial acetic acid, 3% 100.0 ml

Adjust the pH to 2.5. Filter and add a few crystals of thymol. Stable for two months.

**Nuclear Fast Red (Kernechtrot)**

Richard Scientific

**Procedure:**

Use control slide.

1. De-paraffinize and hydrate slides to distilled water.
2. Mordant in 3% acetic acid solution for 3 minutes.
3. Alcian blue solution for 30 minutes.
4. Wash in running water for 10 minutes.
5. Stain with Nuclear Fast Red for 5 minutes.
6. Wash in running water for 1 minute.
7. Dehydrate in 95% and absolute isopropyl alcohol.
8. Clear in xylene and mount.

**Results:**

Weakly acidic sulfated

mucosubstances,

hyaluronic acid, and

silomucins dark blue

Nuclei pink

Acid mucopolysaccharides blue

**Comments:**

Alcian blue 8GX is a water soluble phthalocyanin dye with a blue color because of the copper nucleus. It is a highly selective stain for acid mucopolysaccharides. The basis of its staining activity is not fully known but most likely occurs by salt linkage of the acidic groups of the carbohydrates with the dye.

Selectivity for the acid mucopolysaccharides depends on rather short staining. with longer staining, nearly every tissue component will become blue. With fixed tissue and paraffin sections, the alcian blue will stain connective tissue mucin and epithelial mucin, as well as acid mucopolysaccharides**.**

REFERENCES

Luna, Lee G: *Manual of Histologic Staining Methods of the AFIP*, The C.B. Mosby Co., 1980, pg. 95-96.

Written By: Director Approval:

(Signature and Date) (Signature and Date)

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Histology Supervisor