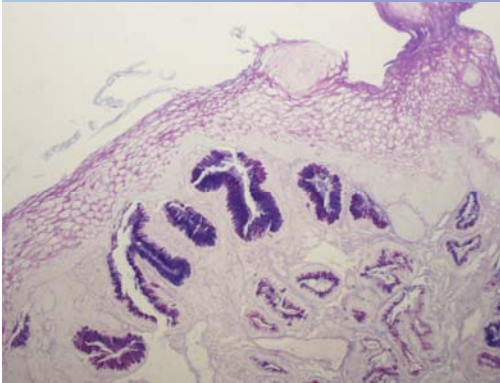


# MIKRO-GRAF

VOLUME 37, ISSUE 1

SUMMER 2008

## INSIDE THIS ISSUE:



Mixture of acidic and neutral mucins

From the President's Desk	2
Note from the Editor	
Spills, Kits and Safety	5
Hematoxylin Shortage?	10
MSH Convention Report	11
2008 MSH News	12
Student Spotlight	14
❖ Coverslipping	
❖ The Habit of Safety	
NSH Convention Update	16
Test Your Knowledge	
Officers & Chairpersons	27

## Muco...Poly...Glyco...What?!!

MICHELLE MARTIN, BS, HT(ASCP)QIHC  
HFHS WYANDOTTE HOSPITAL



Tech Points

**S**ulfated; carboxylated; proteoglycans; glycoconjugates; sialomucins; mucopolysaccharides; glycoproteins: all terms used to describe different types of mucin. In the most basic terms, mucin is the combination of a carbohydrate and a protein. It is produced in a cell by the endoplasmic reticulum and completed in the Golgi apparatus. Single types or mixtures of mucosubstances can be found within connective tissue such as collagen, reticulin and basement membrane. Mucin-rich epithelial cells are found in intestinal and lung goblet cells and mucin cells of the stomach. Mucin is either acidic (negatively charged) or neutral. The special stains used to demonstrate mucin react to this charge.

### ACIDIC MUCINS

Acidic mucins can be strongly or weakly sulfated, carboxylated sialomucins or sulfated sialomucins. Hyaluronic acid is also in the acidic mucin category.

**Strongly sulfated connective tissue mucins**, or **proteoglycans**, are produced by fibroblasts, endothelial cells, osteocytes, chondrocytes and mast cells. They can be differentiated by the presence of various amino acids along with tissue location and are grouped into the following subtypes:

- Chondroitin sulfate A*.....cartilage
- Chondroitin sulfate B*.....aorta, heart valves and the dermis
- Chondroitin sulfate C* .....cartilage, dermis and umbilical cord
- Heparin* ..... mast cells, aorta and cardiac connective tissue
- Keratin sulfate*.....cornea and aging cartilage

Proteoglycans react at low pH values with cationic (positively charged) dyes. Alcian blue pH 1.0 can be used to indicate this type of acidic mucin, which are usually Periodic Acid Schiff (PAS) negative (do not stain with PAS).

**Strongly sulfated epithelial mucins** or **glycoproteins** can sometimes be demonstrated in intestinal goblet cells. These mucins also react at low pH levels with cationic dyes, but differ from proteoglycans by being PAS positive. Mayer mucicarmine can be used to indicate epithelial mucins. To differentiate between epithelial and connective tissue mucins, testicular hyaluronidase is used to digest the connective tissue mucin. The epithelial tissue mucin will not be affected and can be stained with Alcian blue pH 1.0.

(Continued on page 3)

(Continued from page 1)

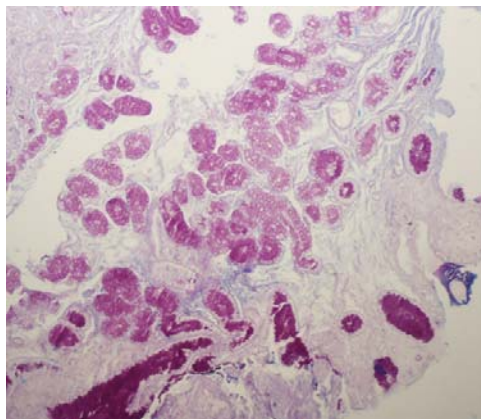
**Weakly sulfated epithelial mucins or sulfomucins** have a wide tissue distribution and are found in goblet cells. Sulfomucins react at a slightly higher pH level with cationic dyes. Alcian blue pH 2.5 and Mayer mucicarmine demonstrate weakly sulfated mucosubstances.

**Carboxylated sialomucins** are primarily epithelial in origin and are either enzyme-labile (easily broken down) or enzyme-resistant.

- ◆ **Enzyme-labile sialomucins** are widely distributed in bronchial submucous glands, submandibular salivary glands and goblet cells of the small intestine. These mucins are found alone or combined with other acidic or neutral mucins.
- ◆ **Enzyme-resistant carboxylated sialomucins** are less wide spread and are found in the mucosa of the large intestine, stomach and bronchus.

The enzyme sialidase is used to digest enzyme-labile sialomucins. The enzyme-resistant carboxylated sialomucins will not be affected and will stain with Alcian blue pH 2.5 and colloidal iron staining techniques. Enzyme-resistant sialomucins are PAS negative.

**Sulfated sialomucins** have been reported in prostatic carcinoma and malignant synovioma (tumor composed of cells similar to those that covering the synovial membrane). Like weakly sulfated mucins, sulfated sialomucins stain with Alcian blue pH 1.0.



**Neutral mucins stained with AB-PAS**

Fibroblasts produce the widely occurring connective tissue mucin

**hyaluronic acid.** Hyaluronic acid is found in synovial fluid and membranes, umbilical cord, early placenta, cardiac connective tissue and the dermis of the skin. Lacking in sulfate radicals this mucin reacts through carboxyl groups. The interaction of the carboxyl group in hyaluronic acid causes staining at pH levels similar to the sialomucins. To distinguish between hyaluronic acid and sialomucin, the enzymes hyaluronidase and sialidase are used respectively.

### NEUTRAL MUCINS

No acidic reactive groups are present in this type of epithelial mucin, which is found in Brunner's glands, most alimentary and respiratory tract goblet cells and prostatic glands. Neutral mucopolysaccharides will stain with PAS or the PAS portion of the Alcian Blue–Periodic Acid Schiff Hematoxylin (AB–PASH) stain.

### MUCIN STAINS

**Periodic Acid Schiff (PAS)** is based on the reactivity of free aldehyde groups of carbohydrates with Schiff reagent. Periodic acid oxidizes the glycol groups, found in some mucins, to free aldehydes. The aldehydes bind with the Schiff dye molecule, producing a compound that turns a bright magenta color when rinsed with tap water. PAS can be used to stain neutral mucosubstances, some epithelial sulfomucins and sialomucins. Ten percent neutral buffered formalin (10% NBF) is the recommended fixative and sections should be 4 to 5 micrometers thick. This stain cannot be used to demonstrate hyaluronic acid or chondroitin sulfate mucins. The strong negative charge from the ionized carboxylate and sulfate groups do not allow for periodic acid oxidation.

While mucopolysaccharides are a complex of different proteins and carbohydrates, tissues also contain simple polysaccharides and other carbohydrates. Glycogen, starch, chitin, and cellulose all stain PAS positive, and must be distinguished from the PAS positive neutral mucins.

- ◆ Glycogen and neutral mucins can be differentiated with the enzymes amylase or diastase. The enzyme will digest the glycogen and tissue will stain PAS negative. Enzyme-resistant neutral mucins will remain PAS positive.
- ◆ Starch from ingested food or glove powder contamination during tissue handling, will stain positively with PAS. Staining can be differentiated by its distinct appearance as polygonal bodies approximately 20 micrometers in diameter.
- ◆ Chitin, found in exoskeletons of insects, will appear as a foreign body and is easily distinguished from cellular components.
- ◆ Cellulose (the structural component of plants) is sometimes seen in the GI tract.

### ACIDIC MUCIN STAINS

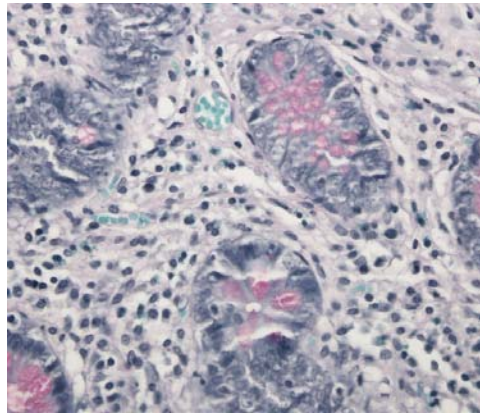
**Alcian Blue (AB)** stains acid mucins, but does not stain neutral mucins. It is a versatile yet specific stain that can be used at various pH's, combined with the PAS technique, or used with the enzyme hyaluronidase. Alcian blue dye, with its positively charged copper ion, is believed to form salt linkages with the acid (negatively charged) groups of the mucopolysaccharides. Tissues fixed in 10% NBF or Bouins fluid work well with this stain, and sections should be cut at 4 to 5 micrometers. *Alcian blue pH 2.5*, made with 3% acetic acid, stains weakly acidic sulfated mucosubstances, hyaluronic acid, sialomucins, sulfomucins and mast cell granules dark blue. Using 0.1N hydrochloric acid to make

(Continued on page 4)

(Continued from page 3)

the Alcian blue stain results in a pH 1.0 staining solution. *Alcian blue pH 1.0* stains sulfated substances a pale blue. To differentiate between epithelial and connective tissue mucins, the enzyme hyaluronidase can be added to the tissue sections to digest the mucosubstances that contain hyaluronic acid and chondroitin sulfates A and C. Two control sections should be used with this technique, one identified as "with digestion" and the other as "without digestion". The non-digested mucosubstances (acid mucopolysaccharides and sialomucins) stain deep blue. Finally, the Alcian blue technique can be combined with the PAS technique to stain both neutral and acidic mucosubstances. The *Alcian Blue-PAS* technique can be used with or without hematoxylin (*AB-PAS* or *AB-PASH*).

The Alcian blue stains the sialomucins, sulfomucins and proteoglycans blue. PAS stains the neutral mucins deep magenta. Structures will appear purple in the presence of both acid and neutral mucins, as in some goblet cells. Nuclei will stain blue if a hematoxylin counterstain is used.



Colloidal Iron

**Colloidal Iron (Muller-Mowry; Hanes)** demonstrates carboxylated and sulfated mucopolysaccharides and glycoproteins. Tissue sections are placed in a solution of ferric chloride, and the ferric iron is attracted to the carboxyl and sulfate groups of the acid mucins and the proteoglycans. The addition of potassium ferrocyanide to the tissue-bound ferric ions produces the blue coloration of the Prussian Blue reaction (ferriferrocyanide). The recommended fixative for the tissue is 10% NBF, but alcoholic formalin or Carnoy's solution can be used, while chromate fixatives should be avoided. Sections should be cut 4 to 5 micrometers. The acid mucins will stain bright blue. This technique can be combined with the PAS technique resulting in the neutral mucins staining a bright magenta color.

**Mucicarmine** is one of the oldest staining techniques and is used primarily for



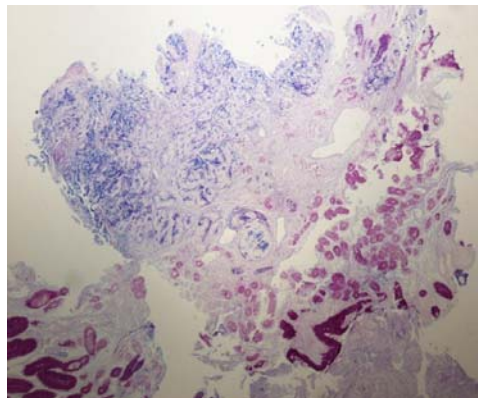
Mucins staining blue with AB-PAS

used as the hematoxylin counterstain, resulting is blue nuclear staining. Metanil yellow is sometimes used with this technique to color the background yellow.

### CLINICAL SIGNIFICANCE

Mucin, under normal circumstances is secreted as a lubricant to protect surfaces from friction and erosion. It is distributed throughout the epithelial and connective tissue. When produced in excessive amounts, several symptoms or diseases may occur.

- ◆ Overexpression of mucin in the lungs can mean a diagnosis of asthma, bronchitis, COPD or cystic fibrosis.
- ◆ Neoplasms with excessive amounts of mucin may be indicative of a carcinoma.
- ◆ Many adenocarcinomas, including cancer of the pancreas, lung, breast, ovary and colon, demonstrate an increased amount of mucin. A pathologist may request the Alcian Blue-PAS technique to determine the presence of mucin in a poorly differentiated adenocarcinoma.
- ◆ Other stains can be used to determine the type of mucin present in the carcinoma.



Invasive Adenocarcinoma

staining epithelial acid mucopolysaccharides. The dye molecule is an aluminum-carmic acid complex. This complex forms a dye lake complex that bonds to the acid mucins. Any well fixed tissue can be used and it should be cut 4 to 5 micrometers. The mucicarmine technique results in acid mucopolysaccharides, carboxylated sialomucins and sulfomucins staining a deep rose color. Nuclei stain black when Weigert hematoxylin is used as the counterstain. Aluminum hematoxylin, such as Mayer or Gill, can also be

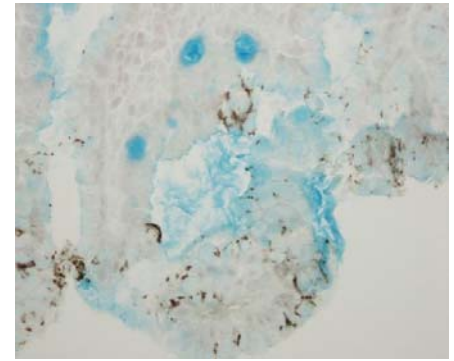
- ❖ Follicular mucinosis, pretibial myxedema, myxoid liposarcoma and some mesotheliomas have excessive hyaluronic acid.
- ❖ Excessive neutral mucins are symptomatic of some stomach carcinomas. Excessive sialomucins are found in some breast and colon carcinomas.
- ❖ Several mucins expressed in ovarian and breast carcinomas have been the focus of diagnostic and therapeutic studies.

(Continued on page 5)

(Continued from page 4)

- ◆ Barrett's esophagus occurs when the squamous epithelium in the lower esophagus becomes damaged due to repeated acid reflux from the stomach. When repaired, these cells are replaced with columnar epithelial cells similar to those found in the small intestine, which include goblet cells containing both sulfated (sulfomucins) and carboxylated (sialomucins) mucopolysaccharides. Alcian blue will demonstrate these mucopolysaccharides.

Mucins: glycoconjugates; proteoglycans; sialomucins; sulfated; carboxylated; mucopolysaccharides: all cellular secretions the human body needs to survive. Mucins lubricate and protect the eye, gastrointestinal tract, respiratory system and the reproductive system. Whether mucin appears blue (Alcian blue; colloidal iron) or red (Schiff's reagent; mucicarmine) the significance of the histotechnician's stain to the pathologist is critical to the healing of the patient.



*H. pylori* with Alcian blue counterstain

TYPE OF MUCIN		LOCATION	STAIN REACTIVITY
ACIDIC	Strongly sulfated connective tissue mucins or proteoglycans	fibroblasts, endothelial cells, osteocytes, chondrocytes, mast cells, cartilage, heart valves and aorta, dermis, umbilical cord, cornea	+ AB pH 1.0 - PAS
	Strongly sulfated epithelial mucins or glycoproteins	some intestinal goblet cells	+ PAS, + Mayer mucicarmine + AB pH 1.0
	Weakly sulfated epithelial mucins or sulfomucins	goblet cells	+ AB pH 2.5 + Mayer mucicarmine
	Carboxylated sialomucins	enzyme-labile: bronchial submucous glands, submandibular salivary glands, small intestine goblet cells	digested with sialidase - PAS
		enzyme-resistant: mucosa of large intestine, stomach, bronchus	+ AB pH 2.5 + Colloidal Iron
	Sulfated sialomucins	prostatic carcinoma, malignant synovioma	+ AB 1.0
	Hyaluronic acid	synovial fluid and membranes, umbilical cord, early placenta, cardiac connective tissue, skin dermis	digested with hyaluronidase + AB 2.5
NEUTRAL		Brunner's glands, alimentary & respiratory tract goblet cells, prostatic glands	+ PAS

**References**

Bancroft, J., Gamble, M: *Theory and Practice of Histological Techniques*. Churchill Livingstone, 2002.  
 Carson, Frieda: *Histotechnology, A Self Instructional Text*. ASCP Press, 1997.  
 Espinoza, C. Modified Mowry's Colloidal Iron Stain. Online image. Retrieved May 2008.  
 from: <http://www.medschool.lsuhsu.edu/pathology/pathist/SURGPATH/special%20stains/Pages/index.htm>  
 Grizzle, W., Fredenburgh, J., Myers, R.: *Advance Special Stains, Combination Stains and Troubleshooting*.  
 Kumar, V., Abbas, A., Fausto, N: *Robbins and Cotran Pathologic Basis of Disease*. Elsevier Saunders, 2005.  
 VanDeGraaff, K., Fox, S: *Concepts of Human Anatomy and Physiology*. Wm. C. Brown Publishers, 1992.  
 Wilkins, S. *Helicobacter pylori*. *MIKRO-GRAF*. 2008; 4:1,3-5.



***Earn 0.5 contact hours of continuing education by reading articles in the Michigan Society of Histotechnologists newsletter MIKRO-GRAF. MSH contact hours can be used for CMP required by ASCP BOR to maintain certification.***

*For previous TechPoint articles/tests, go to the MSH website: <http://www.mihisto.org> Click on Education*

It is the responsibility of the participant to retain their MSH CE certificates as proof of continuing education.

^ ^

**DATE OF ARTICLE:** Summer 2008  
**TITLE:** Muco. . .poly. . .glyco. . .What?  
**AUTHOR:** Michelle Martin, BS, HT(ASCP)QIHC

***DIRECTIONS:***

1. Answer the following questions by circling the one (1) BEST answer for each question.
2. Complete the information required at the bottom of the page.
3. Submit questions & check made out to "**MSH**" (in US funds) to: Peggy Wenk, HTL(ASCP)SLS, 3840 Elmhurst Rd., Waterford, MI 48328

***To earn Continuing Education credit from MSH, completed form must be submitted within three (3) years of original date of the article.***

1. Which of the following stains best demonstrates neutral mucins?  
A. Alcian blue  
B. Colloidal iron  
C. Mucicarmine  
D. Periodic acid-Schiff
2. The Colloidal Iron stain for acid mucins depends upon the **formation** of:  
A. Aldehydes  
B. Chondroitin sulfate  
C. Hyaluronic acid  
D. Prussian blue
3. All of the following organs have cells that routinely produce large amounts of acid and neutral mucopolysaccharides **EXCEPT:**  
A. Colon  
B. Kidney  
C. Lung  
D. Prostate
4. TRUE or FALSE (circle one): Acidic mucins can be stained with routine histology procedures because they have a lot of amino (NH<sub>3</sub>) groups.

***PLEASE PRINT NEATLY***                      DATE and YEAR *Completed/Submitted* Test: \_\_\_\_\_

NAME: \_\_\_\_\_

STREET: \_\_\_\_\_ APT. \_\_\_\_\_

CITY: \_\_\_\_\_ STATE: \_\_\_\_\_ ZIP: \_\_\_\_\_

PHONE: \_\_\_\_\_ Email: \_\_\_\_\_

\_\_\_\_ Yes      \_\_\_\_ No      I am a Michigan Society of Histotechnologists (MSH) member  
\_\_\_\_ Yes      \_\_\_\_ No      I have included a check made out to "**MSH**" in US funds  
   **\*\*FEE:**      \$5.00 for MSH members,      \$10.00 for non-MSH members  
\_\_\_\_ Yes      \_\_\_\_ No      I require a fee receipt for reimbursement from my employer

***Certificate documenting 0.5 hours MSH continuing education (CE) will be mailed to the participant within 4 weeks.***