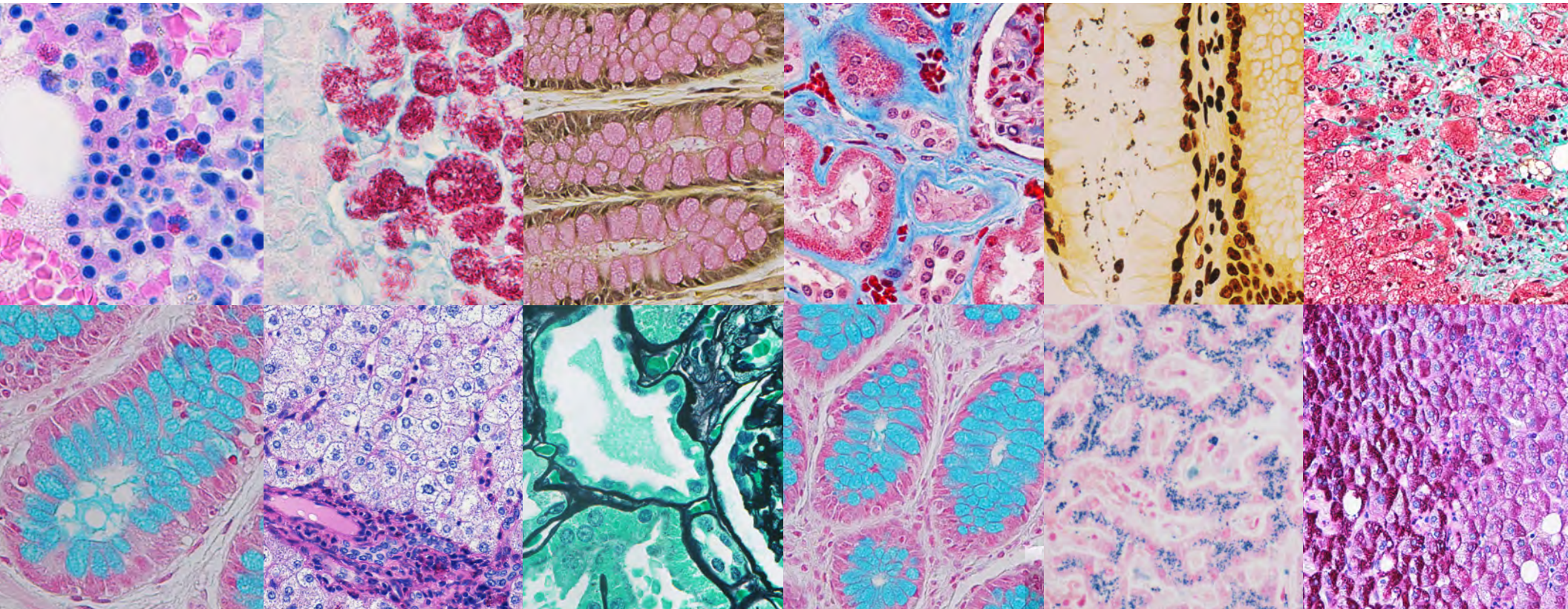
Special Stains

General Reference Guide



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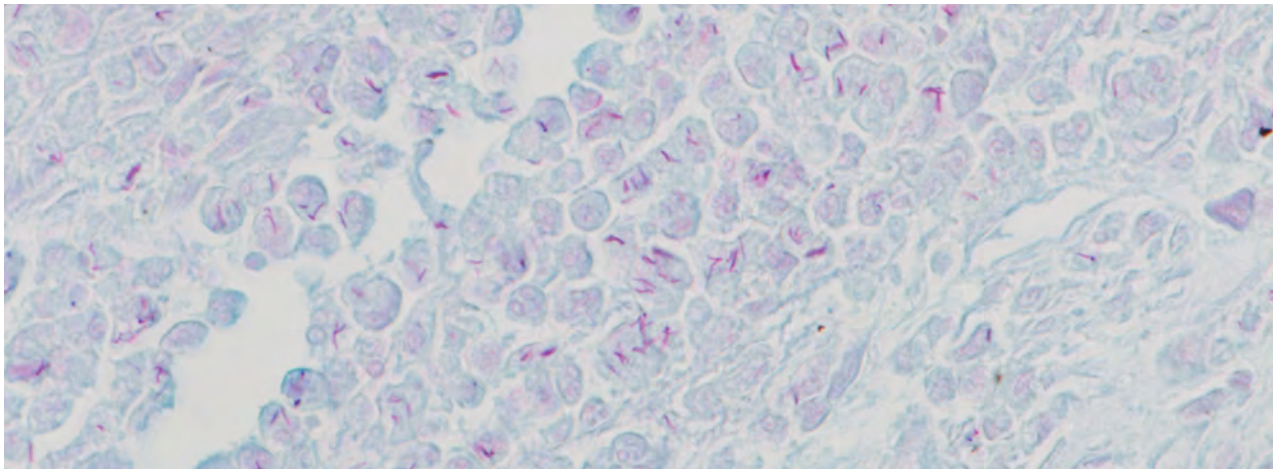

Figure 1. Lung tissue infected with AFB organisms, 600x.

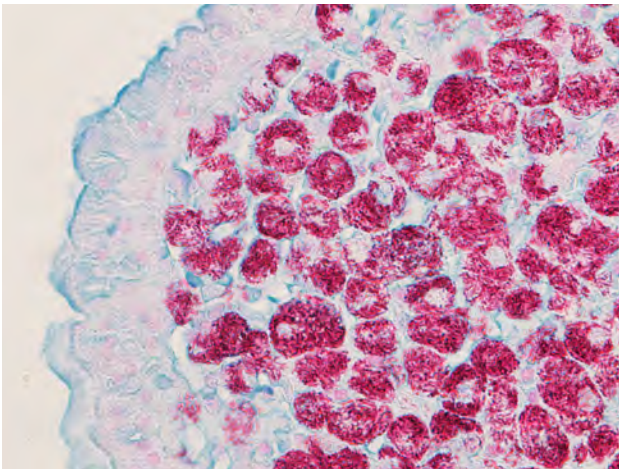

Figure 2. AFB organisms staining on infected colon, 600x.

Purpose

The AFB stain may be used to selectively demonstrate Mycobacteria and other acid-fast organisms or components in formalin-fixed, paraffin-embedded tissue.[1]

Staining principle

The staining reaction is based on the application of pararosaniline in phenol and alcohol (carbol fuchsin), which is taken up by the microorganisms and other tissue components. Decolorizer, an acid alcohol reagent, is applied to remove the color from all tissue elements. The organism's waxy capsule contains a lipid component which is resistant to decolorization by the acid solution (acid fast). The bacteria appear as red patches on lower magnification. At 100X, under oil, they appear as overlapping rod-shaped structures[2] that are sometimes described in literature as "Chinese sticks". Some types of acid-fast bacteria may appear pleomorphic depending on the type of organism and the plane of the section. [3]

Expected results

- Acid-fast bacteria — bright red
- Background — light blue

AFB

Stain basics

Common utility

AFB stain may be used in the visualization of acid-fast bacteria in diseases such as tuberculosis and leprosy.

Tissue controls

A known positive tissue control should be utilized for monitoring the correct performance of processed tissues and test reagents. Ideally, it should be representative of the tissues it is usually used to diagnose. An example of a positive control material would be formalin-fixed, paraffin-embedded human tissue positive for acid-fast bacteria.[3]

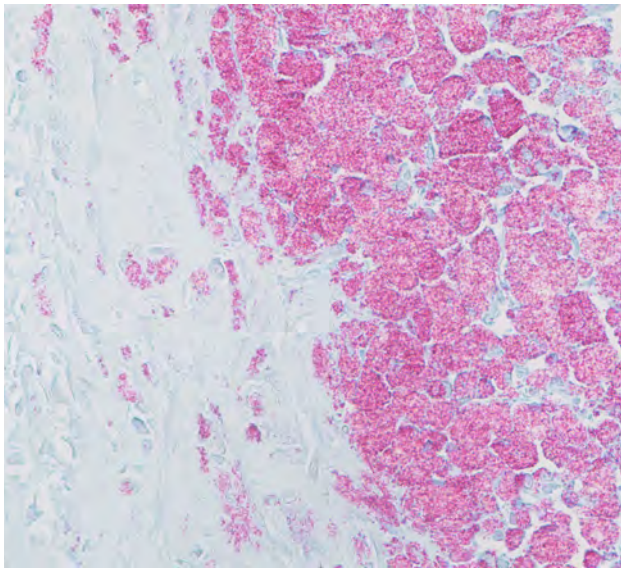

Figure 3. Lung tissue infected with AFB organisms, 400x.

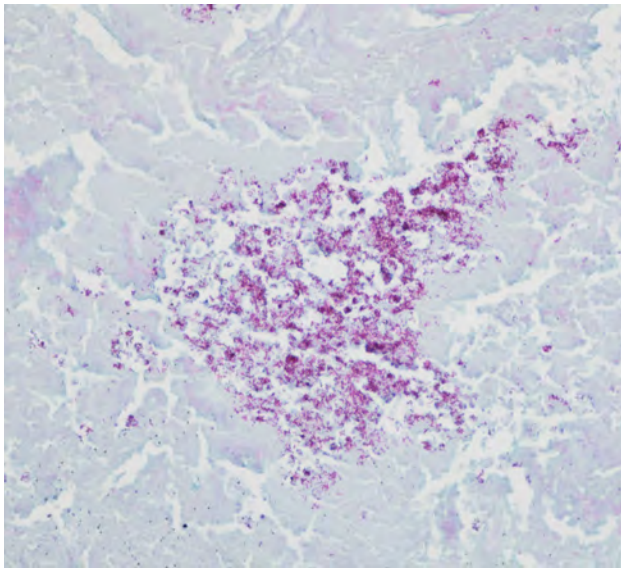

Figure 4. Lung tissue infected with AFB organisms, 200x.

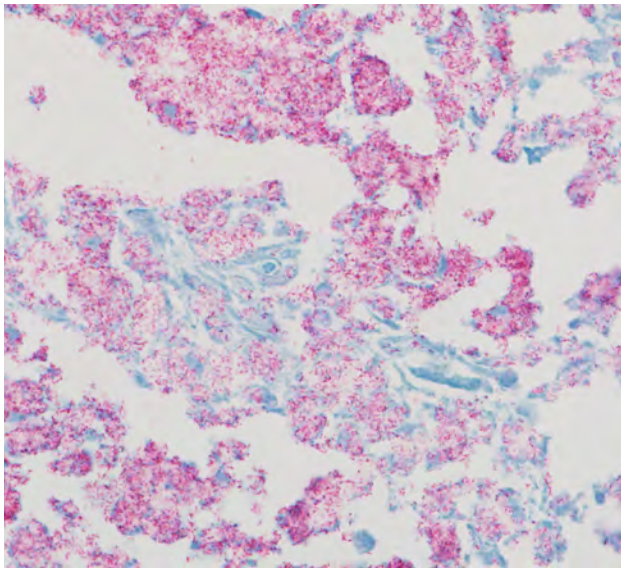

Figure 5. Stomach tissue infected with AFB organisms, 600x.

AFB

Technical notes and references

Technical notes

1. When staining for microorganisms, it is important to ensure that the water bath is scrupulously clean prior to sectioning in order to prevent introduction of extraneous microorganisms onto the specimen section slide. It is recommended not to use water left standing overnight.
2. Cut sections, usually 3–5 µm, and pick the sections up on glass slides.
3. Section thickness may affect quality and intensity of staining. Thinner sections (2-3 µm) may produce lighter staining, but individual bacteria may be easier to discern. Thicker sections (4-5 µm) may produce darker staining due the organisms being stacked upon one another, but individual bacteria may be more difficult to discern.

References

1. Sheehan DC, Hrapchak BB. *Theory and Practice of Histotechnology*. 2nd edition. St. Louis, MO: C.V. Mosby Company; 1980:235-237.
2. Bancroft JD, Gamble, M. T*heory and Practice of Histological Techniques*. 2nd edition. Edinburgh: Churchill-Livingston; 1982:244.
3. Carson F, Hladik C. *Histotechnology:A Self Instructional Text* 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.

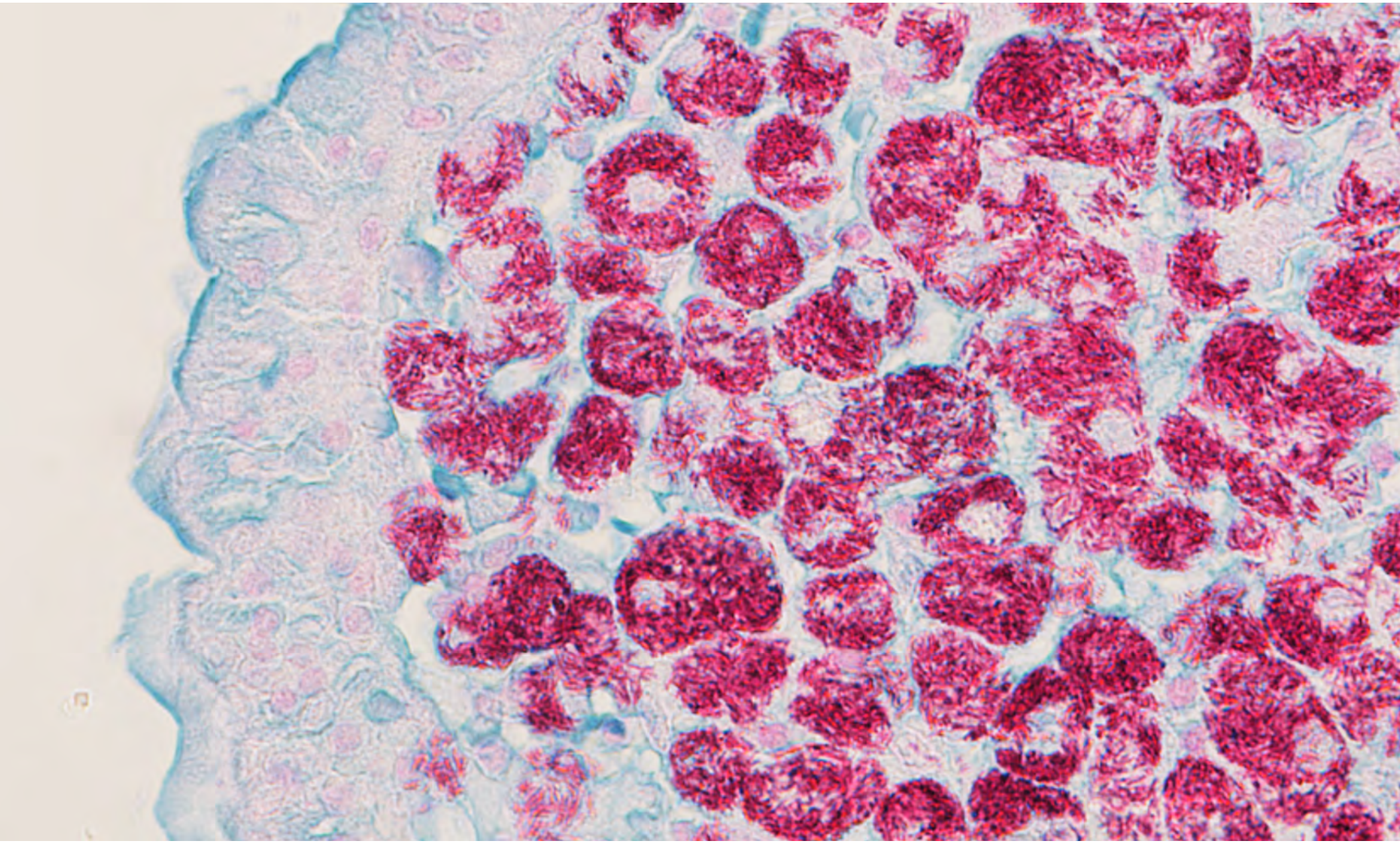

Figure 6. AFB organisms staining on infected colon, 600x.

Alcian Blue

Stain basics

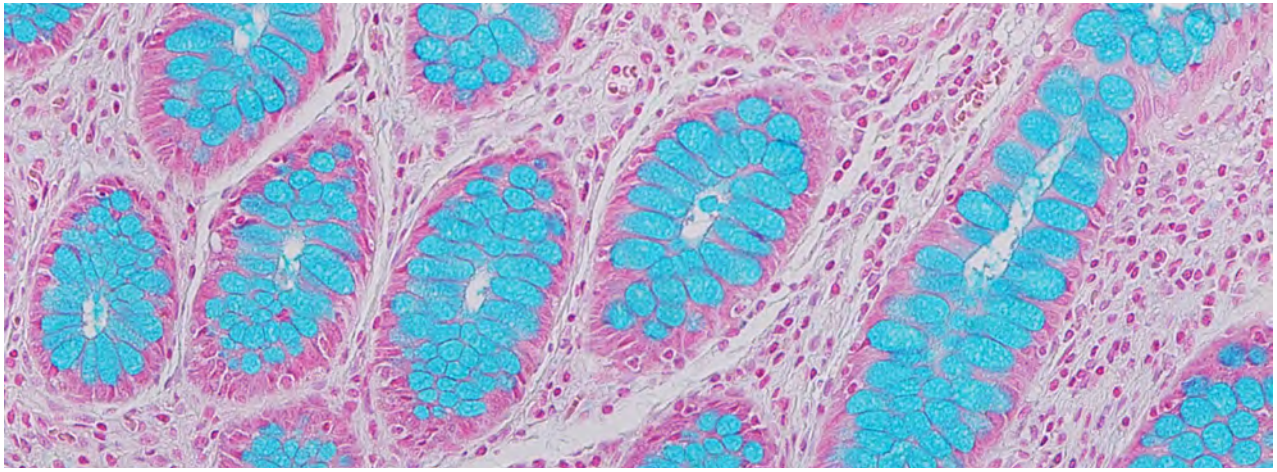

Figure 1. Colon stained with Alcian Blue, 200x.

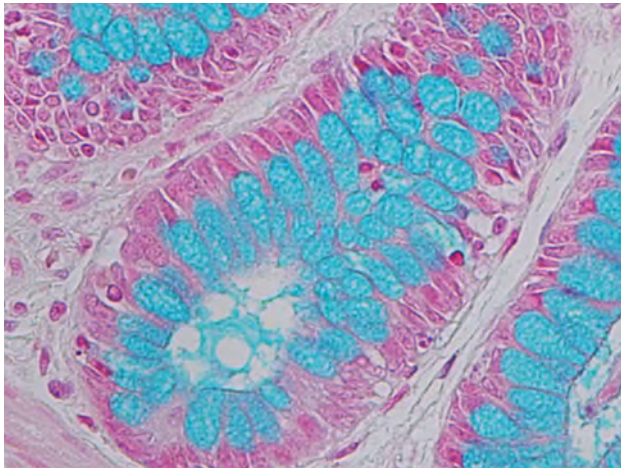

Figure 2. Colon stained with Alcian Blue, 400x.

Purpose

The Alcian Blue stain may be used as a qualitative histologic stain to demonstrate weakly acidic mucopolysaccharides. At pH 2.5, alcian blue stains sulfated and carboxylated acid mucopolysaccharides and sulfated and carboxylated sialomucins and glycoproteins in formalin-fixed, paraffin-embedded tissue.

Staining principle

Alcian dyes belong to the cuprophthalocyanine group, a group of water–soluble polyvalent basic dyes that are blue due to the copper in the molecule. At a pH of 2.5, alcian blue reacts with compounds containing polyanionic charges with sulfuric and carboxylic radicals. The dye appears to be bound by the formation of salt linkages with the acid groups of the acid mucopolysaccharides.[1,2]

Expected results

- Weakly acidic mucosubstances — bright blue/turquoise
- Nuclei stain — pinkish red
- Cytoplasm — pale pink

Alcian Blue

Stain basics

Common diagnostic utility

Alcian Blue may be used to detect cells expressing acid mucopolysaccharides in conditions such as Barrett's Esophagus or other disease processes, or the loss of such cells in certain disease processes in the gastrointestinal tract. The Alcian Blue stain may be helpful in detecting genetic disorders of acid mucosubstance metabolism, and collagen diseases where various acid mucosubstances are increased. The presence of acid mucosubstances tends to decline with advancing age.

Tissue controls

Known positive tissue controls should be utilized for monitoring the correct performance of processed tissues and test reagents. Ideally, it should be representative of the tissues it is usually used to diagnose. Some examples of components that stain positive with Alcian Blue are goblet cells, hyaluronic acid (found in umbilical cord and connective tissue in dermis), salivary glands and gastric lining cells. Tissues containing these components would be appropriate positive controls.

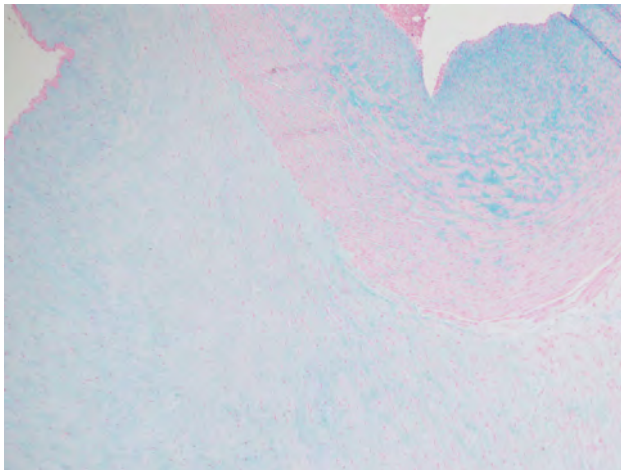

Figure 3. Umbilical cord stained with Alcian Blue. 400x.

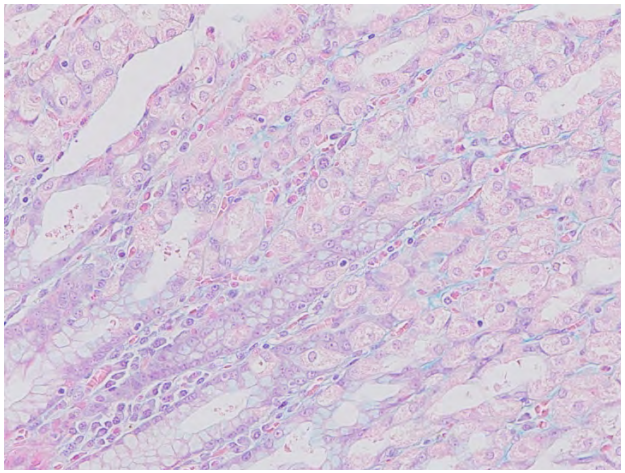

Figure 4. Stomach stained with Alcian Blue showing very weak staining of the gastric mucosa. 200x.

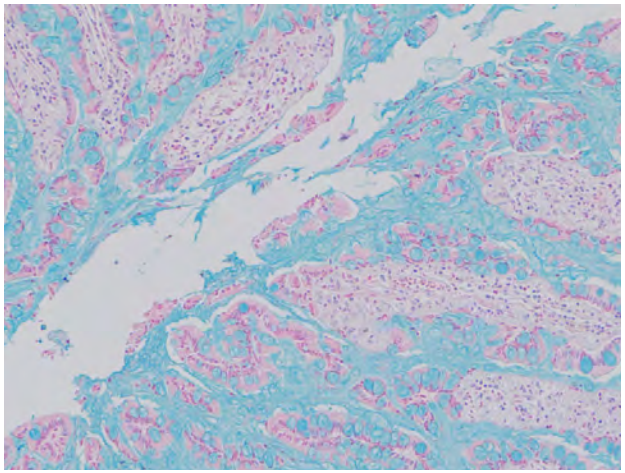

Figure 5. Duodenum stained with Alcian Blue showing strong staining of the goblet cell. 200x.

Alcian Blue

Technical notes and references

Technical notes

1. Section thickness may affect quality and intensity of staining. Cut sections, usually 3–5 µm, and pick the sections up on glass slides.
2. Alcian Blue stain is pH dependent. If the positive control does not stain appropriately, check the quality of the water used to prepare the wash solutions.
3. Make sure that your positive control tissue is known to express acid mucins

References

1. Sheehan DC, Hrapchak BB. *Theory and Practice of Histotechnology*. 2nd edition. St. Louis, MO: C.V. Mosby Company; 1980:172–173.
2. Carson F, Hladik C. *Histotechnology: A Self Instructional Text*. 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.

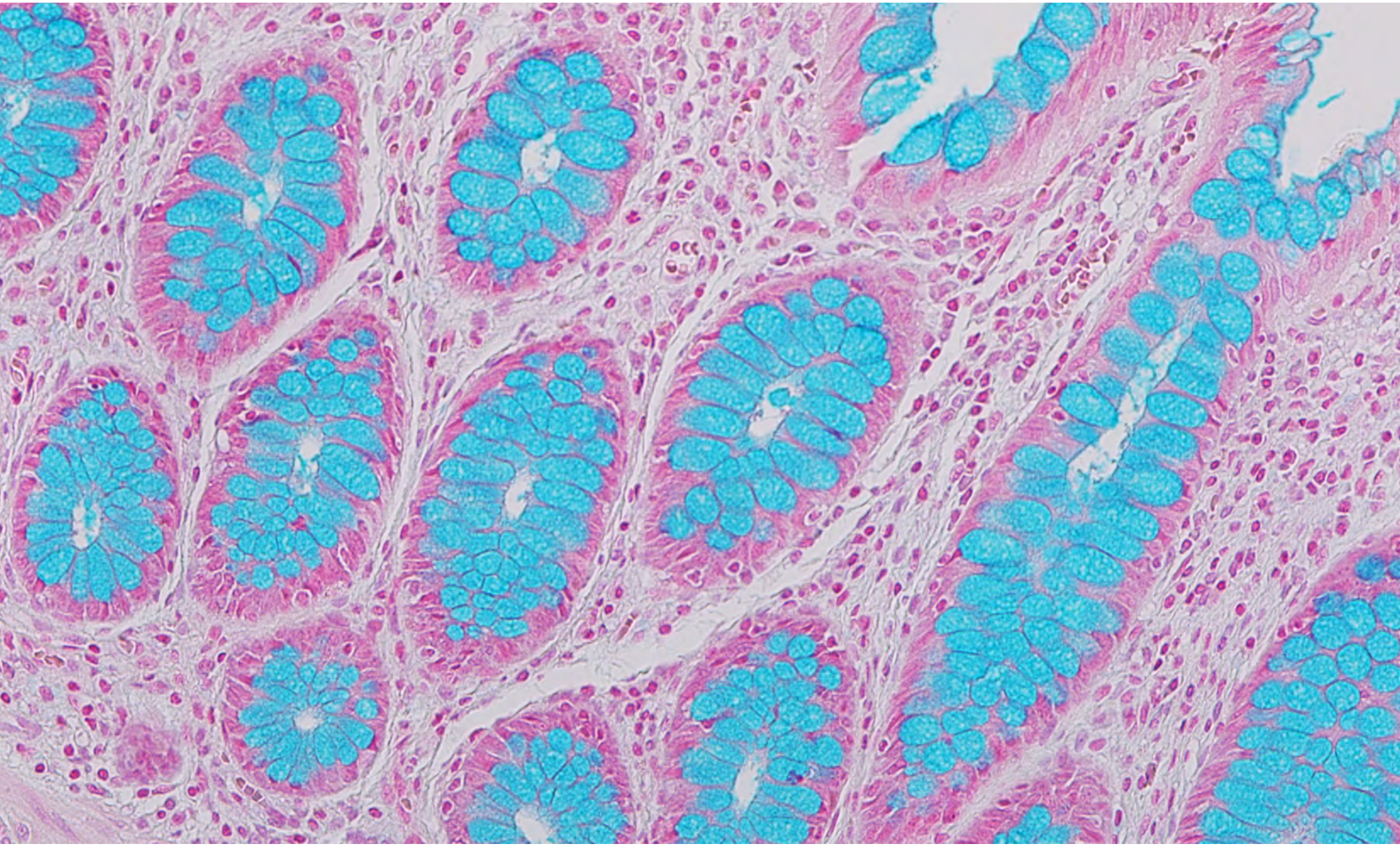

Figure 6. Colon stained with Alcian Blue, 200x.

Alcian Yellow

Stain basics

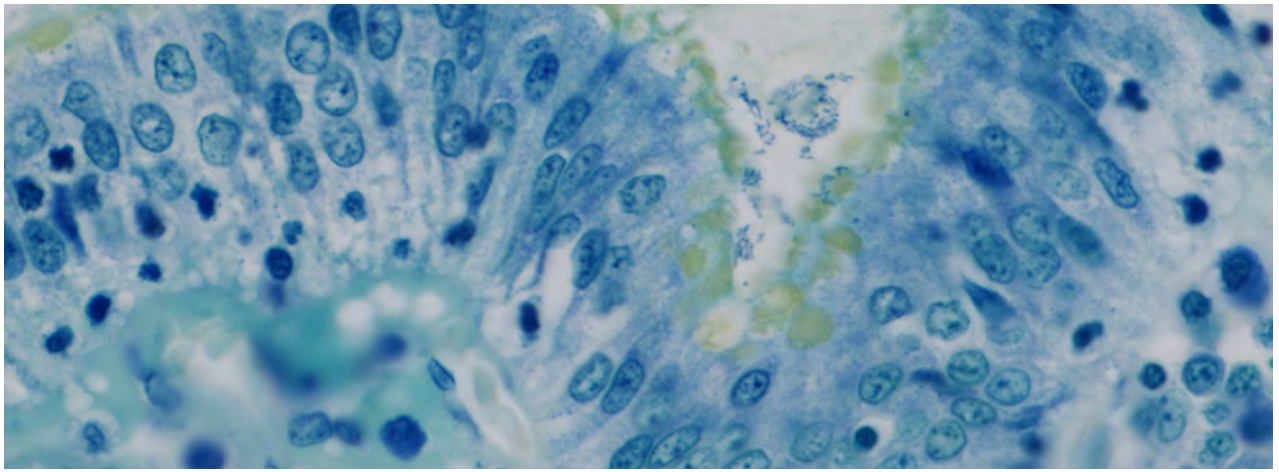

Figure 1. Gastric tissue with H. Pylori stained with Alcian Yellow. 600x.

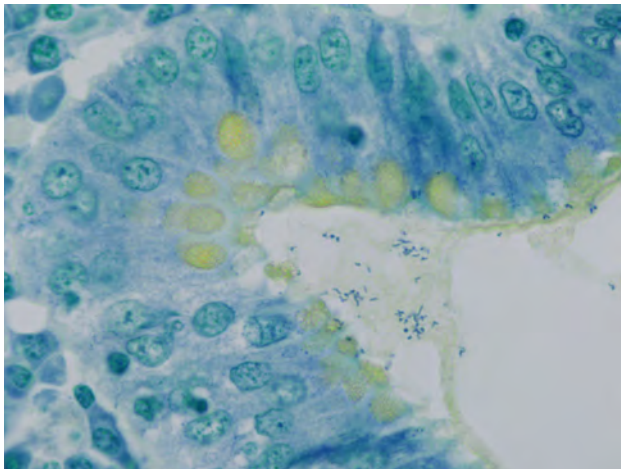

Figure 2. Gastric tissue with H. Pylori stained with Alcian Yellow. 1000x.

Purpose

Alcian Yellow is a qualitative histologic stain for Helicobacter pylori in formalin-fixed, paraffin-embedded tissue.

Staining principle

The gastric mucin is oxidized in aqueous periodic acid and then neutralized with sodium metabisulfite. This renders the subsequent staining with Alcian Yellow (a monoazo dye similar to Alcian Blue) resistant to staining by toluidine. Aqueous Toluidine Blue is then applied, which stains the microorganisms and other tissue components. The final result is blue bacteria on a yellow background. Staining the H. pylori organism with standard hematoxylin and eosin techniques produces a poor result and the special staining process can be lengthy.[1] The typical staining methods for H. pylori are Steiner and Romanowski-type stains.[2] Toluidine Blue has been used as a rapid method for the demonstration of these organisms, but the contrast is generally very poor.

Expected results

- H. pylori organisms — blue
- Background — blue
- Mucin — yellow

Alcian Yellow

Stain basics

Common diagnostic utility

Helicobacter pylori is a gram-negative bacterium that has been demonstrated as the causative organism in some gastric and duodenal ulcers. It is the most common cause of antral gastritis, and is associated with duodenal and gastric peptic ulcer disease, and with the development of distal gastric carcinoma and low grade MALT-type gastric lymphoma. The H. pylori organism has been shown to exist in the acid environment of the stomach and is capable of destroying the neutral mucin secreted by the surface epithelial cells.

Tissue controls

A known positive tissue control should be utilized for monitoring the correct performance of processed tissues and test reagents.[3] Ideally, it should be representative of the tissues it is usually used to diagnose. An example of a positive control material would be formalin-fixed, paraffin-embedded human gastric tissue, positive for H. pylori.[4]

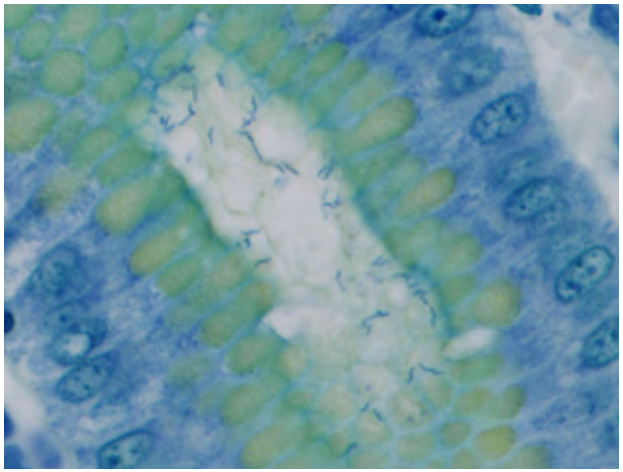

Figure 3. Gastric tissue with H. Pylori stained with Alcian Yellow. 600x.

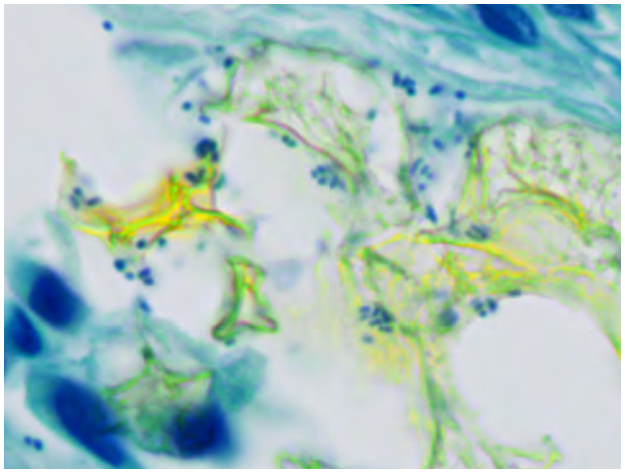

Figure 4. Gastric tissue with H. Pylori stained with Alcian Yellow. 1000x.

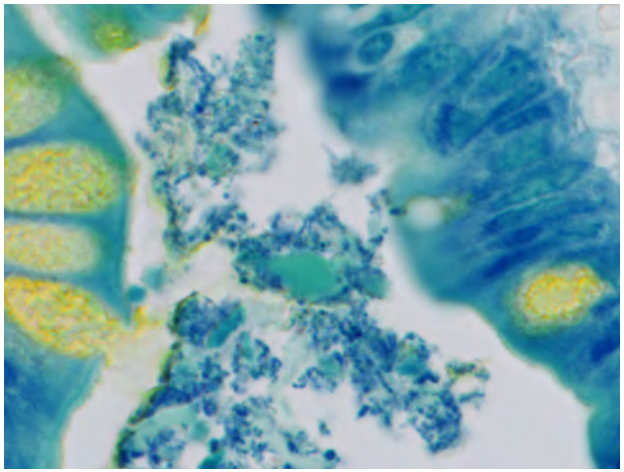

Figure 5. Appendix tissue with micro-organisms stained with Alcian Yellow. 600x.

Alcian Yellow

Technical notes and references

Technical notes

1. Section thickness may affect quality and intensity of staining. Cut sections, usually 3–5 µm, and pick the sections up on glass slides.
2. When staining for microorganisms, it is important to ensure that the water bath is scrupulously clean prior to sectioning in order to prevent introduction of extraneous microorganisms onto the specimen section slide. It is recommended not to use water left standing overnight.
3. Staining intensity and distinct visualization of the microorganisms may be greatly affected by post-instrument time in 95% alcohol. Longer time in 95% alcohol will result in lighter blue and/or yellow staining. This must be tightly controlled and validated however, as over-differentiating in 95% alcohol may result in excessively weak or negative staining.

References

1. Leung JK, Givvon KJ, Vartanian RK. *Rapid Staining method for Helicobacter pylori in Gastric Biopsies*. J Histotechnol. 1996;19(2);131-132.
2. Sheehan DC, Hrapchak BB. *Theory and Practice of Histotechnology*, 2nd edition. St. Louis, MO: C.V. Mosby Company; 1980.
3. Clinical and Laboratory Standards Institute (CLSI). CLSI Web site. http://www.clsi.org/. Accessed November 3, 2011.
4. Carson F, Hladik C. Histotechnology: *A Self Instructional Text*. 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.

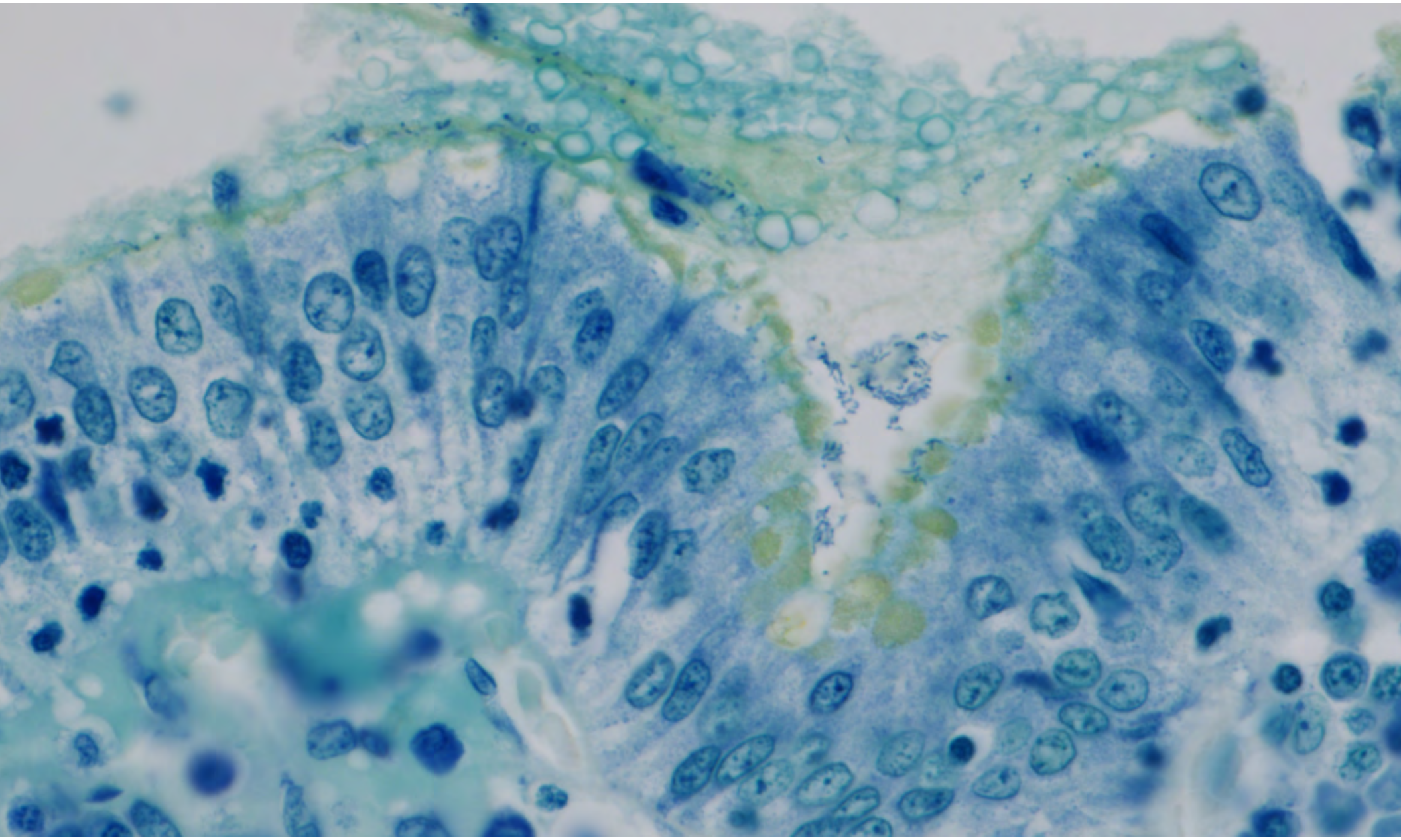

Figure 6. Gastric tissue with H. Pylori stained with Alcian Yellow. 600x.

Congo Red

Stain basics

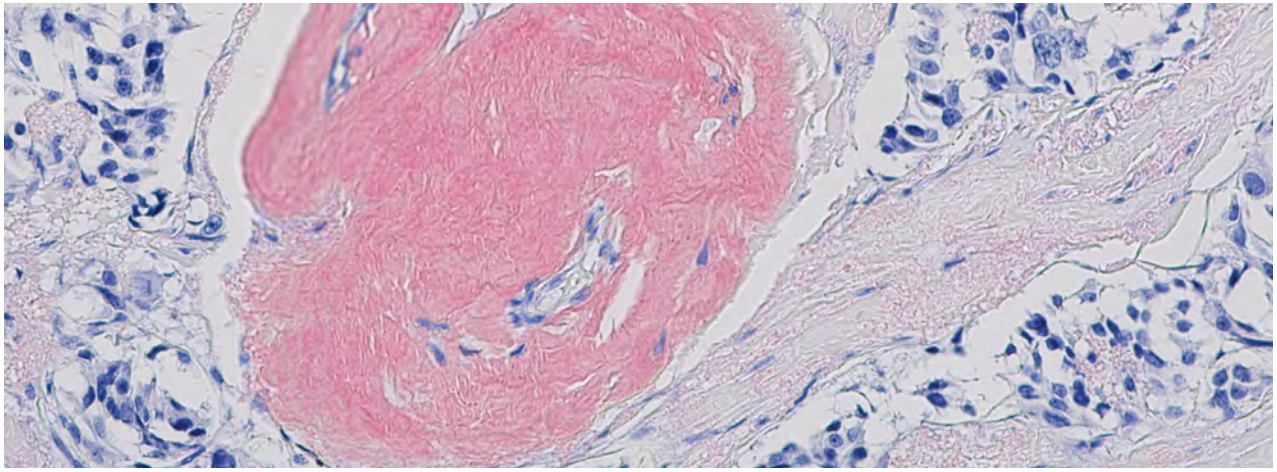

Figure 1. Amyloid in lung stained with Congo Red, regular light microscopy, 200x.

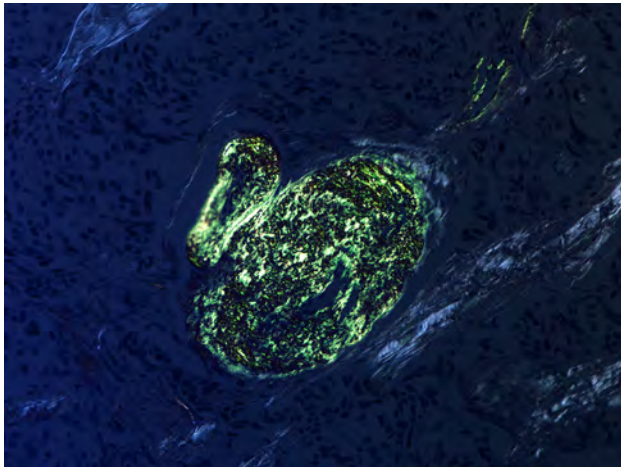

Figure 2. Amyloid in lung stained with Congo Red, polarized light microscopy, 200x.

Purpose

Congo Red stain is used as a qualitative histologic stain to selectively demonstrate amyloid in formalin-fixed, paraffin-embedded tissue. [1]

Staining principle

The staining reaction is based on the application of Congo red, which stains the patterns of atypical proteins (amyloid). The beta-pleated sheets of amyloid are suitable in size and shape to accommodate the Congo red molecules, which are held in the latticework of the beta-pleated sheets.[2] Congo red may also stain unexpected structures such as keratin and elastic and dense collagen fibers. Birefringence is an intrinsic property of the amyloid fibril Congo red complex.[3,4] When examined with polarized light, true amyloid exhibits apple-green birefringence. Without both the red staining of Congo red and the apple-green birefringence under polarization, a definite identification cannot be made.

Expected results

- Light Microscopy:
  - Amyloid — reddish pink
  - Nuclei — blue
- Polarized Light:
  - Amyloid — apple-green fluorscence
  - Background — dark blue/black

Congo Red

Stain basics

Common diagnostic utility

Congo red stain may be used for the definitive identification of amyloid deposits in tissues in the diagnosis of primary (idiopathic) amyloidosis or secondary amyloidosis related to numerous conditions characterized by extracellular protein deposits. The extracellular protein may be localized to one specific site or generalized throughout the body (systemic). Amyloidosis tends to be associated with organ structures such as: peripheral nerves, skin, tongue, joints, heart, lung, liver, spleen, kidneys, liver and adrenals.

Tissue controls

A known positive tissue control should be utilized for monitoring the correct performance of processed tissues and test reagents.[5] An appropriate control tissue for the Congo red stain should contain amyloid deposits. Ideally, it should be representative of the tissues it is usually used to diagnose. The most common tissue controls are amyloid-positive lung or kidney.[6]

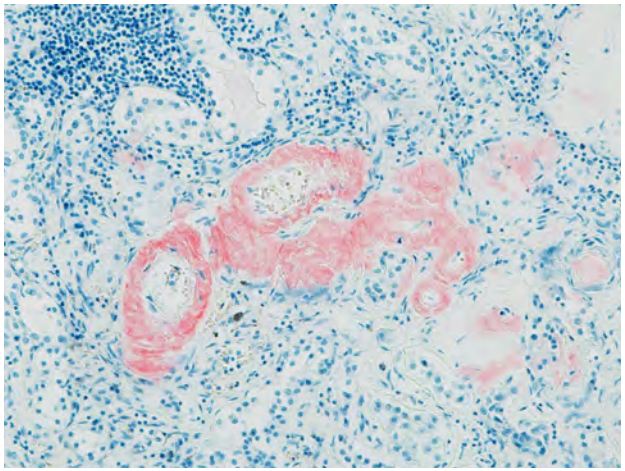

Figure 3. Amyloid in kidney stained with Congo Red, regular light microscopy, 200x.

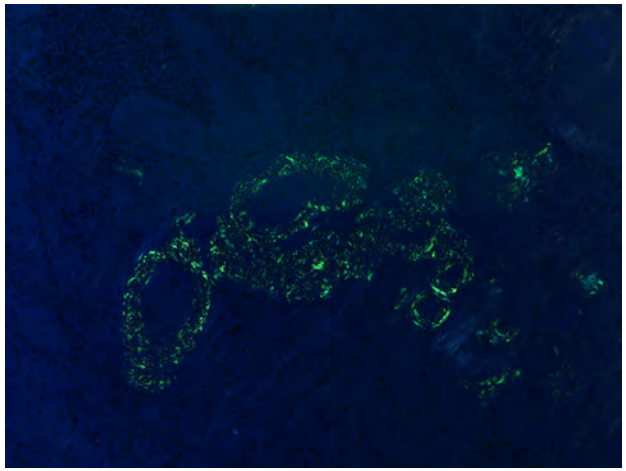

Figure 4. Amyloid in kidney stained with Congo Red, polarized light microscopy, 200x.

Congo Red

Technical notes and references

Technical notes

1. Tissue section thickness is important and may affect quality and intensity of staining.
2. The amyloid fibril/Congo Red complex is a large molecular complex. Unless sections are cut at 5-10 microns, it may be very difficult to visualize the apple-green birefringence under polarized light. This may be especially problematic for routinely sectioned renal biopsies which are normally cut at 3 microns.

References

1. Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology. 2nd edition. St. Louis, MO: C.V. Mosby Company; 1980.
2. Wheater PR, Burkitt HG. Basic Histopathology, 2nd edition. New York: Churchill Livingstone; 1991.
3. Navarro A, Tolivia J, Valle E. Congo Red Method for Demonstrating Amyloid in Paraffin Sections. J Histotechnol. 1999;22(4):305-308.
4. Fredenburgh JL, Grizzle WE. Special Stains: Their Chemical Mechanism. Chicago: ASCP Press; 1998.
5. Clinical and Laboratory Standards Institute (CLSI). CLSI Web site. http://www.clsi.org/. Accessed November 3, 2011.
6. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.

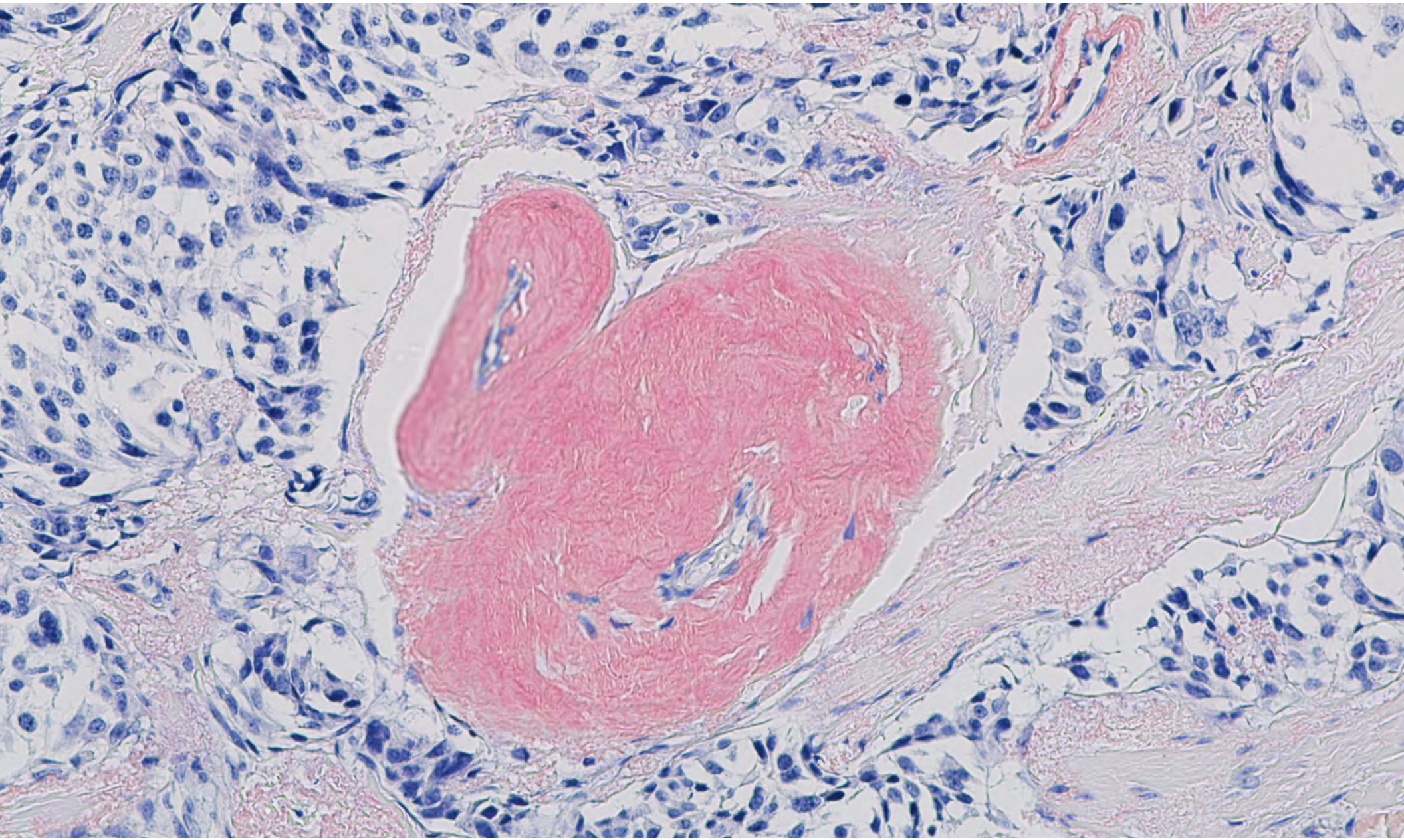

Figure 5. Amyloid in lung stained with Congo Red, regular light microscopy, 200x.

Elastic

Stain basics

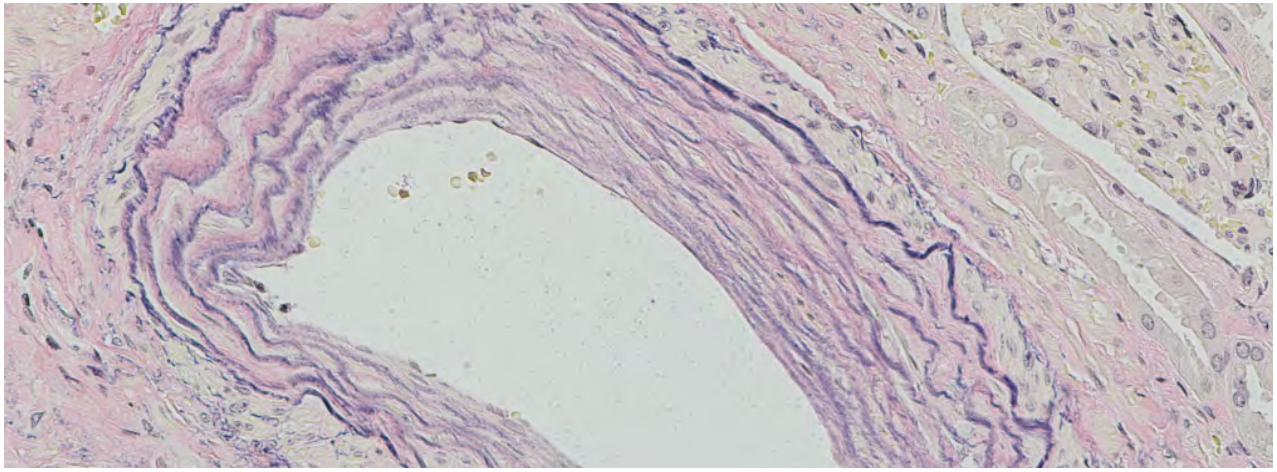

Figure 1. Artery stained with Elastic stain

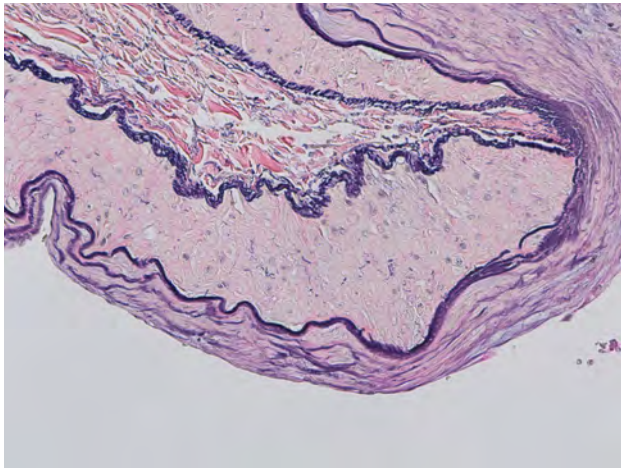

Figure 2. Artery in kidney stained with Elastic stain,

Purpose

The Elastic Staining is used to differentiate elastic fibers from collagen. It is useful in demonstrating changes in elastic fibers in certain disease processes.[1] Depending on the method used, elastic fibers are stained black/purple with the elastic stain, while the collagen is stained red/pink and the other tissue structures are stained yellow with the counterstain.[1]

Staining principle

The staining reaction is based on the affinity towards elastic fibers displayed by resorcin fuchsin. Since the method is not absolutely specific, other structures such as collagen and basal membranes might also stain. Ferric chloride is used as a mordant which binds to elastic fibers. The resorcin-fuchsin (resorcinol and basic fuchsin solution) and the ferric chloride react to form an iron-resorcin lake precipitate. The complex formed from the reaction of the basic fuchsin, resorcinol and ferric chloride (an iron resorcin lake) binds to the elastic fibers, resulting in the blue-black/purple staining of the elastic fibers. The elastic stain is then followed by a Van Gieson counterstain solution of acid fuchsin and picric acid which stains the collagen red and the background yellow.

Expected results

- Elastic — Blue-black/purple
- Collagen — Red
- Background — yellowish

Elastic

Stain basics

Common diagnostic utility

This stain is useful in demonstrating atrophy of elastic fibers in cases of emphysema, and the thinning and loss of elastic fibers in arteriosclerosis and other vascular diseases.[1] The elastic stain has also been used as a possible aid in visualizing pleural effusion in the staging and patient management in non-small cell lung cancer. [2]

Elastic stain may also be used for temporal artery biopsies where it is a standard diagnostic test for temporal arteritis. It is used to illustrate the inflammation and deterioration of the internal elastic lamina.[3]

More recent publications suggest that Elastic stain may be helpful in assessing transmural spread by colorectal carcinoma. Serosal invasion by colorectal carcinoma is generally accepted to have adverse prognostic significance but may be difficult to assess.[4]

Tissue controls

A known positive tissue control should be utilized for monitoring the correct performance of processed tissues and test reagents. An example of a positive control material for Elastic stain would be formalin-fixed, paraffin-embedded human tissue with elastic fibers such as aorta, artery, kidney, lung or skin.[5] Ideally, it should be representative of the tissues it is usually used to diagnose.

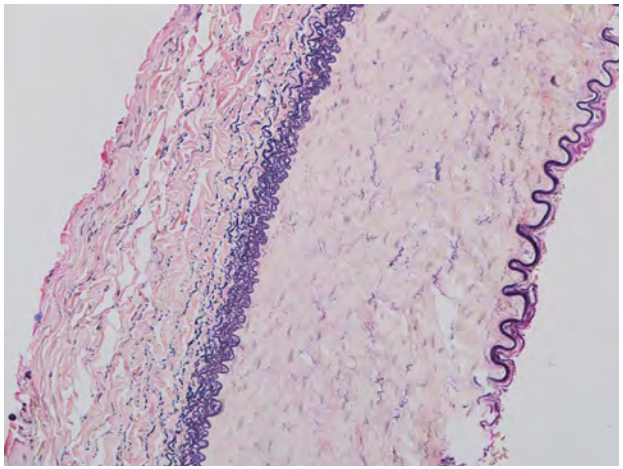

Figure 3. Temporal artery stained with Elastic stain, demonstrating a well-defined internal elastic lamina and a less distinct external elastic lamina separated by a smooth muscle layer. 200x.

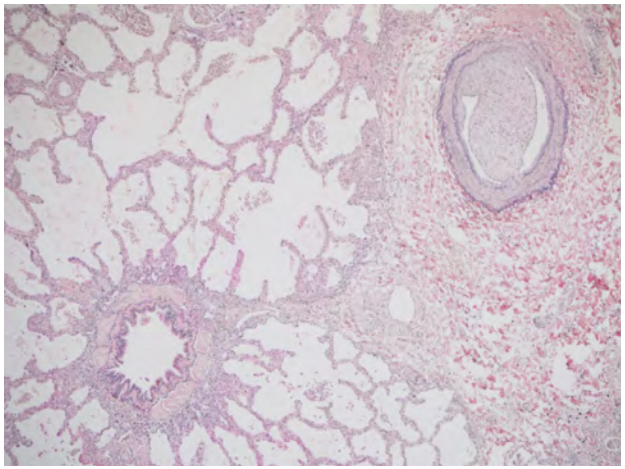

Figure 4. Elastic staining in lung showing a bronchovascular bundle (bronchiole with a single elastic lamina and pulmonary artery containing both internal and external elastic laminae). 200x.

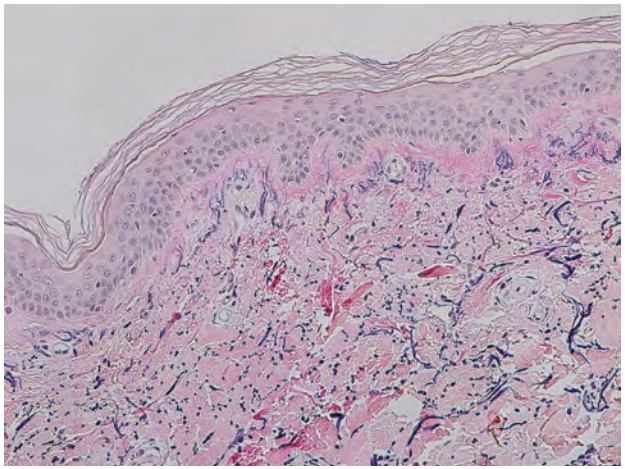

Figure 5. Normal skin stained with Elastic stain, demonstrating loosely arranged elastic fibers in the papillary and densely arranged elastic fibers in the reticular dermis. 200x.

Elastic

Technical notes and references

Technical notes

1. Section thickness may affect quality and intensity of staining. Cut sections, usually 3–5 µm, and pick the sections up on glass slides.
2. The last step in the Elastic staining procedure is the Van Gieson counterstain. Since this is an acidic solution, prolonged overstaining with the counterstain can reduce the elastic staining.
3. Both the elastic staining as well as the counterstain can be greatly affected by post-staining run down procedures. Prolonged time in alcohol solutions is not recommended.

References

1. Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology, 2nd edition. St. Louis, MO: C.V. Mosby Company; 1980.
2. Taube JM, Askin FB, Brock MV, Westra W. Impact of elastic staining on the staging of peripheral lung cancers. Am J Surg Pathol. 2007 Jun;31(6):953-6.
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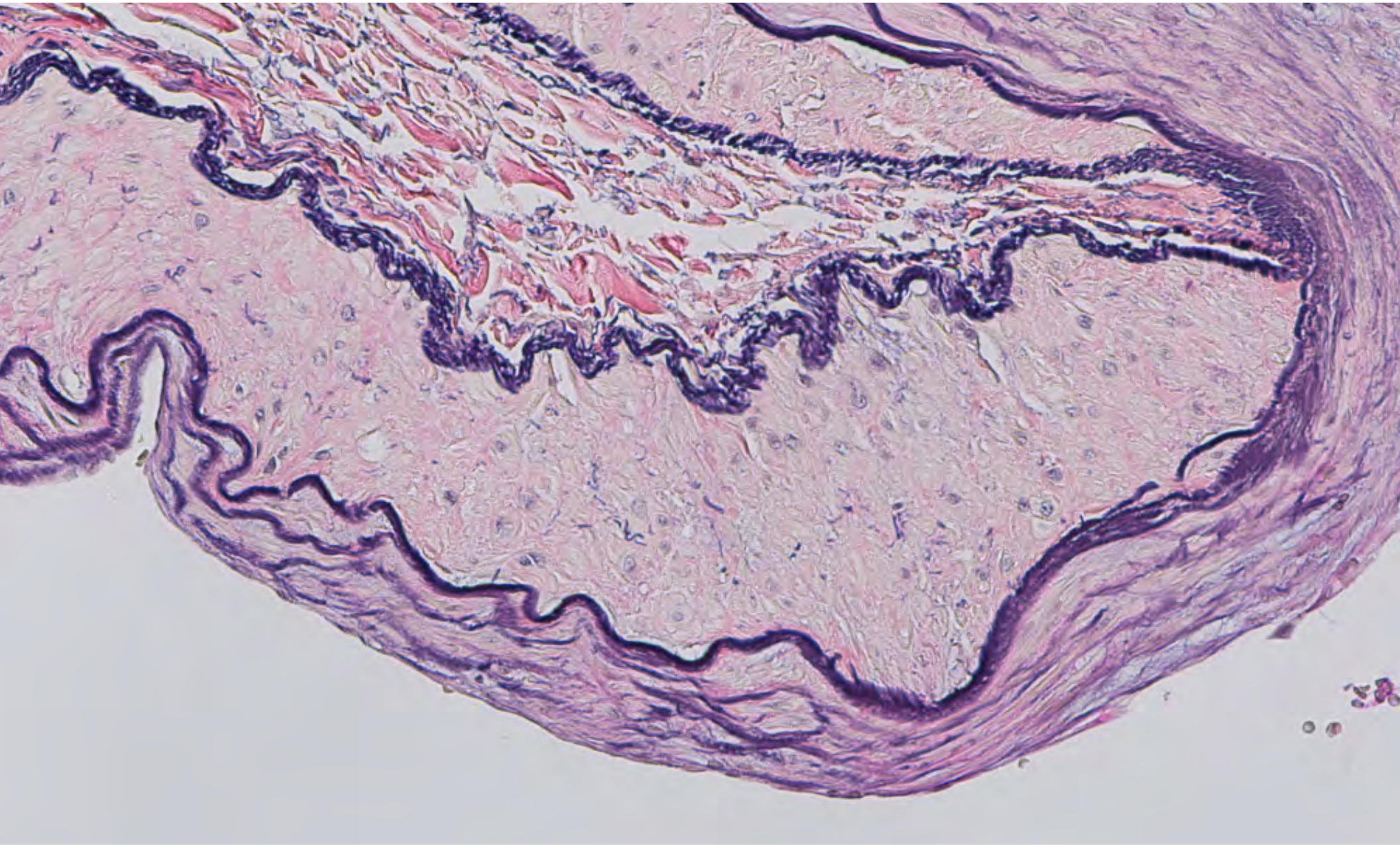

Figure 6. Artery in kidney stained with Elastic stain,

Giemsa

Stain basics

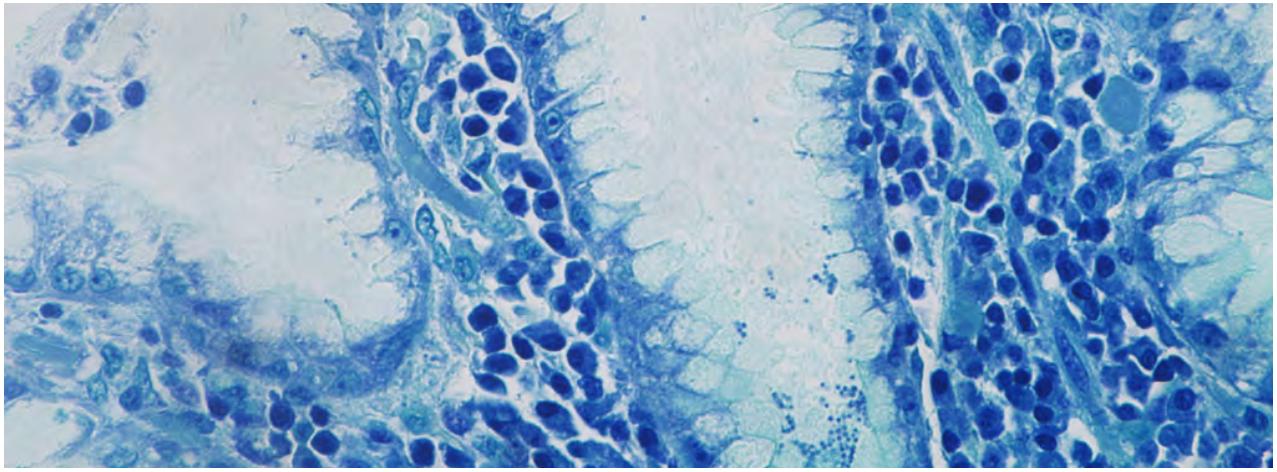

Figure 1. Gastric biopsy stained with Giemsa demonstrating H. Pylori organisms, 600x.

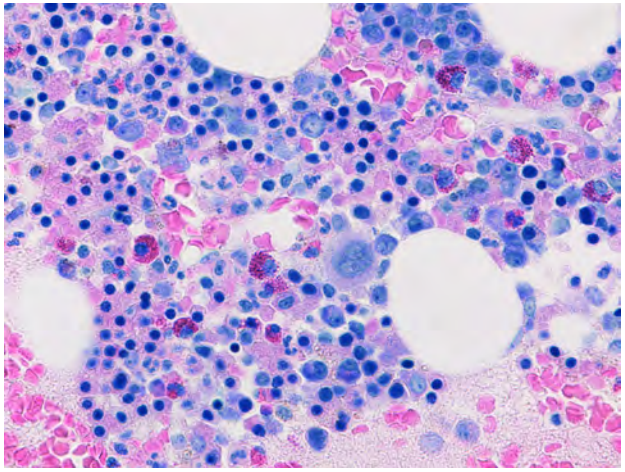

Figure 2. FFPE section of bone marrow clot stained with Giemsa, 600x.

Purpose

In formalin-fixed, paraffin-embedded tissue, Giemsa is used as a qualitative histologic stain to differentiate leukocytes in bone marrow and other hematopoietic tissue (lymph nodes). The stain can also be used to demonstrate some microorganisms,[1] such as Helicobacter pylori.

Staining principle

Giemsa stain is a type of Romanowski stain, which is a polychromatic group of stains formed from the precipitate created by the combinations of solutions of methylene blue and eosin in methanol. These stains were originally intended as a single-step fixation and staining method for blood and bone marrow smears or touch-preps. The staining reaction is based on the differential affinity of cell types for the dyes in the stain. Because of the high degree of dissociation, active molecules (eosin and thiazine dyes) are absorbed by cellular structures very quickly. Differentiation in alcohol renders the staining of the various cell types multi-colored, with the nuclear material staining in various shades of blue and the cytoplasm staining pink.[3] Cellular granules stain red or purple, depending on whether they are acidophilic or basophilic. [1, 2, 6]

Expected results

- Collagen, muscle, bone — pale pink
- Micro-organisms — purplish-blue
- Nuclei — dark blue
- Red blood cells — pink (or green if bone marrow fixed in Zenkers over the weekend)
- Cytoplasm — varying light blue shades
- Basophilic granules — blue/purple
- Eosinophilic granules — red

Giemsa

Stain basics

Common diagnostic utility

Giemsa stain may be used to demonstrate Helicobacter pylori or other campylobacter species in gastric biopsies, [1,5] Donovan bodies and leishmania organisms in tissue sections. It may also be used for the demonstration of mast cells in cutaneous mastocytosis, or for the elucidation of hematopoietic cell lineages in bone marrow biopsies or bone marrow aspirates in diseases involving the bone marrow.

Tissue controls

A known positive tissue control should be utilized for monitoring the correct performance of processed tissues and test reagents. Ideally, it should be representative of the tissues it is usually used to diagnose. Examples of appropriate Giemsa controls include human tissue such as bone marrow, lymph node, or spleen[2] or Helicobacter pylori-positive tissue specimen.

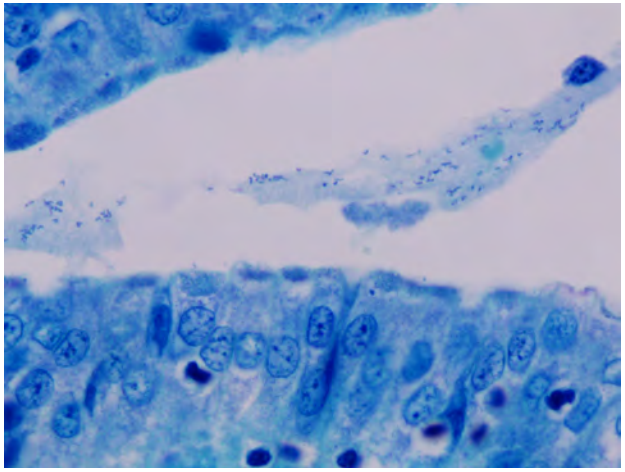

Figure 3. Gastric biopsy stained with Giemsa demonstrating H. Pylori organisms stained deep blue in the gastric pits.

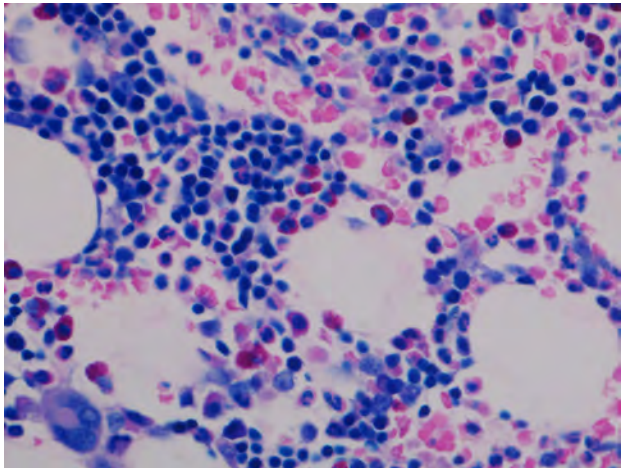

Figure 4. FFPE section of bone marrow trephine stained with Giemsa showing erythrocyte precursor cells with intensely blue nuclei, white blood cell precursor stained with lighter blue nuclei and pink, gray or bluish cytoplasm, bone staining pink, eosinophilic granules staining dark pink/red and basophilic granules staining blue/purple.

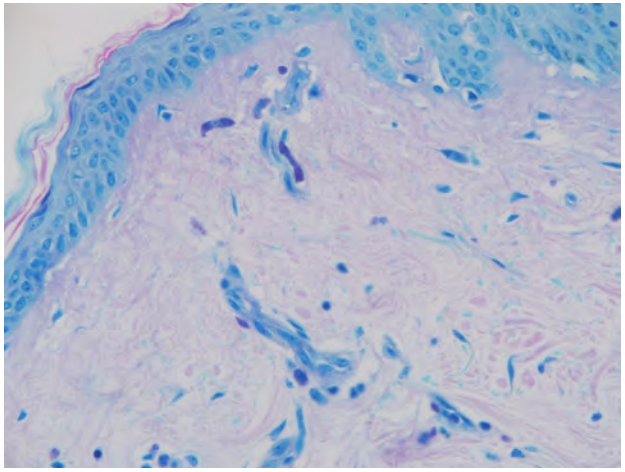

Figure 5. Skin biopsy stained with Giemsa demonstrating dark blue/purple granular mast cells.

Giemsa

Technical notes & references

Technical notes

1. Section thickness may affect quality and intensity of staining. Cut sections, usually 3–5 µm, and pick the sections up on glass slides.
2. When staining for microorganisms, it is important to ensure that the water bath is scrupulously clean prior to sectioning in order to prevent introduction of extraneous microorganisms onto the specimen section slide It is recommended not to use water left standing overnight.
3. Staining intensity and distinct visualization of the microorganisms may be greatly affected by post-instrument time in 95% alcohol. Extending exposure time in alcohol dehydration bath will enhance differentiation but, if overdone, may also decolorize the organisms. Depending on whether staining for H. Pylori or for hematopoietic cells in bone marrow or mast cells in skin, 15-20 seconds in each 95% alcohol bath is a recommended starting point. This must be tightly controlled and validated however, as over-differentiating in 95% alcohol may result in excessively weak or negative staining.
4. Alternately, differentiating in dilute acetic acid will bring out the metachromatic properties of the Giemsa stain. This may be a better option for demonstration of mast cells in skin biopsies. The acetic acid concentration used varies by different authors but generally ranges from 0.1 to 0.5% in deionized/distilled water. The differentiation time will vary according to the staining time, temperature and even section thickness, but it is generally achieved within 30 seconds. This differentiating method primarily removes the blue dye, providing greater contrast for the red dye. (See images below demonstrating differentiation in 0.1% acetic)
5. When fixatives other than neutral buffered formalin are used, red blood cells may appear to stain grayish green.

References

1. Bancroft and Stevens. *Theory and Practice of Histological Techniques*, 2nd edition. Edinburgh: Churchill-Livingston, 1982.
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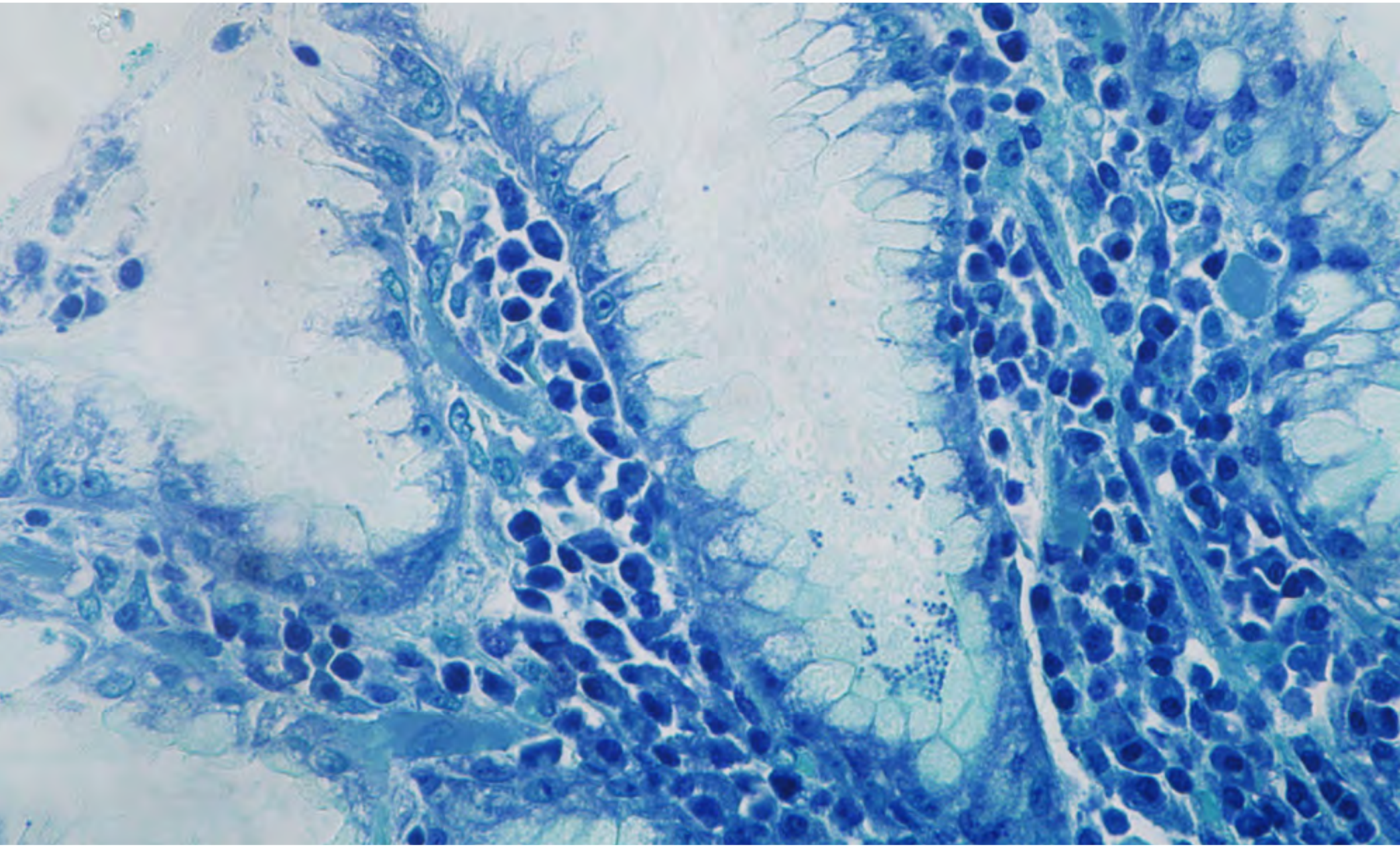

Figure 6. Gastric biopsy stained with Giemsa demonstrating H. Pylori organisms, 600x.

Gram

Stain basics

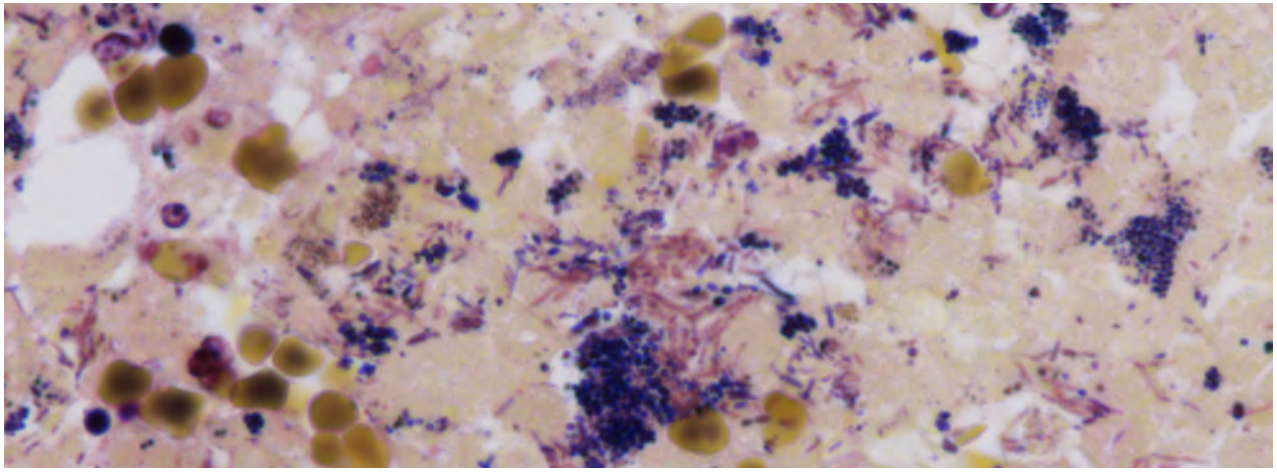

Figure 1. Gram stain with Tarttrazine counterstain showing a mixture of Gram-positive and Gram-negative microorganisms, 1000x. (oil)

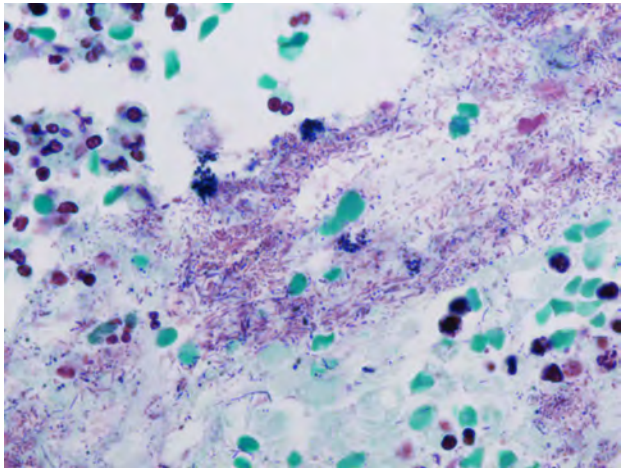

Figure 2. Gram- stain with Light Green counterstain showing a mixture of Gram-positive and Gram-negative microorganisms, 1000x. (oil)

Purpose

Gram stain is a qualitative histologic stain used to classify bacteria broadly into two categories, either gram-negative or gram-positive.

Staining principle

The classification of bacteria as either Gram-positive or Gram-negative is based on the properties of the bacterial cell walls which allow selective retention of the Gram stain. In general, the bacterial cell wall contains a layer of peptidoglycan (an amino acid/carbohydrate polymer). The thickness of this layer is the main factor for how the bacteria will stain with the Gram stain. The cell wall Gram-positive bacteria generally have a thick peptidoglycan layer (20 to 80 nanometers), whereas the cell wall of Gram-negative bacteria generally have a thinner peptidoglycan layer (7 to 8 nanometers). [1] The stain consists of applying an aqueous solution of crystal violet which permeates the peptidoglycan layer and stains both types of organisms blue. This followed by the addition of an iodine solution which complexes with the crystal violet rendering it water insoluble. Differentiation is achieved with the application of an organic solvent. The thinner peptidoglycan layer of the Gram-negative organisms is more readily decolorized than the thicker layer of the Gram-positive organisms, resulting in loss of the blue dye in the Gram-negative organisms. The differentiation step is followed by the addition of a red dye to stain the Gram-negative organisms. [2]

Expected results

- Gram-positive bacteria - blue
- Gram-negative bacteria - red
- Fungal organisms - blue
- Background - yellow (with tratrazine counterstain)

  - green (with methyl green counterstain)

Gram

Stain basics

Common diagnostic utility

The Gram stain may be used to broadly demonstrate the presence of Gram-positive or Gram-negative organisms in miscellaneous tissue infections from various sources and diseases. [3-5]

Tissue controls

A known positive tissue controls should be utilized for monitoring the correct performance of processed tissues and test reagents.[6] An appropriate control tissue for the Gram stain should contain both Gram-positive and Gram-negative microorganisms. Ideally, it should be representative of the tissues it is usually used to diagnose. A common tissue controls for Gram staining is appendix.

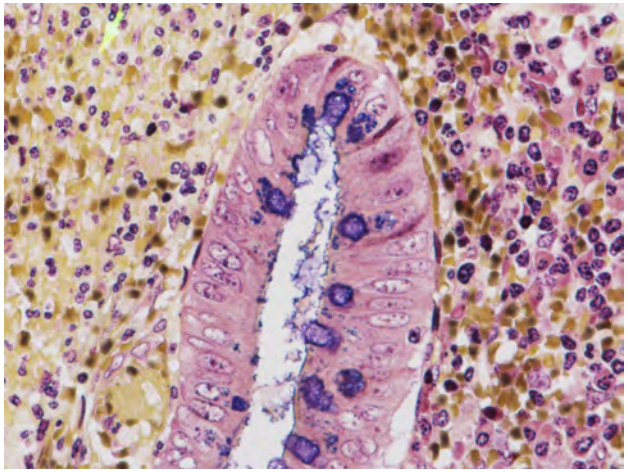

Figure 3. Gram stain with Tarttrazine counterstain on appendix showing mostly gram positive microorganisms, 600x.

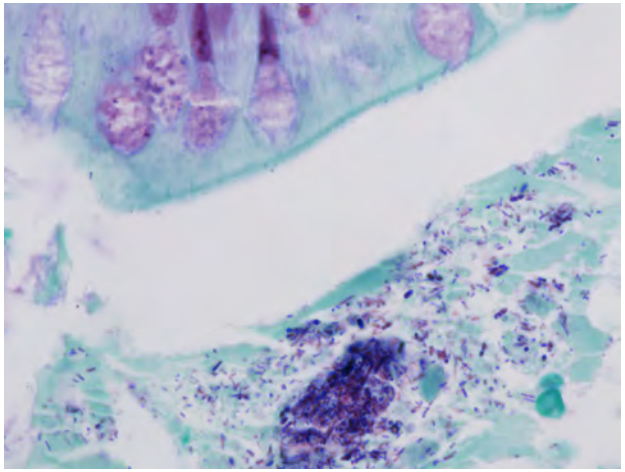

Figure 4. Gram stain with Light Green counterstain on appendix showing a mixture of Gram-positive and Gram-negative microorganisms, 1000x. (oil)

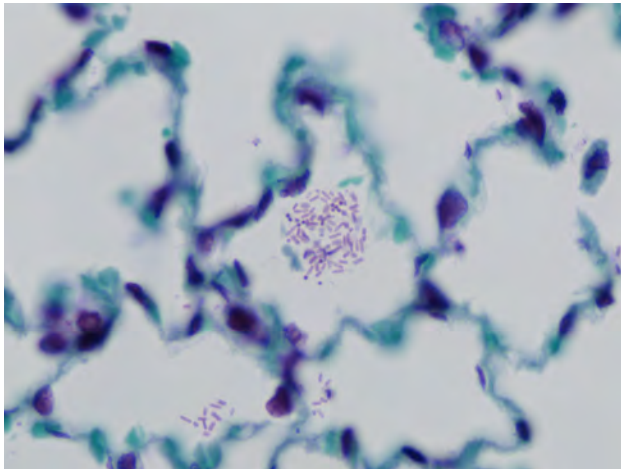

Figure 5. Rat lung engineered control with Gram-positive and Gram-negative organisms, 1000x.

Gram

Technical notes, post-instrument processing and references

Technical notes

1. Section thickness may affect quality and intensity of staining. Cut sections, usually 3–5 µm, and pick the sections up on glass slides.
2. When staining for microorganisms, it is important to ensure that the water bath is scrupulously clean prior to sectioning in order to prevent introduction of extraneous microorganisms onto the specimen section slide. It is recommended not to use water left standing overnight.
3. Antibiotics can affect the cell wall of gram-positive bacteria and therefore affect the staining quality of the Gram stain.
4. Nocardia is a variable gram-positive bacteria which may stain pink to red with some blue to purple segments. It may be better visualized with an alternative staining method.

References

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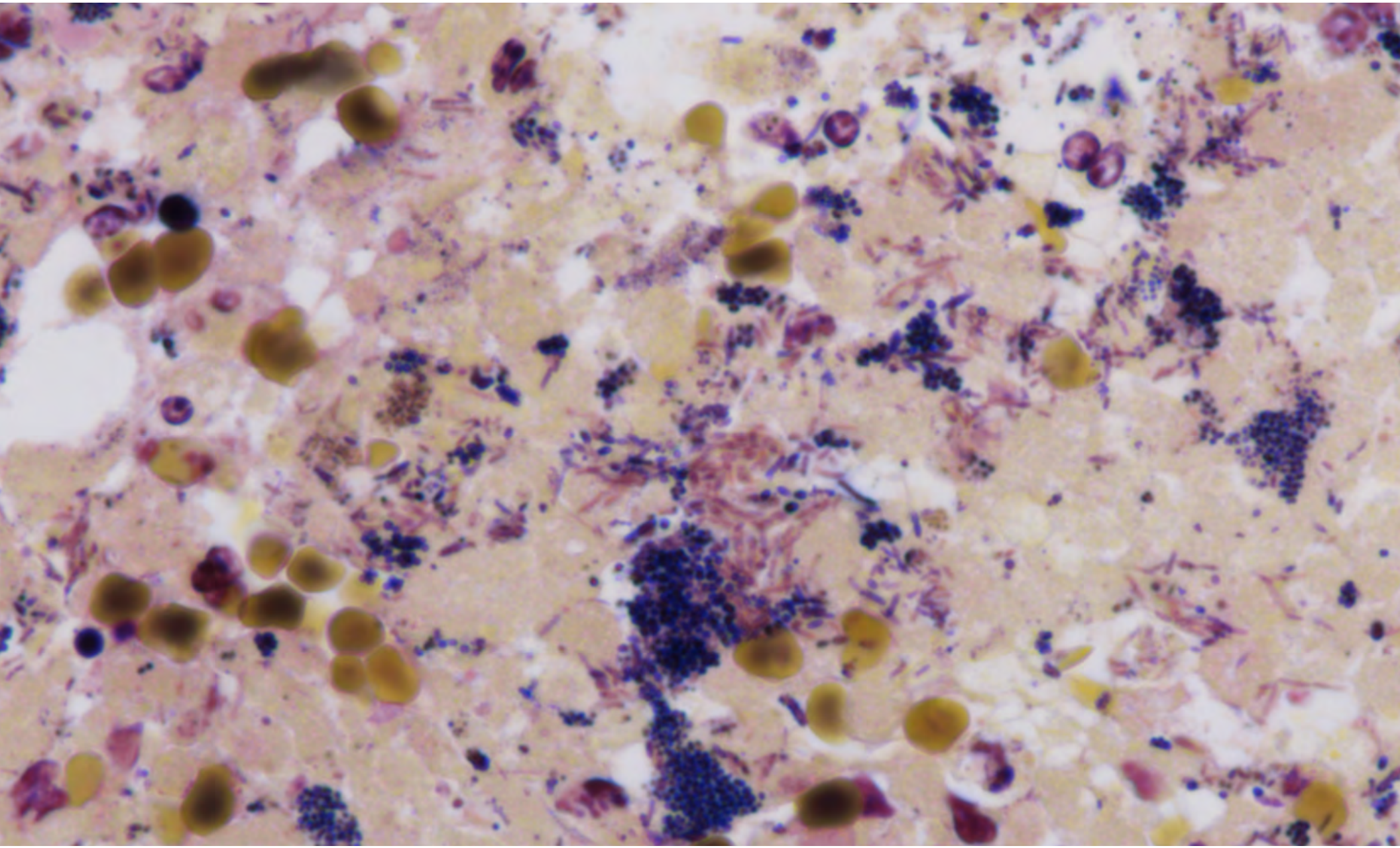

Figure 6. Gram stain with Tarttrazine counterstain showing a mixture of Gram-positive and Gram-negative microorganisms, 1000x. (oil)

GMS

Stain basics

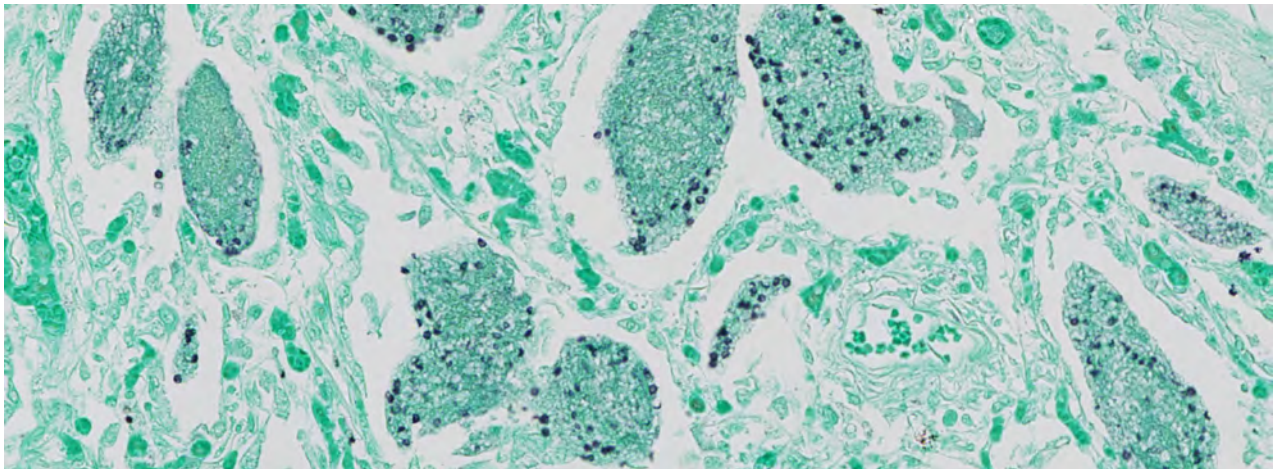

Figure 1. Pneumocystus organisms in lung stained with GMS, 200x.

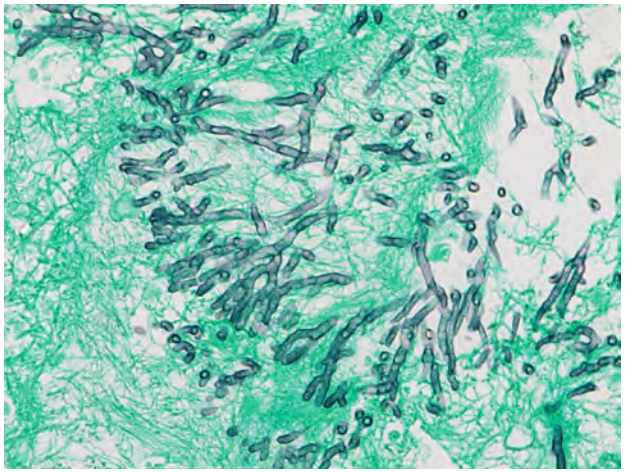

Figure 2. Fungus organisms in testes stained with GMS, 600x.

Purpose

Grocott Methenamine Silver (GMS) stain is intended for use as a qualitative histologic stain to demonstrate polysaccharides in the cell walls of fungi and other opportunistic organisms in formalin-fixed, paraffin-embedded tissue.[1]

Staining principle

The staining reaction is based on aldehyde reduction of silver ions to metallic silver under alkaline conditions.[3] Fungal and microbial cell wall polysaccharides are oxidized to aldehyde groups by chromic acid. This also suppresses weaker background staining of collagen fibers and basement membranes. Excess chromic acid is removed and neutralized with sodium bisulfite. A silver nitrate in an alkaline solution provides the silver ions and alkaline conditions necessary to reduce the silver ions to metallic silver. Subsequent application of gold chloride forms a more stable complex with the silver and removes the yellow tones from the tissue. Sodium thiosulfate stops the reaction and removes any unreduced silver from the section. Light Green is applied as a counterstain to provide a contrasting background.

Expected results

- Organisms — black/gray
- Background — green

GMS

Stain basics

Common diagnostic utility

This stain is primarily used to distinguish pathogenic fungi such as Aspergillus and Blastomyces[1] and other opportunistic organisms such as Pneumocystis pneumonia (PCP) which is caused by Pneumocystis carinii (now reclassified as Pneumocystis jiroveci)[5, 6] in tissue sections.[2]

Tissue controls

A known positive tissue control should be utilized for monitoring the correct performance of processed tissues and test reagents. Ideally, it should be representative of the tissues it is usually used to diagnose. An example of a positive control material would be formalin-fixed, paraffin-embedded human tissue positive for fungus or Pneumocystis carinii (jiroveci) which may be found in lung of immunocompromised patients.[4]

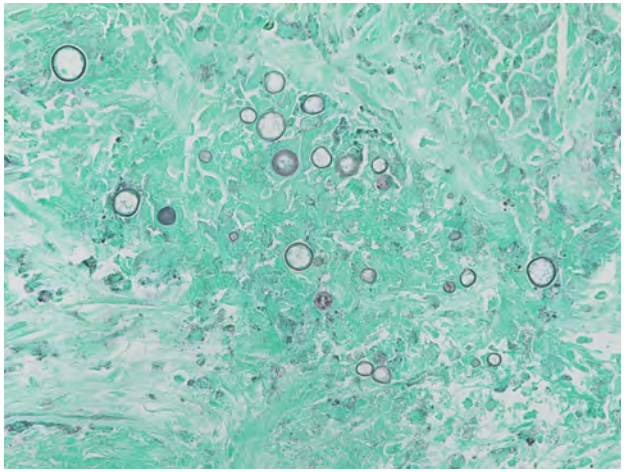

Figure 3. Cryptococcus infected tissue stained with GMS, 400x.

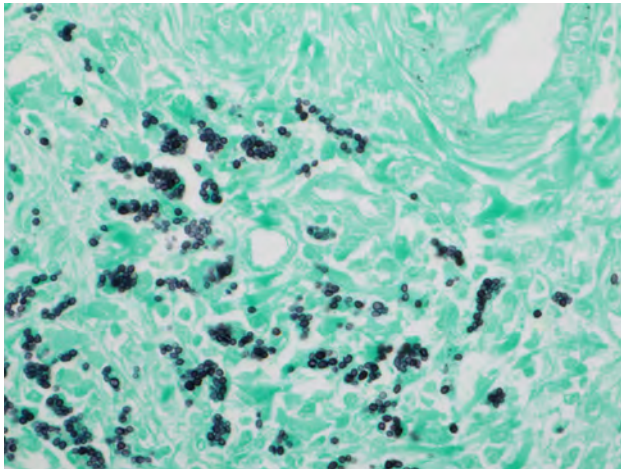

Figure 4. Histoplasma infected tissue stained with GMS, 400x.

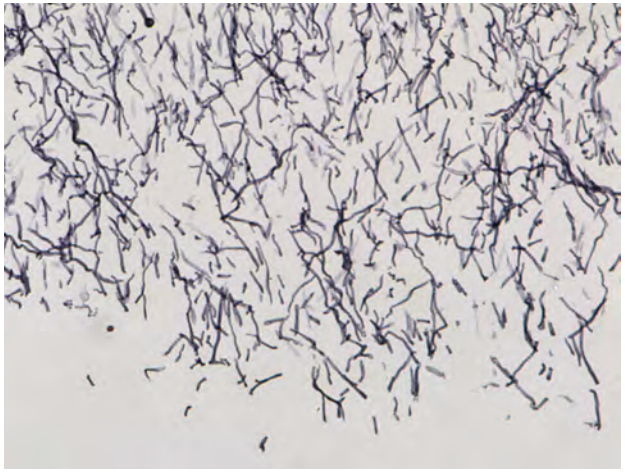

Figure 5. Engineered Sporothrix fungus control stained with GMS, 600x.

Technical notes and references

Technical notes

1. Section thickness may affect quality and intensity of staining. Cut sections, usually 3–5 µm, and pick the sections up on glass slides.
2. When staining for microorganisms, it is important to ensure that the water bath is scrupulously clean prior to sectioning in order to prevent introduction of extraneous microorganisms onto the specimen section slide. It is recommended not to use water left standing overnight.

References

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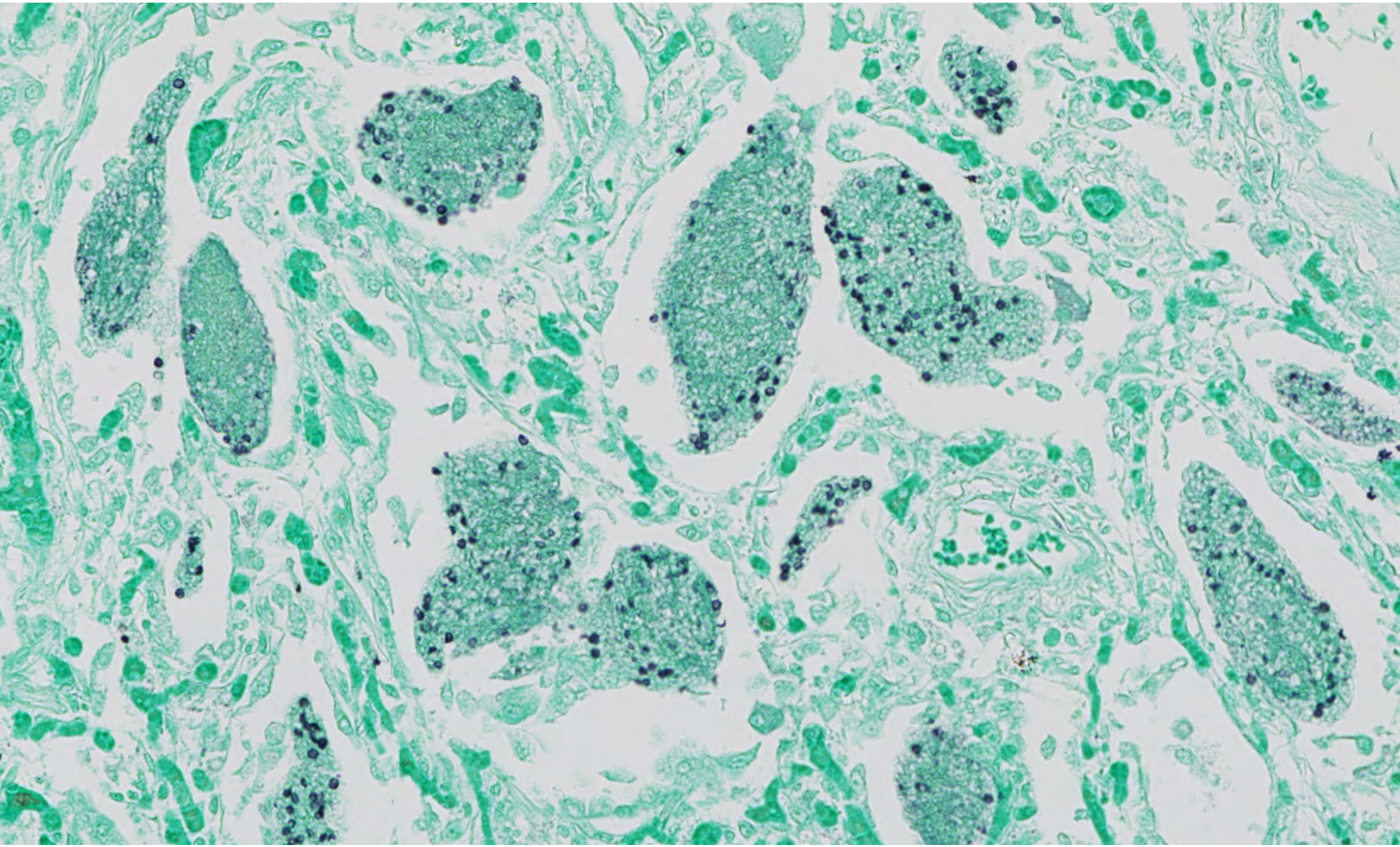

Figure 6. Pneumocystus organisms in lung stained with GMS, 200x.

Iron

Stain basics

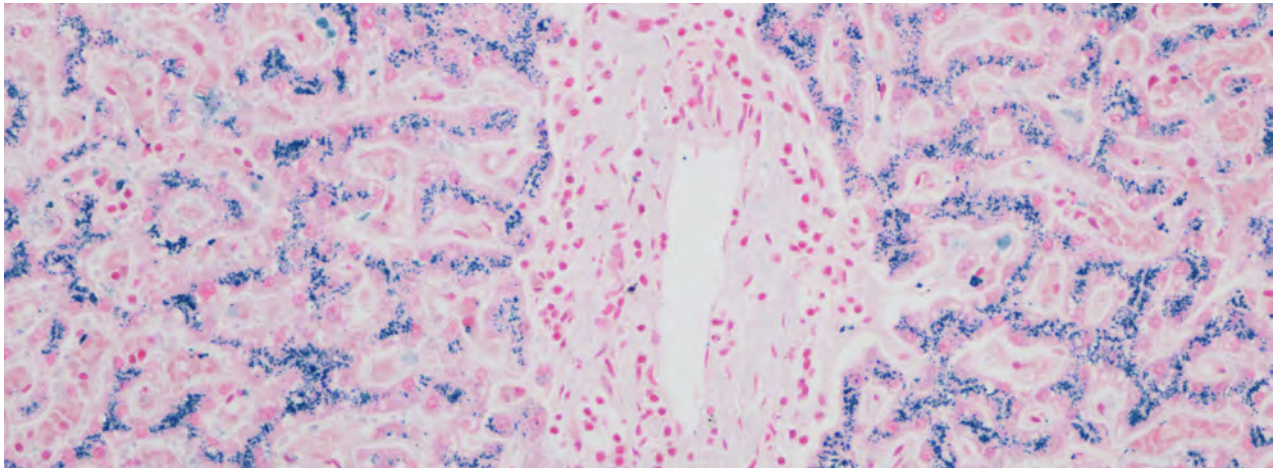

Figure 1. Liver with iron deposits stained with Iron Stain , 200x.

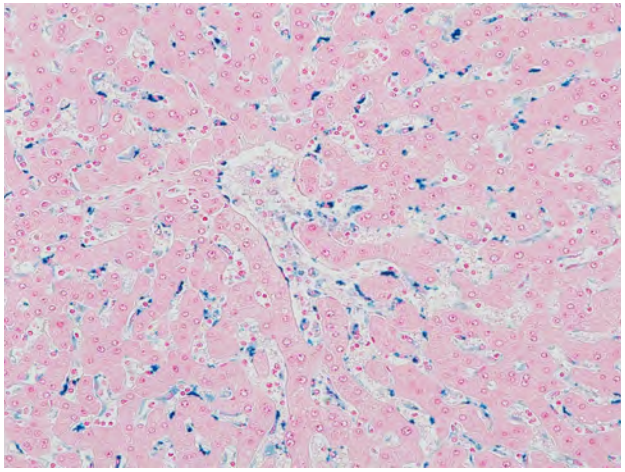

Figure 2. Liver with iron deposits stained with Iron Stain , 200x.

Purpose

Iron stain is intended for use as a qualitative histologic stain to detect iron pigment in bone marrow, tissue with hemochromatosis and hemosiderosis in formalin-fixed, paraffin-embedded tissue.[1]

Staining principle

The iron is separated from protein by hydrochloric acid. The free ferric iron reacts with the potassium ferrocyanide to form an insoluble bright blue ferric ferrocyanide, or Prussian blue. [1, 2, 4]

Expected results

- Iron — deep blue
- Nuclei — Red
- Background — pink

Iron

Stain basics

Common diagnostic utility

Iron is absorbed through the small intestine and transported to the bone marrow. There it is stored as hemosiderin, until erythropoiesis, when it is incorporated into hemoglobin molecules. During erythrocyte destruction at the end of red cell life span, the iron is split off from the hemoglobin in the lymphoreticular system and again stored in the bone marrow as hemosiderin.[5] In the disease states of hemochromatosis and hemosiderosis, excessive amounts of ferric iron are present in the liver, spleen and lymph nodes. Iron may be found at any site where there has been local destruction of red cells, such as hemorrhage sites, infarctions, longstanding congestion and trauma.

Tissue controls

A known positive tissue control should be utilized for monitoring the correct performance of processed tissues and test reagents.[3] Ideally, it should be representative of the tissues it is usually used to diagnose. An example of a positive control material would be formalin-fixed, paraffin-embedded human tissue positive for iron, such as tissue involved in the above-mentioned pathological processes.[4]

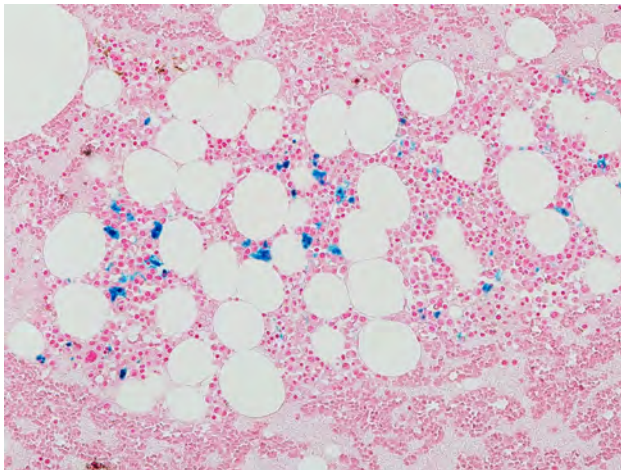

Figure 3. Iron deposits in undecalcified FFPE section of bone marrow clot stained with Iron stain, 200x.

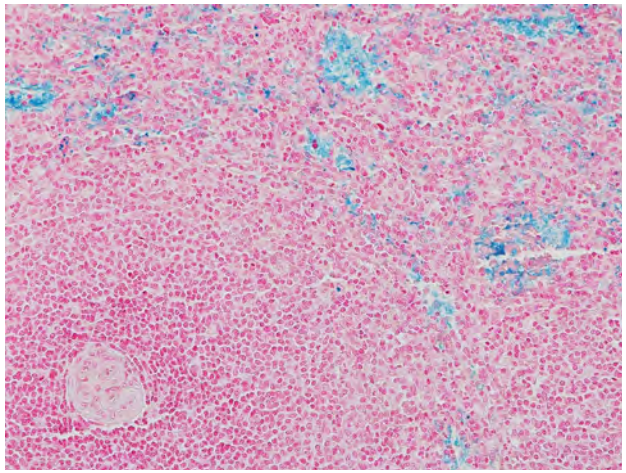

Figure 4. Iron deposits in spleen stained with Iron stain, 200x.

Iron

Technical notes and references

Technical notes

1. Acidic fixatives (including unbuffered formalin) may solubilize the iron in the tissues and cause false negative staining.
2. Iron deposits in the tissue may be removed by decalcification methods. Misleading, false-negative staining may occur. For diagnostic testing on bone marrow samples, iron staining may be more reliable and appropriate on smears of bone marrow aspirates rather than bone marrow trephines.
3. Use distilled water in the water bath.

References

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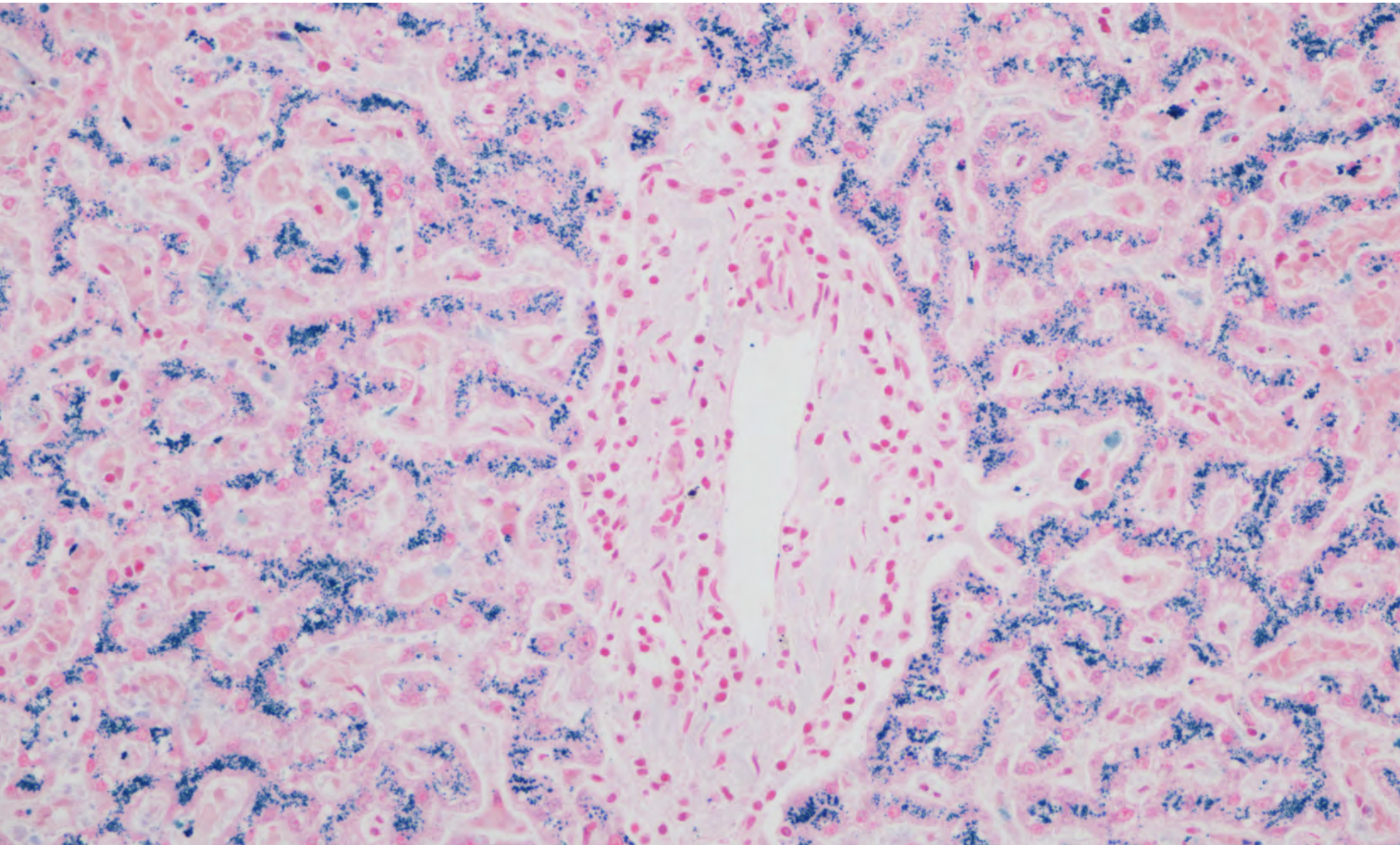

Figure 5. Liver with iron deposits stained with Iron Stain , 200x.

Jones & Jones Light Green

Stain basics

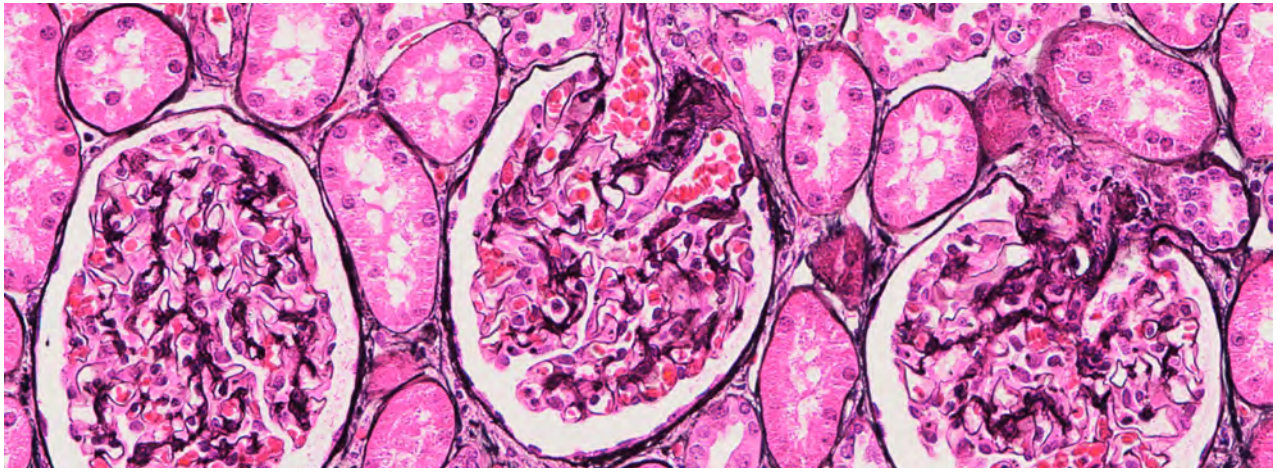

Figure 1. Kidney stained with Jones using Hematoxylin and Eosin counterstain, 200x. H&E

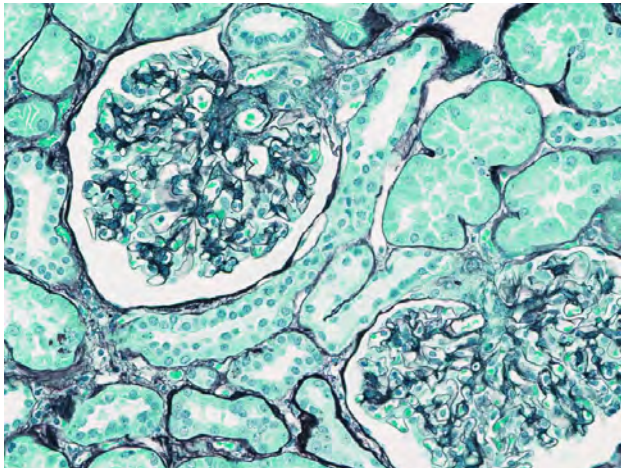

Figure 2. Kidney stained with Jones using Light Green counterstain, 200x.

Purpose

The Jones Methenamine Silver stain is intended for use as a qualitative histologic stain to demonstrate capillary basement membrane in formalin-fixed, paraffin-embedded tissue. [1] The basement membranes appear as black ink drawn lines. The stain is often used to examine the basement membrane of the kidney glomerulus in certain disease states.

Staining principle

The methenamine-silver complex in this stain is used to demonstrate the carbohydrate components of reticular fibers and basement membranes.[2] The periodic acid oxidizes the carbohydrate components of the basement membrane which produce aldehydes. The released aldehydes reduce the silver to a visible metallic silver. The black basement membranes can be counterstained with Hematoxylin and Eosin, or with Light Green.

Expected results

- Basement membranes - black
- Nuclei — Dark Blue
- Background — pink (with H&E counterstain)
- Background — green (with Light Green counterstain)

Jones & Jones Light Green

Stain basics

Common diagnostic utility

The purpose of this stain is primarily to distinguish pathological abnormalities in kidney diseases. The Jones stain demonstrates the spiked glomerular basement membrane (GBM), caused by subepithelial deposits, seen in membranous nephropathy in the autoimmune disease Goodpasture's syndrome, where anti-glomerular basement auto-antibodies result in damage to the basement membrane and inflammation of the capillaries. In diabetic glomerulosclerosis, the basement membrane becomes thickened up to 4-5 times the normal thickness. In nephrotic syndrome the glomerular filtration mechanism is affected, usually in the glomerular basement membrane, causing structural changes. Some symptoms include proteinuria, hypoalbuminaemia, edema and hyperlipidemia.

Tissue controls

A known positive tissue control should be utilized for monitoring the correct performance of processed tissues and test reagents.[3] Ideally, it should be representative of the tissues it is usually used to diagnose. An example of a positive control material would be formalin-fixed, paraffin-embedded human kidney.[4]

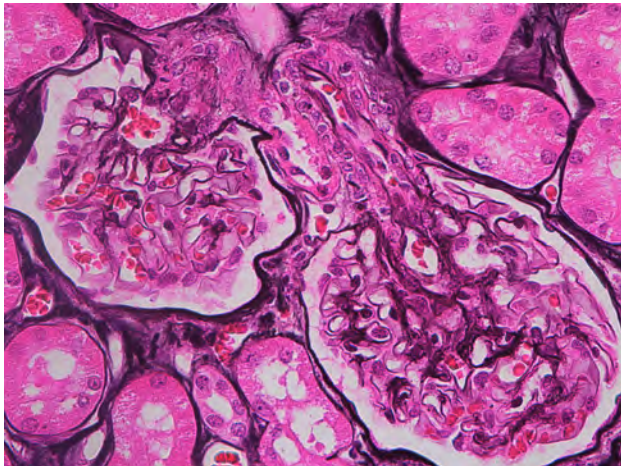

Figure 3. Kidney stained with Jones using Hematoxylin and Eosin counterstain, 400x.

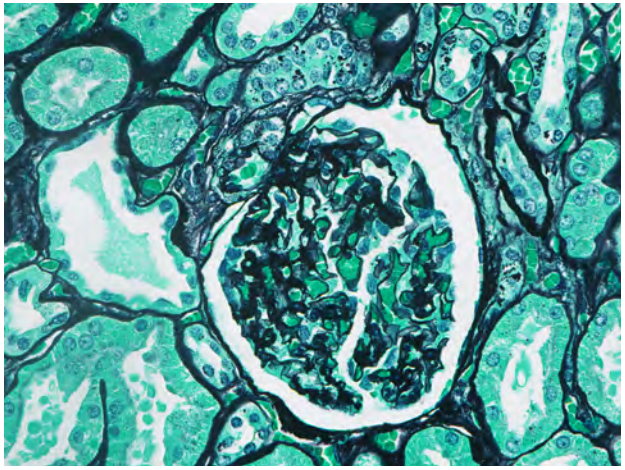

Figure 4. Kidney stained with Jones using Light Green counterstain, 400x.

Jones

Technical notes and references

Technical notes

1. Section thickness may affect quality and intensity of staining. In order to properly visualize the glomerular basement membrane, tissue sections should be very thin. Cut sections preferably at 2 to 3 µm and pick the sections up on glass slides.

References

1. Koski JP. Silver methenamine-borate (MB): Cost reduction with technical improvement in silver nitrate-gold chloride impregnations. *J Histotechnol*. 1981;4:115.
2. Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology. 2nd edition. St. Louis, MO: C.V. Mosby Company; 1980.
3. Clinical and Laboratory Standards Institute (CLSI). CLSI Web site. http://www.clsi.org/. Accessed November 3, 2011.
4. Carson F, Hladik C. Histotechnology: *A Self Instructional Text.* 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.

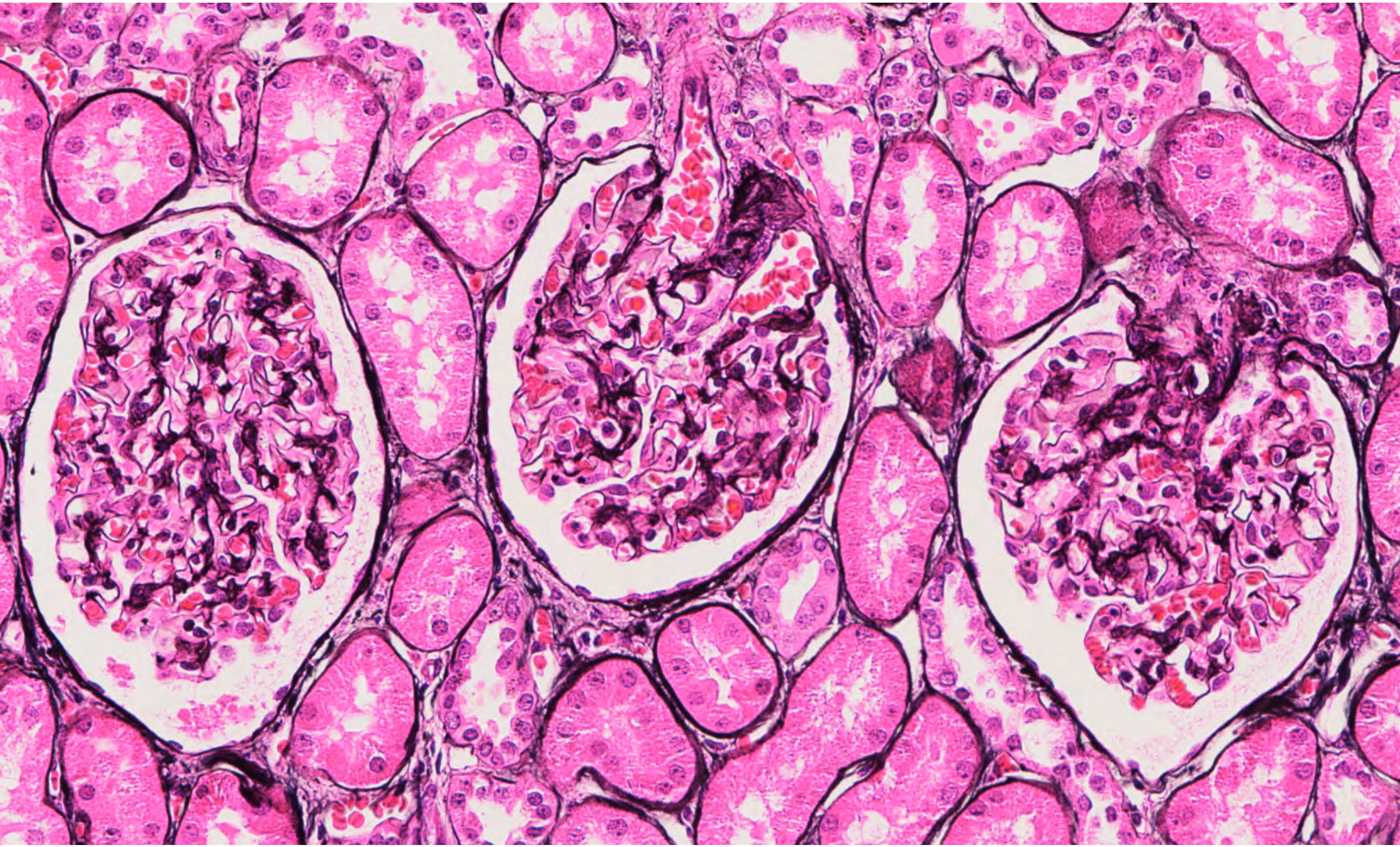

Figure 5. Kidney stained with Jones using Hematoxylin and Eosin counterstain, 200x. H&E

Mucicarmine

Stain basics

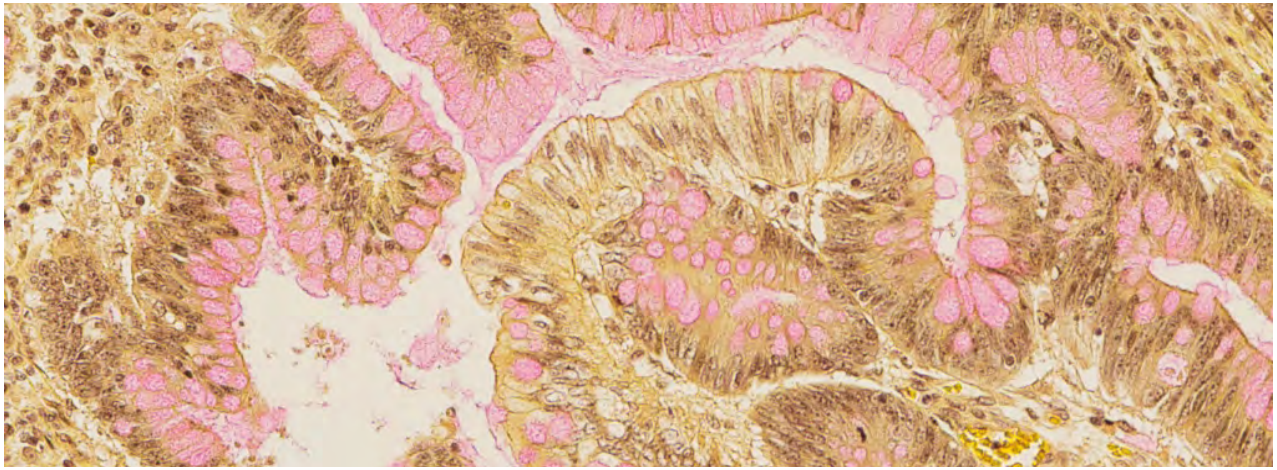

Figure 1. Colon adenocarcinoma stained with Muci stain, 200x.

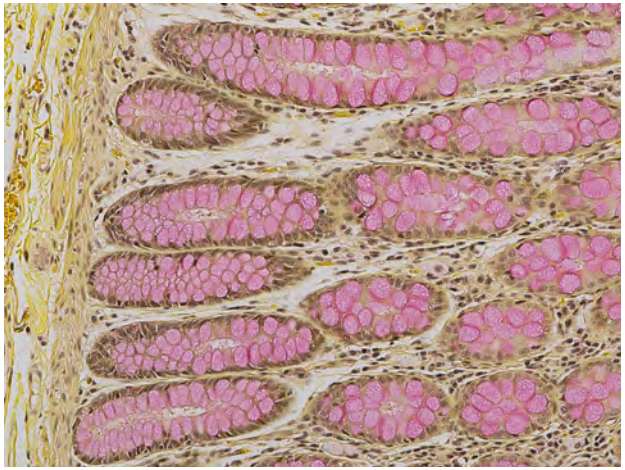

Figure 2. Normal colon stained with Muci stain, 200x.

Purpose

Mucicarmine stain is intended for use as a qualitative histologic stain to detect acid mucopolysaccharides (mucin) in formalin-fixed, paraffin-embedded tissue. Mucin is a secretion of acid mucopolysaccharides and other substances produced by a variety of epithelial and connective tissue cells. In certain inflammatory processes and certain intestinal carcinomas, epithelial cells secrete excess mucin.[1]

Staining principle

The staining reaction is based on the reaction of an aluminum-carmine chelate complex attached to acid groups of mucin. Carmine and aluminum mordant combine to stain the epithelial mucin a deep rose to red color. Although the mechanism is not completely understood, it is believed that the aluminum forms a chelate complex with carmine by dye lake formation, to produce a net positive charge.[1] The complex then attaches to the acid groups of the mucin. Tartrazine counterstain is applied to provide a contrasting yellow background.

Expected results

- Mucin — rose red
- Nuclei — black/gray
- Background/connective tissue — yellow

Mucicarmine

Stain basics

Common diagnostic utility

Mucicarmine stain detects mucins of epithelial origin. Fibroblastic or connective tissue mucins may stain poorly.[2] The purpose of this stain is primarily to determine the site of a primary tumor or to distinguish undifferentiated mucin-negative squamous cell lesions from mucin-positive adenocarcinomas.[1] As is the case with current manual stains, mucicarmine stain may not be appropriate for detection of metastatic cancers of the stomach.[3] The stain may also be used as an aid in the identification of Cryptococcus neoformans, a pathogenic fungus containing mucin in its capsule.

Tissue controls

A known positive tissue control should be utilized for monitoring the correct performance of processed tissues and test reagents.[4] An appropriate control tissue for the Mucicarmine stain should contain sialomucins. Ideally, it should be representative of the tissues it is usually used to diagnose.[5] The most common tissue controls are tissues that contain normal colonic mucosa, adenocarcinomas or Cryptococcus infected tissue.

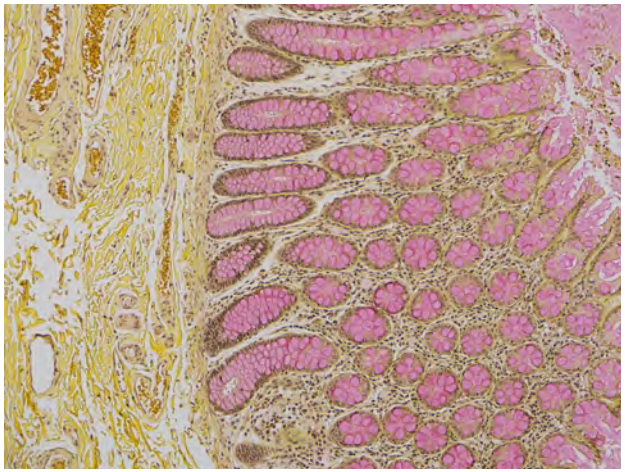

Figure 3. Normal colon stained with Muci stain, 100x

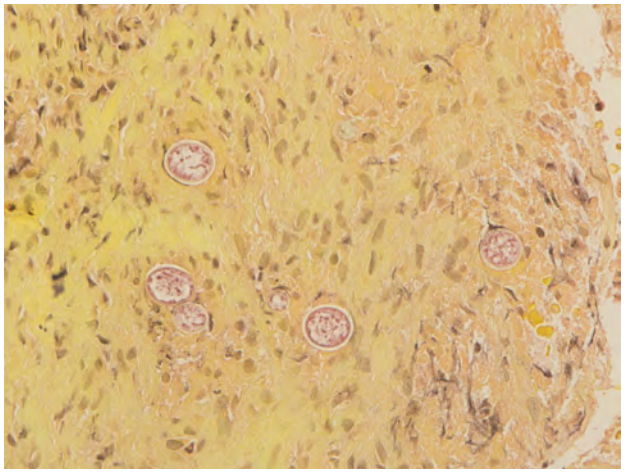

Figure 4. Cryptococcus organisms in bowel stained with Muci stain, 200x.

Mucicarmine

Technical notes and references

Technical notes

1. Section thickness may affect quality and intensity of staining. Cut sections, usually 3–5 µm, and pick the sections up on glass slides.

References

1. Sheehan DC, Hrapchak BB. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis, MO: C.V. Mosby Company; 1980.
2. Thompson SW. *Selected Histochemical and Histopathologic Methods*. Springfield, IL: CC Thomas; 1966:453.
3. Silverberg SG, Delellis RA, Frable WJ. *Principles and Practice of Surgical Pathology and Cytopathology*. New York, NY: Churchill Livingstone Inc.;1997:51.
4. Clinical and Laboratory Standards Institute (CLSI). CLSI Web site. http://www.clsi.org/. Accessed November 3, 2011.
5. Carson F, Hladik C. Histotechnology:*A Self Instructional Text* 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.

PAS

Stain basics

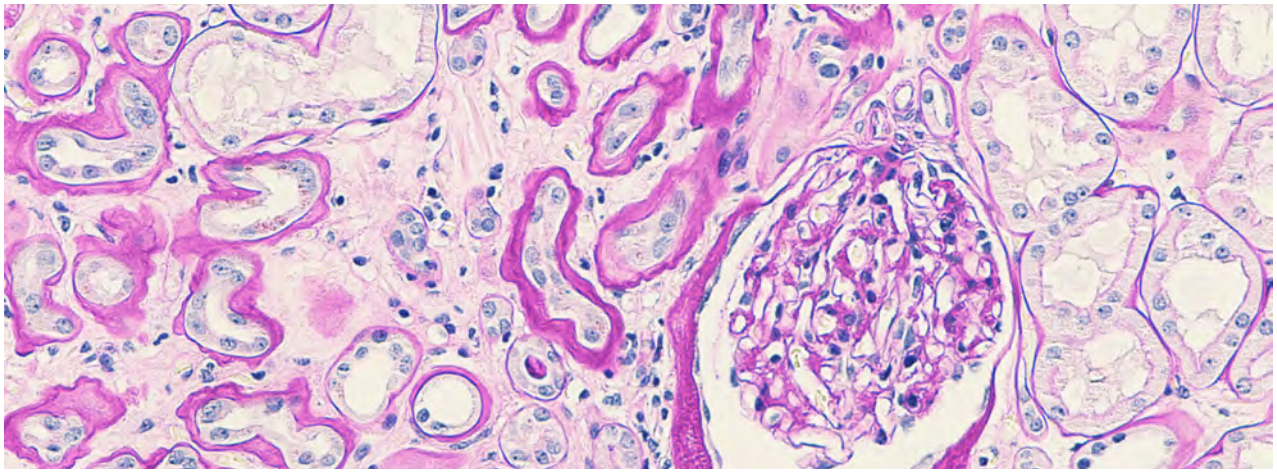

Figure 1. Kidney showing glomerular basement membrane stained with PAS stain

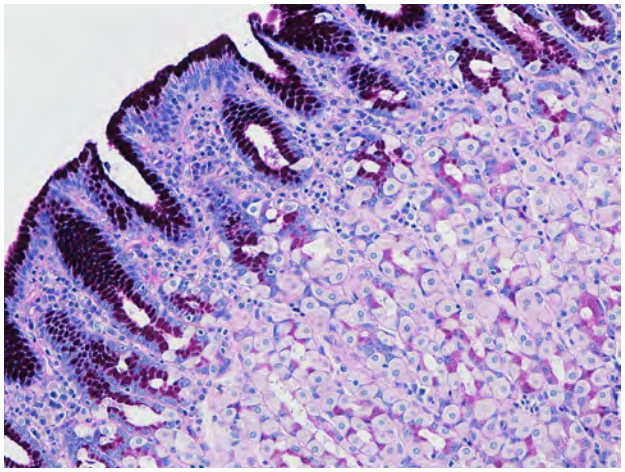

Figure 2. Stomach showing intense mucosal staining with PAS stain, 200x.

Purpose

PAS stain may be used as a qualitative histologic stain in formalin-fixed, paraffin-embedded tissue to demonstrate the presence of neutral polysaccharides in cellular substances and structures such as glycogen, starch, cellulose, glycolipids, glycoproteins and many other carbohydrates. It is useful as a stain for basement membranes, plasma membrane stain and carbohydrate secretions and granules.

Staining principle

The staining reaction is based on the reactivity of Schiff's regent with aldehyde groups. Aldehyde groups are produced in polysaccharides though the oxidation of glycol groups by periodic acid.[5] This is followed by selective staining of the aldehyde groups by a colorless Schiff's Reagent. Schiff's Reagent forms a colorless dialdehyde compound that is transformed to the magenta colored final staining of the glycol-containing cellular components.[5]

Expected results

- Neutral polysaccharides, glycogen, fungus and basement membrane — bright magenta
- Nuclei — dark blue
- Background and other tissue components — depend on combination of stain used.

PAS

Stain basics

Common diagnostic utility

PAS stain may be used to demonstrate reticular fibers, basement membrane, fungus and neutralmucopolysaccharides.[1] PAS stain may also be used to aid in distinguishing a PAS-positive secreting adenocarcinoma from an undifferentiated PAS-negative squamous cell carcinoma.[2] It may also be useful for the detection of glycogen in certain liver diseases. The basic PAS stain may be combined with other adjunctive staining reagents for the better elucidation of certain cellular and tissue components.

Tissue controls

A known positive tissue control should be utilized for monitoring the correct performance of processed tissues and test reagents.[4] Ideally, it should be representative of the tissues it is usually used to diagnose.[3] An example of a positive control material would be formalin-fixed, paraffin-embedded human kidney, a liver tissue specimen containing containing glycogen, and marrow.

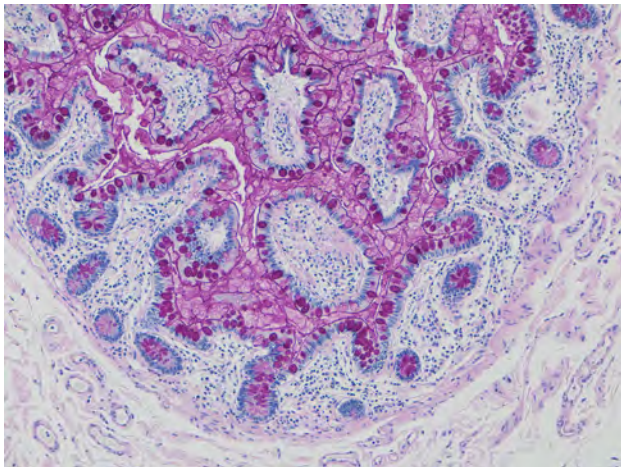

Figure 3. Duodenum showing strong mucosal staining with PAS stain, 200x.

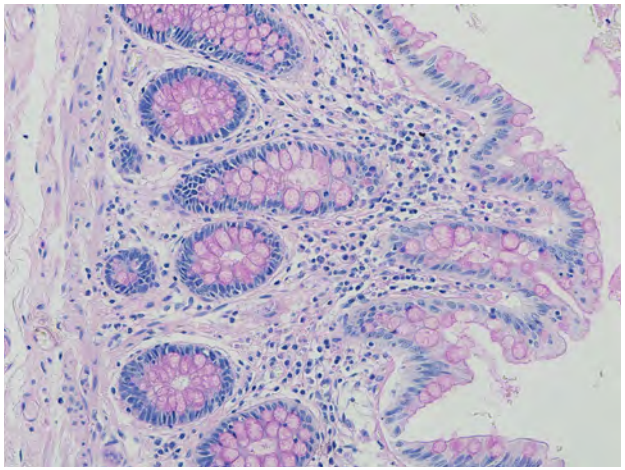

Figure 4. Colon showing lighter mucosal staining with PAS stain, 200x.

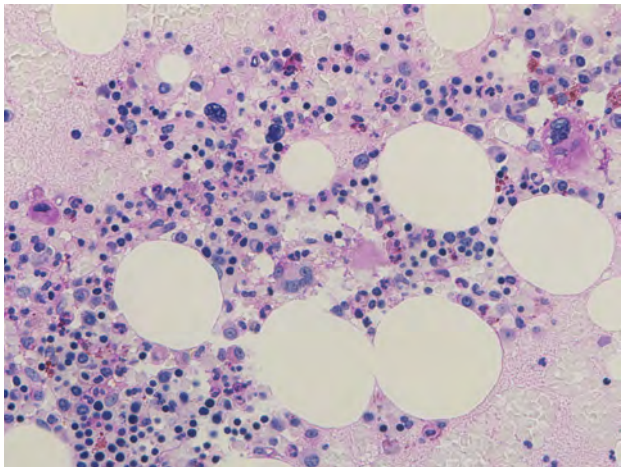

Figure 5. Section of FFPE Bone marrow clot showing PAS positive cells staining with PAS stain, 400x.

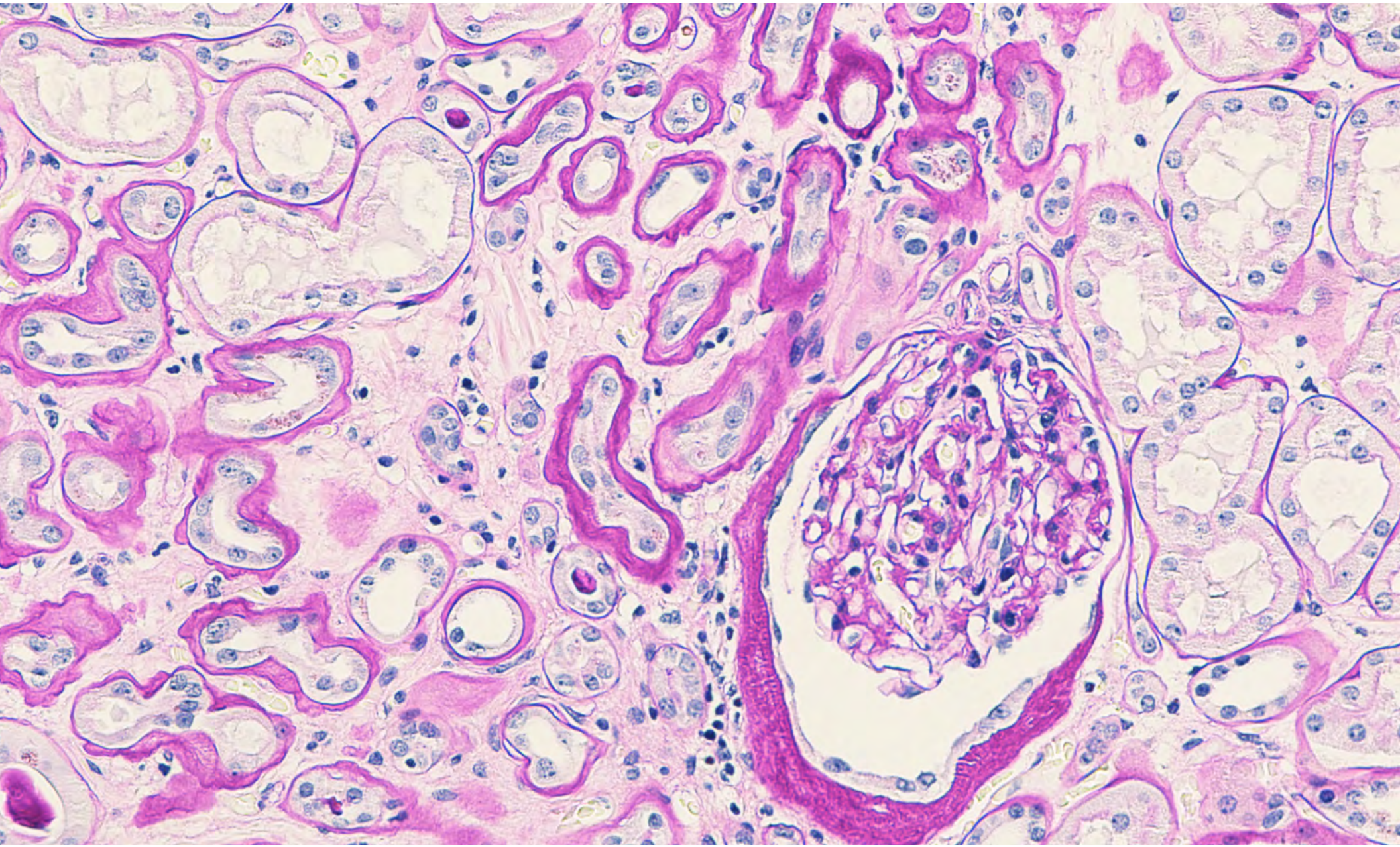

Figure 6. Kidney showing glomerular basement membrane stained with PAS stain

PAS/Diastase

Stain basics

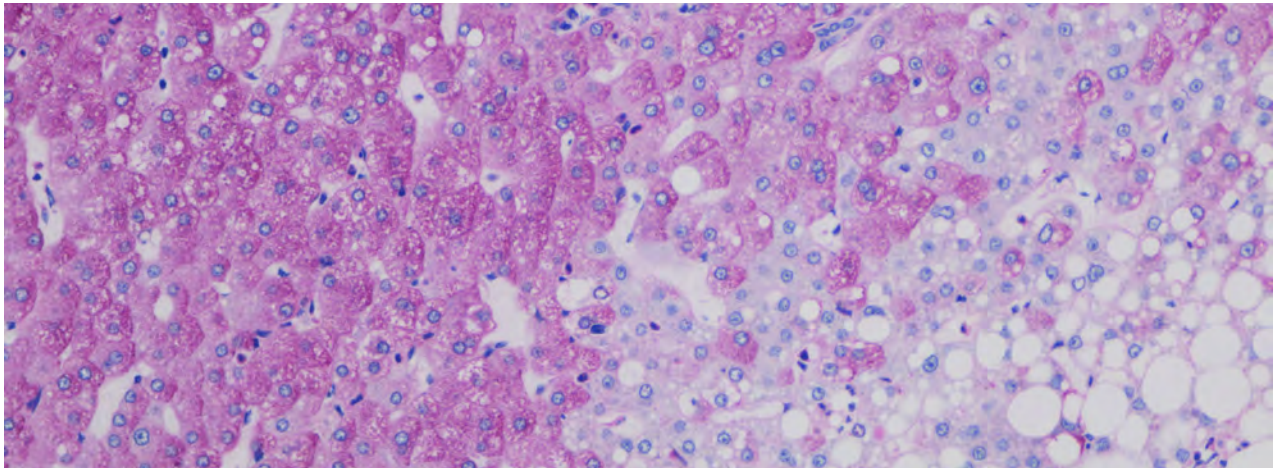

Figure 1. Glycogen storage disease in liver showing fairly strong magenta staining with PAS stain, 200 x

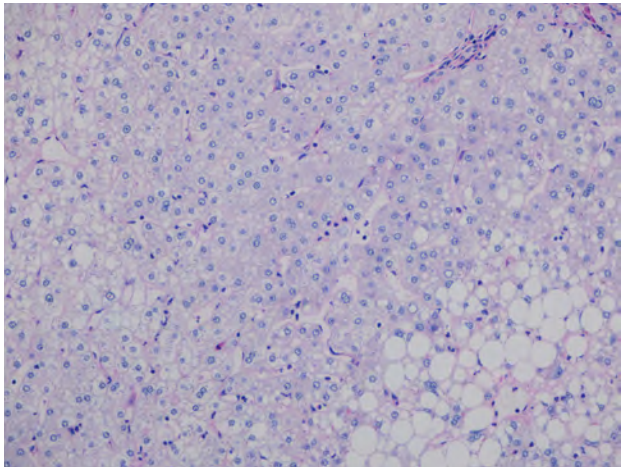

Figure 2. Glycogen storage disease in liver showing no staining magenta staining with PAS stain after digestion with Diastase, 200 x

Purpose

The PAS stain in combination with enzymatic digestion with Diastase is used to aid in the differentiation of glycogen form other neutral polyscaccharides in tissues.

Staining principle

The PAS stain may be combined with diastase reagent for the demonstration of glycogen. The PAS stain uses periodic acid to oxidize glycols to aldehydes. Schiff's reagent forms a colorless dialdehyde compound that is transformed to the fuschia-colored final staining of glycol containing cellular components.[1]

In the combined PAS/Diastase stain, two parallel sections are run. One section is digested with diastase prior to staining with the PAS reaction. The Diastase digests away the glycogen before PAS staining occurs. The second is stained with the PAS reaction without digestion. The diastase removes any glycogen in the tissue thereby confirming the presence of glycogen by its presence in the undigested section and its absence in the digested section.

Expected results

- Neutral polysaccharides, glycogen, fungus and basement membrane - bright magenta
- Nuclei – dark blue
- Background and other tissue components – depend on combination of stain used.

PAS/Diastase

Stain basics

Common diagnostic utility

Diastase is useful as an aid in the diagnosis of glycogen storage disease[1]

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Tissue controls

A known positive tissue control should be utilized for monitoring the correct performance of processed tissues and test reagents.[4] Ideally, it should be representative of the tissues it is usually used to diagnose.[3] An example of a positive control material would be formalin-fixed, paraffin-embedded human liver sample from glycogen storage disease.

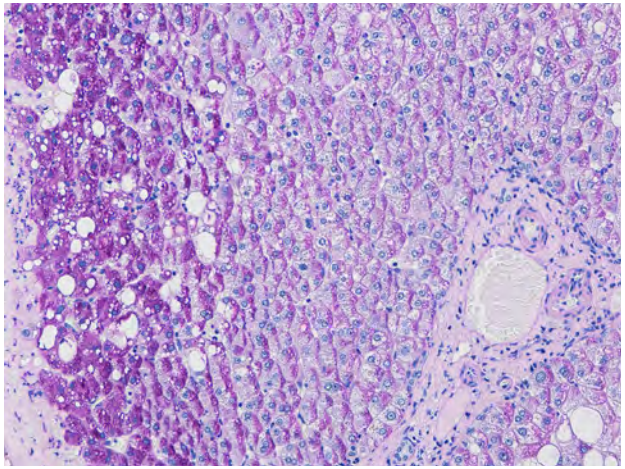

Figure 3. Liver with glycogen stained with PAS, 200x.

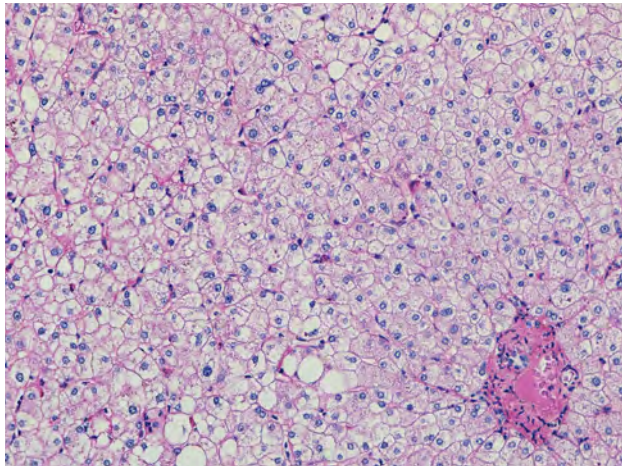

Figure 4. Liver showing absence PAS (glycogen) staining following digestion with diastase, 200x.

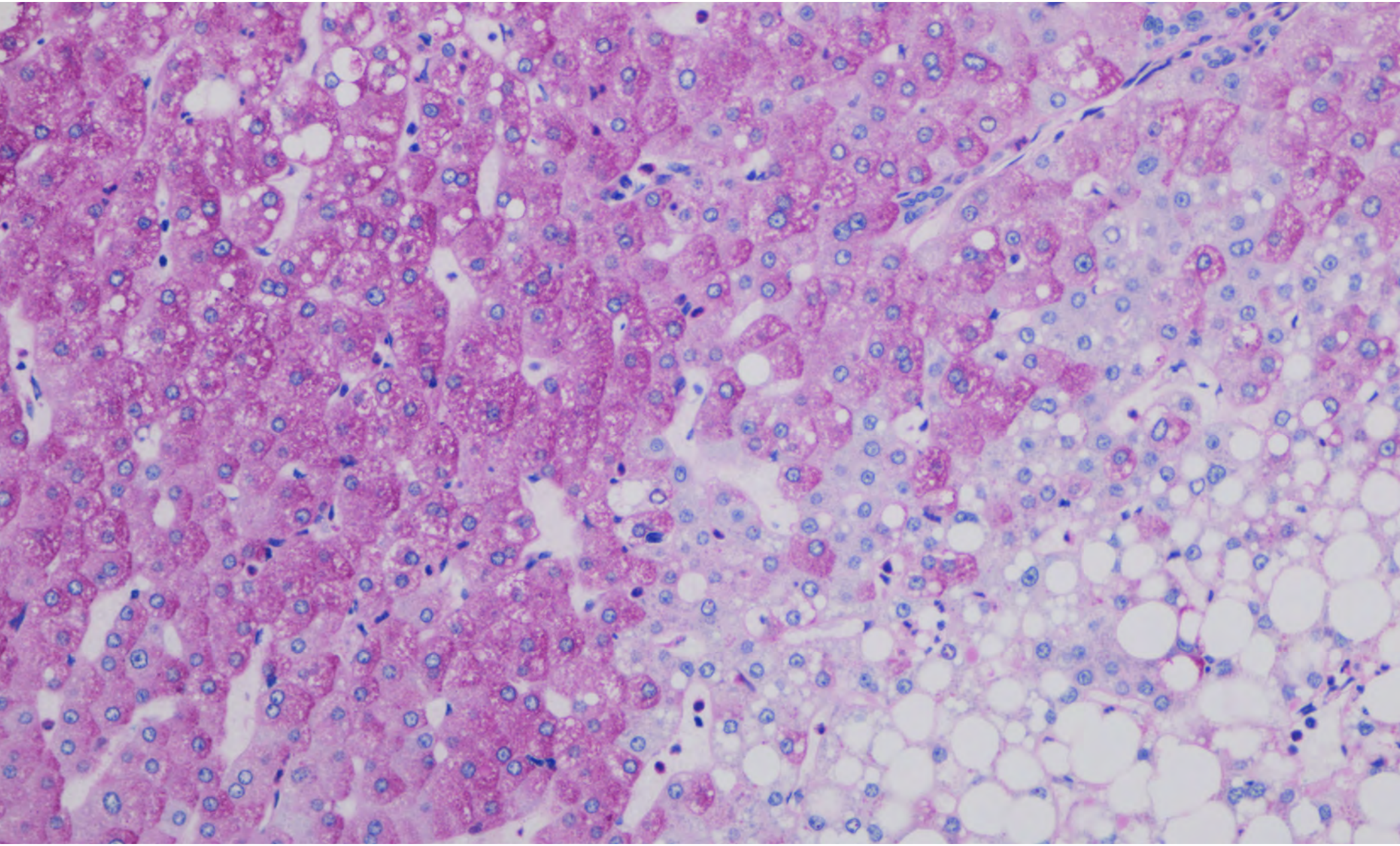

Figure 5. Glycogen storage disease in liver showing fairly strong magenta staining with PAS stain, 200 x

PAS/Alcian Blue

Stain basics

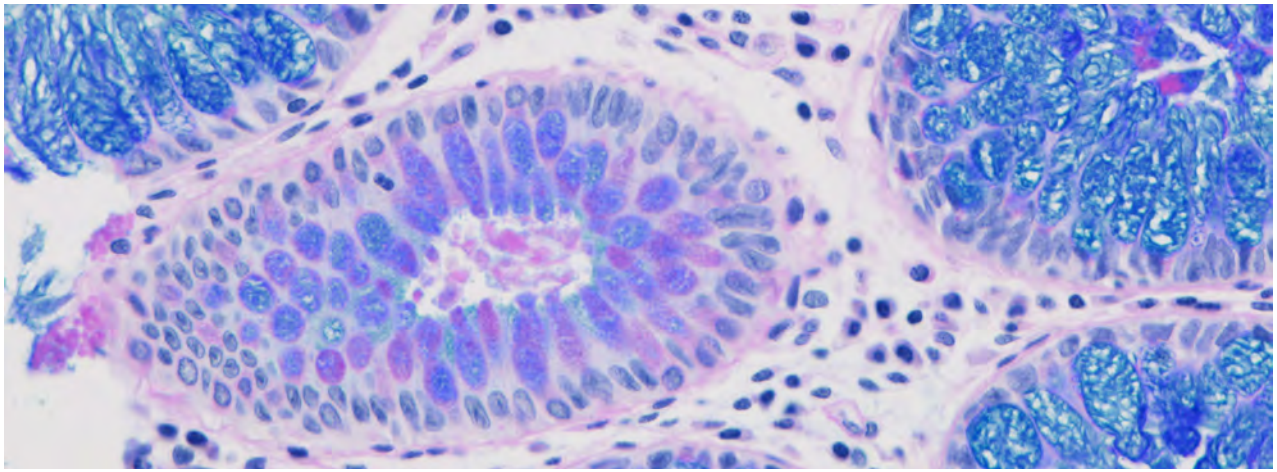

Figure 1. Adenocarcinoma stained with PAS and Alcian Blue. Sialomucins that have acid and neutral mucopolysaccharides stain purple. Mucosa that have only neutral mucins will stain red.

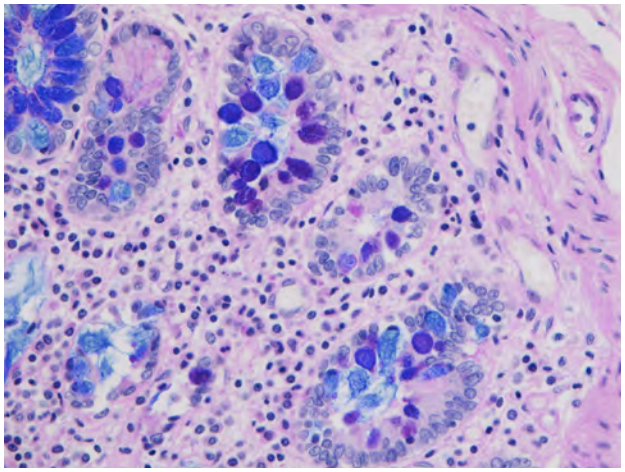

Figure 2. Bowel stained with PAS + Alcian blue, 600x

Purpose

The PAS stain may be combined with Alcian Blue as a qualitative histologic stain to differentiate acid mucopolysaccharides (mucins) from neutral mucopolysaccharides

Staining principle

The staining reaction is based on the oxidation of glycol to aldehyde followed by selective staining of the aldehyde groups by Schiff's reagent. The acidic Alcian Blue reagent differentially stains acid mucins and neutral mucopolysaccharides.

Expected results

- Neutral mucins - magenta with Schiff reaction from PAS
- Acid mucins - blue with Alcian Blue
- Neutral+Acid mucins - purple with both PAS and Alcian Blue

PAS/Alcian Blue

Stain basics

Common diagnostic utility

The PAS/Alcian Blue stain may be used for the discrimination of acid mucins from neutral sialomucins in the detection of intestinal metaplasia in chronic gastritis, Barrets Esophagus and other GI abnormalities.

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Tissue controls

A known positive tissue control should be utilized for monitoring the correct performance of processed tissues and test reagents.[4] Ideally, it should be representative of the tissues it is usually used to diagnose.[3] An example of a positive control material would be formalin-fixed, paraffin-embedded GI tract tissue such as stomach and duodenum, and small bowel.

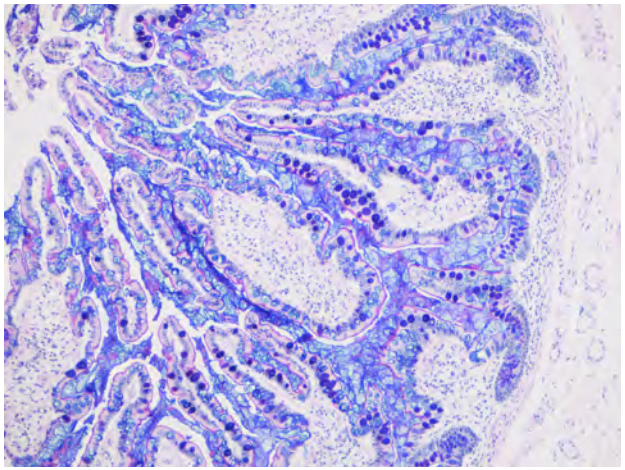

Figure 3. Stomach stained with PAS/Alcian Blue, 100x.

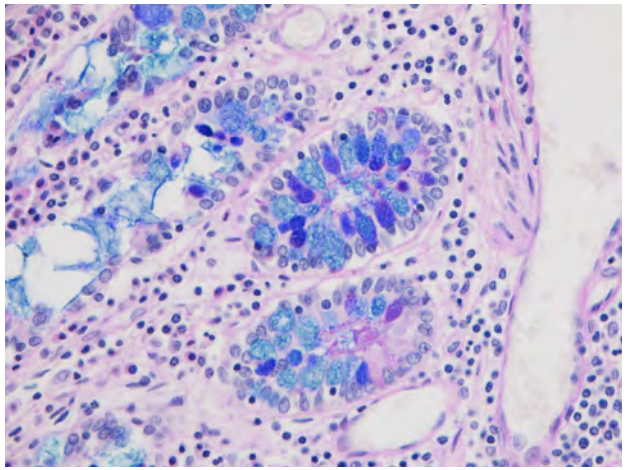

Figure 4. Colon stained with PAS/Alcian Blue, 400x.

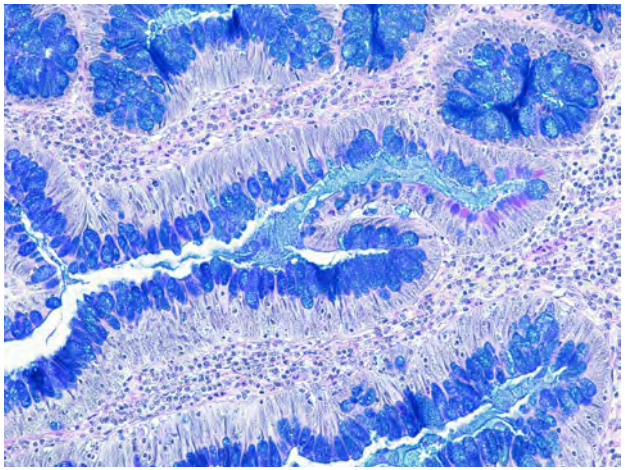

Figure 5. Colon carcinoma stained with PAS/Alcian Blue, 200x.

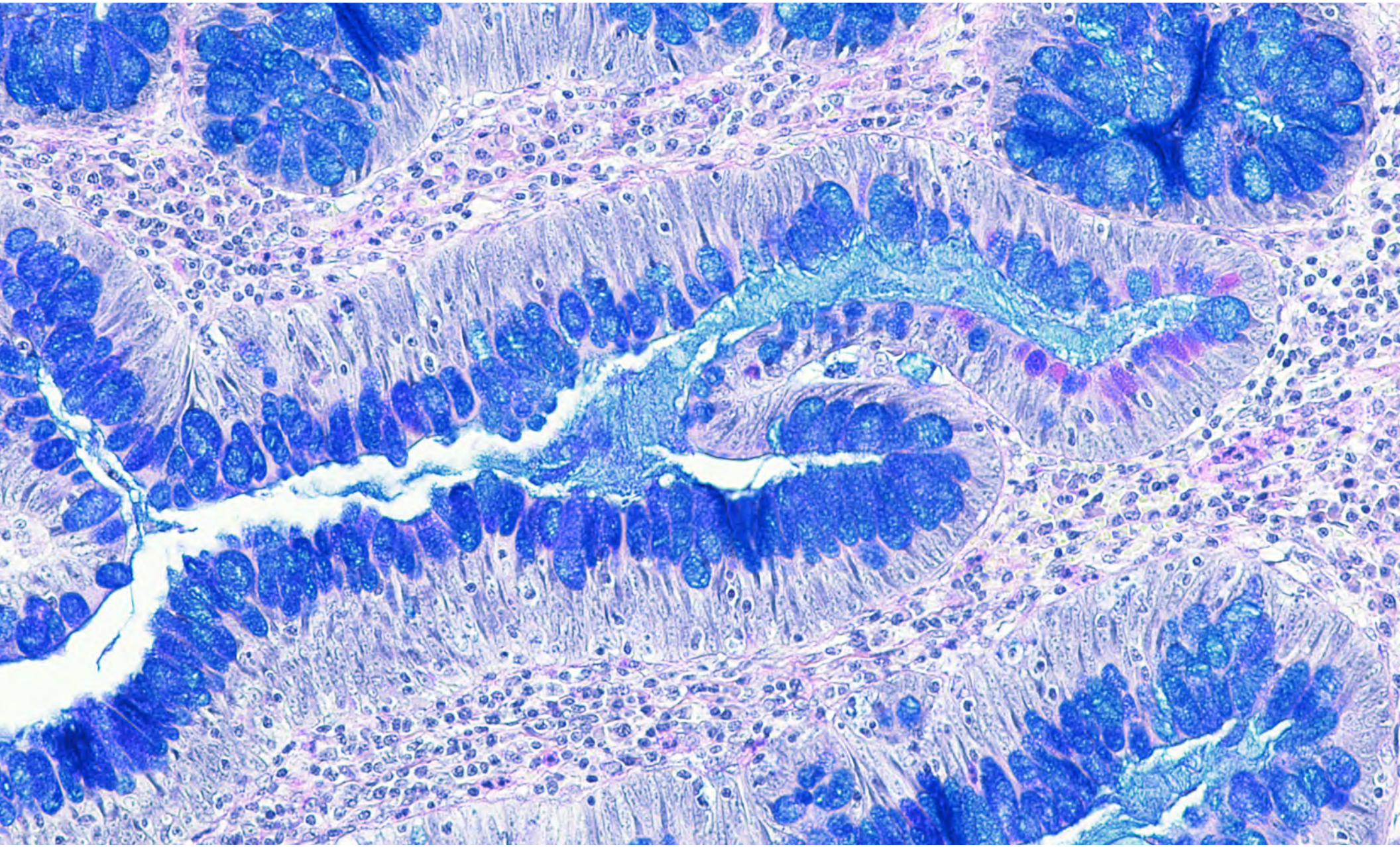

Figure 6. Colon carcinoma stained with PAS/Alcian Blue, 200x.

PAS/Light Green

Stain basics

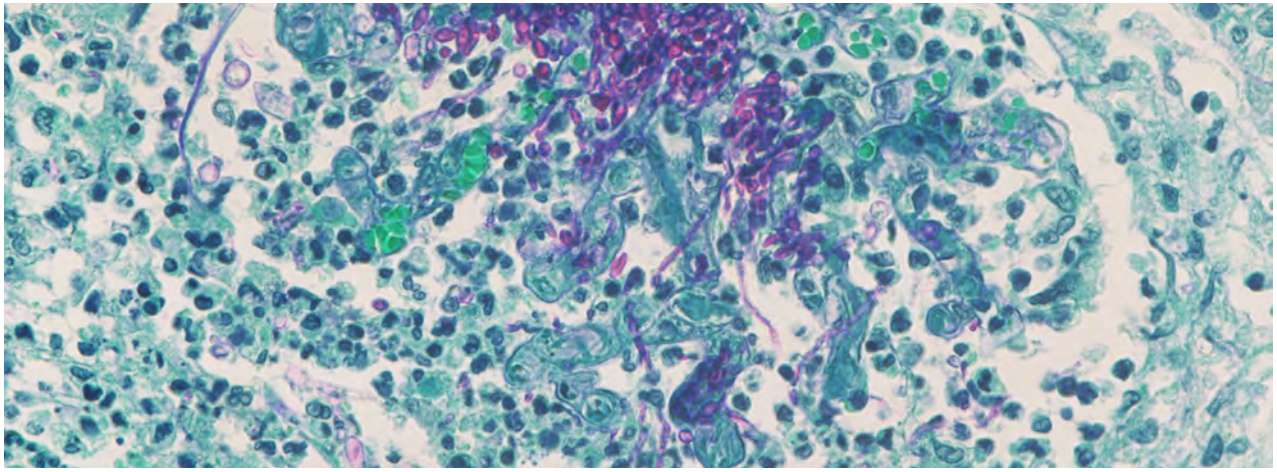

Figure 1. Stomach showing intense mucosal staining with PAS stain, 200x.

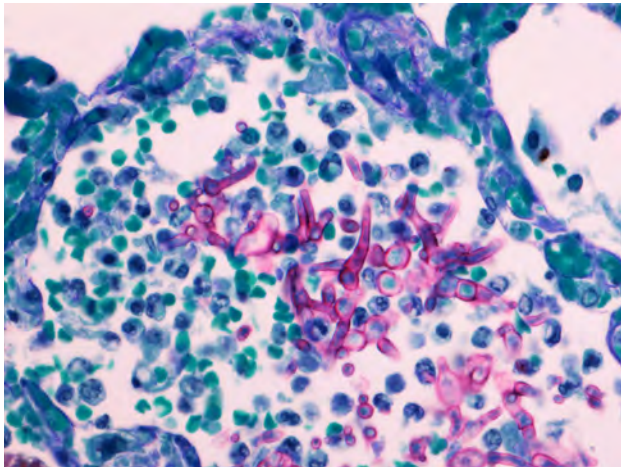

Figure 2. Bowel stained with PAS/Light green showing showing intense magenta staining of mucin with PAS stain , 200x

Purpose

The PAS stain in combination with Light Green counterstain may be used used as qualitative histologic stain for detection of fungus in formalin-fixed, paraffin-embedded tissue.

Staining principle

The PAS stain uses periodic acid to oxidize glycols to aldehydes. Schiff's Reagent forms a colorless dialdehyde compound that is transformed to the magenta-colored final staining of the glycol containing cellular components.[1] The Light Green counterstain stains the background light green providing improved contrast to magenta.

Expected results

- Fungus - magenta
- Other tissue components - medium green
- Red blood cells - bright green
- Nuclei - Blue with hematoxylin (optional)

PAS/Light Green

Stain basics

Common diagnostic utility

PAS may also be combined with light Green as a counterstain to aid in the visualization of fungus in tissue sections. The light green stains the connective tissue components, combining with the PAS stain to give them a purple color. The fungus is not staining with Light Green thus allowing better discrimination of the organisms from the other PAS positive tissue components.

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Tissue controls

A known positive tissue control should be utilized for monitoring the correct performance of processed tissues and test reagents.[4] Ideally, it should be representative of the tissues it is usually used to diagnose.[3] An example of a positive control material would be formalin-fixed, paraffin-embedded tissue known to contain fungus or known to contain neutral polysaccharides, such as colon.

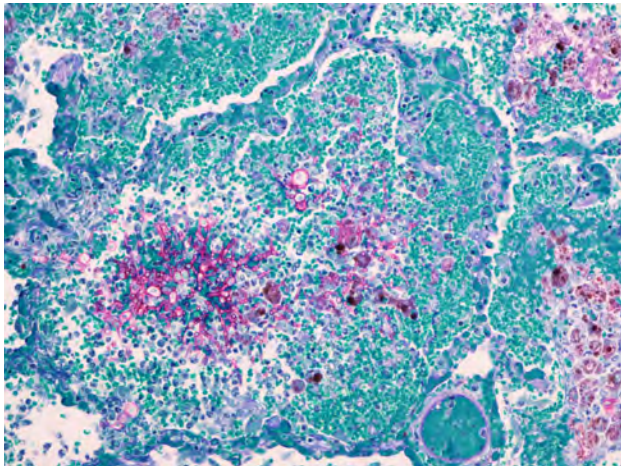

Figure 3. Fungus in lung stained with PAS/Light Green, 200x.

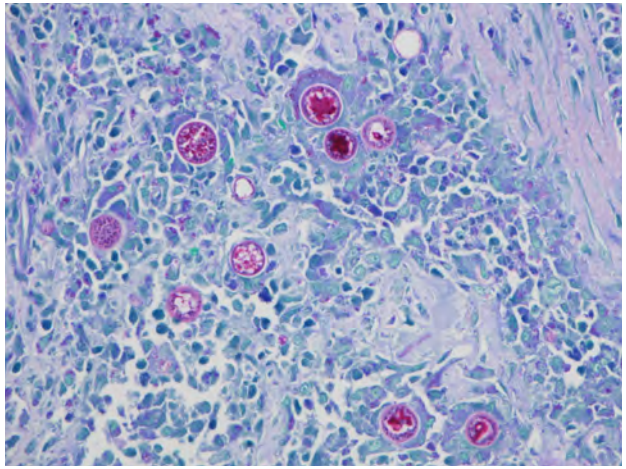

Figure 4. Intestinal tissue with Cryptococcus organisms stained with PAS/Light Green, 400x.

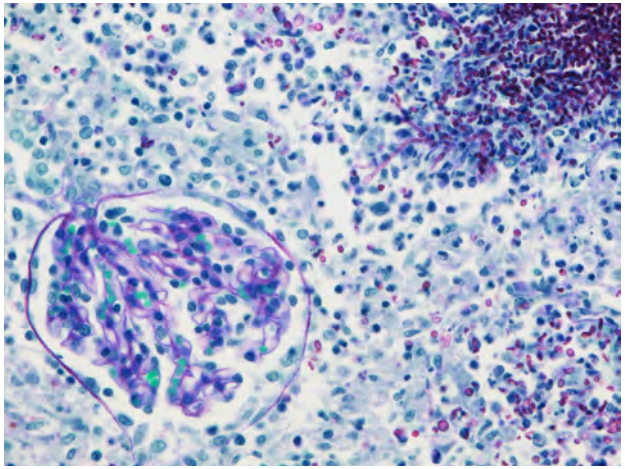

Figure 5. Fungal organisms in kidney stained with PAS/Light Green, 400x.

PAS, PAS/Diastase, PAS/Light Green, PAS/Alcian Blue

Technical notes and references

Technical notes

1. Section thickness may affect quality and intensity of staining. In order to properly visualize the glomerular basement membrane in kidney biopsies, tissue sections should be very thin. Cut sections, preferably, at 2 to 3 µm, and pick the sections up on glass slides.
2. When staining for microorganisms, it is important to ensure that the waterbath is scrupulously clean prior to sectioning in order to prevent introduction of extraneous microorganisms onto the specimen section slide. Therefore, it is recommended not to use water left standing overnight.
3. Alcian Blue stain is pH dependent and therefore sensitive to fluctuations of the deionized or distilled water used for the wash steps. Careful monitoring of water quality is recommended.

References

1. Thompson SW. Selected Histochemical and Histopathological Methods. Springfield; CC Thomas; 1966:
2. Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology. 2nd edition. St. Louis, MO: C.V. Mosby Company; 1980.
3. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
4. Clinical and Laboratory Standards Institute (CLSI). CLSI Web site. http://www.clsi.org/. Accessed November 3, 2011.
5. Hotchkiss RD: A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. Arch Biochem. 1948;16:131.

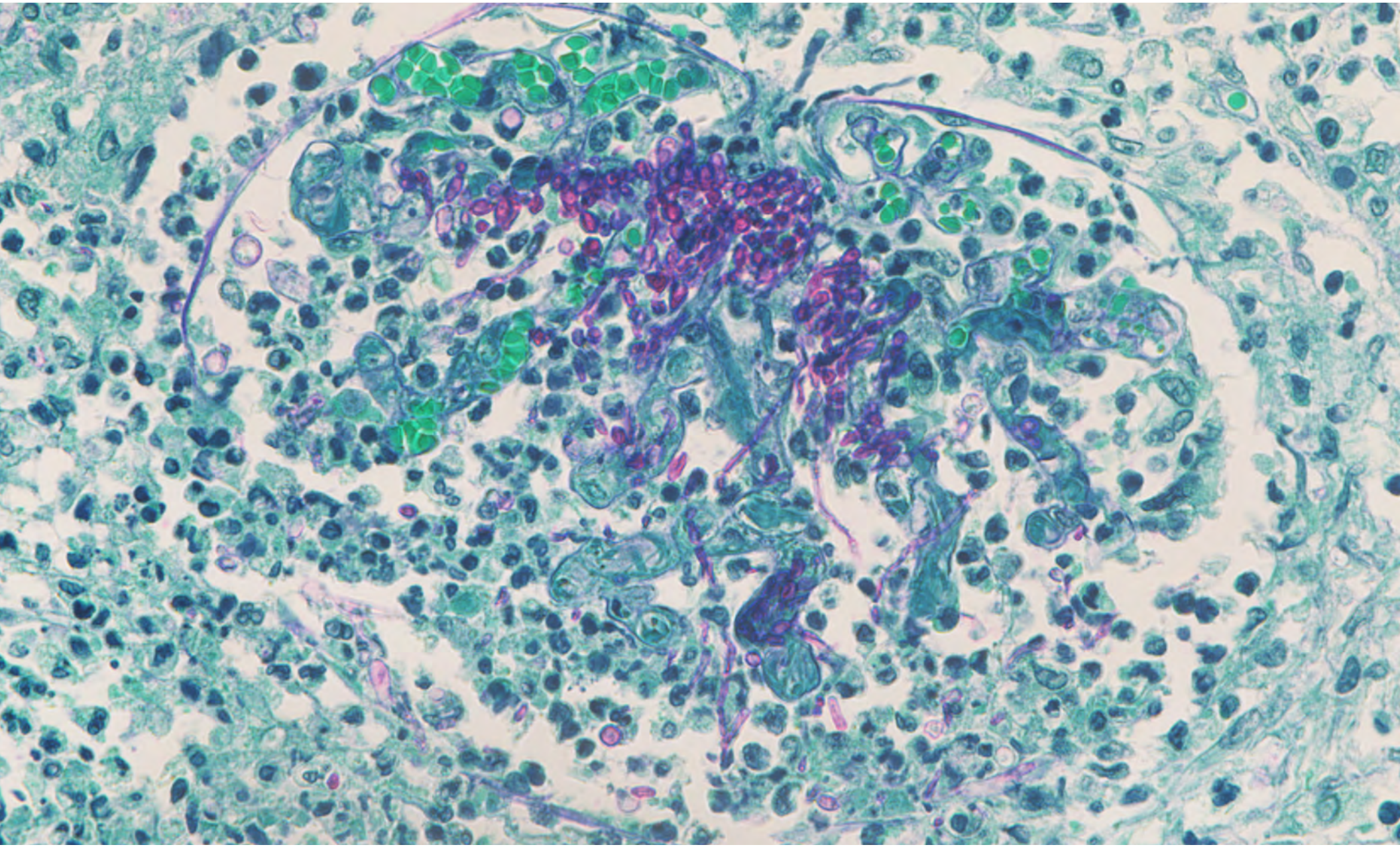

Figure 6. Stomach showing intense mucosal staining with PAS stain, 200x.

Reticulum

Stain basics

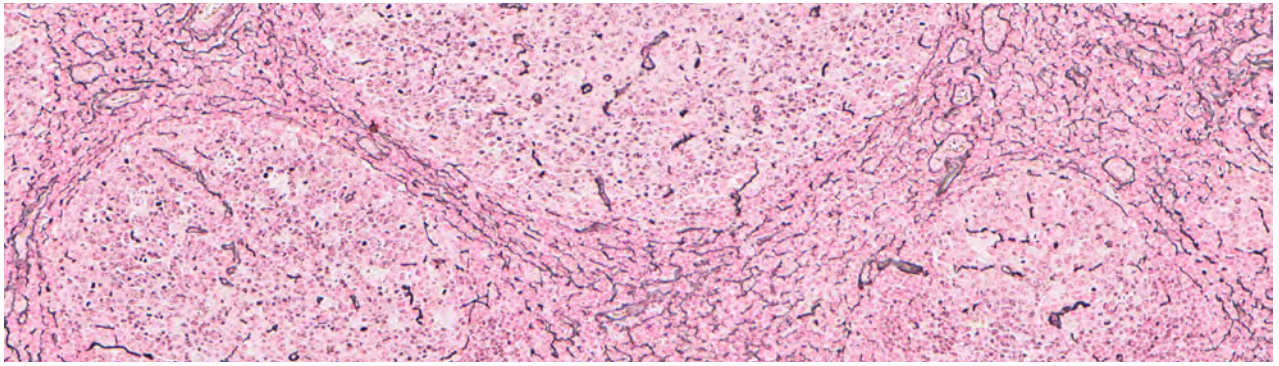

Figure 1. Tonsil stained with Retic stain

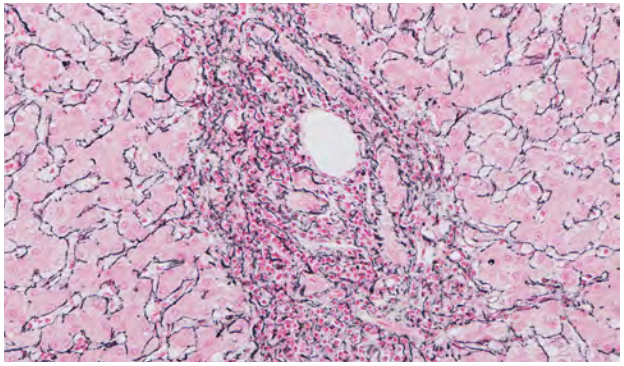

Figure 2. Liver stained with Retic stain demonstrating continuous fine reticular meshwork around hepatic cords in the perisinusoidal space.

Purpose

The Reticulum stain is intended for use as a qualitative histologic stain to demonstrate reticular fibers in formalin-fixed, paraffin-embedded tissue. In certain tumors, reticulum is located in a characteristic position in relation to the actual tumor cells. Reticulum stain can be used to show disease states in organs such as the liver, spleen and kidney by demonstrating reticular patterns[1] not normal to the organ. In normal liver, the fibers are well defined strands, but in necrotic or cirrhotic liver, the fibers have discontinuous patterns.

Staining principle

Reticular fiber is composed of one or more types of very thin and delicately woven strands of type III collagen. These strands build a highly ordered cellular network and provide a supporting network. Many of these types of collagen have been combined with carbohydrate. They are reactive with Periodic Acid-Schiff (PAS) and are also argyrophilic (affinity for silver). They react with silver stains where they selectively reduce silver salts to metallic silver.

The carbohydrates are oxidized with an acidic solution of potassium permanganate and produce reactive aldehyde groups. This followed by an aqueous oxalic acid solution which bleaches the brown color left by the potassium permanganate. The oxalic acid is followed by an iron alum which impregnates the fibers and forms complexes with the aldehyde groups. Following the iron alum, ammoniacal silver is added which replaces the complexed iron. The silver is reduced to a black precipitate by exposure to 10% neutral buffered formalin, resulting in metallic silver impregnation of the fibers. Gold chloride and sodium thiosulfate are then used to tone the color of the fibers and remove excess silver, respectively.

Expected results

- Reticular fibers — black
- Collagen — gray
- Nuclei/background — red to pink

Reticulum

Stain basics

Common diagnostic utility

Reticular fibers composed mainly of type III collagen form a type of connective tissue called reticulin. It is made up of fine fibers cross-linked to form a meshwork.[4] It acts as support system in soft tissue organs such as the spleen, lymph nodes and tonsils, liver and bone marrow.[5] Reticulum stain may be helpful in the identification of changes in the reticular structure in certain disease processes such as primary hepatocellular carcinoma and liver cirrhosis. It may also be used in the diagnosis of diseases involving the bone marrow. Reticular fibers in the bone marrow are formed by fibroblasts and are normally few — mainly perivascular and periendosteal. They may be increased or reduced in many bone marrow disease conditions. The reticulin stain has even been helpful in identifying Cryptococcus fungus in bone biopsies.[6]

Tissue controls

A known positive tissue control should be utilized for monitoring the correct performance of processed tissues and test reagents. Ideally, it should be representative of the tissues it is usually used to diagnose.[3] An example of a positive control material would be formalin-fixed, paraffin-embedded human tissue with defined reticular fibers such as liver, spleen, kidney or lymph nodes.[2]

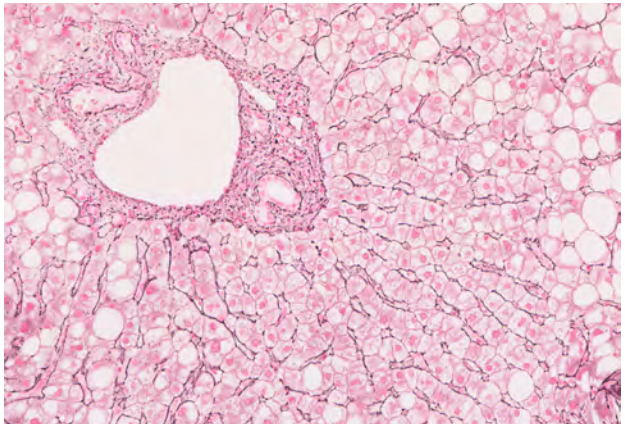

Figure 3. Liver biopsy stained with Retic stain demonstrating reticular fiber network.

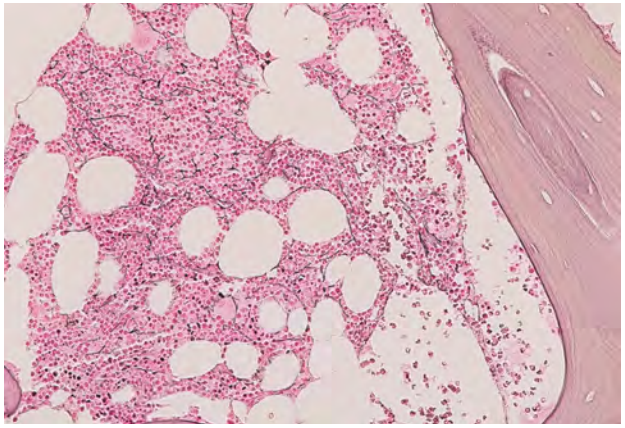

Figure 4. Bone marrow trephine stained with Retic stain demonstrating periendosteal reticular fibers

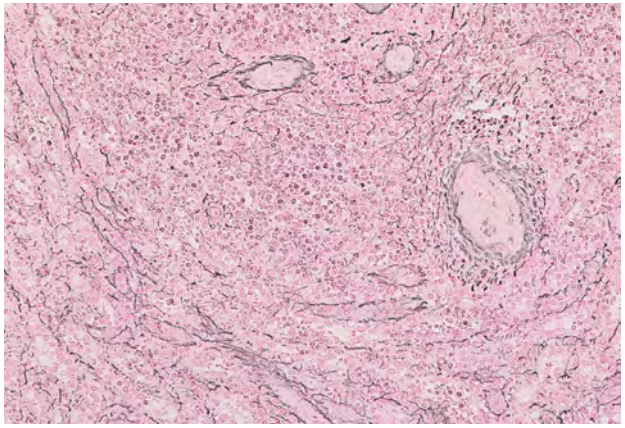

Figure 5. Normal spleen stained with Retic stain demonstrating the reticular fiber network of the normal splenic architecture

Reticulum

Technical notes and references

Technical notes

1. Section thickness may affect quality and intensity of staining. Cut sections, usually 3–5 µm, and pick the sections up on glass slides.

References

1. Sheehan DC, Hrapchak BB. *Theory and Practice of Histotechnology,* 2nd edition. St. Louis, MO: C.V. Mosby Company; 1980:181-188.
2. Carson F, Hladik C. Histotechnology: *A Self Instructional Text* 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
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6. Jasmina Ahluwalia, Gurjeewan Garewal, Reena Das, Kim Vaiphei. The reticulin stain in bone marrow biopsies – beyond marrow fibrosis. *British Journal of Haematology*, Volume 123, Issue 3, page 379, November-I 2003

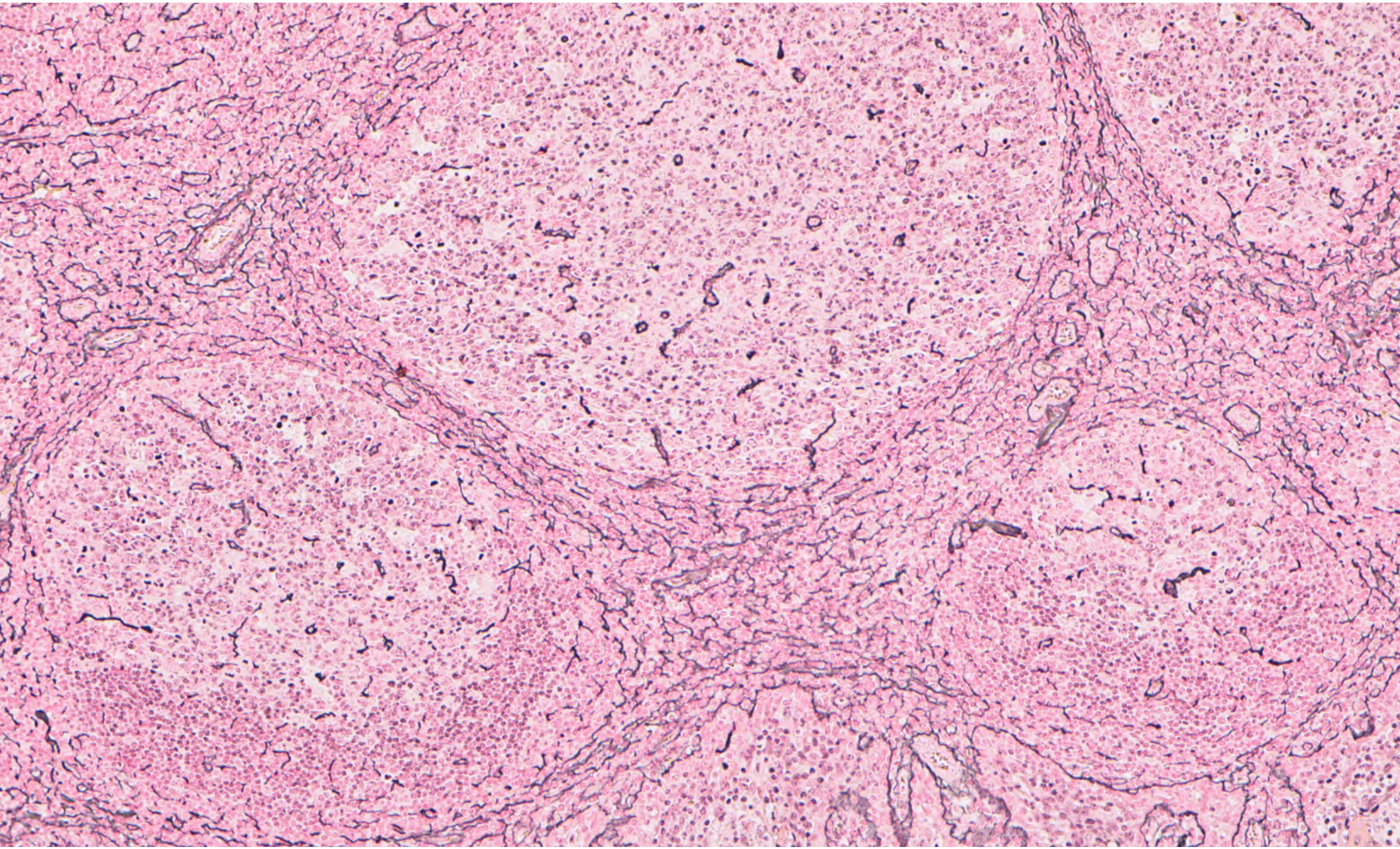

Figure 6. Tonsil stained with Reticulum stain

Steiner

Stain basics

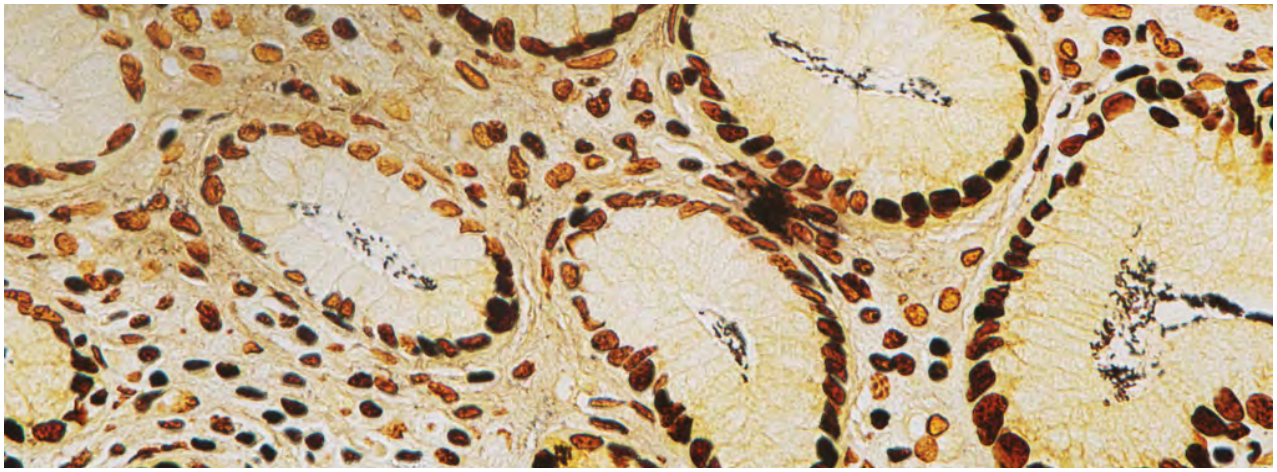

Figure 1. Gastric Biopsy positive for H. pylori, 400x.

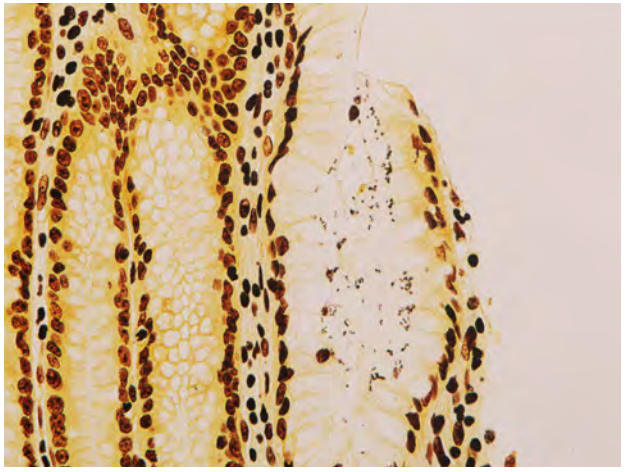

Figure 2. Gastric biopsy H. pylori, 400x.

Purpose

Steiner stain is a qualitative histologic stain used to study specific argyrophilic microorganisms in formalin-fixed, paraffin-embedded tissue.[1]

Staining principle

The ability to visualize organisms in tissue is important in the diagnosis of many diseases: however the stains routinely used (Gram, PAS, GMS, AFB and others) are ineffective for many organisms. Silver impregnation techniques are successful in demonstrating several of these organisms. These techniques have been reviewed by Garvey.[5] The staining reaction is based on the impregnation of microorganisms with silver nitrate and the reduction of silver ions to metallic silver by hydroquinone.[2] The microorganisms are demonstrated using a sensitizer that enables the uptake of the silver faster than the surrounding tissue. Silver nitrate impregnates the microorganisms. The sections are exposed to the developer, which allows the silver ions to be reduced to black metallic silver and other tissue elements to a golden yellow to tan color.

Expected results

- Organism— black Cytoplasm - pale pink
- Background — yellow/amber

Steiner

Stain basics

Common diagnostic utility

Steiner stain may be used as a qualitative histologic silver stain to aide in the identification of the causative organisms of diseases such as syphilis, some gastric ulcers (H. pylori), Lyme disease, Legionnaire's disease, cat scratch fever and others in formalin fixed, paraffin embedded tissue. [1,5]

Tissue controls

A known positive tissue control should be utilized for monitoring the correct performance of processed tissues and test reagents.[3] Ideally, it should be representative of the tissues it is usually used to diagnose.[4] An example of a positive control material would be formalin-fixed, paraffin-embedded human tissue with Helicobacter pylori organisms in gastric ulcer, or other tissue samples known to be positive for organisms suitable for staining with Steiner.

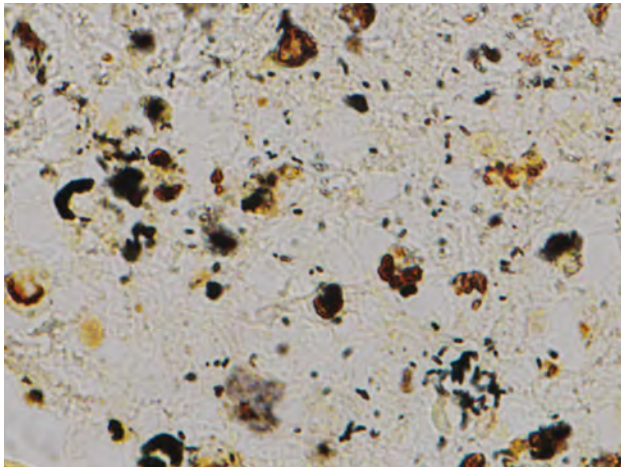

Figure 3. Organisms stained black with Steiner stain in Legionnaire's disease infected tissue control, 600x.

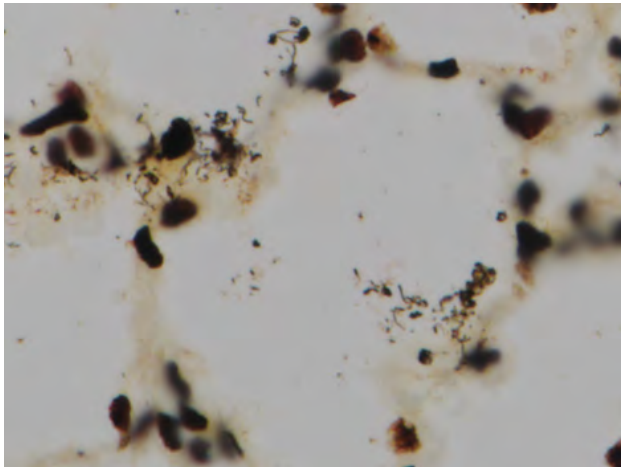

Figure 4. Spirochetes organisms stained black with Steiner stain in lung, 400x.

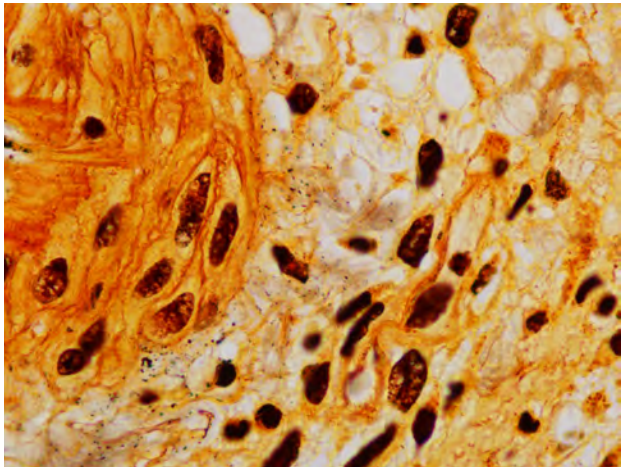

Figure 5. Cat scratch organisms stained black with Steiner stain in infected tissue control, 400x.

Steiner

Technical notes and references

Technical notes

1. Section thickness may affect quality and intensity of staining. Cut sections, usually 3–5 µm, and pick the sections up on glass slides.
2. When staining for microorganisms, it is important to ensure that the water bath is scrupulously clean prior to sectioning in order to prevent introduction of extraneous microorganisms onto the specimen section slide. It is recommended not to use water left standing overnight.

References

1. Lillie RD, Editor. H.J. Conn's Biological Stains, 9th ed. Lippincott Williams and Wilkins Company, Baltimore, 1977.
2. Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology, 2nd Edition. C.V. Mosby Company, St. Louis, 1980, p 190.
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4. Carson FL. Histotechnology: A Self Instructional Text, 2nd Edition. ASCP Press, Chicago, 1996.
5. Winsome Garvey, Silver Impregnation Techniques to Identify Spirochetes and Other Bacteria. The Journal Histotechnology. Sept 1996;19(3):203-209.

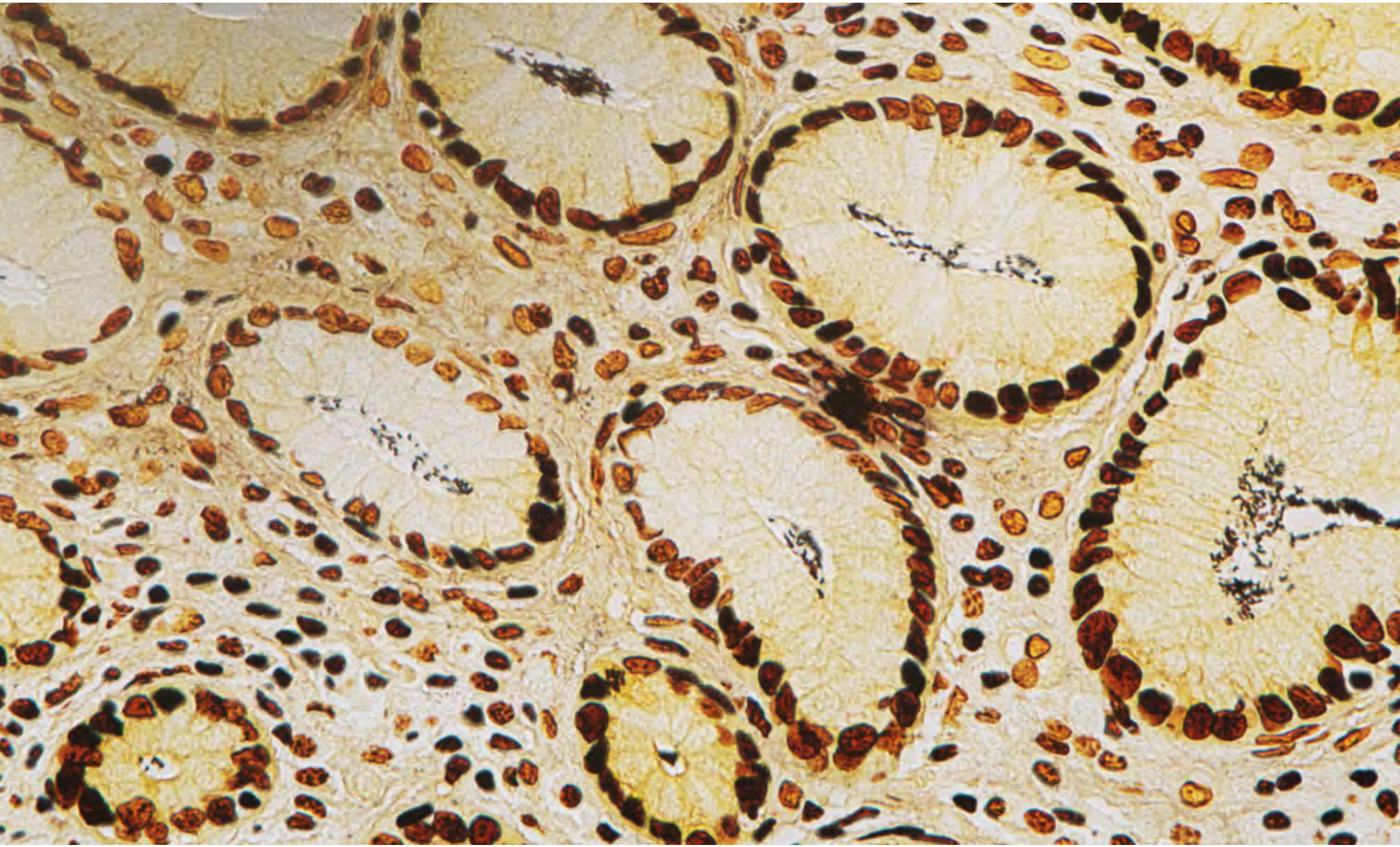

Figure 6. Gastric Biopsy positive for H. Pylori with Steiner stain, 400x.

Trichrome (Masson)

Stain basics

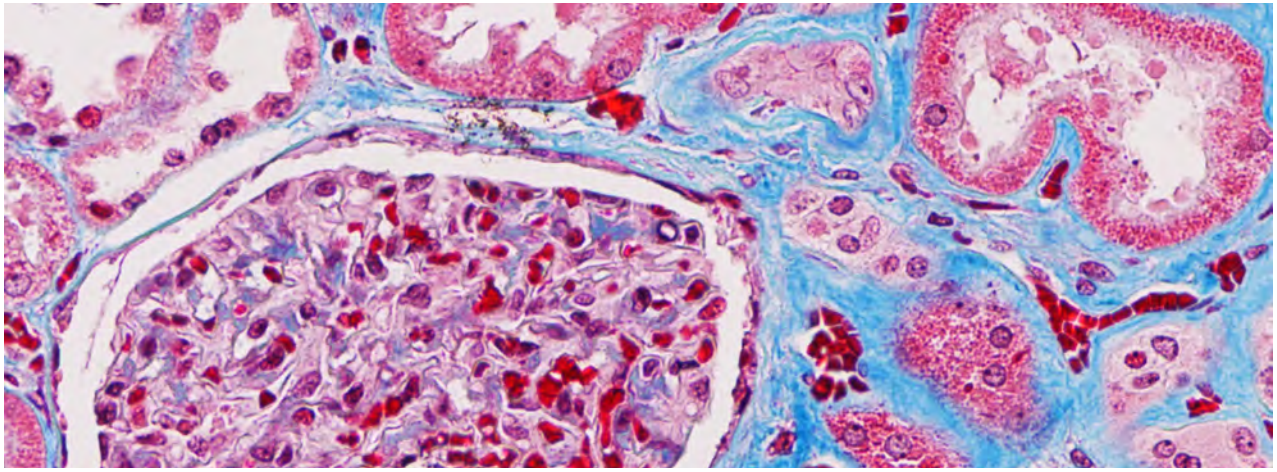

Figure 1. Kidney stained with Trichrome Blue, 200x.

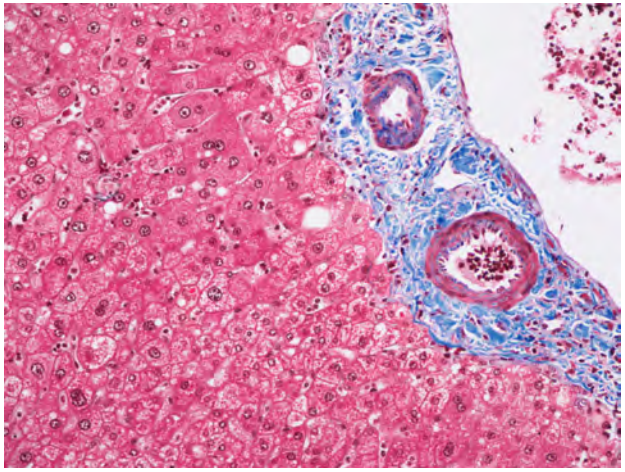

Figure 2. Liver stained with Trichrome Blue, 200x.

Purpose

Trichrome stains are used to differentiate collagen from muscle tissue.[1] Implicit in its name: the Trichrome stain uses three colored dyes to selectively stain certain tissue components. The first color is from the black of Weigert's iron hematoxylin which is used to stain the nuclei. The second dye is a red acidic dye that colors the muscle cells and the cytoplasm of liver cells, among other tissue components. The final color is a blue or green dye which is used to stain the connective tissue and collagen.

Expected results

- Nuclei — varying shades of black/gray
- Red blood cells — vibrant dark red
- Muscle — vibrant medium red
- Cytoplasm — medium shade of muted red
- Collagen — blue

Trichrome Blue

Stain basics

Staining principle

The first trichrome stain has been attributed to Mallory.[1-3] It used a solution of acid fuchsin to stain the nuclei and muscle red, followed by a mordant (phosphomolybdic acid), and finally a solution of Orange G and Methylene Blue to stain erythrocytes orange and collagen blue. Modifications which include a nuclear stain were introduced by Masson and Gomori, and by Lillie.[3] Weigert's iron hematoxylin is used the nuclear stain with all three modifications due to its ability to withstand decolorization by the subsequent acid dyes. Masson Trichrome is a multi-step trichrome stain in that the plasma stain and the collagen stain are applied sequentially. In Gomori's modification, the plasma stain dye(s) and the collagen stain dye(s) are combined and applied in a single-step.

It is thought that Trichrome stains function based on the principles of acid-base chemistry and dye displacement, using the permeability and affinity of the various tissue components for the different acid dyes to produce differential staining of the selective tissue components.[4]

In the Masson Trichrome, the nuclear stain from the iron hematoxylin is followed by staining with a red acid dye and solution, which is used to ultimately stain the muscle and cytoplasmic tissue components. This sometimes referred to as the plasma stain. The plasma stain is usually made up of one or more of the following dyes — Acid Fuchsin, Ponceau de Xylidine and Biebrich Scarlet - in glacial acetic acid and distilled water.

The excess red dye is washed off with a rinse in water and is followed by "negative" staining with a polyacid; typically phosphotungstic or phosphomolybdic acid. Although this mechanism is not well understood, it thought that the polyacid binds preferentially to various tissue components, displacing the red dye. Some tissue structures retain the red dye more strongly than others and resist decolorization with the polyacid. In descending order, the following components tend to resist the removal or substitution of the red dye by phosphomolybdic or phosphotungstic acid: erythrocytes, eosinophil granules, keratin, fibrin, muscle, cytoplasm, bone and tendon, collagen and areolar connective tissue.[4]

The plasma stain is followed by the collagen (fiber) stain, which is usually a green dye (such as Fast Green) or blue dye (such as Aniline Blue) in a weak acetic acid solution. The fiber stain will more easily displace the polyacid before it displaces the plasma stain. The excess fiber stain is rinsed with a weak acetic acid solution. This serves to accentuate the color balance between the plasma stain and the fiber stain.

Trichrome Blue

Stain basics

Common diagnostic utility

Trichrome stains are useful for indicating fibrotic change; that is, an increase in collagen like that which occurs in cirrhosis of the liver and pyelonephritis. Trichrome stains can be useful for distinguishing histologic changes that occur in neuromuscular diseases. They are also useful for differentiating tumors that originated in muscle cells from those that originated in fibroblasts. Trichrome stains are included as standard practice in diagnostic panels for renal, liver biopsies and muscle biopsies (such as cardiac biopsies).

Tissue controls

A known positive tissue control should be utilized for monitoring the correct performance of processed tissues and test reagents. An appropriate control tissue for the Trichrome stain should contain collagen and smooth muscle, such as colon, liver, esophagus or skin. Ideally, it should be representative of the tissues it is usually used to diagnose. The most common tissue controls are (normal) kidney, (normal) liver and (normal) muscle.

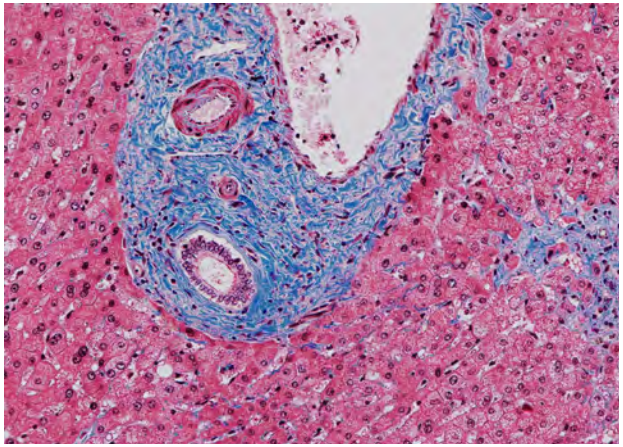

Figure 3. In liver, the cytoplasm of the hepatocytes, the smooth muscle component of the vessel walls, and the red blood cells should stain red in increasing intensity. The collagen component of the liver triad, a small amount of collagen in the portal tracts, and a small rim of collagen in the luminal rim of the hepatic venules should stain blue.[2] 200x.

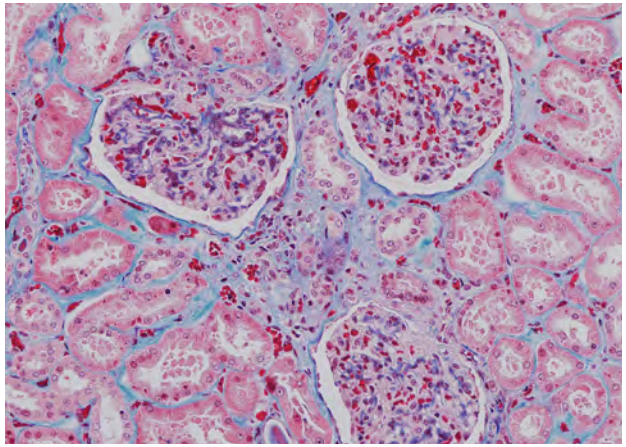

Figure 4. In kidney, the cytoplasm of the cells in the tubules, the smooth muscle component of the vessel walls, and the red blood cells should stain red in increasing intensity. The collagen in between the tubules, as well as in the glomerular basement membrane should stain blue. 200x.

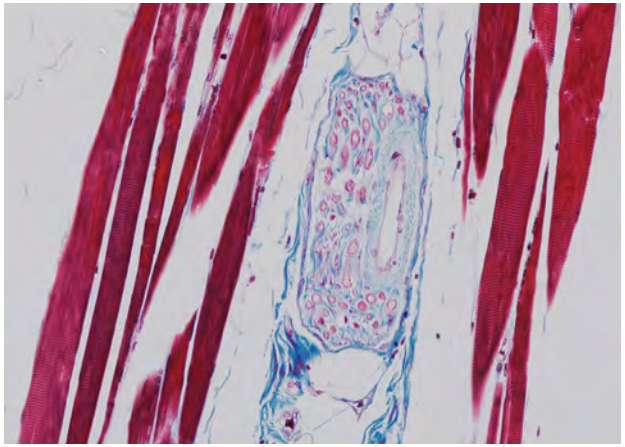

Figure 5. In muscle, the cytoplasm of the muscle cells should stain red with very clear striations. The smooth muscle components of the vessel walls and the red blood cells should stain red in increasing intensity. The collagen in between the myocytes and the connective tissue structures should stain blue. 200x.

Trichrome Blue

Technical notes and references

Technical Note

1. 10% Neutral Buffered Formalin is not the ideal fixative for trichrome staining as it does not confer the optimal acid/base properties to the tissue components. For this reason, Bouin's is applied to tissue sections as an online secondary fixative to intensify the final coloration. If the original fixation of the tissue was especially problematic, a Bouin's pretreatment is recommended.
2. Tissue section thickness is important and may affect quality and intensity of staining. Sections cut at 5-6 microns hold the colored dyes much better than thin (2-3 micron) sections. Since renal (kidney) biopsies and liver biopsies must be cut very thin in order to provide better diagnostic clarity, they may stain light & pale compared to thicker sections.
3. Trichrome Mordant is a critical factor in achieving staining balance between the plasma stain and the fiber stain. Reducing Trichrome Mordant time will leave more red dye and reduce the uptake of the blue dye. Conversely, increasing Trichrome Mordant time will differentiate the red dye and increase the uptake of the blue dye.
4. Some tissue displays a reddish cast in the cell nucleus. This usually stains dark brown-black with Iron Hematoxylin. Additionally, some tissues, such as glandular epithelium (secretory) in the uterus and in the G.I. tract can stain decidedly reddish.

References

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Trichrome Green

Stain basics

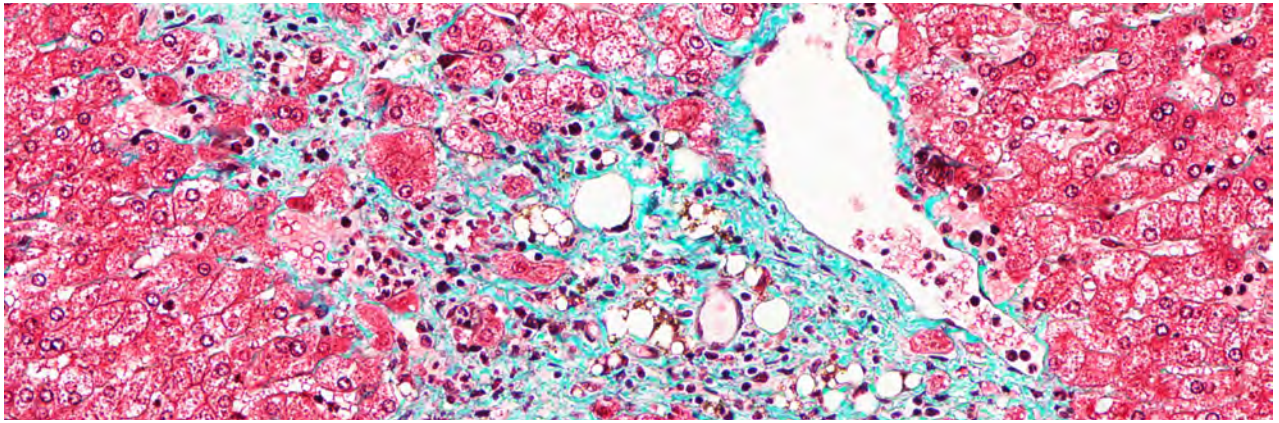

Figure 1. Liver stained with Trichrome Green, 200x.

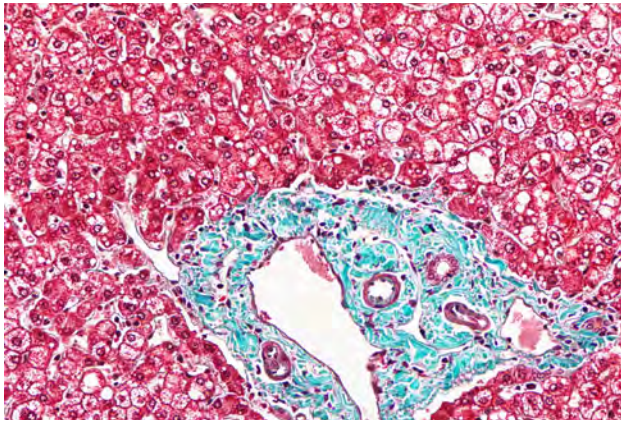

Figure 2 . Liver stained with Trichrome Green, 200x.

Purpose

Trichrome stains are used to differentiate collagen from muscle tissue.[1] Implicit in its name, the Trichrome stain uses three colored dyes to selectively stain certain tissue components. The first color is from the black of Weigert's iron hematoxylin which is used to stain the nuclei. The second dye is a red acidic dye that colors the muscle cells and the cytoplasm of liver cells, among other tissue components. The final color is a blue or green dye which is used to stain the connective tissue and collagen.

Expected results

- Nuclei — varying shades of black/gray
- Red blood cells — vibrant dark red
- Muscle — vibrant medium red
- Cytoplasm — medium shade of muted red
- Collagen — green

Trichrome Green

Stain basics

Staining principle

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Trichrome Green

Stain basics

Common diagnostic utility

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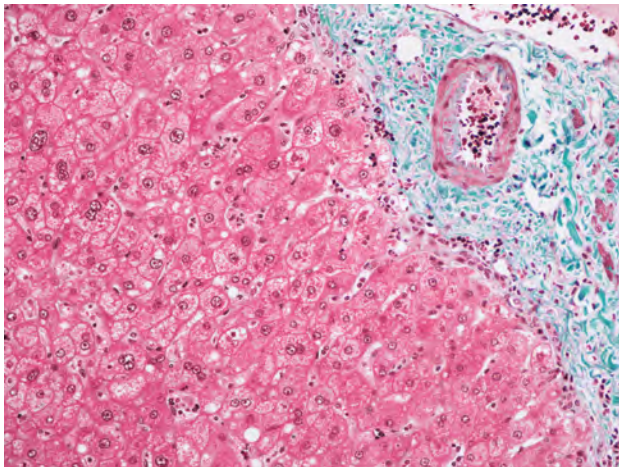
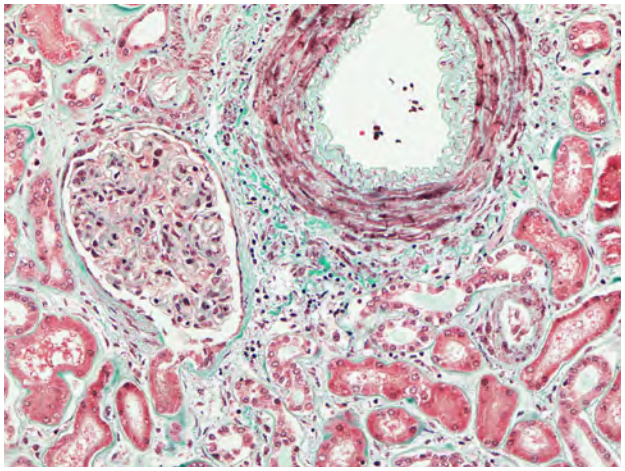


Figure 3. In liver, the cytoplasm of the hepatocytes, the smooth muscle component of the vessel walls and the red blood cells should stain red in increasing intensity. The collagen component of the liver triad a small amount of collagen in the portal tracts, and a small rim of collagen in the luminal rim of the hepatic venules should stain green.[2] 200x.



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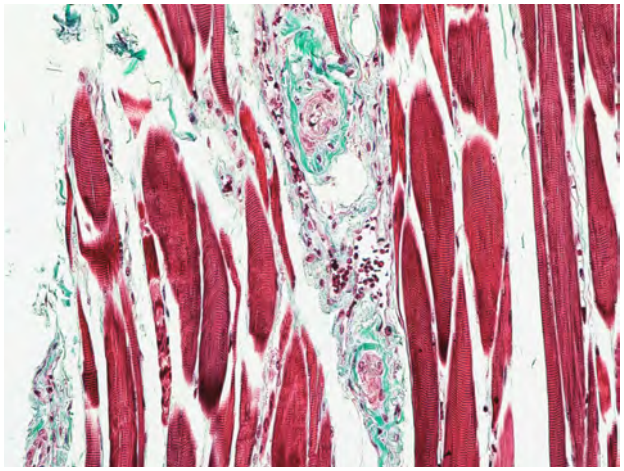

Figure 5. In muscle, the cytoplasm of the muscle cells should stain red with very clear striations. The smooth muscle components of the vessel walls and the red blood cells should stain red in increasing intensity. The collagen in between the myocytes and the connective tissue structures should stain green. 200x.

Trichrome Green

Technical notes and references

Technical notes

1. 10% Neutral Buffered Formalin is not the ideal fixative for trichrome staining as it does not confer the optimal acid/base properties to the tissue components. For this reason, Bouin's is applied to tissue sections as an online secondary fixative to intensify the final coloration. If the original fixation of the tissue was especially problematic and the online fixation in Bouin's is insufficient, off-line Bouin's pretreatment is recommended. It is recommended that the Bouin's should be preheated for 15 minutes at 60°C. Once the slides are added to the Bouin's reagent, incubate for another 1 hour at 60°C.
2. Tissue section thickness is important and may affect quality and intensity of staining. Sections cut at 5-6 microns hold the colored dyes much better than thin (2-3 micron) sections. Since renal (kidney) biopsies and liver biopsies must be cut very thin in order to provide better diagnostic clarity, they may stain light and pale compared to thicker sections.
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