Special Stains Review

BSC 45th Annual Conference
NSH Convention – San Antonio, 2026

Group Special Stains by tissue element they demonstrate. Keep in mind there is some overlapping.

- Carbohydrates
- Connective tissue
- Pigments & Minerals
- Microorganisms
- Nerve tissue

ASCP content guideline lists 33 procedures. They mention the list is not all inclusive, but if you know those well, that is an excellent headstart!

For each procedure, it helps to list the following:

- Target tissue element
- Pathology (HTLs)
- Mode of action
- Reagents and its purposes
- Expected color results
- Troubleshooting

Build a stain profile for each procedure where you gather all that information.

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Staining Procedure:

Detects (tissue element):	Pathology (differential diagnosis):

Mode of action:

Positive control:	Positive control:

Reagents and its purposes

Reagent name, percentage	Purpose	Notes

Expected Color results (include image):

Troubleshooting:

Image	Problem	Cause	Solution/Prevention

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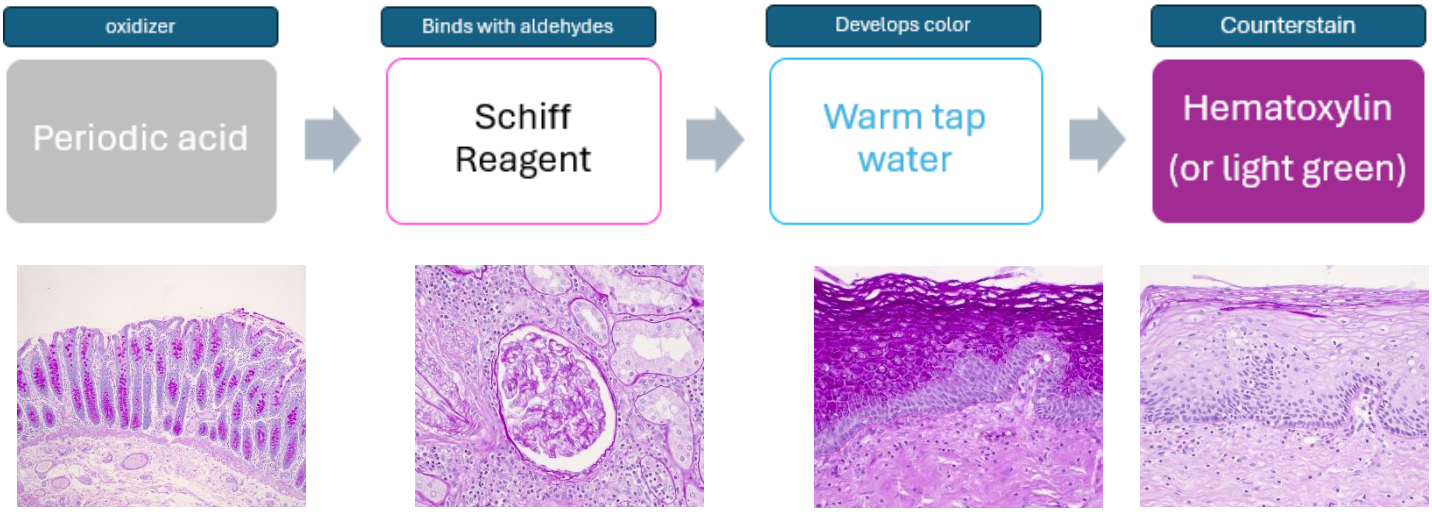
PAS – Periodic Acid-Schiff

Demonstrates	Sugars (carbohydrates) found in glycogen*, neutral mucins, BM and fungal walls**
Pathology	Depends on tissue (glycogen storage diseases, tumor differentiation, glomerular diseases, mycosis
Results	PAS +: Magenta Nuclei: Bluish purple Background: lavender/light pink or green
Common problems	No staining of PAS+ tissue elements, overstained with counterstain
Positive control	Internal control - tissue with glycogen (liver), BM (kidney), Mucin (GI), fungus



Notes:

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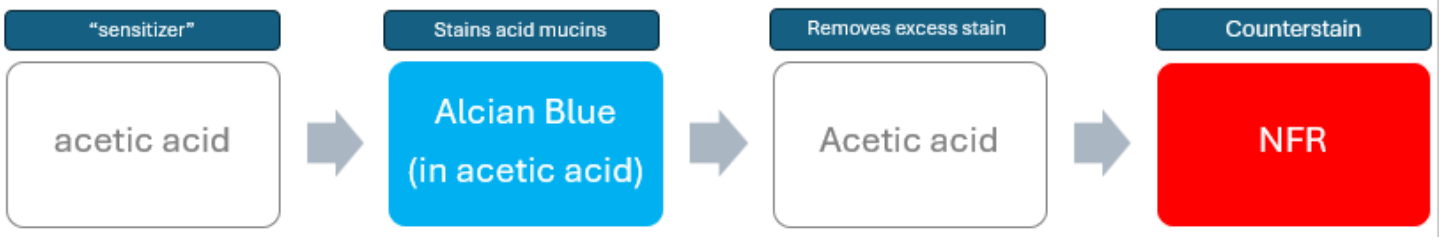
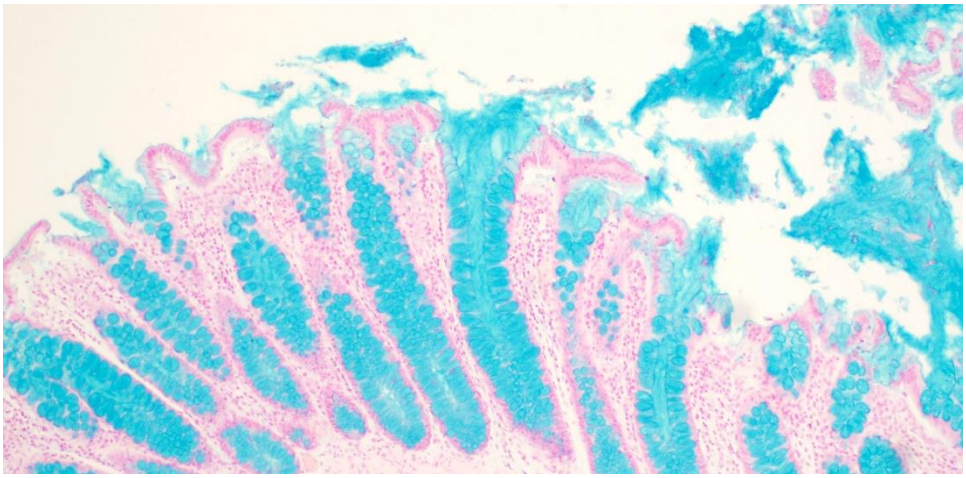
Alcian Blue pH 2.5

Demonstrates	Acid mucins
Pathology	Differential diagnosis (adenocarcinoma x other tumors)
Results	Mucins: Blue Nuclei: Red Background: light pink*
Common problems	Faint staining, nonspecific blue stain, over/under stained with counterstain, white film
Positive control	Internal control - tissue with acid mucins (colon)





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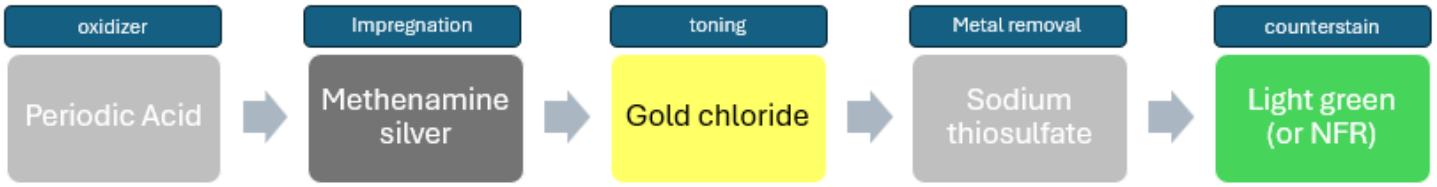
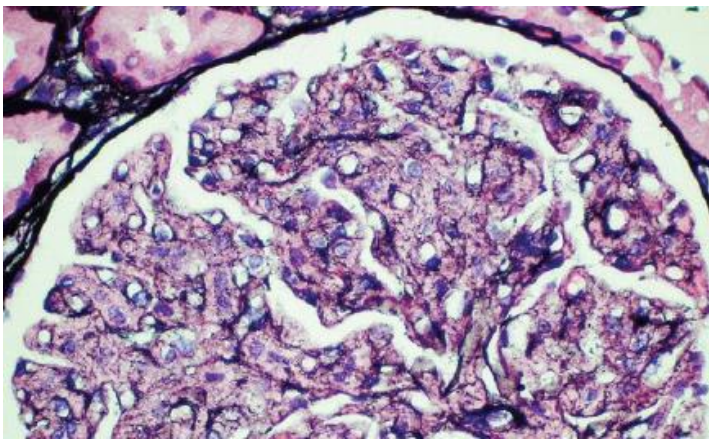
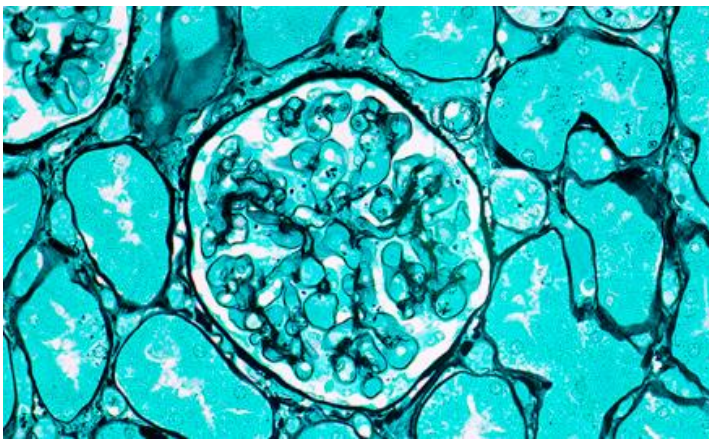
PAMS/Jones

Demonstrates	Basement (usually glomerular) membranes
Pathology	Glomerular diseases
Results	Basement membrane: Black Everything else: shades of green or pink
Common problems	Basement membrane not stained, excessive nonspecific stain
Positive control	Internal control: kidney







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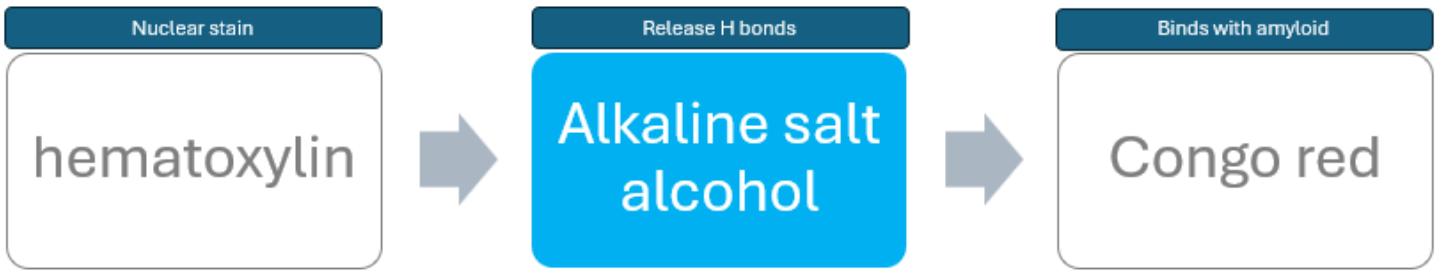
Congo Red

Demonstrates	Amyloid
Pathology	Amyloidosis
Results	Amyloid: pinkish red (birefringent – apple green) Nuclei: bluish purple
Common problems	Faint staining, incorrect birefringence color
Positive control	NO healthy tissue internal control - you need tissue with amyloid



Notes:

Test your knowledge!

https://create.kahoot.it/share/carbohydrate-stains-bsc-nsh-2026/053384c6-e2a3-48f2-8169-bdc05fc5bbbd

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Connective Tissue Stains

Collagen - Masson Trichrome, Gomori 1-step trichrome

Elastic fibers – Verhoeff vanGieson, Aldehyde Fuchsin, Orcein

Reticulin – Several silver procedures

Basement membrane (carbohydrate) – PAMS, PAS, Jones

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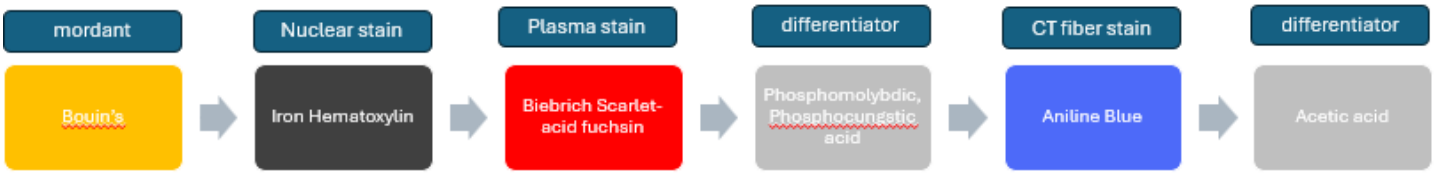
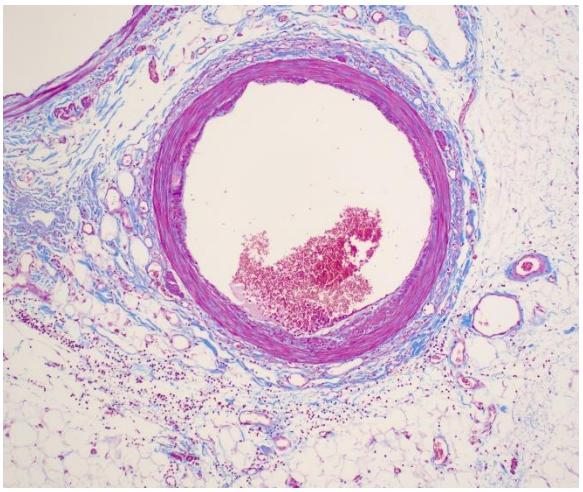
Masson Trichrome

Demonstrates	Collagen x smooth muscle
Pathology	Fibrotic changes
Results	Collagen: Blue Smooth muscle (cytoplasm): red nuclei: grayish/black
Common problems	Poor contrast, muscle stained blue
Positive control	Internal control - tissue with smooth muscle and collagen (GI, skin)





Expected color results:

Notes:

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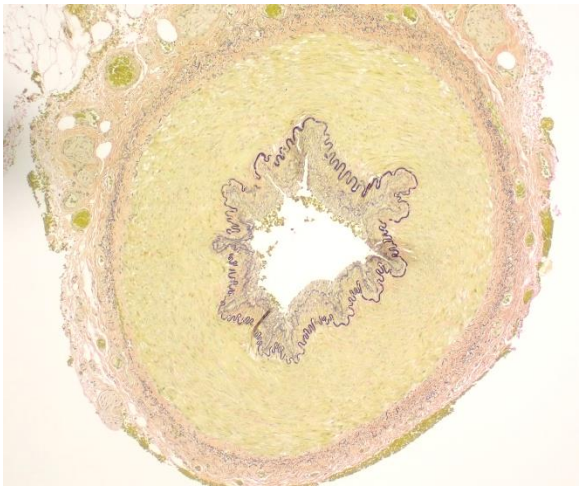
Verhoeff van Gieson

Demonstrates	Elastic fibers, secondarily - collagen x smooth muscle
Pathology	Arterial conditions, tumors (broke through elastic lamina)
Results	Elastic fibers: BLACK Collagen: RED Smooth muscle (cytoplasm): YELLOW
Common problems	No elastic fibers, poor contrast, muscle light yellow/rose
Positive control	Internal control - tissue with elastic fibers (arteries, skin)



Expected color results:

Notes:

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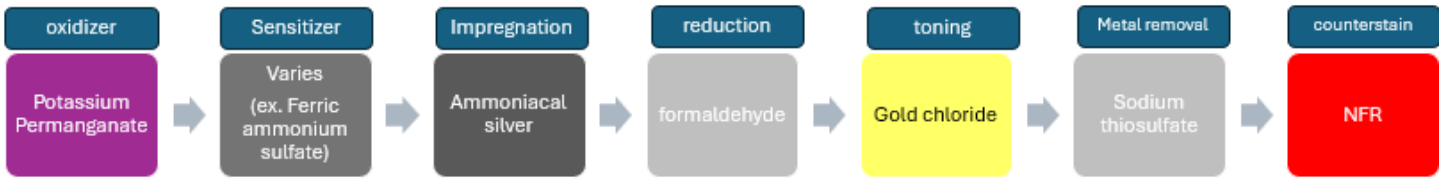
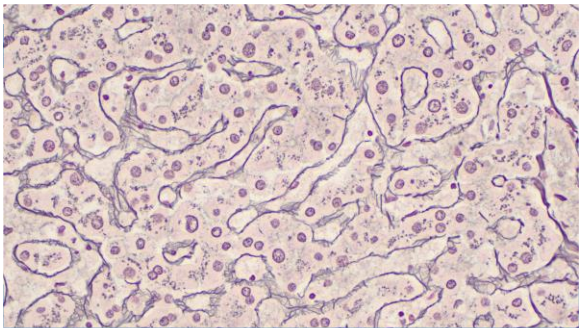
Reticulin

Demonstrates	Reticular fibers (type III collagen)
Pathology	Differential diagnosis for certain tumors, liver & bone marrow architecture
Results	Reticular fibers: Black Everything else: shades of pink
Common problems	Fibers not stained, fibers look like a string of pearls, excessive nonspecific stain, tissue falls off, milky slide
Positive control	Internal control - tissue reticular fibers (liver, bone marrow)





Expected color results

Notes:

Test your knowledge!

https://create.kahoot.it/share/connective-tissues-carbs-and-amyloid/777a129a-6718-472e-9ce4-4f85ac58cd40

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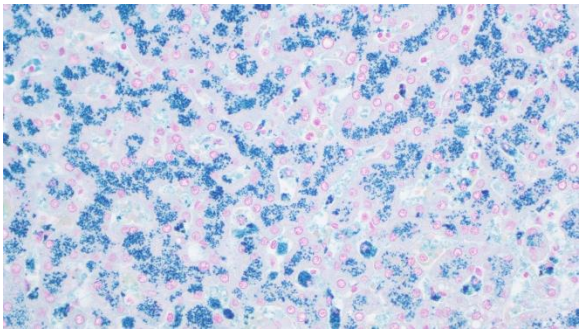
Pigments & Minerals

Fontana-Masson (argentaffin granules, melanin), Prussian Blue, Gomori Methenamine Silver (urates), Hall's (bile).

Procedure examples: Prussian Blue reaction (Do not confuse this with colloidal iron), Fontana Masson (also review Schmorl for reducing substances)

Prussian Blue for ferric iron

Demonstrates	Ferric (Fe3+) iron, hemosiderin (blood broken down)
Pathology	Hemorrhage, hemochromatosis
Results	Iron deposits: BLUE Background: shades of pink
Common problems	No iron demonstrated, nonspecific staining, milky white film on slides
Positive control	Internal control - tissue with iron (spleen, bone marrow)



Expected color results:

Notes:

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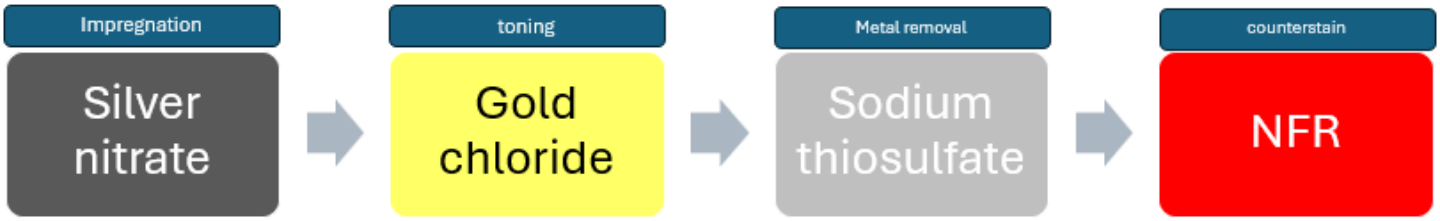
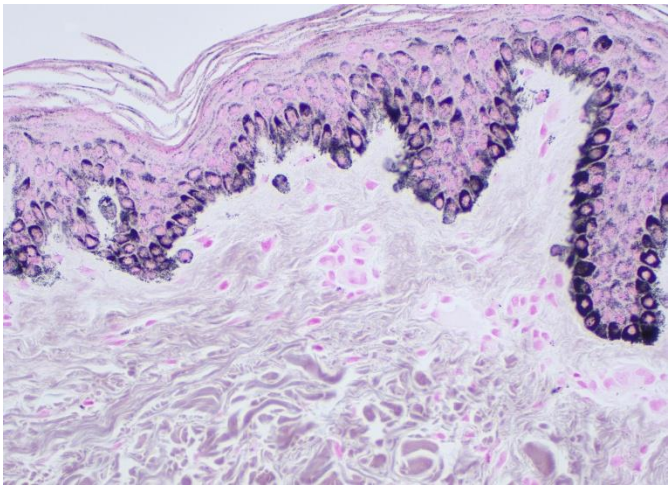
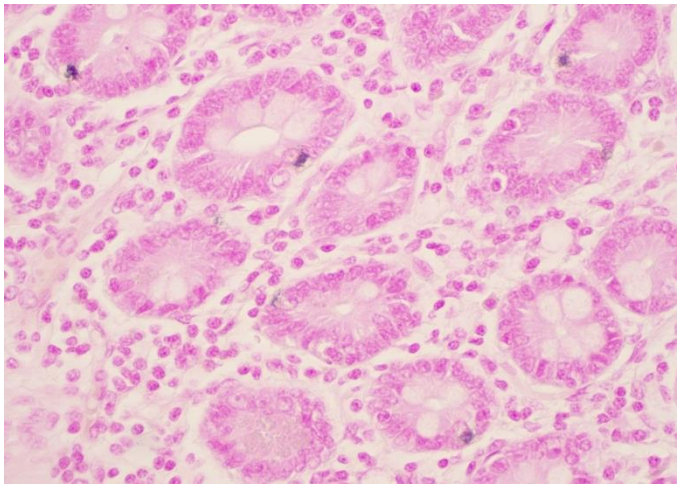
Fontana Masson (for reducing substances)

Demonstrates	Reducing substances such as MELANIN and argentaffin granules
Pathology	Differential diagnosis for certain tumors, Hirschsprung's disease
Results	Reducing substances: Black Everything else: shades of pink
Common problems	No staining, background stain, milky white film over slide
Positive control	Internal control - tissue reticular fibers (liver, bone marrow)







Expected color results:

Notes:

Test your knowledge!

https://create.kahoot.it/details/8442dd86-10cd-439a-9729-5e67789fc328

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Microorganisms

Procedures: Acid Fast Bacteria (Kinyoun, Ziehl-Neelsen, Fite, auramine-rhodamine), Gram, Grocott Methenamine silver (GMS, fungus), Giemsa, Alcian Yellow

What are we looking for?

1. Fungus – PAS, GMS, CAS
2. Gram-positive & gram-negative bacteria – Brown & Brenn, Brown & Hopps
3. Acid fast bacteria – Ziehl Neelsen, Kinyoun, Fite (Carbol fuchsin methods)

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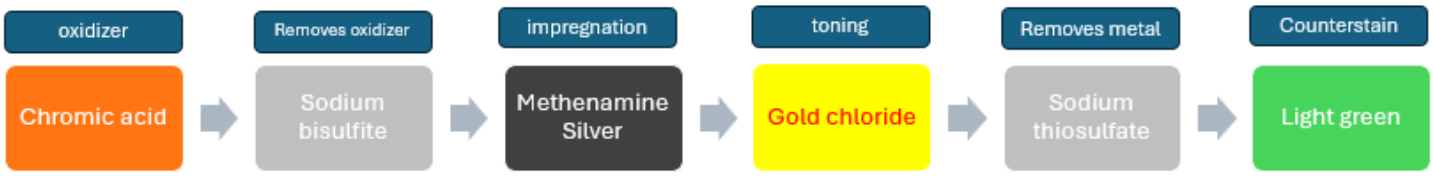
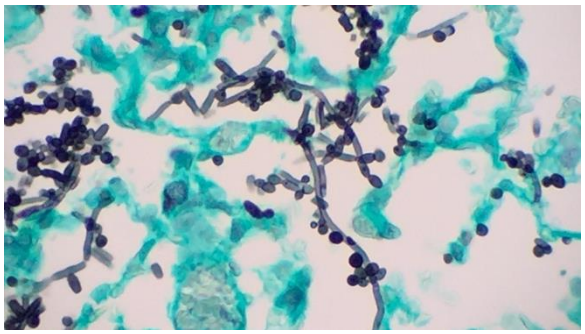
Grocott Methenamine Silver - GMS

(do not confuse with Gomori Methenamine silver for urates)

Demonstrates	Fungal walls
Pathology	mycosis
Results	Fungus: black outline, lighter center Background: green
Common problems	Silver stains are finicky. No staining, too much stain, precipitate
Positive control	Tissue with fungus





Expected color results:

Notes:

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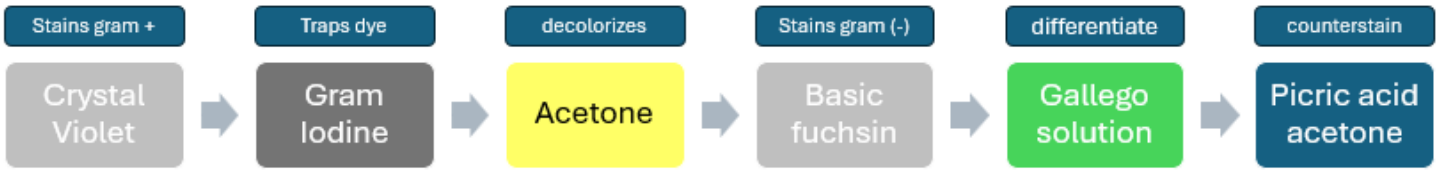
Brown & Hopps (Carson 5th edition)

Demonstrates	Bacteria differentially (gram positive and gram negative)
Pathology	Infections
Results	Gram positive: blue blue-black Gram negative: red Background: yellow
Common problems	Overdecolorization, overstaining
Positive control	Tissue with both gram positive and gram negative bacteria



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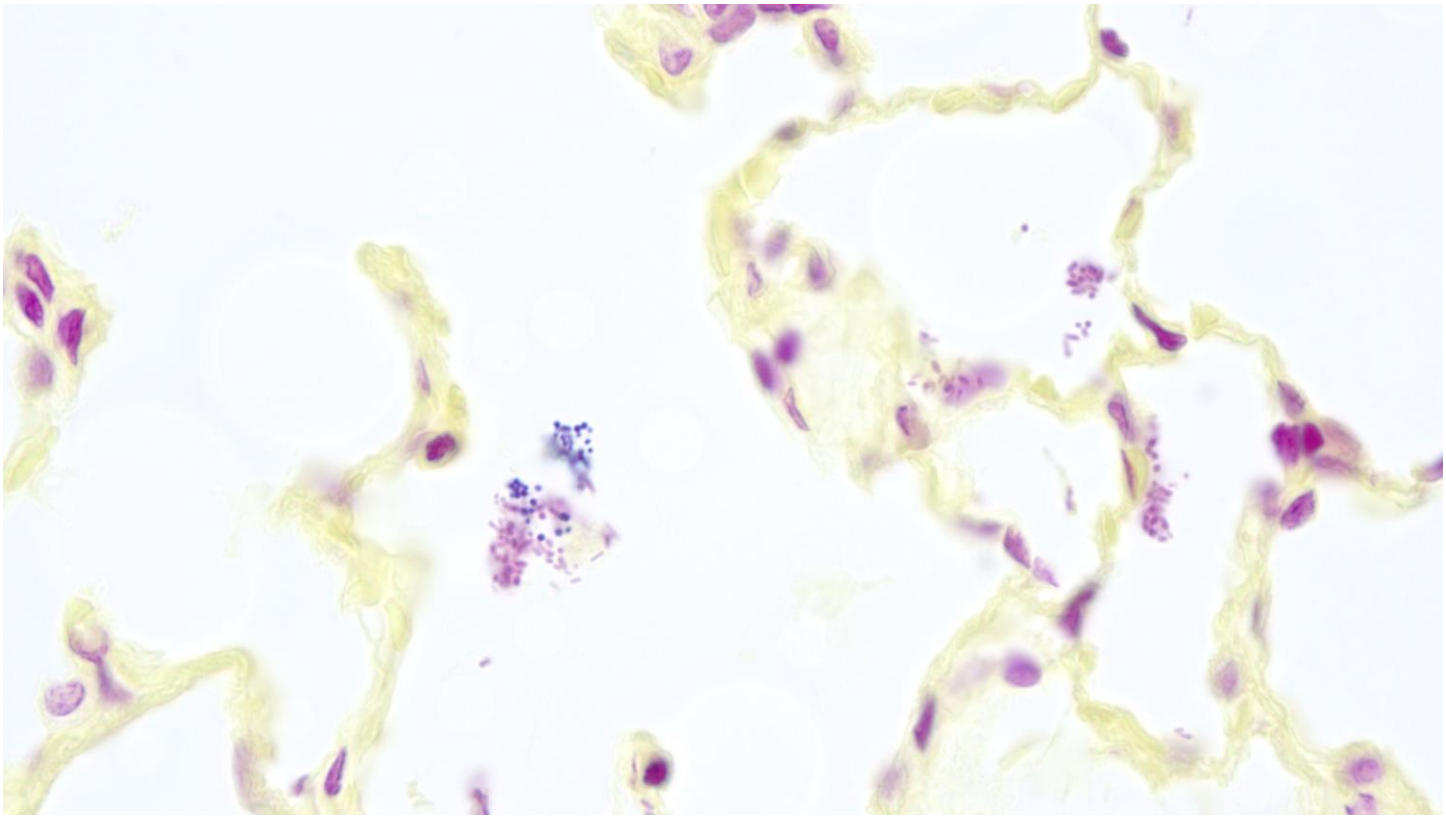

Expected color results:

Notes:

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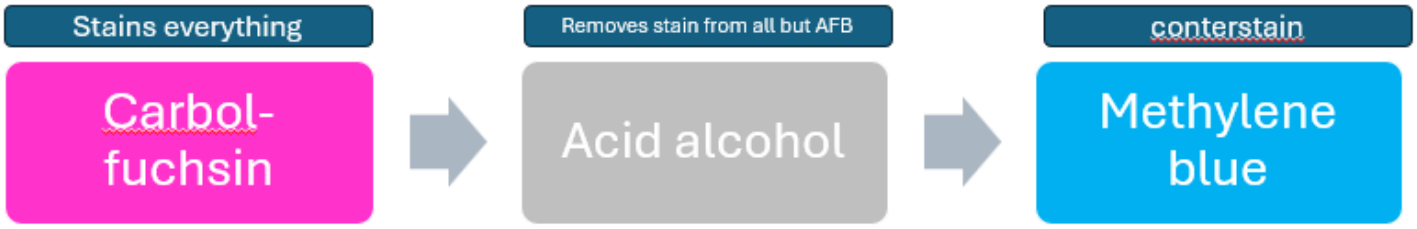
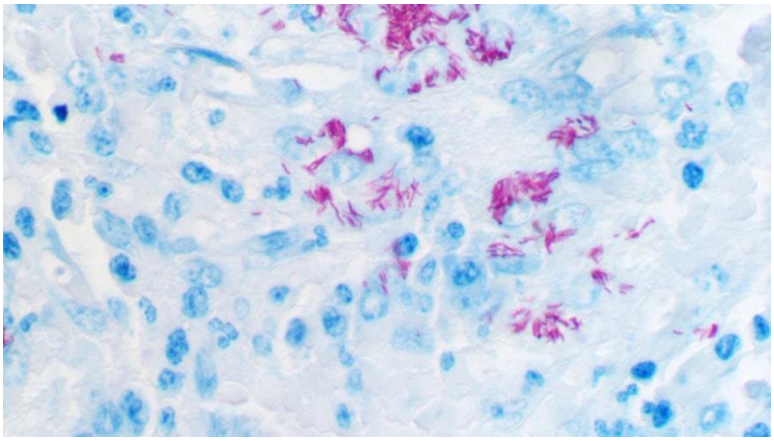
Ziehl-Neelsen - Acid Fast Bacteria (AFB)

Demonstrates	Acid Fast Bacteria
Pathology	Tuberculosis, leprosy
Results	AFB: RED Nuclei: blue Background: light blue
Common problems	Faint or no positive stain (overdifferentiation), overstained with methylene blue
Positive control	Tissue with acid fast bacteria





Expected color results:

Notes:

Test your knowledge!

https://create.kahoot.it/share/microorganisms/f6b37b03-e5dc-4778-aaed-c9ac1900add7

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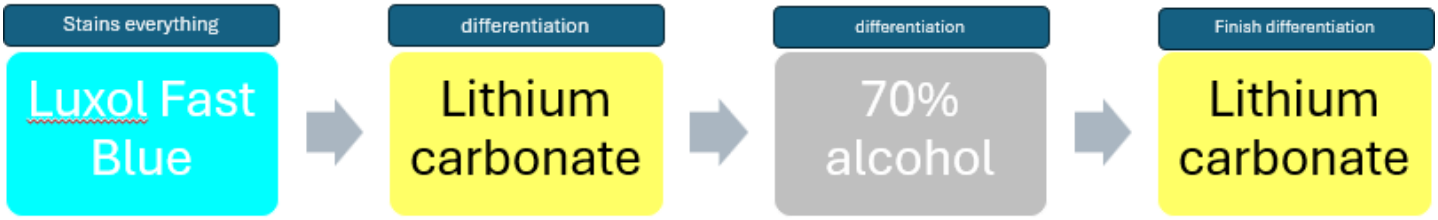
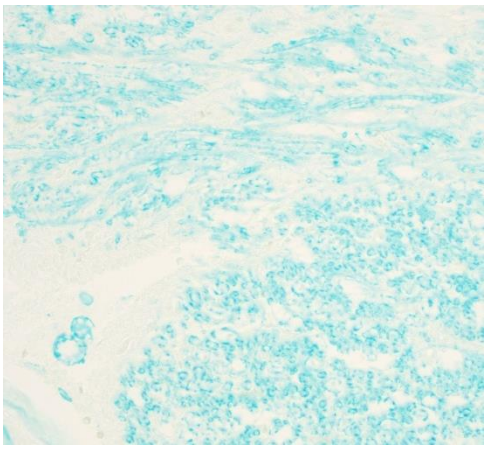
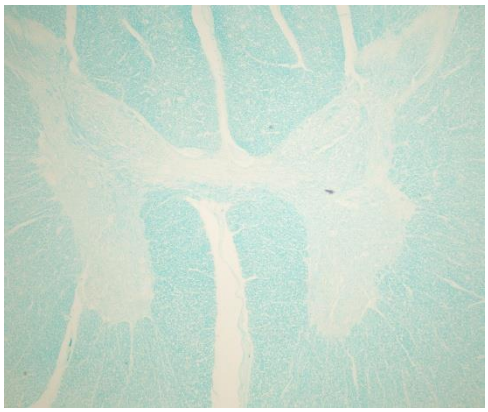
Nerve Stains

- Luxol Fast Blue - Myelin
- Cresyl (not Crystal) Violet - Nissl substance
- Bielschowsky - Nerve fibers, neurofibrillary tangles
- Cajal – Astrocytes (replaced by IHC)

Demonstrates	myelin
Pathology	Wallerian degeneration, demyelination
Results	Myelin: turquoise Everything else: unstained
Common problems	No staining (over differentiation), overstaining (under differentiation)
Positive control	Internal control: cross section of spinal cord







Expected color results:

Notes:

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Cresyl Violet

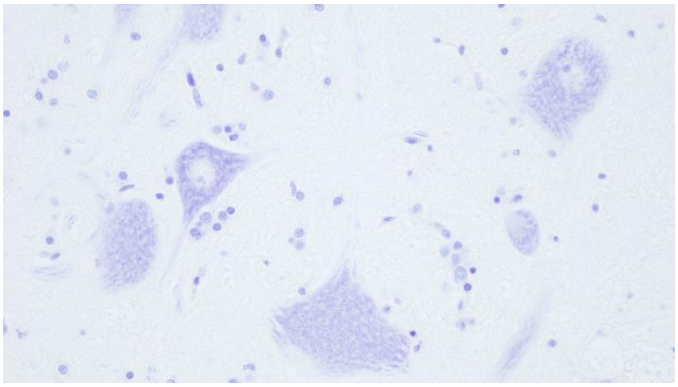
Demonstrates	Acidic tissue elements (RNA, DNA, Nissl substance)
Pathology	Chromatolysis
Results	Nissl substance: PURPLE Background: unstained
Common problems	Over staining, poor contrast
Positive control	Internal control - tissue with neuron cell bodies (spinal cord)

Stains Nissle substance and nuclei

Acidified Cresyl Violet (acetate)

Start dehydration

95% alcohol



Expected color results:

Notes: (Review combined techniques such as LFB+ CV and LFB+ H&E)

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Common Question Structures

When using the [procedure name], [tissue element] are stained:
a blue
b orange
c red
d green

In order to suppress background and nonspecific staining, a *[solution name]* solution frequently contains:
a reagent A
b reagent B
c reagent C
d reagent D

The *[procedure name]* is useful for the demonstration of:
a tissue element A
b tissue element B
c tissue element C
d tissue element D

A section of a *[tissue element]* has been stained with the *[procedure]*. Microscopic examination shows *[color results]*. The significance of these results is that the:
a section was overstained in reagent X
b staining in reagent Y solution was prolonged
c reagent Z was too dilute
d stain results are as expected

The technique shown below demonstrates:

image

a tissue element A
b tissue element B
c tissue element C
d tissue element D