



Tikrit University  
College of Veterinary Medicine

# Special Stains

Subject name: Practical Pathology

Subject year: 2024-2025

Lecturer name: Thamer J. Shihab

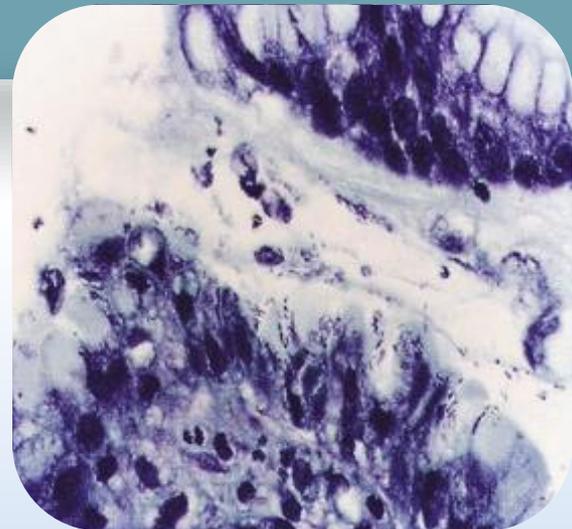
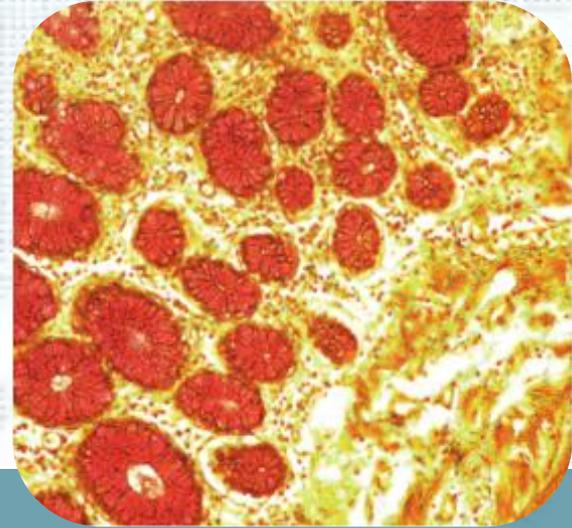
Academic Email:

[dr.thamer.vet@tu.edu.iq](mailto:dr.thamer.vet@tu.edu.iq)

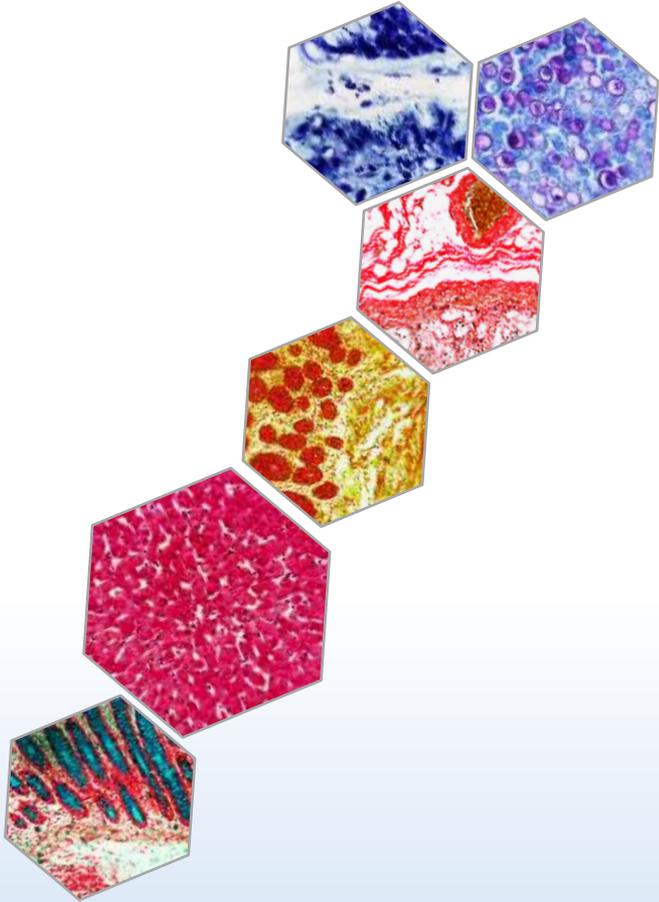


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# Special Stains

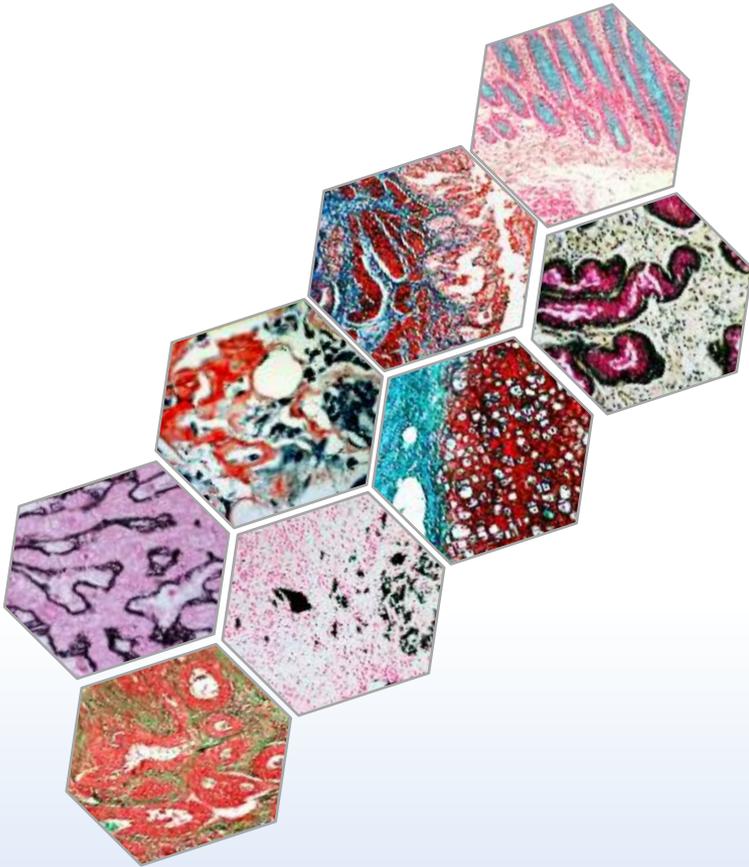


# Special Stains



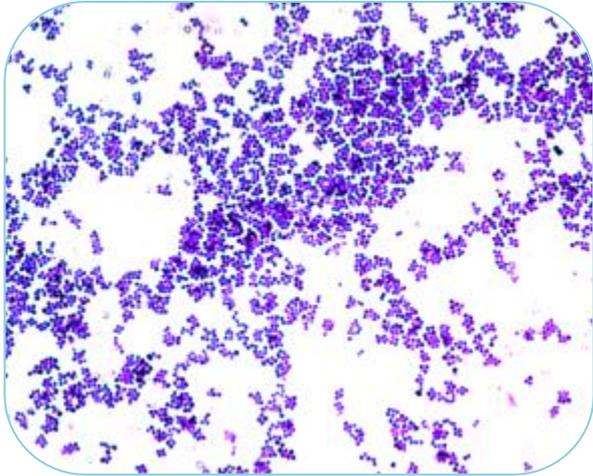
- Used in addition to H & E staining to selectively stain cells and cellular components
- Used when needed
- **Gives information on:**
  - Presence of certain class of molecules
  - Their localization
  - Number of molecules present

# Classification



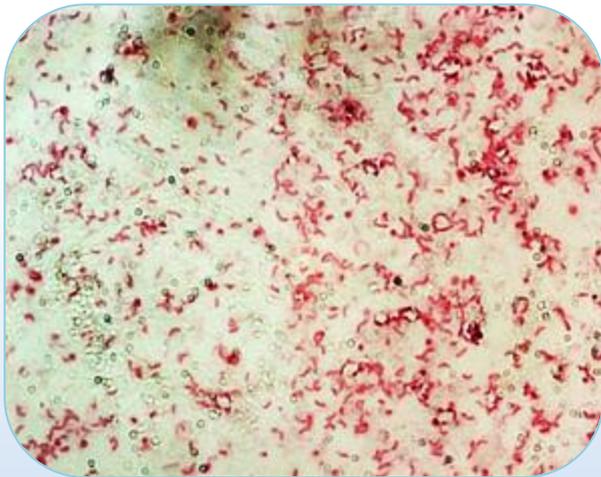
- **Can be grouped into:**
  - Stains for detection of microorganisms
  - Connective tissues and lipids
  - Carbohydrates
  - Amyloid
  - Minerals, pigments and miscellaneous

# Stains for the detection of Microorganisms

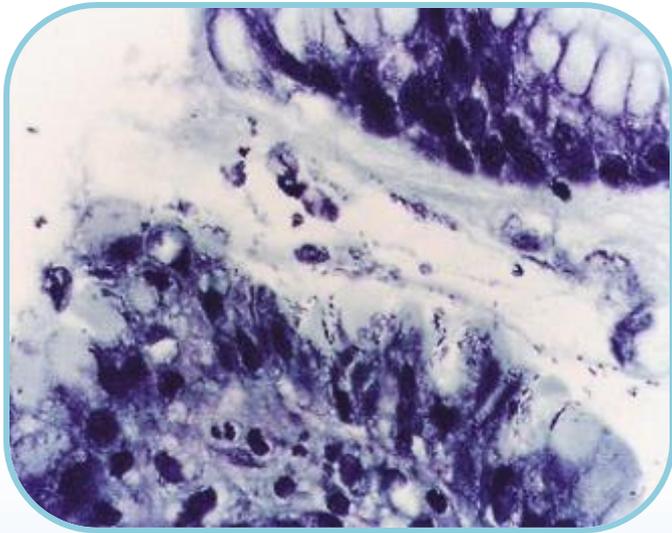


- **Gram Staining :**

- Used to stain both bacilli and cocci
- Basic classification of bacteria are based on this staining
  - Bacteria with large deposits of peptidoglycan in their cell walls retain methyl violet and are termed Gram positive
  - Bacteria with large deposits of lipids and lipopolysacharrides are termed Gram negative

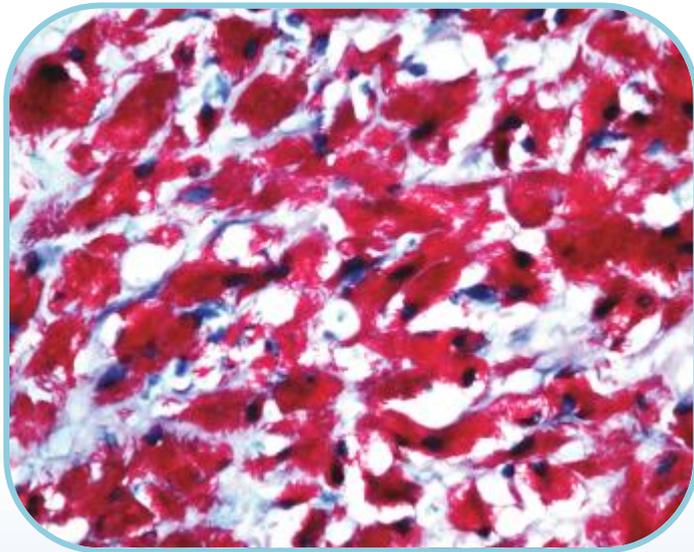


# Stains for the detection of Microorganisms



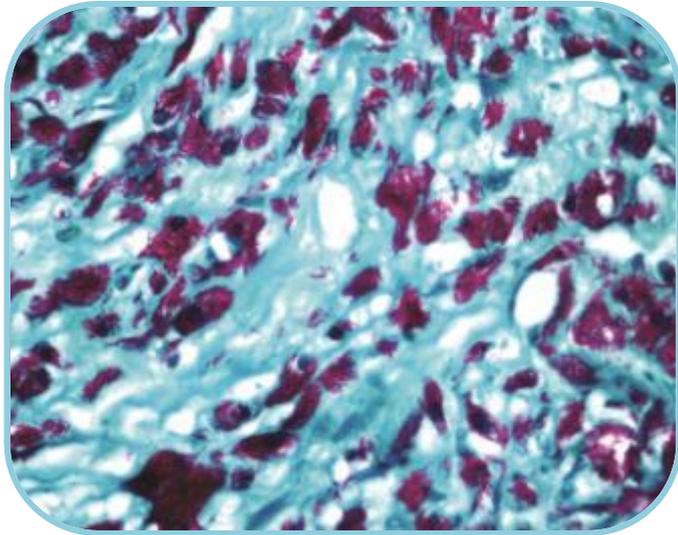
- **Giemsa Stain :**
  - Used to stain bacteria and protozoa, H. pylori, rickettsia and chlamydiae
- **Type of staining:**
  - Bacteria stains blue
  - cytoplasm stains from pink to rose and nuclei blue
  - Eisonophils are also easily detected

# Stains for the detection of Microorganisms



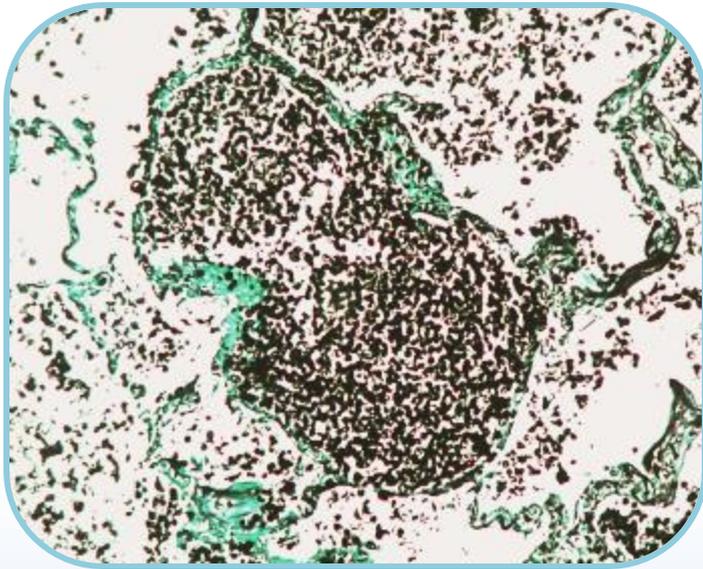
- **Acid Fast Blue**
  - Acid fast refers to cell walls containing high lipid content (mycolic acid and long chain fatty acids)
  - These bind to Carbol-fuchsin dye after decolorization
  - Used to stain Mycobacteria, oocysts of *Cryptosporidium parvum*, *Cyclospora*, *Isospora*; also hooklets of cysticerci
  - Acid fast cells stain Red and non acid fast cells stain Blue

# Stains for the detection of Microorganisms



- **Acid Fast Green**
  - Used for the detection of Mycobacterium spp
  - Stains Acid fast bacteria red while the background Stains green

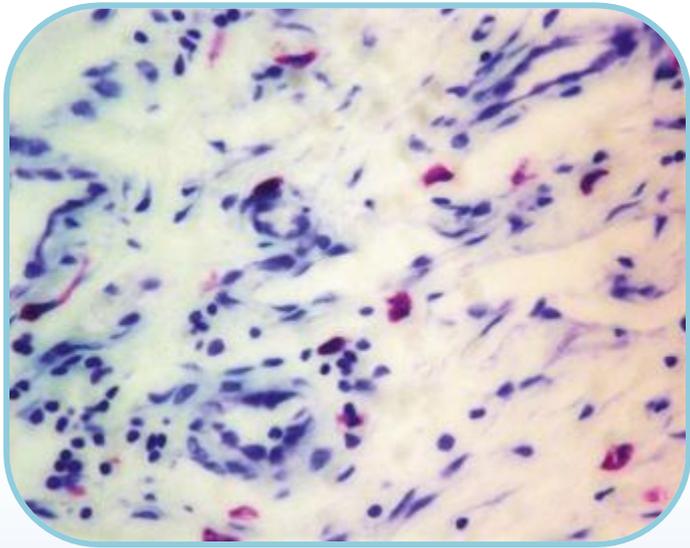
# Stains for the detection of Microorganisms



- **GMS Staining**

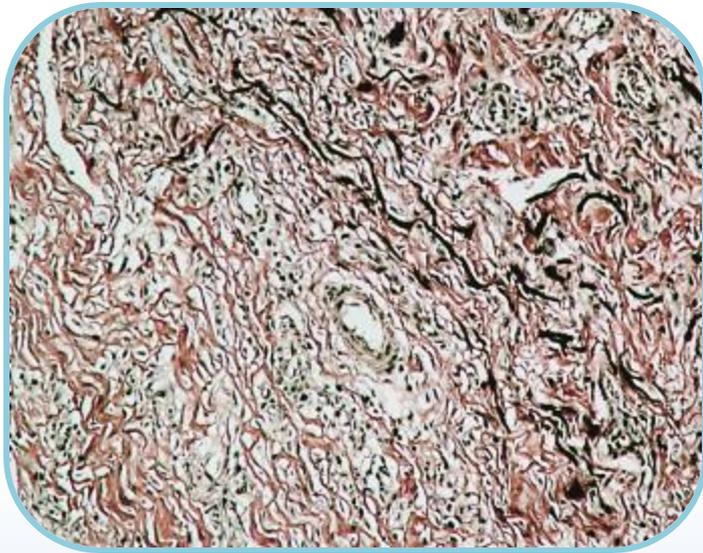
- Used for the detection of fungi in tissue sections
- Argentaffin reaction forms the basis for the identification of fungi
- Stains fungi, *Pneumocystis carinii*, *histoplasma* spp Black, inner parts of mycelia and hyphae old rose, *leishmania* spp, *toxoplasma* spp negative, mucin dark grey, background pale green

# Stains for the detection of Connective Tissue



- **Toluidine Blue**
  - Used to stain mast cells
  - These cells are widely distributed in connective tissue
  - Mast cells stain Red-purple (Metachromatic staining) and the background stain blue (orthochromatic staining)

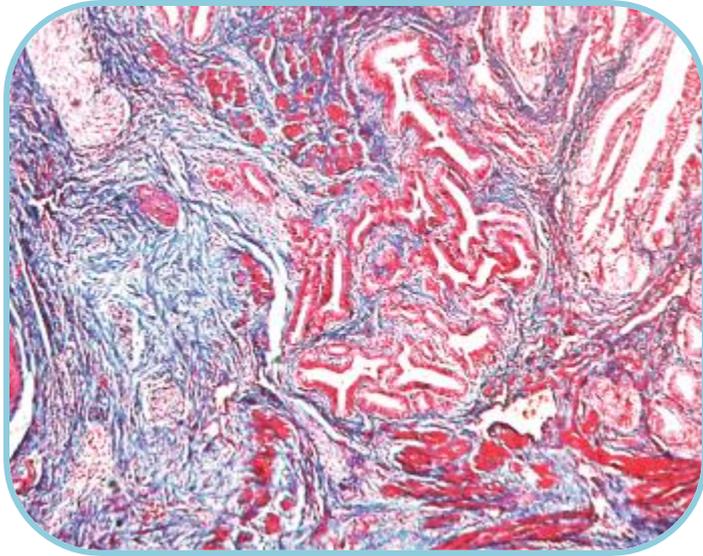
# Stains for the detection of Connective Tissue



- **Elastic Stain**

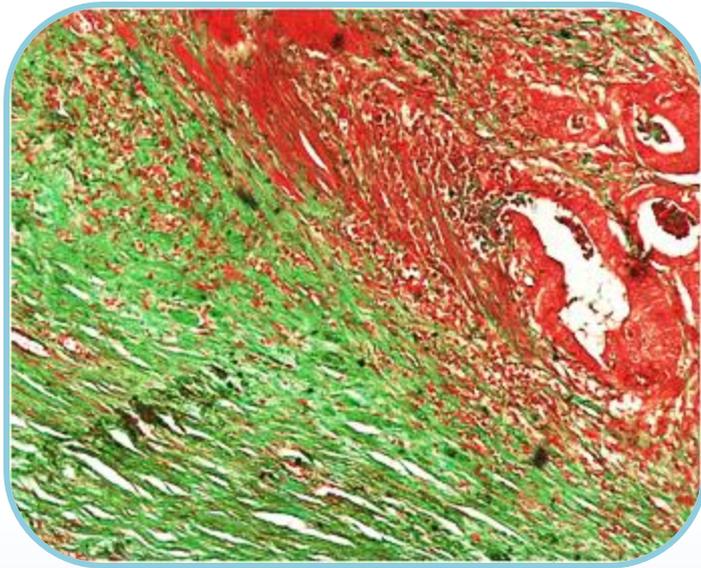
- Used to stain elastic fibers
- Based on the affinity of elastin for hematoxylin complex
- Retains dye longer than other tissues elements
- Elastin stains dark brown/ black where as nucleus stains black

# Stains for the detection of Connective Tissue



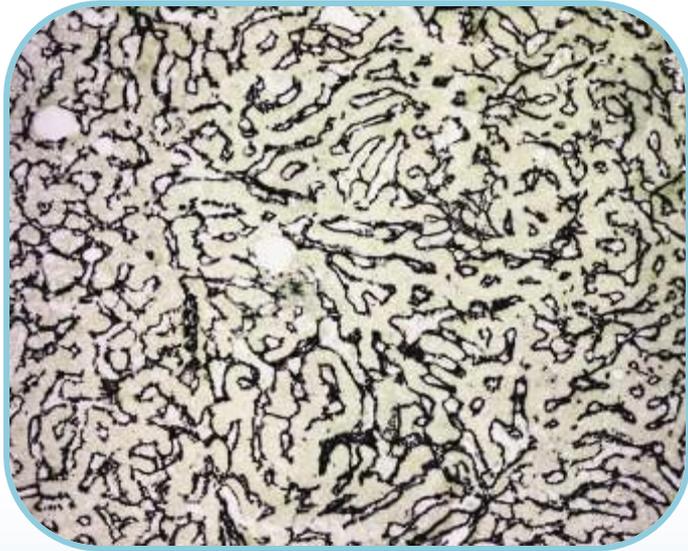
- **Gomoris Trichrome Blue**
  - Used to distinguish collagen from muscle tissue
  - Stains nucleus collagen blue, muscle, keratin and cytoplasm red and nuclei grey/blue/black

# Stains for the detection of Connective Tissue



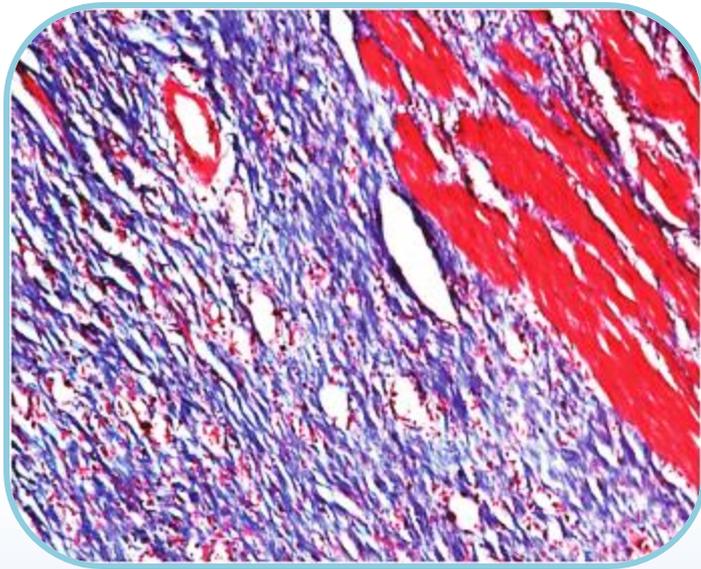
- **Gomori's Trichrome Green**
  - Useful in the study of diseases of connective tissue and muscle characterized by fibrotic and dystrophic changes and to differentiate between collagen and smooth muscle in tumors
  - Stains Nuclei(Blue), Collagen(Green), Muscle Fiber(Green)

# Stains for the detection of Connective Tissue



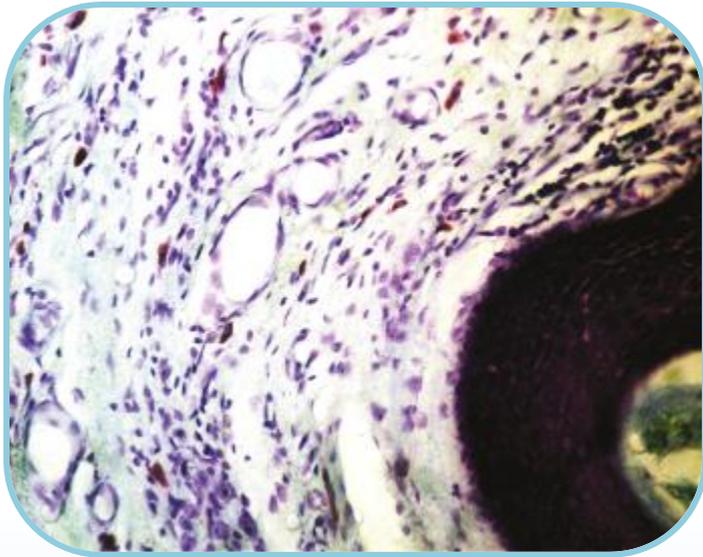
- **Reticulin no counter stain**
  - Used for the identification of Reticular fibers
  - Used for the diagnosis of carcinomas, Sarcomas, lymphosarcomas
  - Reticulin stains black with out any counter stain

# Stains for the detection of Connective Tissue



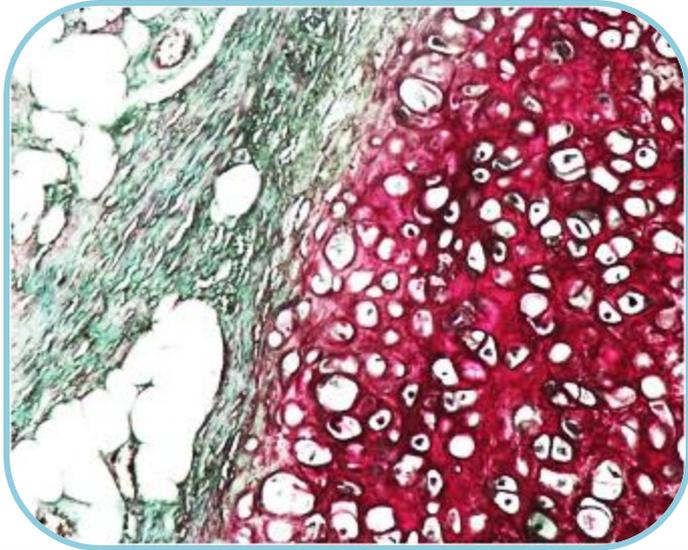
- **Massons Trichrome Stain**
  - Used to differentiate between collagen and smooth muscle in tumor
  - Increase of collagen in diseases such as Cirrhosis.
  - Stains Nuclei black, cytoplasm, muscle, erythrocytes red and collagen Blue

# Stains for the detection of Connective Tissue



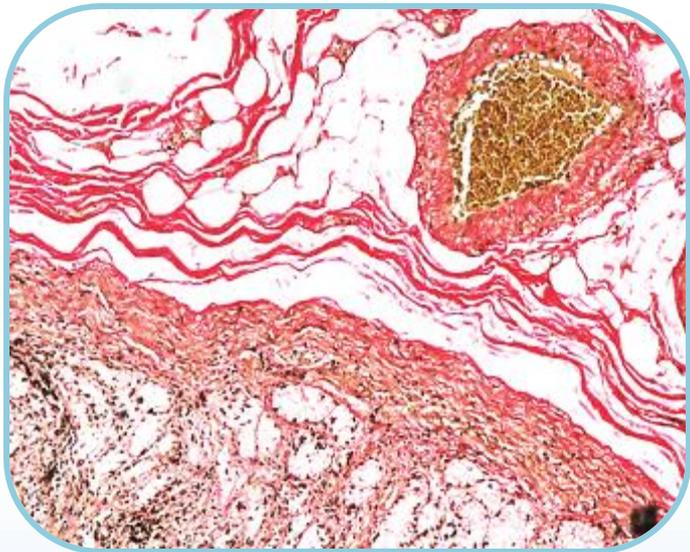
- **Azure A stain**
  - Used for the visualization of mast cells basophils and eisonophils
  - Stains Mast cell granules, sulphated and carboxylated mucins purple and Nuclei blue

# Stains for the detection of Connective Tissue



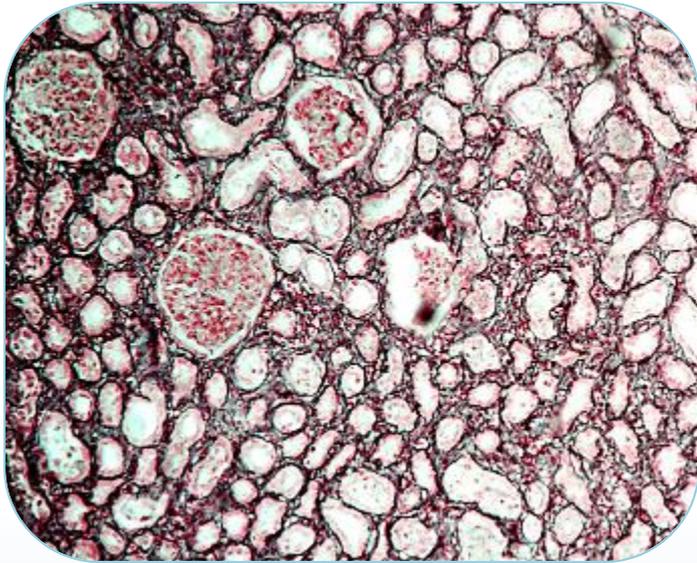
- **Safranin O staining**
  - Used for the detection of cartilage, mucin and mast cell granules
  - Stains Nuclei black, Cytoplasm bluish green, Cartilage, mucin, mast cell granules orange to red

# Stains for the detection of Connective Tissue



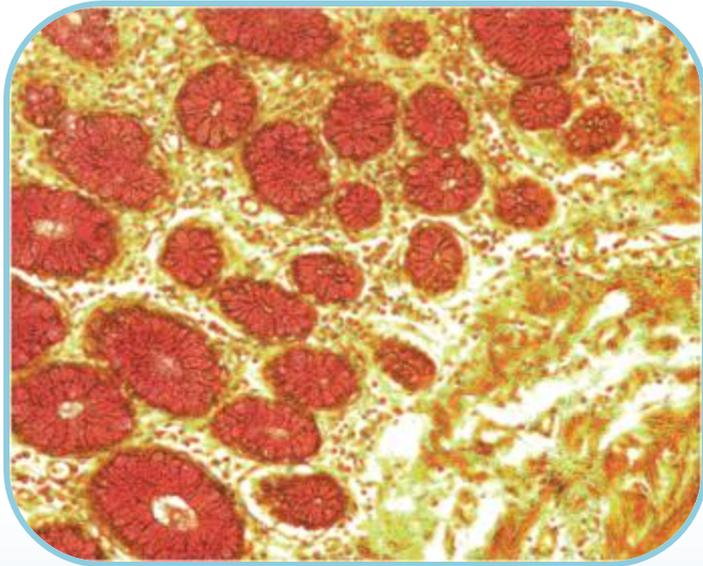
- **Van Gieson Stain:**
  - Used to differentiate collagen and smooth muscle
  - Can be used to demonstrate the presence of collagen in pathological conditions
  - Stains nuclei blue, Collagen bright red, Cytoplasm, muscle, fibrin and red blood cells yellow

# Stains for the detection of Connective Tissue



- **Reticulin Nuclear Fast Red:**
  - Used to identify reticulin fibers
  - Can be used for differential diagnosis of tumors such as carcinomas, sarcomas and lymphosarcomas
  - Stains reticulin black with a pink to rose background

# Stains for the detection of Carbohydrates



- **Mucicarmine Stain**
  - Used to detect epithelial mucin
  - Exhibits strong staining of epithelial mucins where as fibroblastic mucin show a poor staining
  - Stains mucin in shades of red

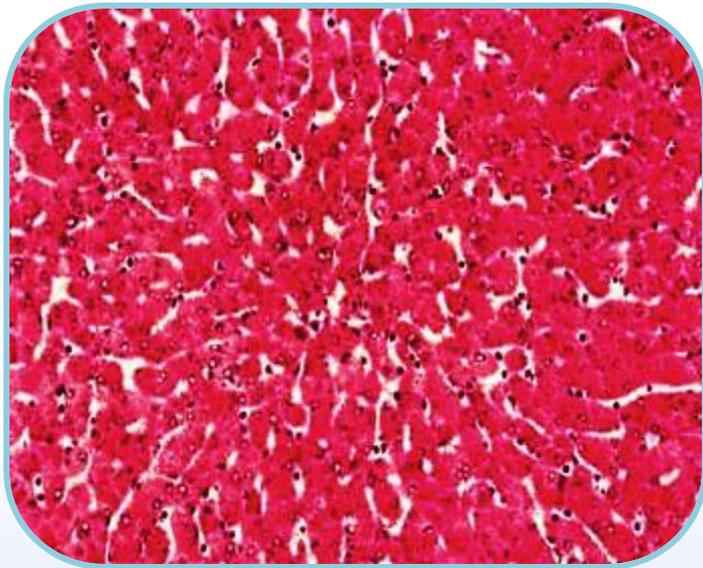
# Stains for the detection of Carbohydrates



- **Alcian Blue**

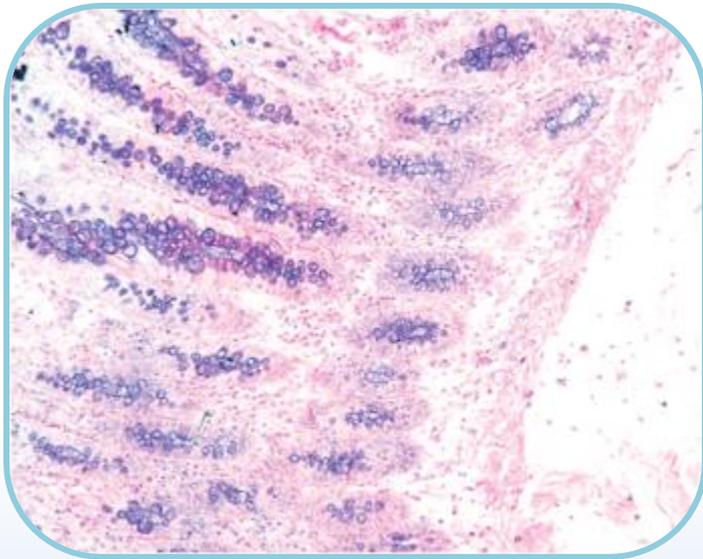
- Stains acid mucins and mucopolysaccharides
- Copper in the stain is responsible for the blue stain
- Strongly acidic muco substances stain blue, nuclei pink to red and cytoplasm pale pink

# Stains for the detection of Carbohydrates



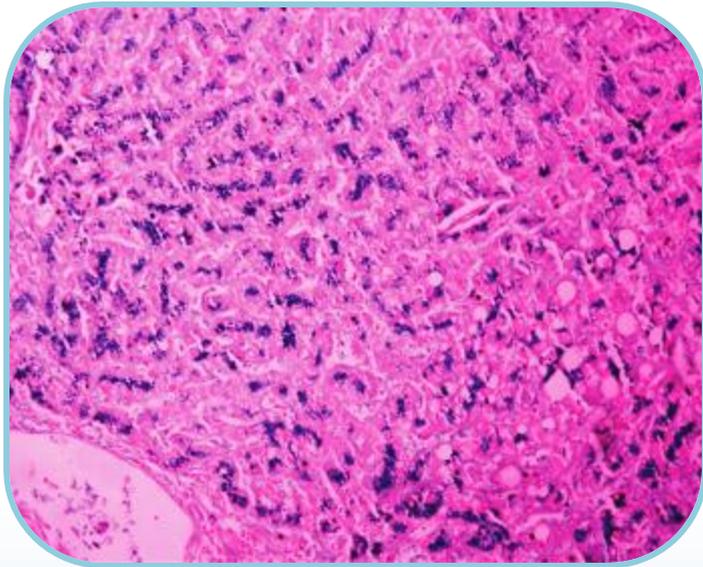
- **Acid-Schiff**
  - Used to detect glycogen, glycoproteins, mucopolysaccharides, basement membrane and mucin
  - Based on the reaction of the free aldehyde group of monosaccharides with Schiff's reagent
  - PAS stains glycogen, mucin, mucoprotein, and glycoproteins magenta. The nuclei will stain blue. Collagen will stain pink.

# Stains for the detection of Carbohydrates



- **Alcian Blue PAS**
  - Combination of Alcian Blue and PAS technique
  - Demonstrates both acidic- neutral and mixtures of acidic and neutral mucins
  - Stains acid mucopolysaccharides blue and Neutral polysaccharides magenta

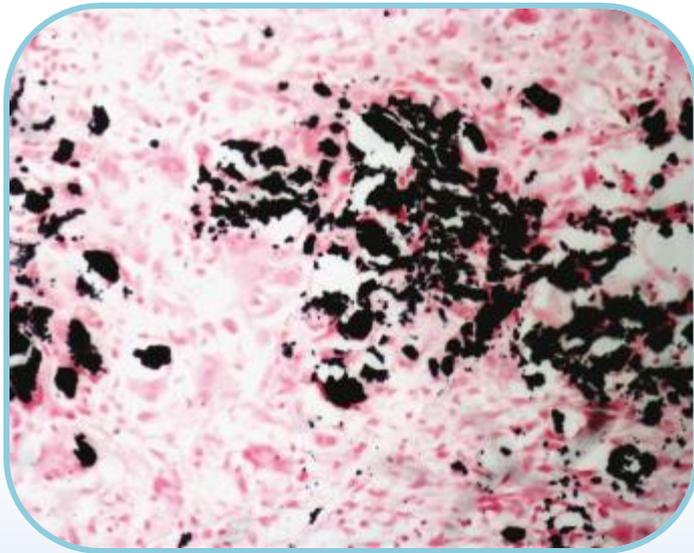
# Stains for the detection of Minerals



- **Iron Stain**

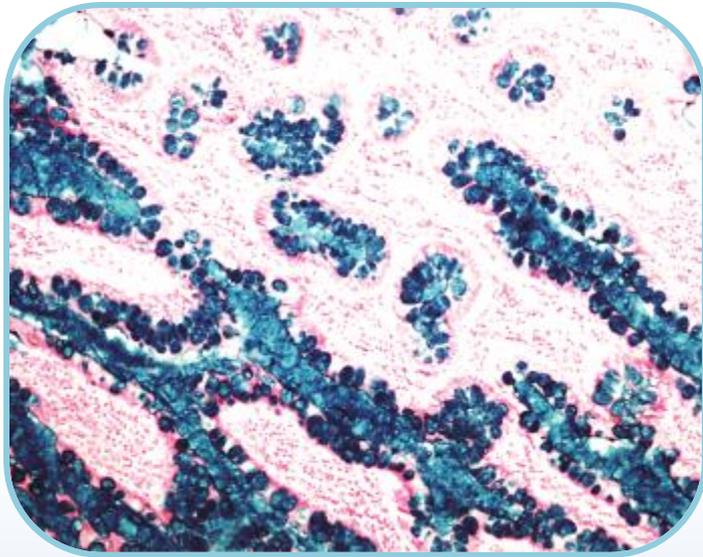
- Used to detect iron in specimens
- Ferric iron present in tissues react with ferrocyanide to form insoluble prussian blue dye
- Ferric iron stains bright blue, nuclei Red and cytoplasm stains pink

# Stains for the detection of Minerals



- **von Kossa Stain:**
  - Used for demonstrating calcium or its Salts and is not specific for calcium
  - Tissue sections are treated with silver nitrate solution, the calcium is reduced by the strong light and replaced with silver deposits, visualized as metallic silver
  - Stains Calcium salts black, Nuclei red, Cytoplasm pink

# Stains for the detection of Minerals



- **Colloidal Iron:**
  - Used demonstrate carboxylated and sulfated mucopolysaccharides and glycoproteins.
  - Stains Acid mucopolysaccharides and sialomucins deep blue, Nuclei Pink-red and Cytoplasm pink



# Molecular Pathology Workflow Solution

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