National University-Sudan

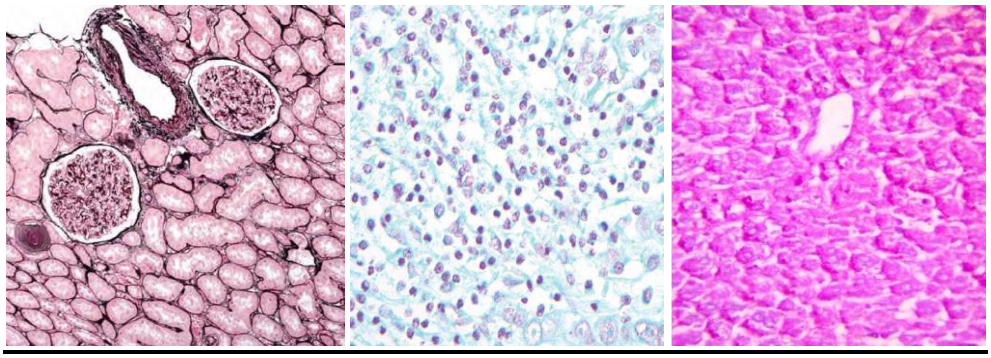
Faculty of Medical Laboratory Sciences

Student Practical Manual
Histopathology and Cytology Department

Third Year, Semester (5)
Cytological and Histopathological Techniques
(MLS-CYTO-314)

Student Name: ……...

ID: ……**Batch**…….....…...



Practical NO.1

Harris's hematoxylin and Eosin

Aim:

To demonstrate nuclei and cytoplasm

Principle of hematoxylin:

Hematoxylin its self is not a stain, haematein is the major oxidation product, haematein is an acid dye have a poor affinity for tissue, so mordant substance is added to form mordanted dye, mordanted dye react with the tissue to form tissue mordant dye complex(acid base reaction)

Solutions:

1Harris's Hematoxylin

2.5 g hematoxylin……..stain

25 ml absolute ethanol……solvent

500 ml D.W……solvent

0.2 g sodium iodate……..oxidizing agent

50 g potassium alum……..mordant

25 glacial acetic acid……..sharpen cytoplasmic stain

2- Eosin Y:

1 g eosin Y……Counter stain

100 ml D.W……solvent

0.05 ml GAA……...sharpen cytoplasm stain.

Small amount of crystal thymol or few drops of formalin …preservative

Method:

1. Wash mouth by water
2. Scrape cells from buccal mucosa by wooden spatula
3. Smear directly on a microscopic glass slide
4. Fix the smear immediately while it's wet in 95% ethanol
5. Hydrate in 90% alcohol for 2 min
6. Hydrate in 80% alcohol for 2 min
7. Hydrate in 70% alcohol for 2 min
8. Rinse in water for 2 min
9. Stain in Harris Hematoxylin for 5 min
10. Differentiate in 0.5 % aqueous hydrochloric acid for 10 second or in 1% acid alcohol for 5-10 second
11. Rinse in water
12. Blue in running tap water for 10 min.
13. Counter stain in eosin Y for 1-3 min.
14. Dehydrate, clear and mount.

Results:

Nuclei: Blue

Cytoplasm: Pink

Others: Pink-Red

Notes:

1. Duration of staining with hematoxylin:
2. Bluing of Hematoxylin:
   In some places, the tap water is not sufficiently alkaline, or is even acid, and unsatisfactory for bluing hematoxylin, so Scott tap water or alkaline solution can be used.
3. Eosin y used commonly as counter stain because it colors back ground by colors vary from pinkish to red

Practical NO.2

Weigert's Hematoxylin and Eosin

Aim:

To demonstrate nuclei and cytoplasm

(Connective tissue section)

Principle of hematoxylin:

Hematoxylin is self is not a stain, haematein is the major oxidation product, haematein is an acid dye have a poor affinity for tissue, so mordant substance is added to form mordanted dye, mordanted dye react with the tissue to form tissue mordant dye complex(acid base reaction)

Solutions:

1- Weigert's Hematoxylin (nuclear stain)

A) 1% alcoholic hematoxylin:

1 g hematoxylin……..stain

100 ml alcohol……solvent

B) Iron solution:

4ml 30% ferric chloride

1ml conc Hcl

95ml D.W

Working solution: Take equal volume from A & B.

2- 1% acid alcohol(differentiation fluid):

99 ml of 70% alcohol

1ml of conc Hcl

3- Eosin Y(counter stain):

- 1 g eosin Y…….Counter stain

- 100 ml D.W……solvent

-0.05 ml GAA……..sharpen cytoplasm stain.

-Small amount of crystal thymol or few drops of formalin …preservative

Method:

1- Take section to water.

2- Stain with Weigert's hematoxylin for 10-15min.

3- Rinse in water.

4- Differentiate in 1% acid alcohol.

5- Wash in water 5 mins.

6- Counter stain in eosin Y for 1-3 min.

7- Dehydrate, clear and mount.

Results:

Nuclei: Blue black.

Cytoplasm: Pink

Others: Pink-Red

Notes:

1. The working solution must be violet black if its brown discards it and prepare new one.

Practical NO.3

Feulgen Reaction

Aim:

For demonstration of Deoxyribonucleic acid (DNA)

Fixation:

Not critical but not Bouin's.

Solutions:

1-1M HCL (Hydrolysis):

Concentrated HCL…….. 8.5 ml

D.W……...……. 91.5 ml

2-Schiffs reagent:

Basic Fuchsin…..…… Stain

D.W ……..…… Solvent

Potassium metabisulphite…….. Reducing agent

Conc HCL…….…….…… Maintain PH

Activated Charcoal……..…. Remove any excess Sulpher

3-1% Light green (counter stain)

Principle:

Mild acid hydrolysis employing 1M HCL at 60^0c, is used to break down the purine deoxyribose bond, the resulting exposed Aldehydes are then demonstrated by the use of Schiff's reagent.

Method:

1. Bring section to water.
2. Rinse sections in 1M HCL at room temperature for 1 min.
3. Place sections in 1M HCL at 60^0c for 8 min (according to fixative used).
4. Rinse in 1M HCL at room temperature for 1 min.
5. Transfer sections to Schiff's reagent for 45 min.
6. Rinse sections in R.T W for (5-10) min.
7. Counter stain in 1% Light green for 1-2 min.
8. Wash in D.W.
9. Dehydrate, clear and mount.

Results:

DNA: red- purple.

Cytoplasm: green.

Notes:

1. Bouin's fixative is not suitable as it causes over hydrolysis.
2. Decalcification, strong inorganic acid should be avoided, and organic acid for short time can be used.
3. Optimum hydrolysis time should be recommended.
4. The hydrolysis time is important, and the correct time for the fixative must be used.
5. The 1M HCL should be preheated to 60˚C.
6. 5M HCL at room temperature may be used but it need longer time that at 60 ˚C.

Practical NO.4

Methyl Green – Pyronin

Aim:

For the demonstration of DNA and RNA

Fixation:

Carnoy preferred but formalin acceptable

Solutions:

Methyl green pyronin Y

- 2% aqueous methyl Green (chloroform extracted)……..... 9 ml
- 2% aqueous pyronin y…….……..…….. 4 ml
- Acetate buffer pH 4.8……...……...… 23 ml
- Glycerol…..……..… 14 ml

Mix well before use.

Preparation of acetate buffer pH 4.8:

Stock A: 0.2 M acetic acid:

1.2 ml glacial acetic acid in 100 ml of D.W

Stock B: 0.2 M sodium acetate:

1.64 g of sodium acetate in 100 ml of D.W

Composition of buffer:

8 ml of A + 12 ml of B

Principle:

Methyl green specificity is attributed to the spatial alignment of the NH_2 groups of the dye to the phosphate radicals on the DNA, pyronin lack this spatial affinity and any negatively charged tissue will stain red.

Method:

1. Bring section to water.
2. Rinse in acetate buffer solution pH 4.8.
3. Place in staining solution for 25 min.
4. Rinse in acetate buffer solution pH 4.8.
5. Blot, dry.
6. Rinse in 93% Alcohol, then in Absolute Alcohol.
7. Clear and mount.

Results:

DNA: green-blue.

RNA: red.

Notes:

1. PH and staining solution concentration, final dehydration optimizes.
2. Methyl green is an impure dye containing Methyl violet removed by washing in chloroform.
3. Some mucous cells may be stained by pyronin.
4. Acid decalcification is said to interfere with the technique (to solve, by adjusting the two dyes the methyl green increased and the pyronin y decreased).
5. The final dehydration is also important, and washing in water should be avoided.

Practical NO.5

Periodic Acid Schiff's Reaction (PAS)

Aim:

For demonstration of Glycogen

Fixation:

Bouins is the best.

Solutions:

1. **1%periodic acid.**
2. **Schiff's reagent.**
    - Basic fuchsin……..……..stain.
    - D.W…….……..solvent.
    - Potassium metabisulphite……..…….….Reducing agent.
    - Conc HCL.…… PH.
    - Activated Charcoal……....Remove any excess Sulpher.
3. **Mayer's Hematoxylin (nuclear counter stain).**
    - 1 g hematoxylin……..stain
    - 1000 ml D.W……solvent
    - 0.2 g sodium iodate……..oxidizing agent
    - 50 g potassium alum……..mordant-
    - 1 g citric acid……..sharpen nuclear stain
    - 50 g chloral hydrate……preservative

Principle:

1%Periodic acid will bring about oxidative cleavage of the Carbon to Carbon bond in 1-2 glycol or their amino or alkyl-amino derivatives, to form dialdehydes. Then these aldehydes will react with Schiff's reagent to form magenta color compound.

Method:

1. Bring section to water.
2. Oxidize in 1% P.A for 5 min.
3. Wash well in different changes of D.W
4. Place in Schiff's reagent for 15 min.
5. Wash in R.T W for (5-10) min.
6. Stain nuclei with M.H and bluing.
7. Wash in water, rinse in absolute alcohol.
8. Clear and Mount.

Results:

Glycogen: Red/ Magenta.

Nucleus: Blue.

Notes:

1. Periodic acid is the oxidant of choice.
2. Other oxidants like potassium permanganate and chromic acid substitute periodic acid
3. They tend to over oxidize the formaldehyde to carboxylic acid which result in a weak Schiff's reaction.
4. Tap water after Schiff's solution to intensify the color of reaction.

Practical NO.6

PAS with Diastase

Aim:

To specify the demonstration of Glycogen by PAS

Solutions:

1. **1%diastase:**
    - Diastase: 1g
    - D.W: 100 ml
2. **1% periodic acid.**
3. **Schiff's reagent.**
4. **Mayer's hematoxylin.**

Principle:

Diastase enzyme break down the glycosidic bonds (converting the glycogen into glucose units), the resulting glucose units dissolve in water so that the Schiff's reaction appear negative after employing of PAS staining procedure.

Method:

1. Dewax two positive control and two test sections and bring to distilled water.
2. Treat one test section and one positive control section, for 1 hour at 37^0c in diastase solution.
3. Wash in R.T.W for (5-10) min.
4. Stain all sections with the PAS technique for glycogen.
5. Dehydrate, clear and mount.

Results:

Presence of glycogen will be evidenced by loss of staining after enzyme treatment when compared to the untreated sections.

Treated section: colorless.

Untreated section: Red.

Glycogen present in sections.

Notes:

1. In addition to glycogen, starch is also digested but at a lower rate.
2. The type of fixation will affect digestion, as Gender or glutaraldehyde resist diastase digestion and longer times will be needed.
3. Celloidinization of sections prior to digestion may slow down or inhibit the enzyme action.

Practical NO.7

Alcian Blue

Aim:

For demonstration of Acid mucins and connective tissue mucins

Preparation:

1. **1% Alcian blue.**
    - Alcian blue……. 1g
    - 3% acetic acid……..…… 100ml
2. **1% Eosin or neutral red (counter stain).**

Principle:

The Alcian blue is cationic dye, and at pH 2.5 bond via electrostatic linkages with polyanionic molecules within tissues (acid mucins and connective tissue mucins).

Method:

1. Bring section to water.
2. Treat with working solution for 30 min.
3. Wash in running tap water for 5 min.
4. Counter stain with 0.5% Neutral Red or Safranine or Eosin for 1-2 min.
5. Wash in D.w.
6. Dehydrate, clear and mount.

Results:

Acid Mucins, proteoglycans and hyaluronic acid: Blue

Nucleus or other structures: according to counter stain in use

Notes:

1. It is important to counter stain with a weak solution of Neutral Red to avoid masking the Alcian blue staining.
2. It is best to filter the staining solution before uses it, particularly if the age of solution is more than month or so old.
3. Avoid celloidinization of slides as the Alcian dyes are strongly retained by the celloidin.
4. 3% of glacial acetic acid uses to adjust the ph of staining solution at 2.5.
5. Van Gieson solution may be used as a counter stain.
6. Alcian blue at different Ph level may differentiate the sub types of acid mucins.

Practical NO.8

Colloidal iron technique

Aim:

For demonstration of acid mucopolysaccharides

Principle:

The ferric cations in a colloidal ferric oxide solution are attracted and bound to acidic mucins; the ferric ions bound to the mucins are then treated with potassium ferrocyanide to form bright blue deposits of ferric ferrocyanide or Prussian blue.

Solutions:

Stock colloidal iron solution:

29% ferric chloride…… 4.4 ml

D.W…… 250 ml

Working solution:

Stock colloidal iron solution……..……. 20 ml

Deionized water……..… 15 ml

Glacial acetic acid……...… 5 ml

Prepare just prior to use.

12% Acetic acid solution

Potassium ferrocyanide hydrochloric acid solution:

A. 5% potassium ferrocyanide solution.
B. 5% Hydrochloric acid solution.

Take equal volume from A and B mix just prior to use.

Van Gieson solution:

1% acid fuchsin…….. 5 ml

Saturated picric acid……... 95 ml

Method:

1. Take section to D.W
2. Rinse in 12% acetic acid solution for 1 min.
3. Cover the section with the working colloidal iron solution for 30 min.
4. Rinse in 12% acetic acid solution 2 or 3 changes each 3 min.
5. Place in the potassium ferrocyanide hydrochloric acid solution for 20 min.
6. Rinse in R.T.W for 5 min.
7. Counter stain with van Gieson solution for 5 min.
8. Dehydrate in absolute alcohol, clear, and mount.

Result:

Acid mucopolysaccharides: bright blue

Collagen: red

Muscle and cytoplasm: yellow

Notes:

1. The PH of the staining solution is critical.
2. Nuclear fast red or eosin may be used as a counter stains.

Practical NO.9

Combined alcian blue PAS

Aim:

To differentiate neutral mucins from acidic mucins within a tissue section

Also it may be used as a broad means of detecting mucins

Preparation:

1%Alcian blue.

Stain the acid mucopoly saccharides

1% periodic acid

Oxidizing agent

Schiff's reagent

Main stain

Mayer's hematoxylin

Counter stain

Principle:

The section is first stained with Alcian blue (stains all acid mucins even those are PAS positive), then the subsequent treatment with PAS staining procedure demonstrate only the neutral mucins.

Method:

1. Bring section to water.
2. Stain with Alcian blue at pH 2.5 for 30 min.
3. Wash in water and then wash again in D.W
4. Oxidize in 1% P.A for 5 min.
5. Wash well in different changes of D.W
6. Place in Schiff's reagent for 20 min.
7. Wash in R.T W for (5-10) min.
8. Stain nuclei with M.H and bluing.
9. Wash in water, rinse in absolute alcohol.
10. Clear and Mount.

Results:

Acid Mucins: Blue

Neutral Mucin: Red/ magenta

Notes:

1. It is important to stain only lightly with Hematoxylin, as otherwise it may be difficult to distinguish B/w this and the Alcian blue staining.
2. Ehrlich's Hematoxylin should be avoided as a counter stain, as it well also stains some mucins.
3. It's very important to apply alcian blue first then followed by PAS.
4. Glands that contain acid and neutral mucins stain purple.

Practical NO.10

Highman's Congo red

Aim:

For the demonstration of Amyloid

Solutions:

Solution (a): Congo red working solution.

o.5% Congo red in 50% alcohol

Solution (b): Differentiator.

o.2%KOH in 80% Alcohol

Principle:

Congo red is an acidic diazo dye; the staining of Amyloid by Congo red is through hydrogen bonding formation between the amyloid and Congo red as opposed to the electrochemical bonds formed between the dye and most tissue components.

Method:

1. Bring section to water.
2. Stain with working solution for 5 min.
3. Differentiate in 0.2% KOH for (3-10) seconds.
4. Wash in water.
5. Stain nucleus with Alum hematoxylin.
6. Bluing in R.T.W.
7. Dehydrate, Clear and Mount.

Results:

Amyloid, elastic fibers, eosinophilic granules: orange Red

Nucleus: Blue

Notes:

1-Differentiation in step ‘’3’’ can be arrested in water and resumed if necessary-over differentiation can occur.

2-The solutions are relatively stable and the method affords a high degree of selectivity in practical hands.

3-This is simple method, has found wide application.

Practical NO.11

Weigert's Van Gieson

Aim:

For selective demonstration of muscles, collagen fibers, fibrin, and erythrocytes

Solutions:

Van Gieson solution:

1. Saturated aqueous picric Acid.
   (Small dye molecule)
2. 1% acid Fuchsin. 1 gm in 100 ml D.W
   (Large dye molecule)

Take 10 ml of 1% acid fuchsin and add to 90 ml Picric acid.

Weigert's hematoxylin (Nuclear stain)

A. 1% alcoholic hematoxylin
B. Iron solution:

30% aqueous ferric chloride…….....4 ml

HCL (Conc)……..……1 ml

D.W……95 ml

Take equal parts of solution A and B and mix them immediately before use.

Principle:

Collagen fibers have strong affinity for high molecular weight of dyes, while muscle, RBCs, and fibrin have affinity for low molecular weight of dyes (according to the tissue permeability and dye size molecule).

Method:

1-Bring section to water.

2-Stain nucleus with Weigert's solution for (10-15) min

3-Wash in water for (7-10) min

4-Stain with V.G working solution for (3-5) min

5-Blot with Filter Paper

6-Dehy, Clear and Mount.

Results:

Nucleus: Black color.

Collagen fiber: Red color.

R.B.Cs, Muscles: Yellow color.

Notes:

1. Washing in water after V.G should be avoided, the color balance being impaired by this.
2. Nuclear staining should be before application of V.G.
3. Celloidin sections washed in D.W. after V.G solution.
4. Differentiation step after Weigert's hematoxylin is not needed because picric acid in vangieson solution does this.

Practical NO.12

Masson Trichrome Technique

Aim:

For selective demonstration of muscles, collagen fibers, fibrin, and erythrocytes

Solutions:

Nuclear stain:

Weigert's Hematoxylin

Cytoplasmic stain:

1% Acid Fuchsin in 1% Acetic Acid

Fiber stain:

2% Light Green in 1% Acetic Acid or

2% Fast Green in 1% Acetic Acid or

2% Methylene Blue in 1% Acetic Acid

Differentiation:

1% Aqueous PMA or PTA

Principle:

Collagen fibers have strong affinity for high molecular weight of dyes, while muscle, RBCs, and fibrin have affinity for low molecular weight of dyes.

Method:

1. Bring section to water.
2. Stain Nucleus with Weigert's .H for (10-15) min
3. Wash in water.
4. Differentiate with 1% Acid Alcohol.

5. (Rinse) Keep in R.T.W for 5 min.
6. Stain with Cytoplasmic stain for (3-5) min.
7. Wash.
8. Differentiate with 1% aqueous PMA or PTA until the collagen decolorize while the RBCs and Muscle still stain or differentiate for 5 minutes.
9. Drain.
10. Stain with Fiber stain for (3-5) min.
11. Treat with 1% Acetic Acid for (1-2) min, to remove any excess of light green.
12. Dehydrate, Clear and Mount.

Results:

Collagen Fiber: Green.

RBCs, Muscle: Red.

Nucleus: Black.

Notes:

1-If a section is first treated with PMA or PTA solution and then with a low concentration of a dye in the same solution, the dye will color nothing but the erythrocytes.

2-PTA is unstable at a pH greater than about 2.

Practical NO.13

Verhoeff's method

Aim:

For demonstration of Elastic Fibers

Preparation:

Solution "A": 5% Alcoholic Hematoxylin.

Solution "B": 10% Ferric chloride.

Solution "C": Lugol's Iodine.

Working solution

Solution "A": 20 ml

Solution "B": 8ml

Solution "C": 8ml

Add in the above order, mixing between additions.

Counter stain:

Van Gieson solution:

- Saturated aqueous picric Acid.
- 1% acid Fuchsin. 1 gm in 100 ml D.W

Take 10 ml of 1% acid fuchsin and add to 90 ml Picric acid.

Or: 1% Eosin.

Principle:

Elastic Fibers are highly linked with disulphide Bridges, iodine oxidizes disulphide bonds to anionic sulphonic acid derivatives, and these derivatives react with mordanted hematoxylin to form black color.

Method:

1. Bring section to water.
2. Stain section with Verhoeff's solution for (15-20) min.
3. Wash with water.
4. Differentiate with 2% $Fecl_3$, until Elastic fiber appears black color.
5. Wash in D.W.
6. Rinse in 95% Alcohol to remove iodine deposit from back ground.
7. Counter stain with eosin for (2-3) min.
8. Dehydrate, Clear and Mount.

Result:

Elastic Fiber: black.

Collagen fibers: Red.

Muscles, cytoplasm, fibrin, and RBCs: yellow

Notes:

1. Prepared working solution has a usable life of only (2-3) hours, satisfactory results have been obtained using solution up to 48 hours old.
2. The differentiation step is critical to the success of this method, so control this step by microscope and washing to stop the reaction of differentiation solution, so will give good results and to prevent loss of fiber by over differentiation.
3. Removal of mercury pigment is unnecessary, this being carried out by the iodine in the staining solution.

Practical NO.14

Gordon and sweet

Aim:

For demonstration of reticular fibers

Solutions:

Silver solution

- 10% $AgNo_3$
- 3% NaOH
- Concentrated Ammonia

Working solution:

- To 5ml of 10% Ag No_3, add ammonia drop by drop until the precipitate first formed is dissolve, then add 5 ml of 3% Na OH, and redissolves by using ammonia drop by drop till the solution retain fine opalescence, if not, you can add a drop of 10% Ag No_3.
- Make up the volume 50 ml with D.W; means complete the volume to 50 ml by adding distilled water.

1% potassium permanganate (oxidizing agent)

1% oxalic acid (bleaching agent)

2.5% iron alum (sensitized agent)

10% formalin (reducing agent)

5% sodium thiosulphate (removes the unreduced silver)

Principle:

Reticular fiber reduces ammonical silver solution to metallic silver with help of reducing agent (argyrophilic).

Method:

1. Bring section to water.
2. Oxidize with 1% potassium permanganate for 5 min.
3. Wash well in D.W.
4. Bleach with 1% oxalic acid until the brown color become colorless.
5. Wash well in D.W.
6. Treat with 2.5% iron alum for 15 min.
7. Wash well in D.W.
8. Treat with silver solution for 30 seconds to 1 min.
9. Was well with D.W.
10. Reduce with 10% formalin for 2 min.
11. Wash well.
12. Treat with 5% sodium thiosulphate for 5 min.
13. Wash well.
14. Counter stain as desire, either 1% neutral red, Safranin or Eosin.
15. Dehydrate, Clear and Mount.

Results:

Reticular fiber: Black.

Other: Red.

Notes:

1. A short treatment with iron alum of less than 5 min. gives less staining of nuclei.
2. The use of Coplinjars for the silver solution greatly reduces the possibility of precipitation on the slide.
3. Silver solution, mainly Alkaline frequently causing sections to become detach from the slide, adhesive media must be used.

Practical NO.15

Perl's Prussian blue Method

Aim:

For the demonstration of haemosiderin and Inorganic Iron

Solutions:

Perls solution

Stock solution:

A: 2% K. Ferrocyanide.

B: 2% Hcl.

Working solution:

Mix equal parts of solution A and B just immediately before use.

Eosin: as a counter stain.

Principle:

2% Hcl removes the masking of ferric iron to form ferric hydroxide, and then ferric hydroxide will react with K. Ferrocyanide to form ferric-ferrocyanide (Prussian blue).

Method:

1. Bring section to water and rinse in D.W.
2. Treat section with Perls' solution for (10-20) min.
3. Wash well in D.W
4. Counter stain with Eosin (or neutral red or Safranin) for 1 min.
5. Dehydrate, Clear, and Mount in D.P.X.

Results:

Haemosiderin and Ferric salts: Blue color.

Other pigment: Their natural color.

Other tissue structures: pink to Red color.

Notes:

1. It is better to keep the two stock solutions made up separately and stored in the refrigerator, but must be stored for prolonged periods, this will ensure that the solutions retain their viability.
2. A positive control is used with all test sections.
3. Avoid the use of acid fixatives; chromates will also interfere with the presentation of Iron.
4. Necessary to vary the staining time

Practical NO.16

Schmorl's Reaction

Aim:

For demonstration of Melanin pigment

Solutions:

Stock solution:

A: 0.4% aqueous K.Ferricyanide

B: 1% aqueous Ferric Chloride or Ferric sulphate

Working solution:

Add 4ml of K.Ferricyanide to 30ml of Fe Cl_3, and mix gently.

Principle:

Melanin reduces potassium ferricyanide to ferrocyanide, and then ferrocyanide will react with ferric chloride to form ferric ferrocyanide dark/ blue in color (Prussian blue).

Method:

1. Bring section to D.W
2. Treat with working solution from 5-10min.
3. Wash in R.T.W for 5 min, so as to remove any ferricyanide remains.
4. Lightly counter stain with 1% eosin (or o.5 % neutral red or Safranine) for 1-2 min.
5. Dehydrate, Clear and Mount.

Results:

Melanin: Dark/Blue color.

Nuclei: Red color.

Notes:

1. The time for the reaction to take place depends on the substance to be demonstrated, with melanin generally reacting more quickly than lipofuscins.
2. When choosing a control section, remember that melanin reduces the ferric-ferricyanide more quickly than other reducing substances, so control which could match the test should be used.
3. This modification is preferred to the more traditional method because it is easier to control and gives less background staining.

Practical NO.17

Masson Fontana

Aim:

For demonstration of melanin pigment

Solutions:

Silver solution

Stock solution:

10% Ag No_3

Ammonia

5% Na. thiosulphate

Working solution:

1. To 20 ml of 10% silver nitrate, add ammonia drop by drop until a fine precipitate remains.
2. Add 20 ml of D.W to the above solution, and keep in a dark bottle in cold place.

Principle:

Melanin directly reduces silver to metallic black silver without help of reducing agent (Argentaffin).

Method:

1. Bring section to water.
2. Treat section with working solution for (3-4 min) at 56^0 c in water bath or oven.
3. Wash well in several changes of D.W.
4. Treat with fix with 5% sodium thiosulphate for 1min.
5. Wash in R.T.W for 3 min.
6. Lightly counter stain with neutral red, Safranin, or eosin for 1min.
7. Wash, Dehydrate, Clear and Mount.

Result:

Melanin: Black color.

Nucleus: Red color.

Notes:

1. Prolonged exposure to 56^0c, may give rise to a fine deposit over the section.
2. Friable material may need to be coated with celloidin as the ammonia in the silver solution could lead to sections lifting off the slide.
3. Adhesive media uses in preparation of section to avoid detach of tissue from slide.
4. Only use clean glassware, as the silver solution will react with any residual contaminant left on glassware.

Practical NO .18

Silver nitrate method

Aim:

For AgNOR protein sites

Solutions:

50% silver nitrate solution:

Silver nitrate……..….. 50 g

Distilled water……..100 ml

Gelatin solution:

Gelatin……2 g

Formic acid……1 ml

Distilled water……100 ml

Working solution:

Silver nitrate solution…….2 parts by volume

Gelatin solution……...1 part by volume

Mix immediately before use.

Principle:

AgNOR protein sites directly reduce silver to metallic silver without help of reducing agent (Argentaffin).

Method:

1. Take section to water.
2. Rinse in D.W.
3. Incubate in freshly prepared working solution for 45 min at room temperature.
4. Wash in D.W for 1 min.
5. Dehydrate, clear, and mount.

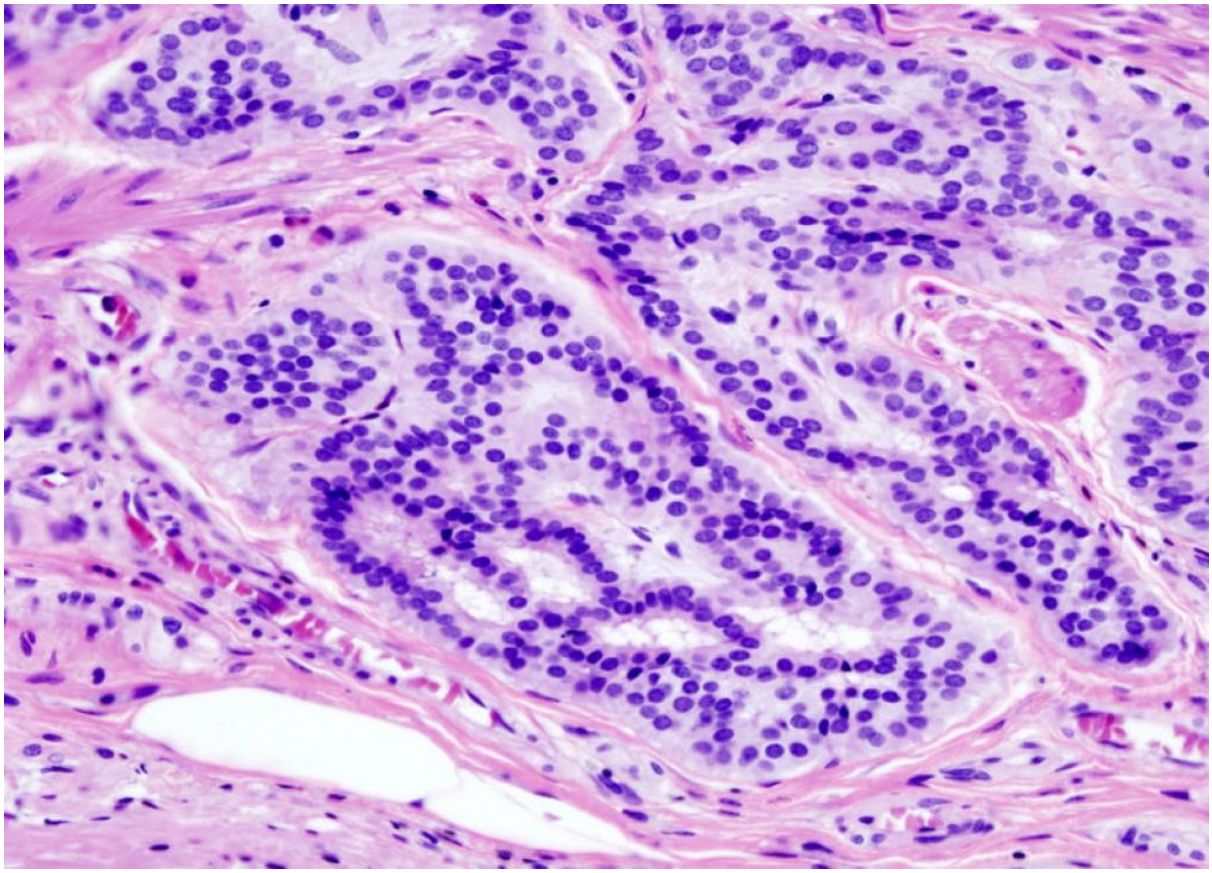
Results:

AgNOR sites: Intranuclear black dots

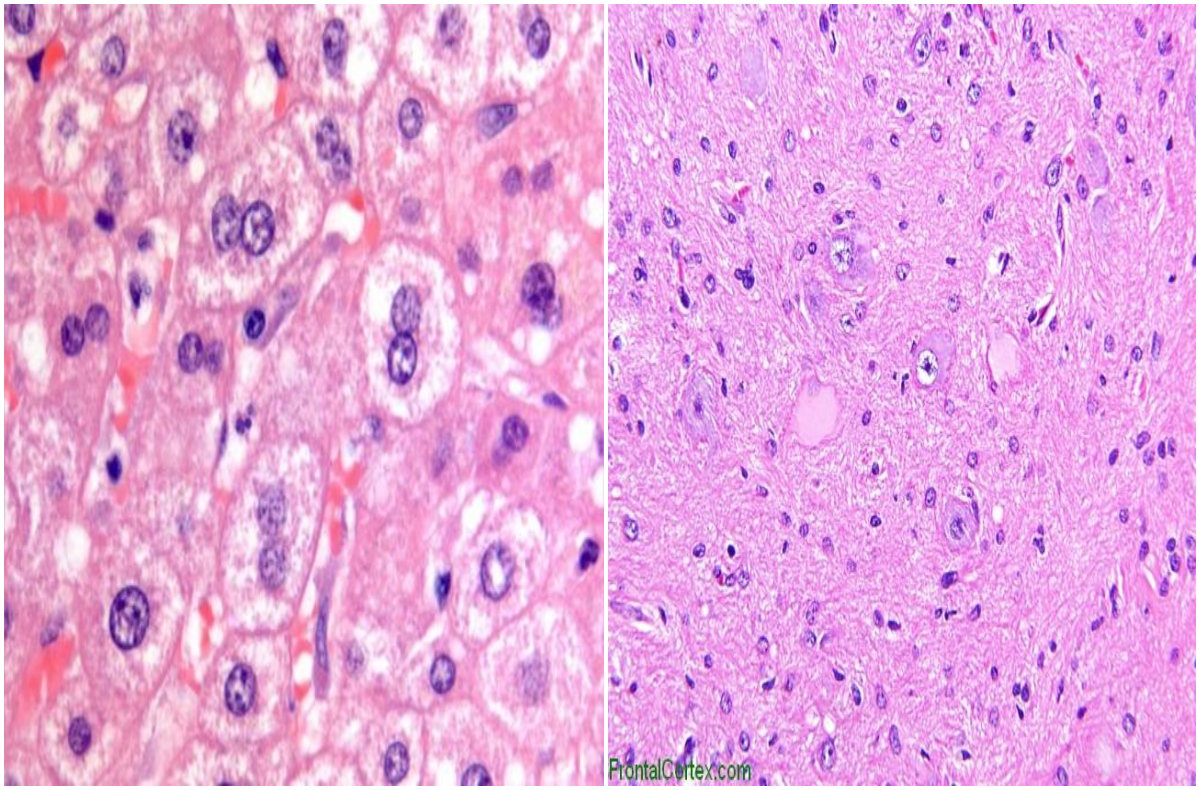
Background: pale yellow

Notes:

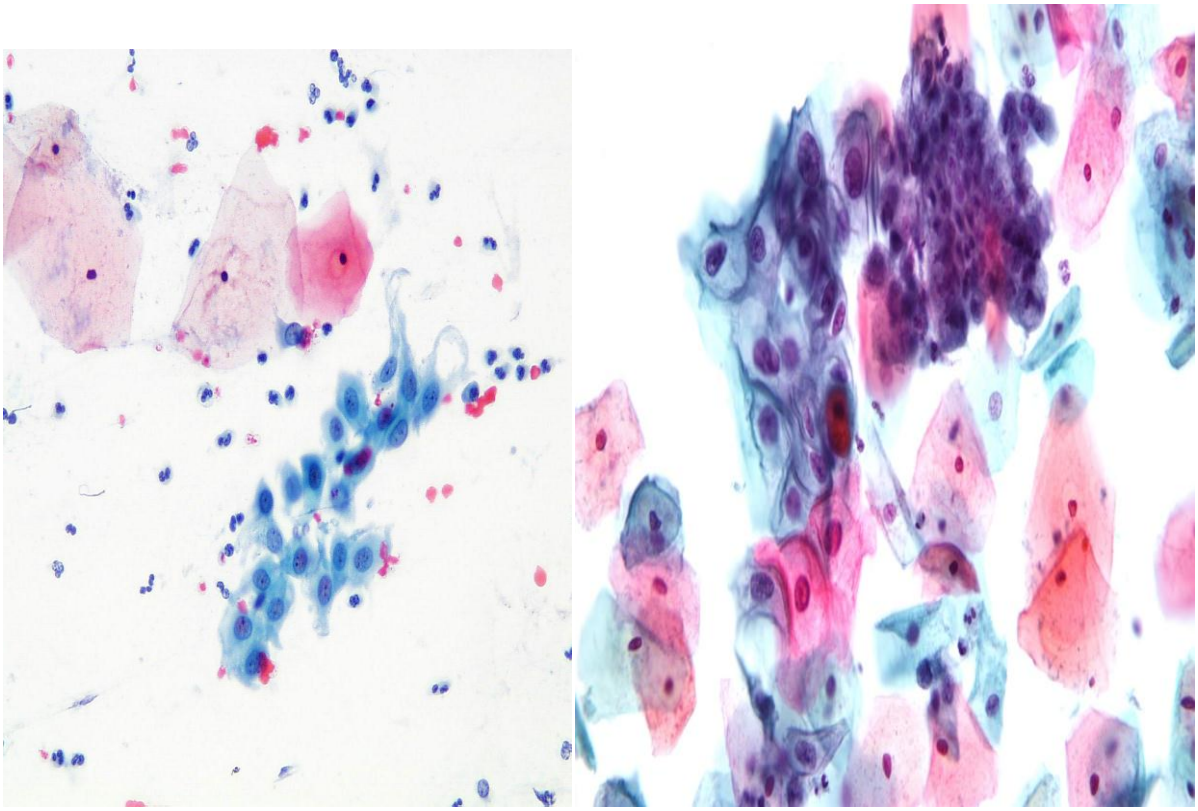
1. Section may be lightly counter stain with neutral red or carmalum.
2. Section may be toned using 1% gold chloride.
3. The working solution must be used immediately.



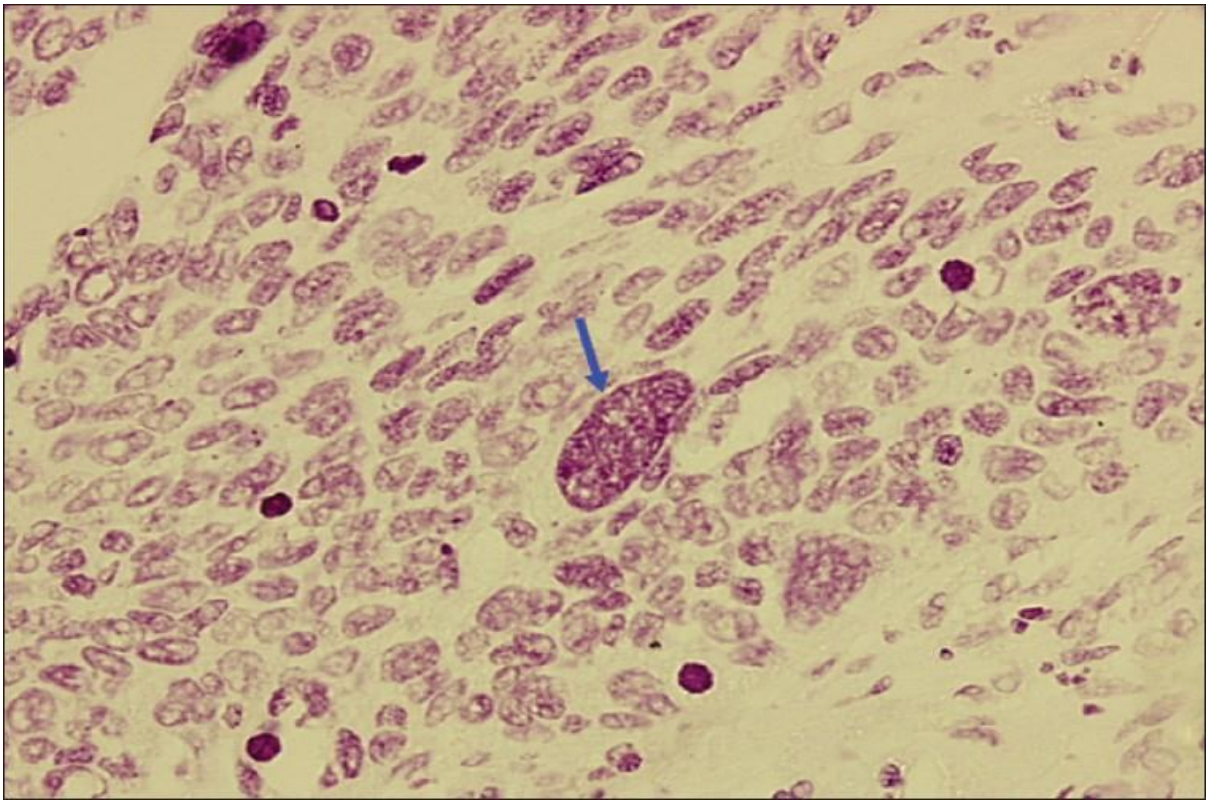
Hematoxylin and eosin: nucleus blue, others stained pink to red.



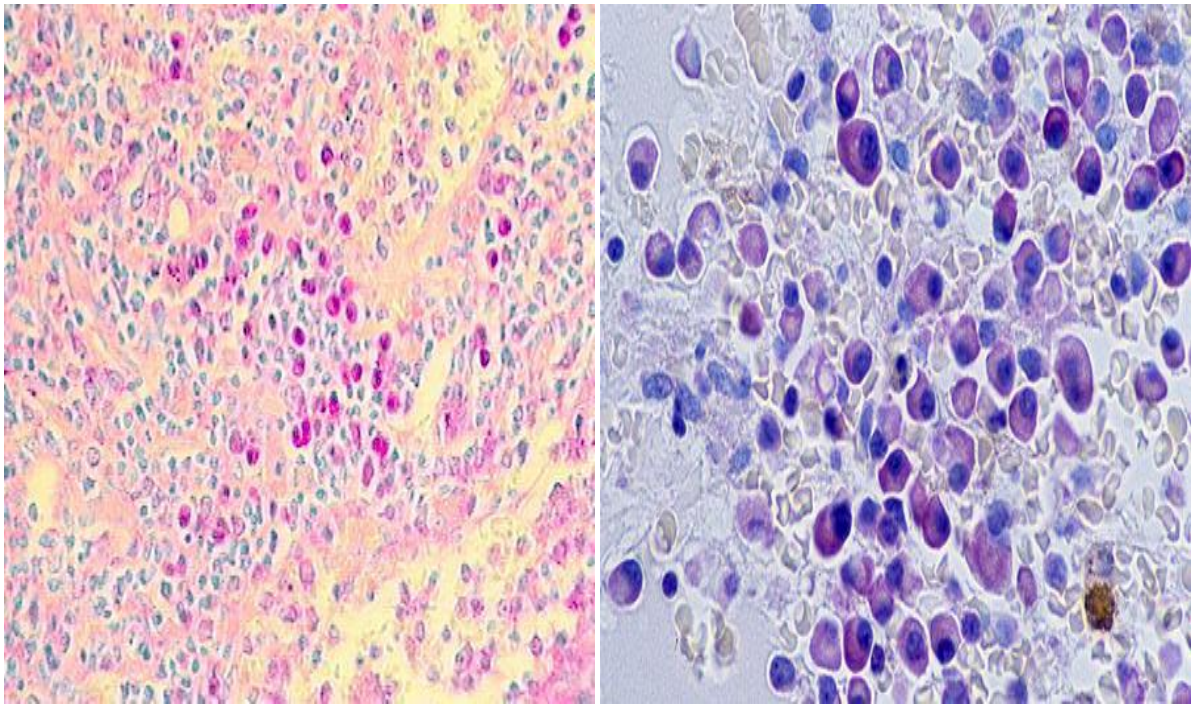
Hematoxylin and eosin: nucleus blue, others stained red.



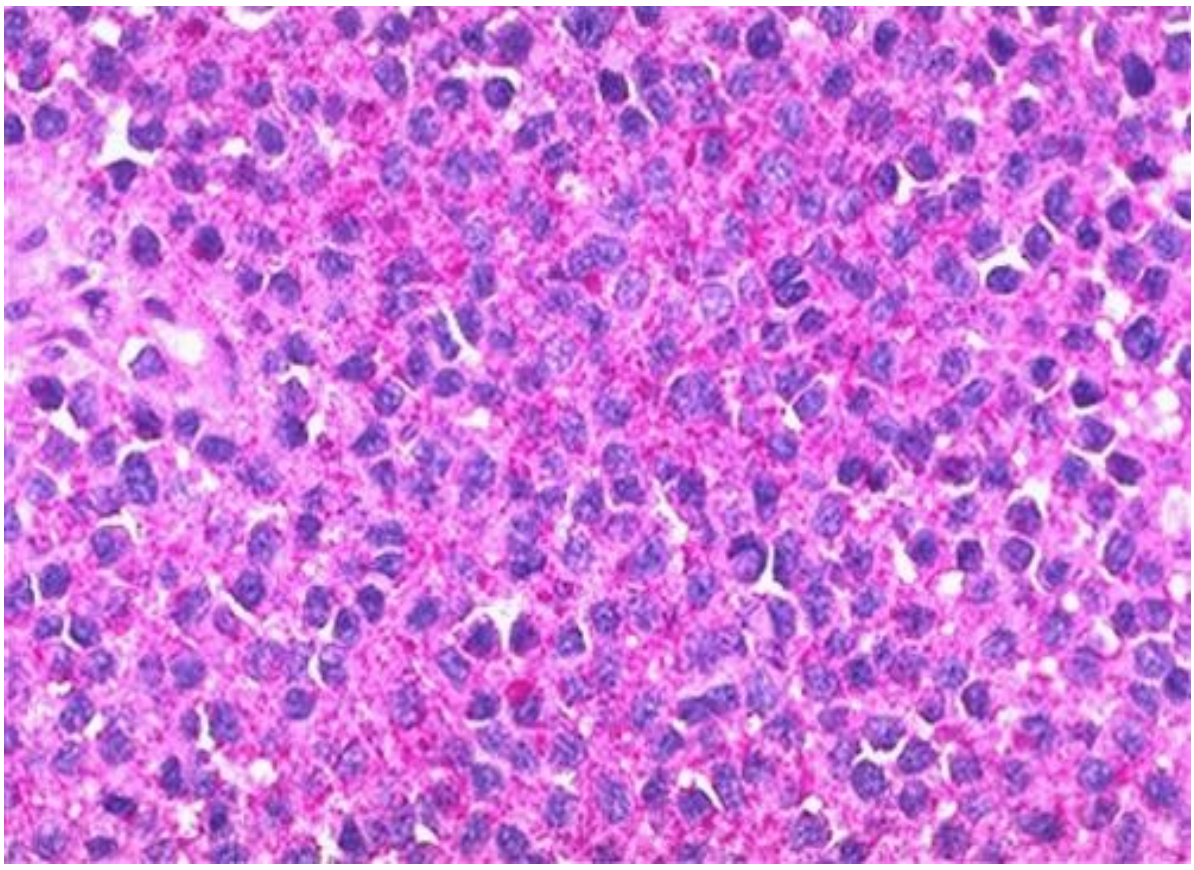
Papanicolaou stain: nucleus blue, cytoplasm of immature cells green, cytoplasm of mature cells pink



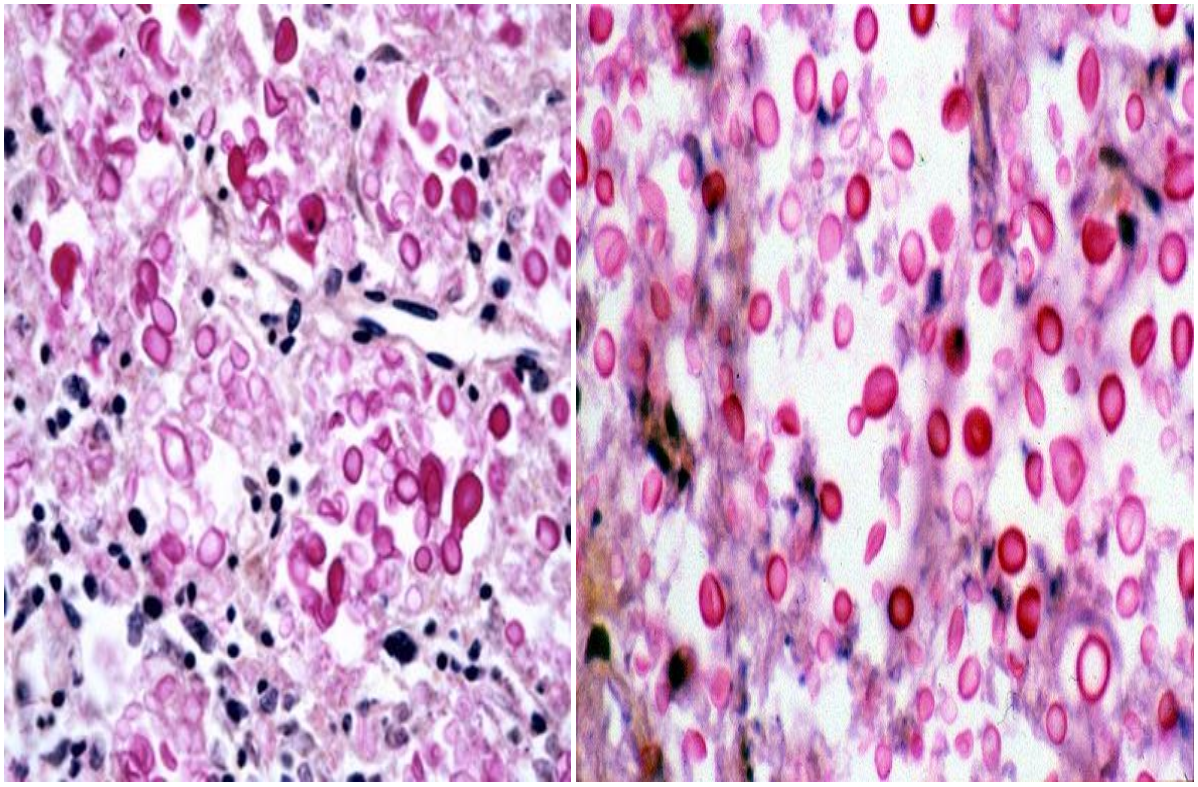
Feulgen reaction: DNA stains magenta



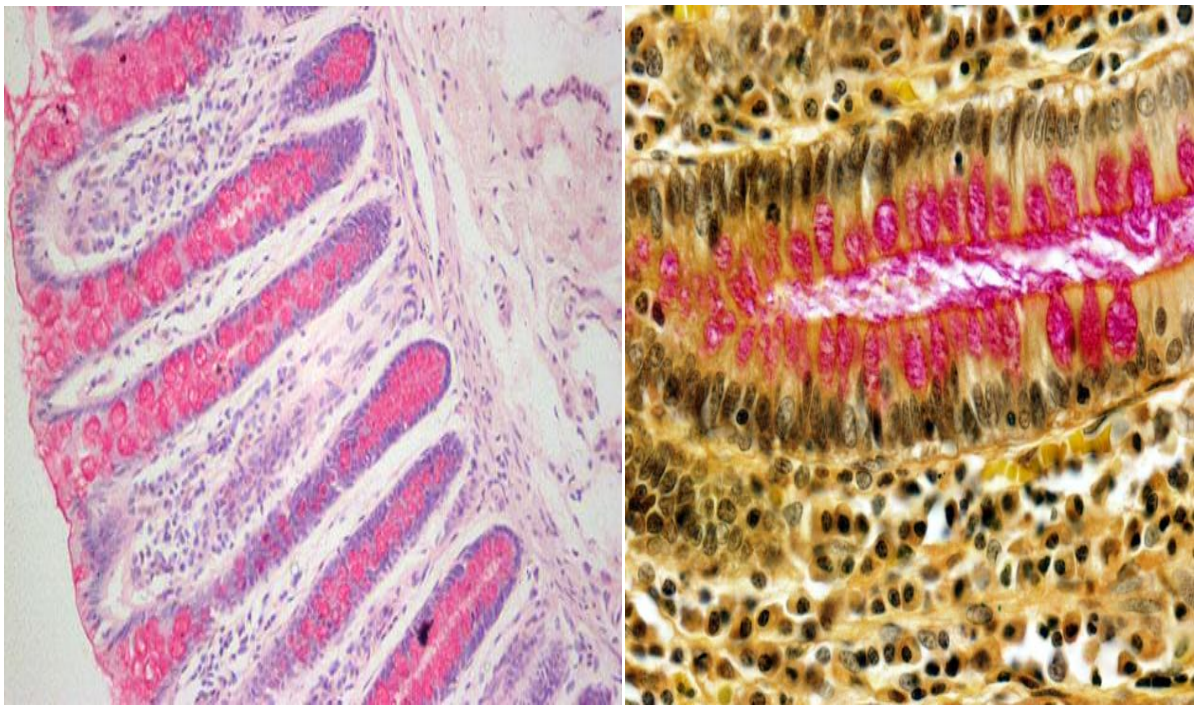
Methyl green pyronin: DNA green, RNA red



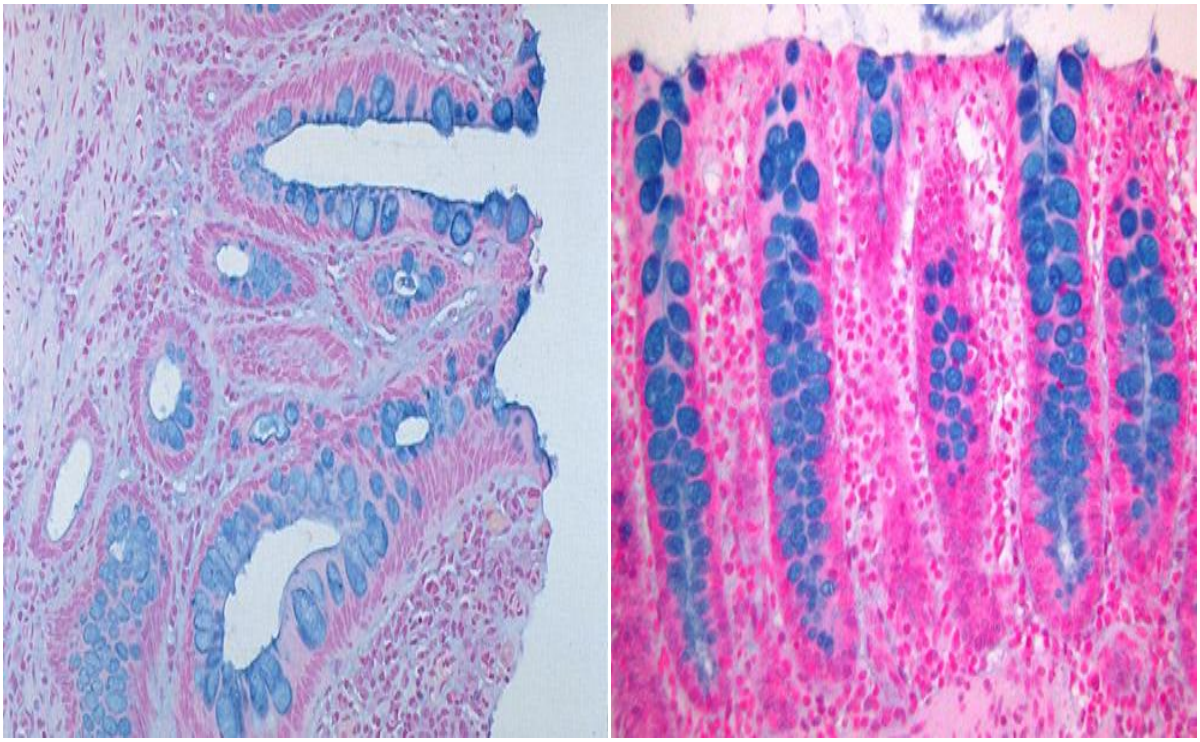
Periodic acid Schiff's reaction: glycogen magenta, nucleus blue



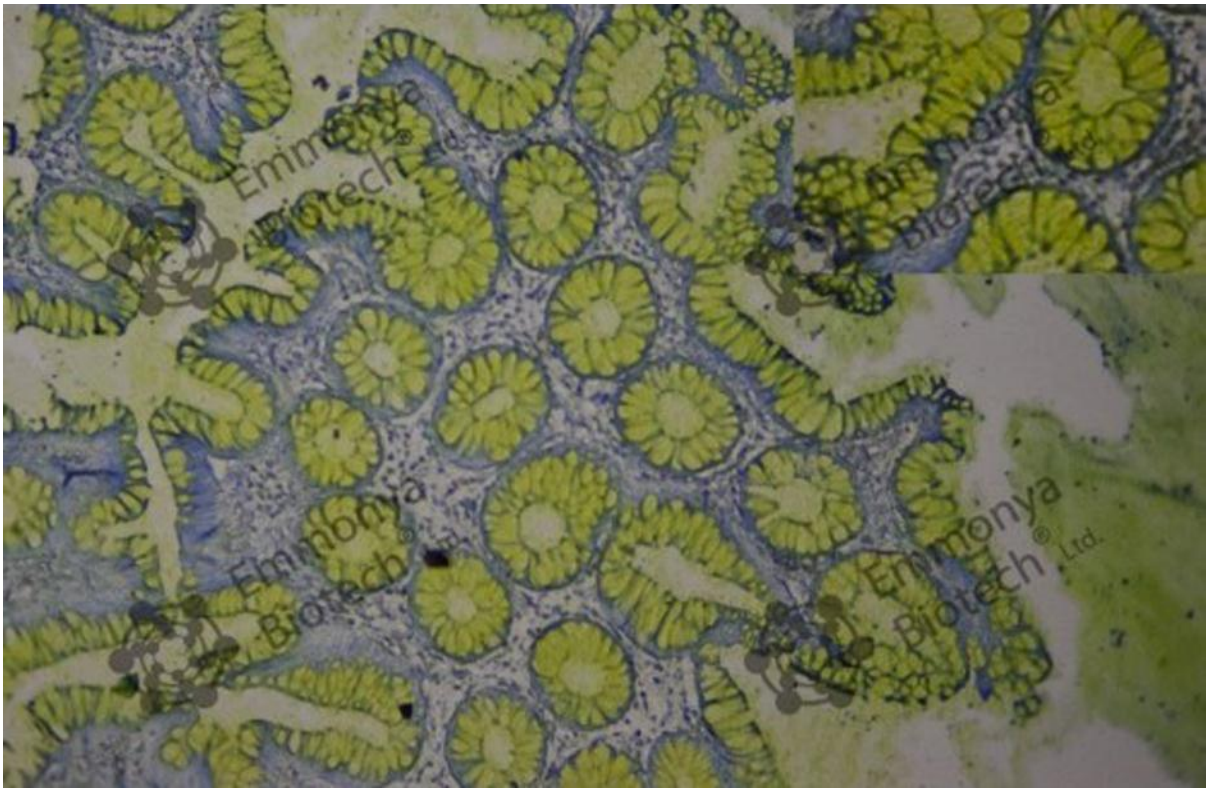
Mucicarmine stain: Cryptococcus neoformans stained red



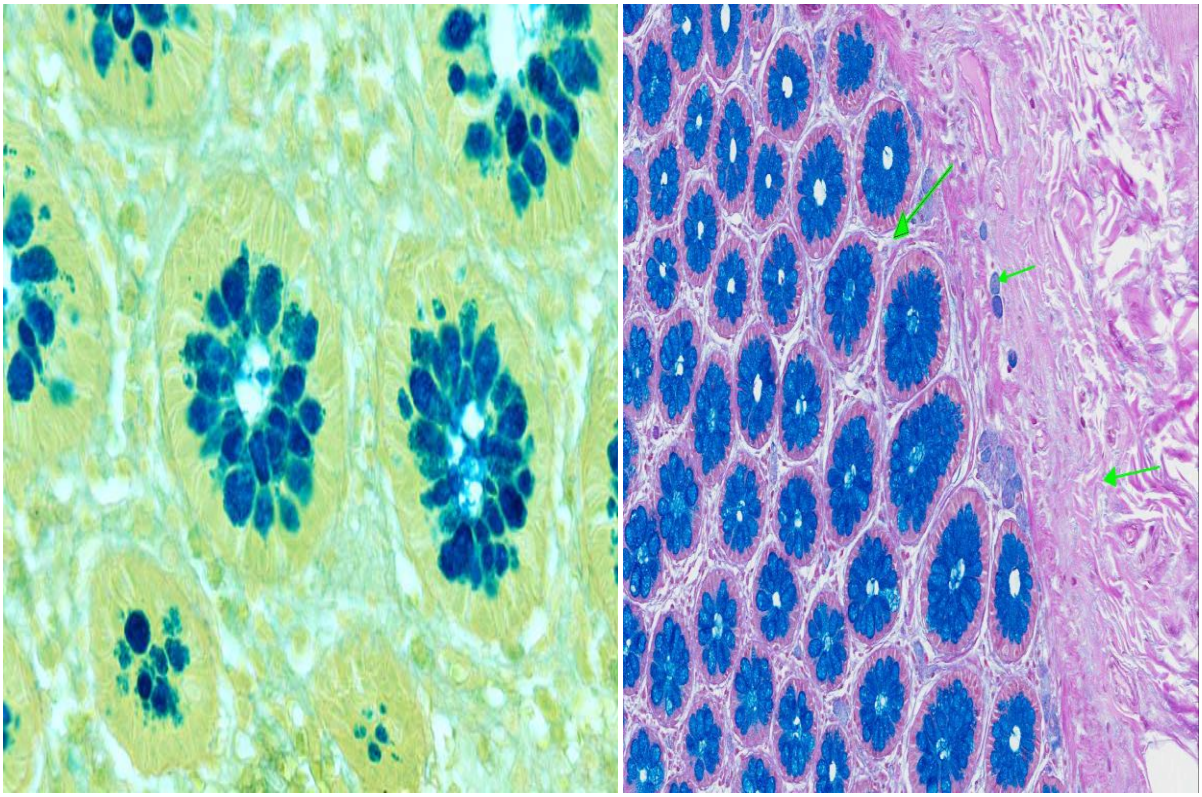
Mucicarmine staining technique: acid mucopolysaccharides stained red



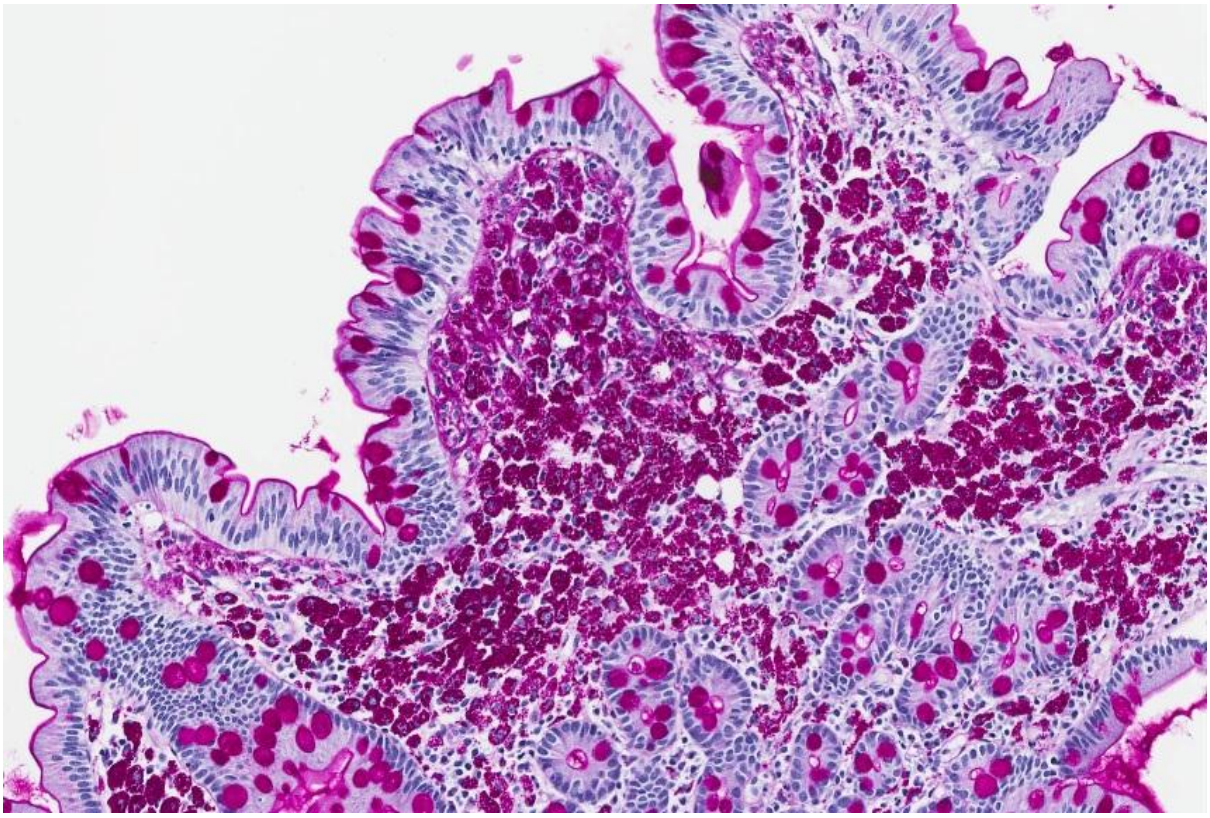
Alcian blue at ph 2.5: acid mucopolysaccharides stained blue



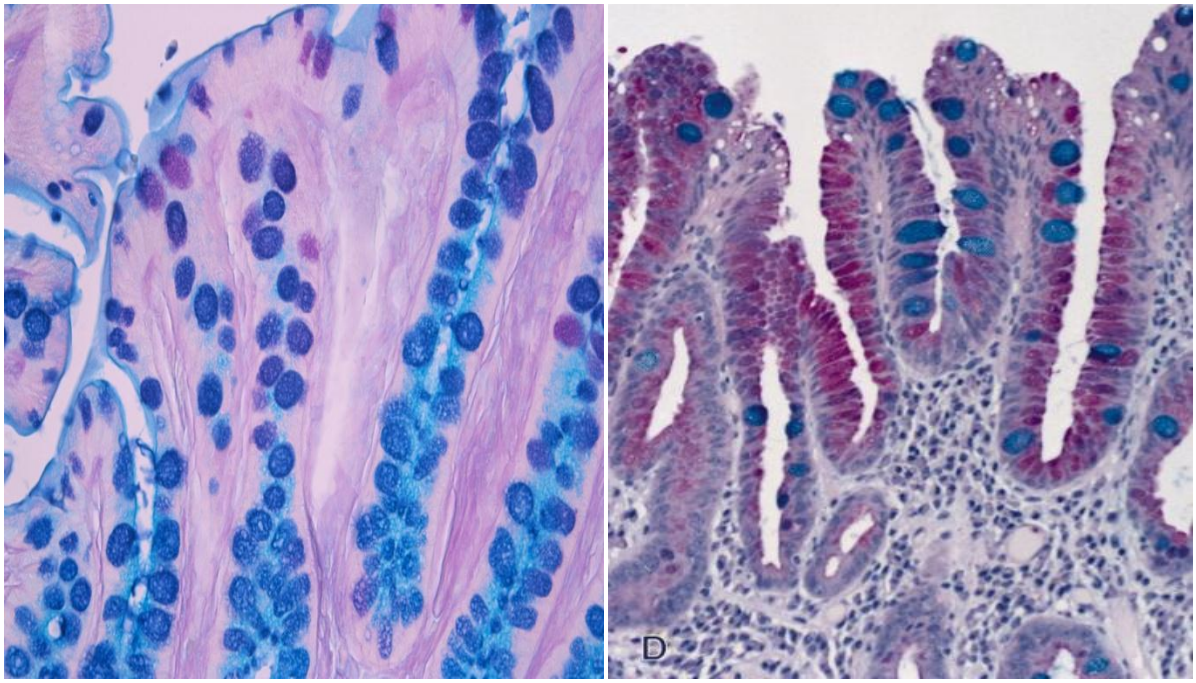
Alcian yellow: strongly sulfated acid mucins stained yellow



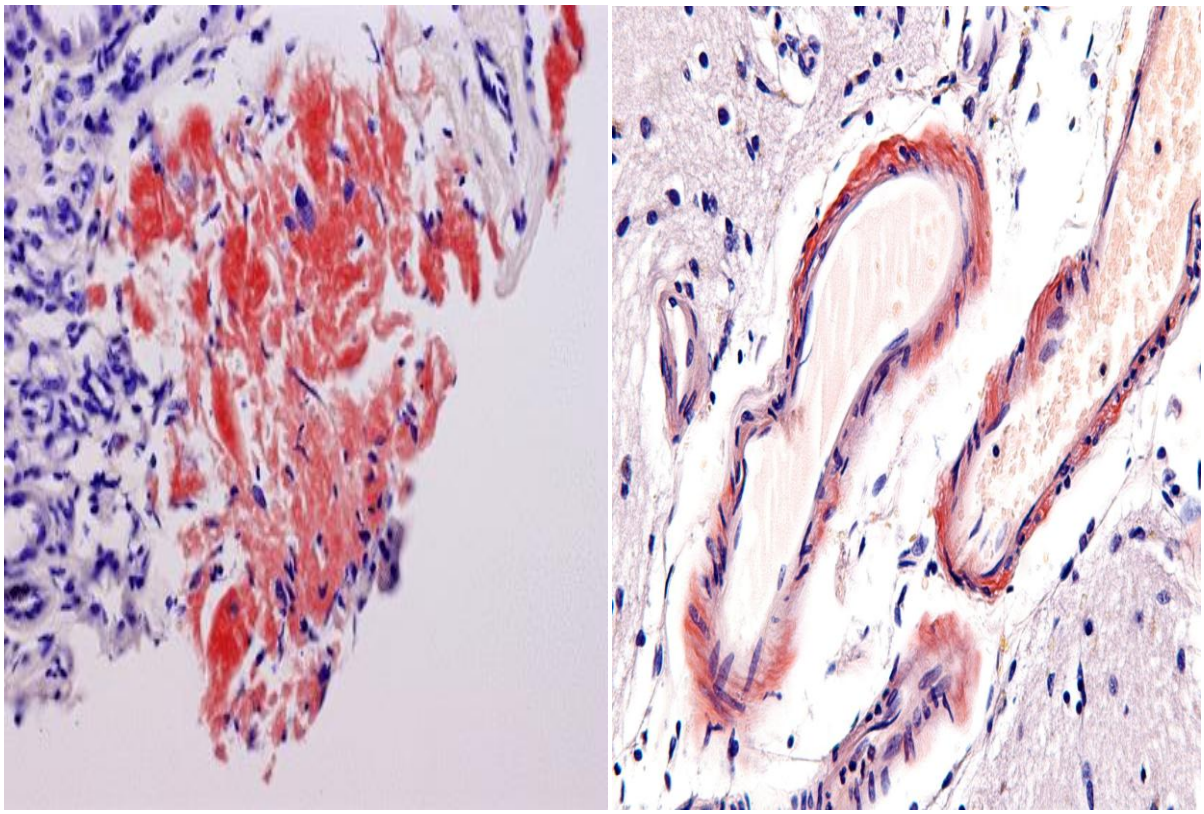
Colloidal iron technique: acid mucopolysaccharides stained blue



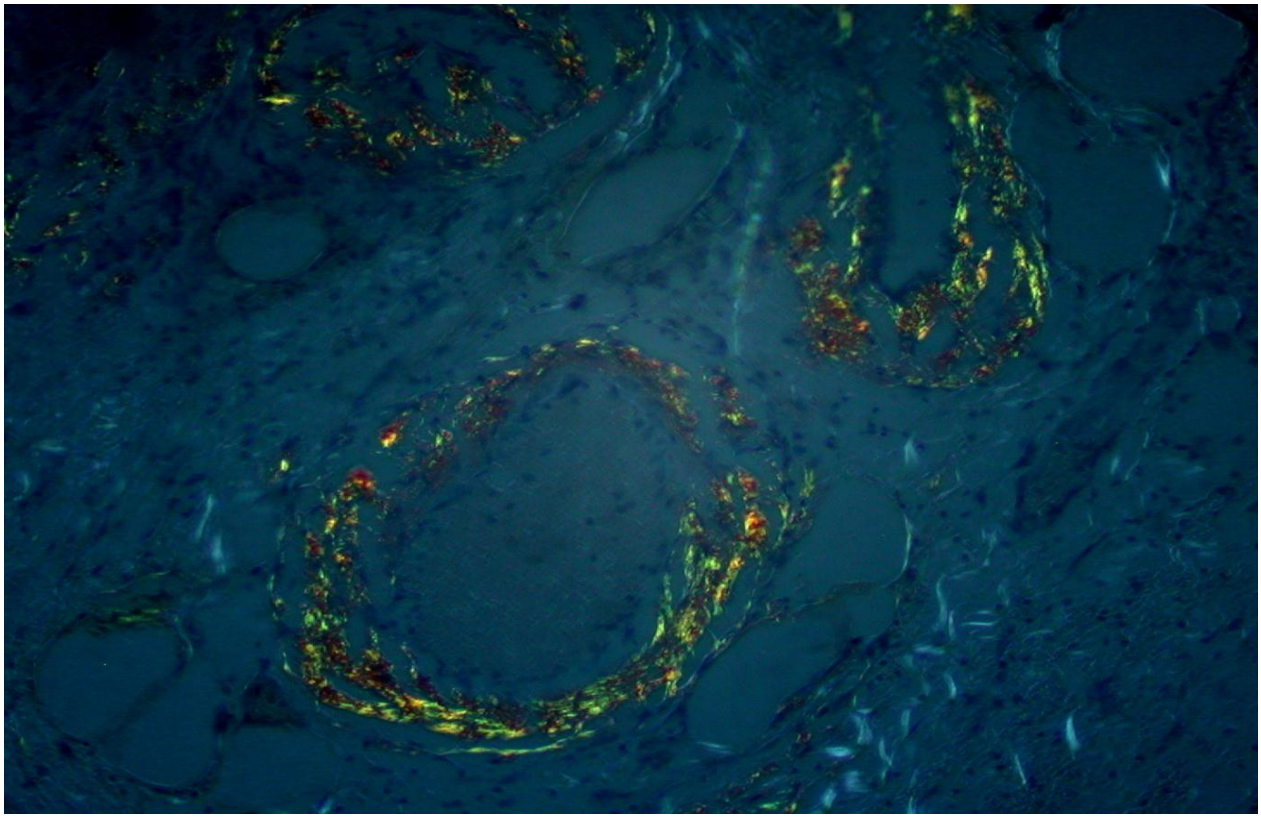
Periodic acid Schiff's reaction: Neutral mucins stained magenta



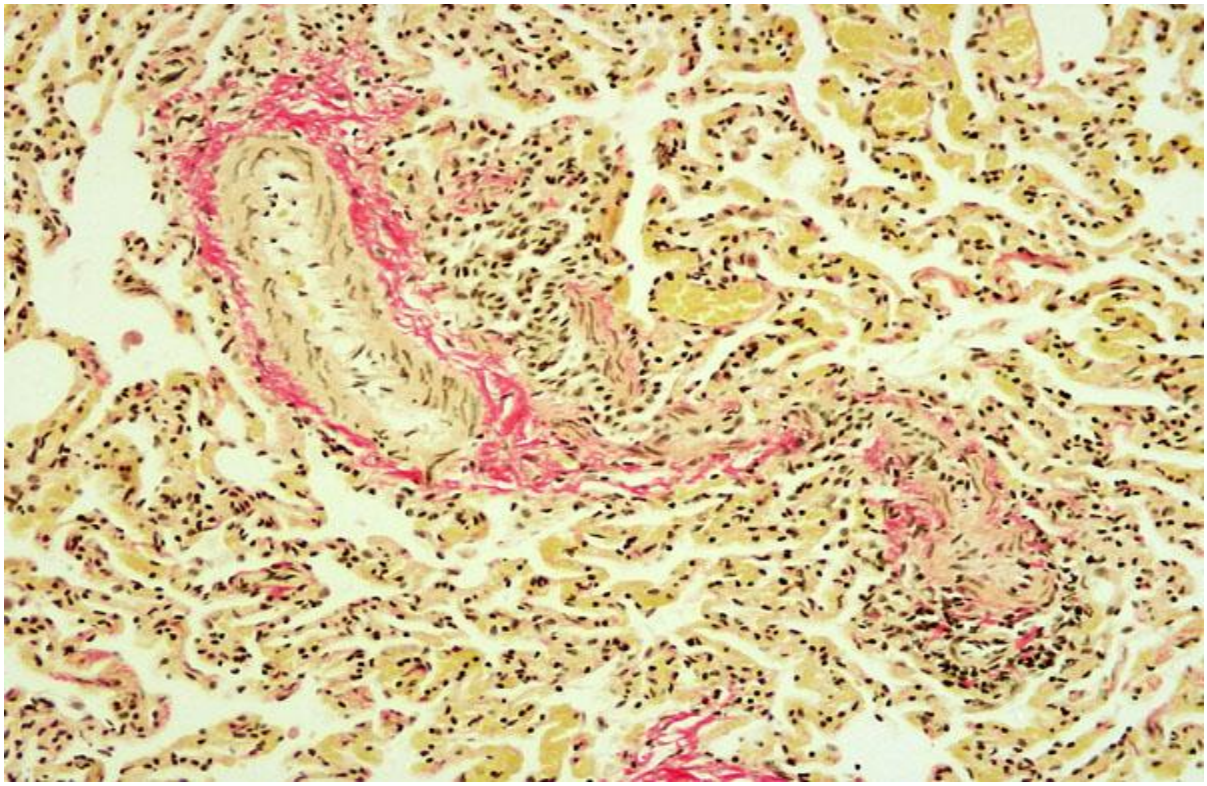
Combined alcian blue PAS: acid mucins blue, neutral mucins magenta



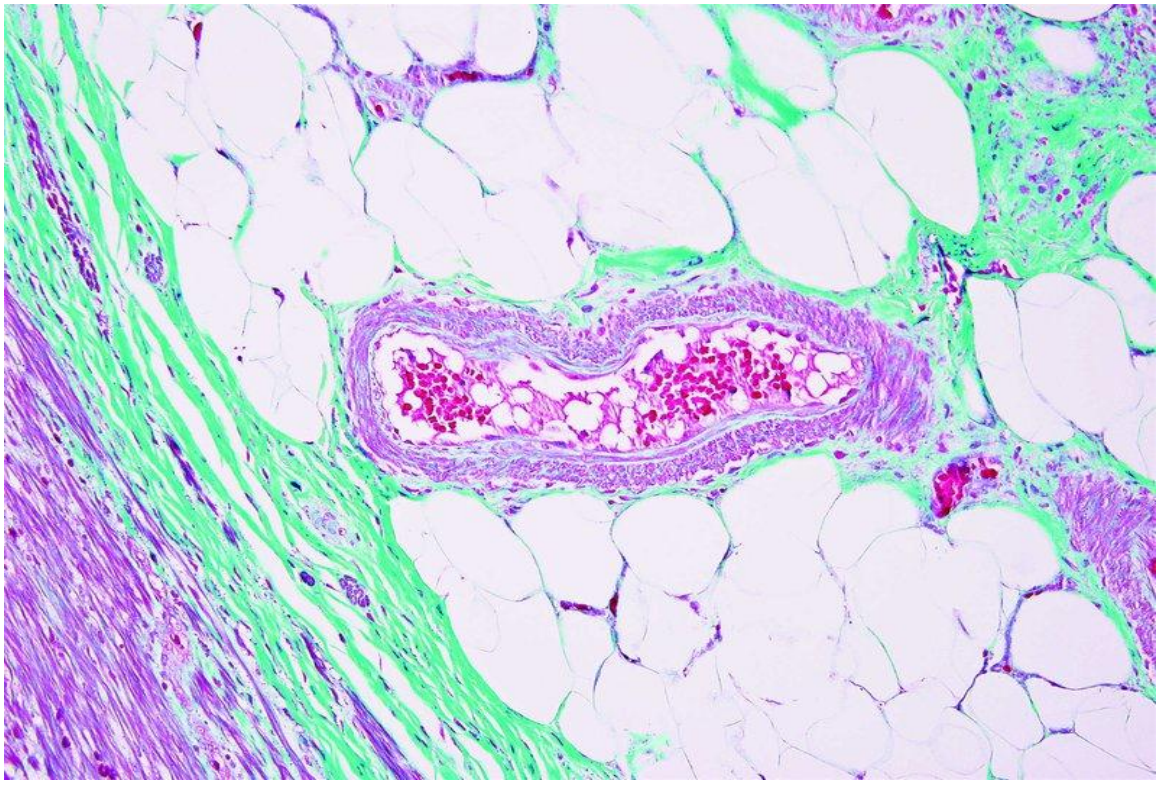
Highman’s Congo red: Amyloid orange red, nucleus blue



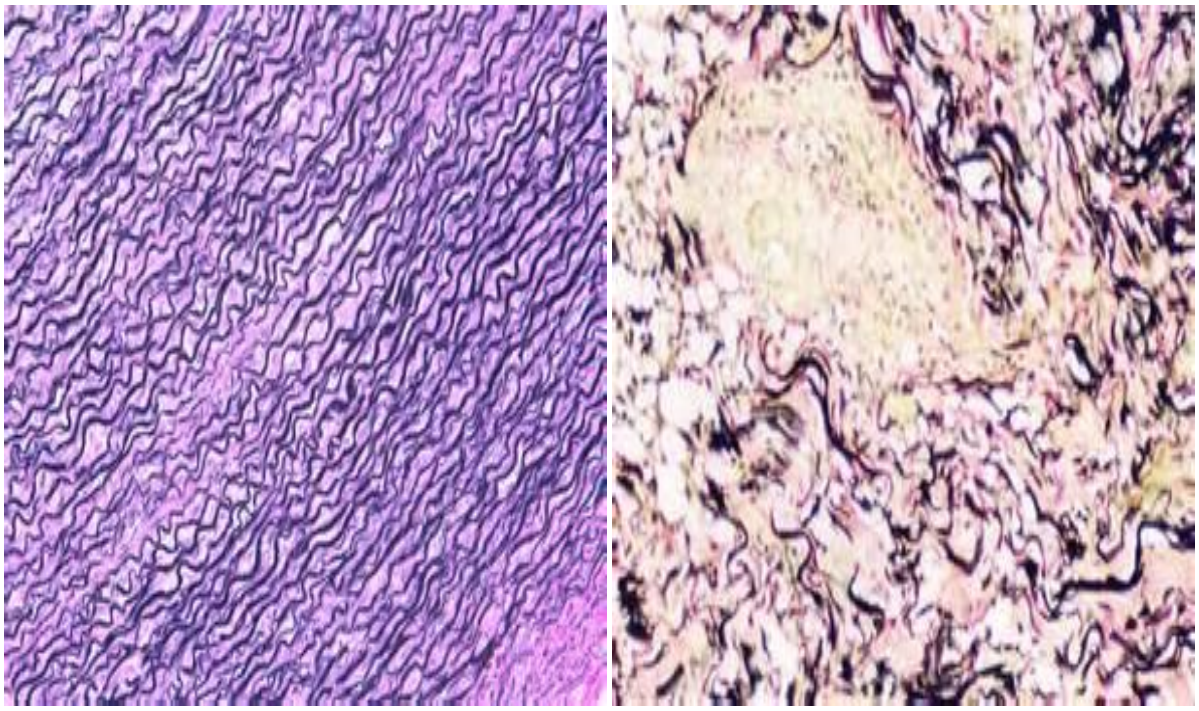
Congo red view with polarized light: Amyloid apple green



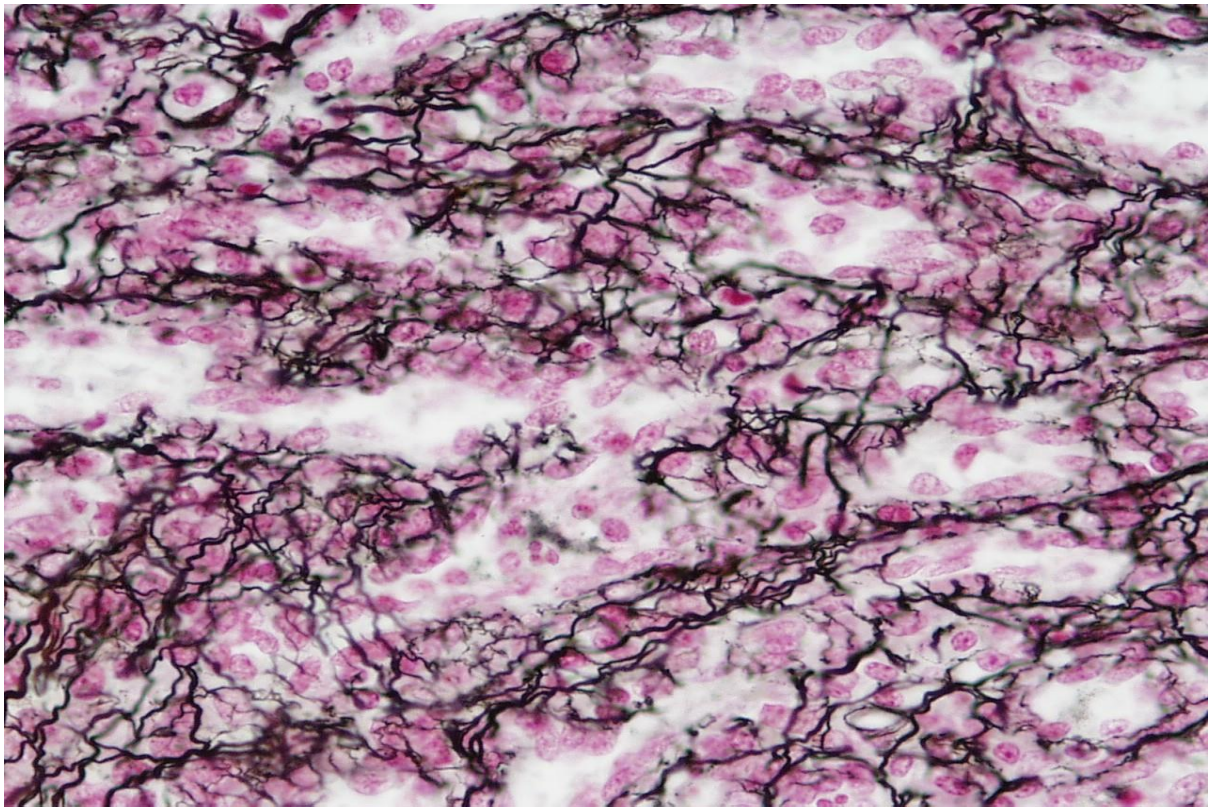
Weigert's van Gieson: collagen fibers red, nucleus black, muscles yellow



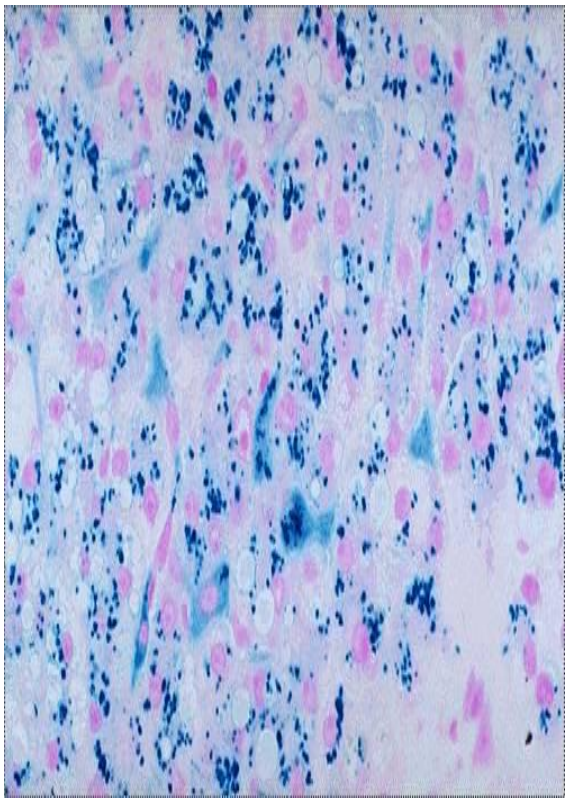
Massons tri chrome: collagen fibers green, muscles and RBCs red, nucleus black

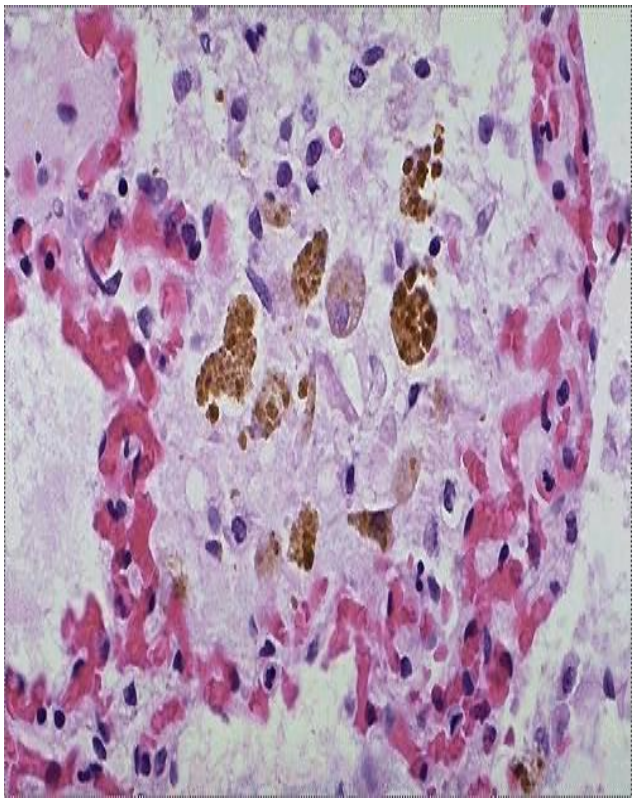


Verhoeffs hematoxylin: Elastic fibers appear black

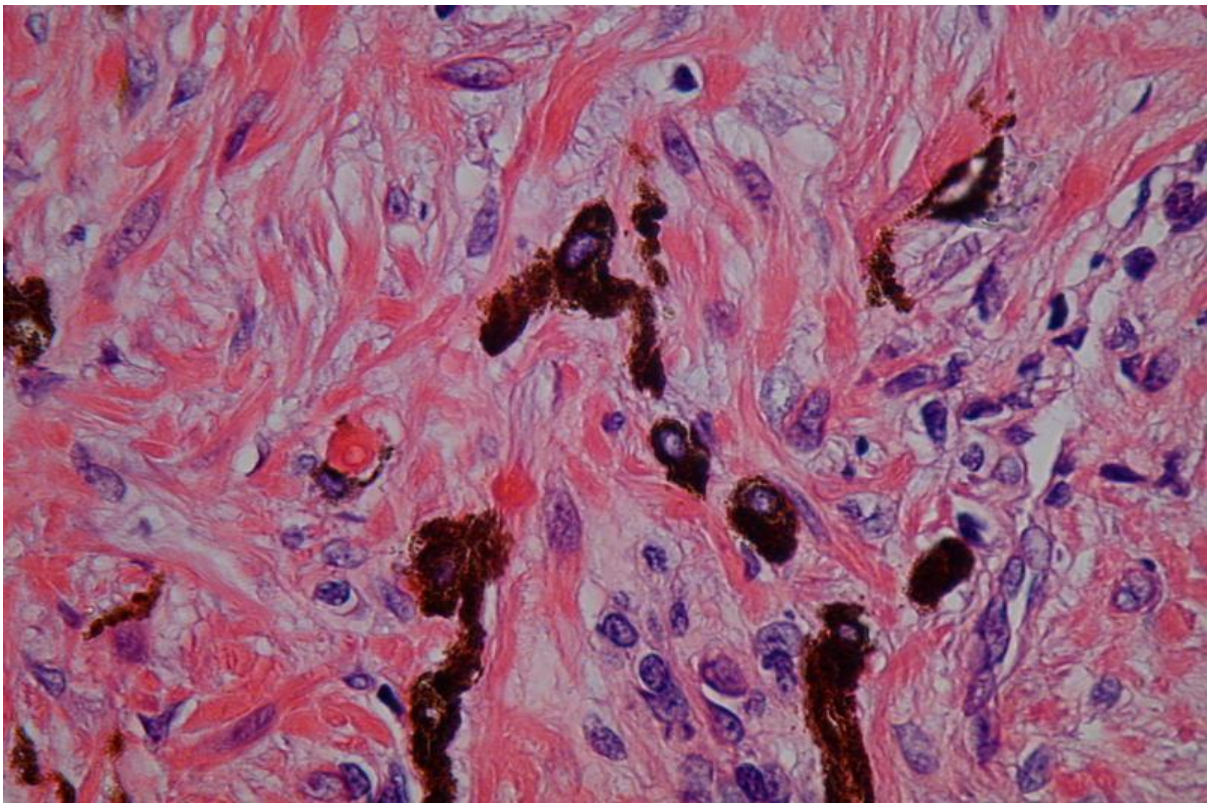


Gordon and sweet: reticular fibers black, nucleus red

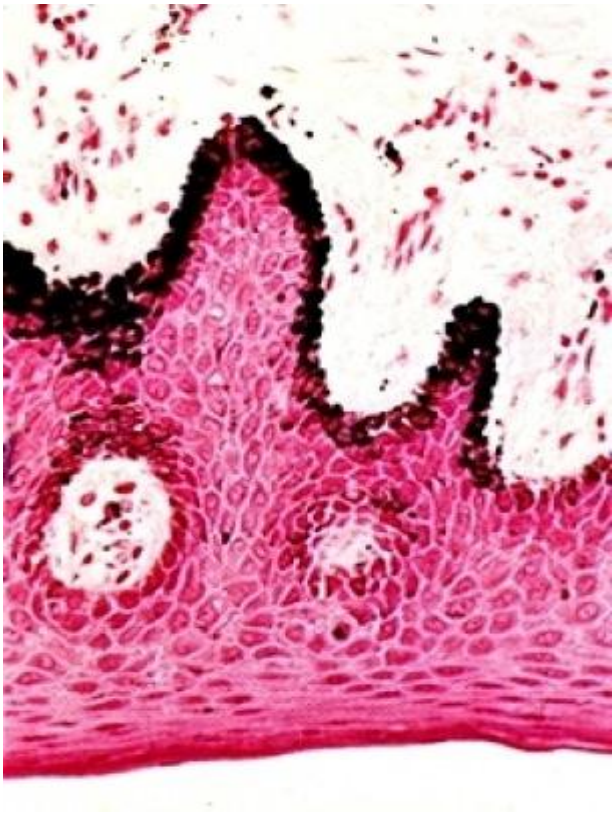


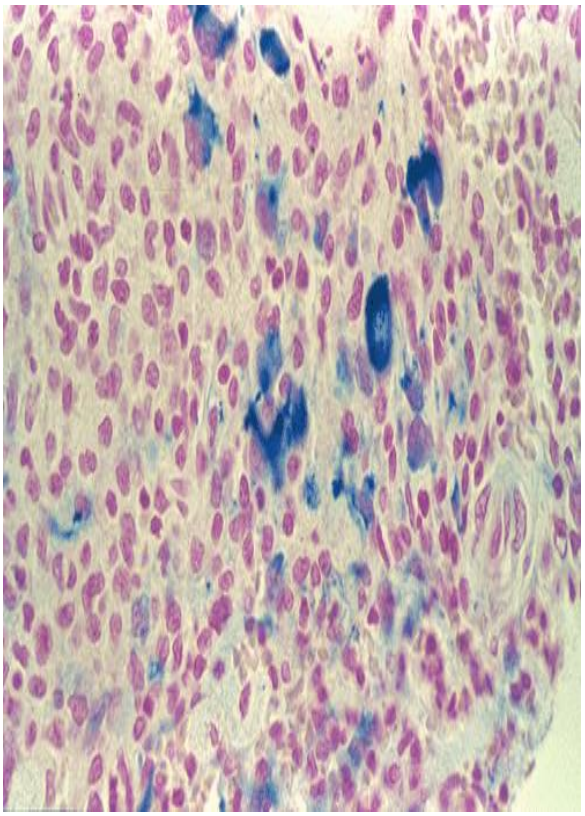


Perls Prussian blue: Hemosiderin (blue deposits), Right H&E hemosiderins brown yellow

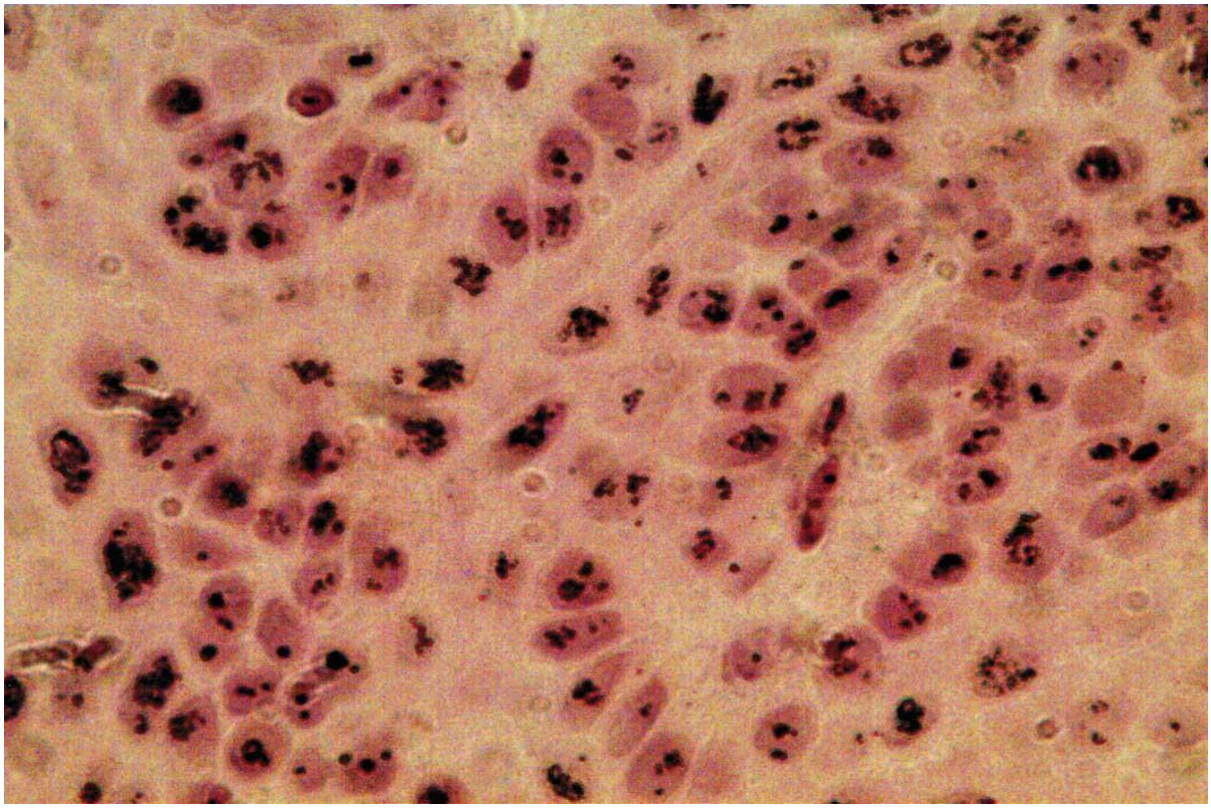


Masson Fontana: melanins black

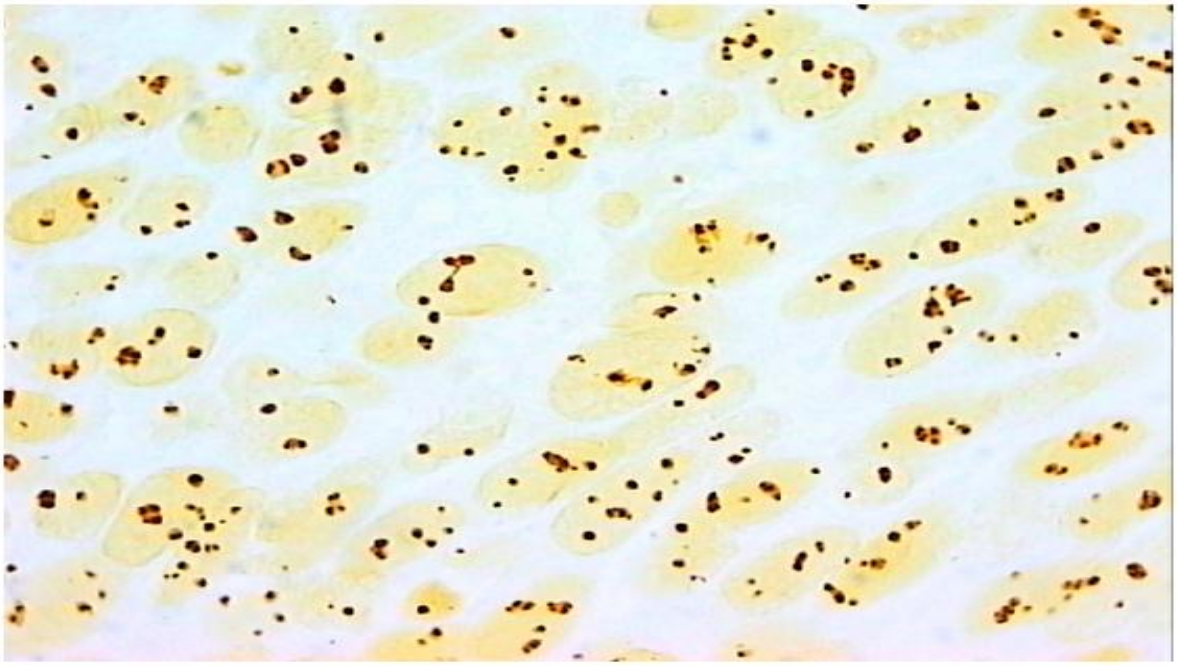




Masson Fontana: melanins black Schmorl’s reaction: melanins blue deposits



Silver technique: AgNOR protein sites black



Silver technique: AgNOR protein sites black