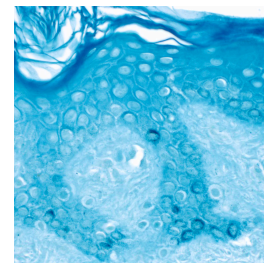


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🌐 Ferric ferricyanide reduction test or Schmorl's stain in modification of Lillie (diffuse endocrinocytes)

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Egor Kuzmin¹

¹Sechenov University

European Society for Pi...



Egor Kuzmin

Sechenov University

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We use this protocol and it's working

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Abstract

Schmorl's ferrocyanide reduction technique stands as a cornerstone histochemical method for detecting diverse reducing compounds. When ferric ions (Fe^{3+}) are reduced to the ferrous state (Fe^{2+}), they react with ferricyanide ions to form insoluble Berlin blue. This reductive capacity is exhibited by melanocyte granules, enteroendocrine cell contents, thyroid colloid, lipofuscin deposits, and numerous other biological substances. The method is particularly valuable for comprehensive identification of gastrointestinal endocrine cells. Its key advantages include procedural simplicity and rapid execution compared to argentaffin, argyrophil, and chromaffin techniques.

Guidelines

- Ferric chloride (FeCl_3) may be substituted with ferric sulfate ($\text{Fe}_2(\text{SO}_4)_3$).
- Solutions must be prepared immediately before use due to rapid precipitation and subsequent loss of activity.
- Elevated temperature dramatically increases reaction kinetics but significantly reduces the stability of the staining complex.

Materials

1. Ferric chloride, FeCl_3
2. Potassium ferricyanide, $\text{K}_3[\text{Fe}(\text{CN})_6]$
3. Hydrogen peroxide, H_2O_2
4. Acetic acid, CH_3COOH
5. Ortho-xylene, $\text{C}_6\text{H}_4(\text{CH}_3)_2$
6. Ethanol 100% or isopropanol 100%
7. Distilled water

Safety warnings

- ❗
 - **Formalin** is a volatile compound classified as carcinogenic.
 - **Xylene, isopropyl alcohol**, and ethyl alcohol are volatile substances hazardous to human health, especially following long-term exposure. Procedures utilizing these solvents require a ventilated fume hood and the use of medical gloves (e.g., nitrile).

Before start

Autopsy specimens were fixed by immersion in neutral buffered formalin for 48 hours. Processing involved a standard protocol utilizing a graded series of isopropyl alcohols, followed by paraffin embedding. Histological blocks were cut on a microtome to yield 3- μ m sections. Sections were placed on silane-coated adhesive glass slides for secure adhesion. All staining reagents were freshly prepared immediately before staining commenced.

1 Introduction

The Ferric Ferricyanide Reduction Test

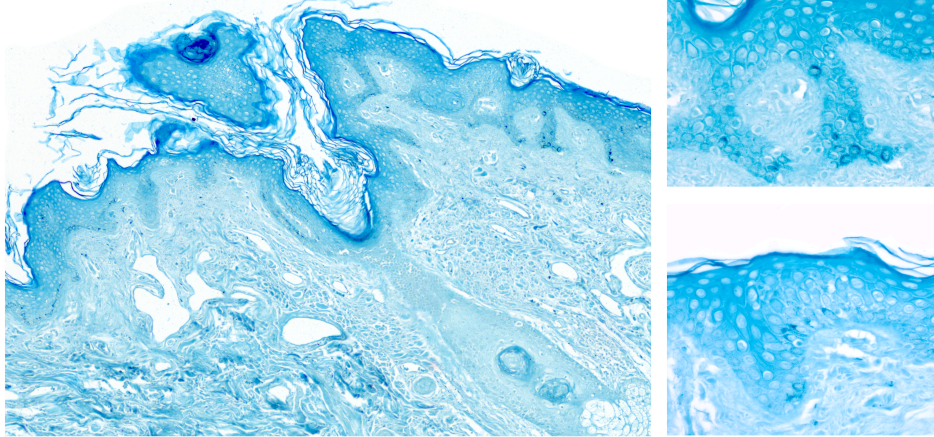


Fig. 1. Schmorl's ferricyanide staining on human skin histological sections. In the basal layer of the epidermis, melanin granules within melanocytes are stained. Prior to staining, melanin was decolorized using peroxide bleach.

Preparation of solutions

2 Working staining solution (Lillie's Modified Solution) 40 mL

Prepare fresh immediately before use

Mix

 30 mL of freshly prepared 1% **ferric chloride** (FeCl_3) solution

 4 mL of freshly prepared 1% **potassium ferricyanide** ($\text{K}_3[\text{Fe}(\text{CN})_6]$) solution

 6 mL distilled water

Use immediately after preparation. Protect from light. Avoid heating. Unfit for use after several hours.

2.1 Alternative option of the staining solution 100 μL

Mix

 75 mL of freshly prepared 1% **ferric chloride** (FeCl_3) solution



🧪 10 mL of freshly prepared 1% **potassium ferricyanide** ($K_3[Fe(CN)_6]$) solution

🧪 15 mL distilled water

Protocol

1h 15m 30s

3 STARTING POINT:

3.1 Dewaxing, rehydration, rinsing in distilled water. Rinse in pre-warmed distilled water.

25m

1. ortho-xylene 5 min
2. ortho-xylene 5 min
3. absolute ethanol or isopropanol 5 min
4. 50% ethanol or isopropanol 5 min
5. distilled water 5 min

4 STAINING:

4.1 Treat with 1% hydrogen peroxide for 5 minutes. (Not obligatory)

5m

Used in melanocyte staining to bleach melanin.

After peroxide treatment, rinse sections thoroughly to prevent reaction with the staining solution.

4.2 Place sections in the staining solution for 10 minutes at room temperature.

10m

The staining procedure is protocol-specific and may require extended exposure to the staining solution. Staining under minimal light is recommended to prolong reagent shelf life. Some protocol variations permit heating using a water bath, incubator, or microwave oven.

The time for the reaction to take place depends on the substance to be demonstrated, with melanin generally reacting faster than lipofuscin.

4.3 Rinse in 1% acetic acid solution.

30s

4.4 Nuclear counterstaining. (Not obligatory)

10m

For counterstaining, the original Lillie protocol recommends using an acidified fuchsin solution followed by differentiation in 1% acetic acid.

Alternatively, Nissl's neutral red can be used with differentiation in acidified alcohol.

5 FINAL STEP:

6 Rinse, dehydrate, mount in resin medium.

25m

1. distilled water 5 min
2. 50% ethanol or isopropanol 5 min
3. absolute ethanol or isopropanol 5 min
4. ortho-xylene 5 min.
5. ortho-xylene 5 min.
6. moun

Results

7 **Blue staining:** melanin, argentaffin cells, chromaffin, some lipofuscins, thyroid colloid and bile

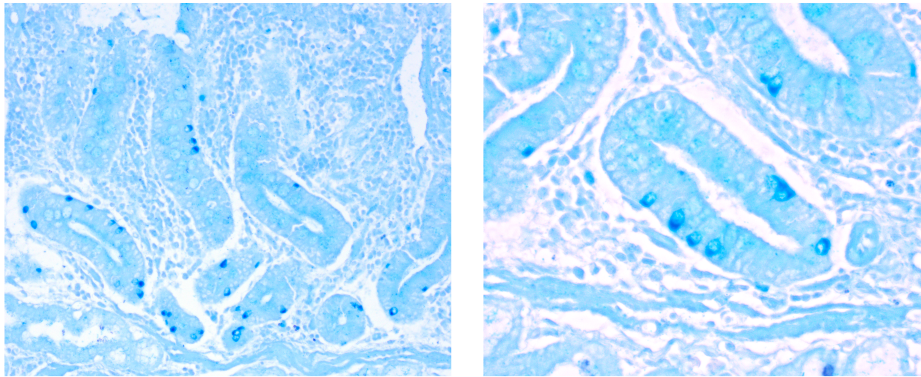


Fig.2. Macrophotograph of bovine small intestine stained by Schmorl's method. Granules of crypt enteroendocrine cells are stained blue.

Protocol references

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