STAINING PROTOCOL OF IRON OF BONE MARROW

Small amounts of ferric iron are found normally in bone marrow. Any ferric ion (+3) present in the tissue combines with the ferrocyanide and results in the formation of a bright blue pigment called Prussian blue, or ferric ferrocyanide. This is one of the most sensitive histochemical tests and will demonstrate even single granules of iron in blood cells.

- Fix the air-dried bone marrow specimen in methanol for 15 min.
- Dry the slides in room temperature.
- Mix equal parts of hydrochloric acid (0.2 mol/L HCl) and potassium ferrocyanide prepared immediately before use.
- Immerse slides in this solution for 15 min in room temperature.
- Immerse in water for 20 min and wash with distilled water.
- Counterstain with aqueous solution of eosin or safranin 1gr/L for 10-15 sec.
- Wash with water. Let them dry.

Results:

1. Iron (ferric form) ➔ bright blue
2. Nuclei ➔ red
3. Cytoplasm ➔ pink

Fe negative

Fe positive