

J-603, JOHNSON'S METHOD FOR IRON MICROINCINERATION

Fixation: 10% Buffered Neutral Formalin (F-113)

Embedding: Paraffin @ 6 microns

PROCEDURE:

- A. Microincineration
1. Place unstained paraffin section uncovered on a sheet of asbestos and heat cautiously, using the outer cone from the flame of a Fisher or Meker Burner. Keep flame in motion and heat the slide as uniformly as possible.
 2. Continue the heating until the specimen, which turns brown the white, has almost completely disappeared. Immediately cover the slide with and inverted pan, (e.g. a pie pan) and allow the slide to cool gradually – to rapid cooling may cause the slide to break.
 3. After the slide has cooled, the completely incinerated specimen is examined under strong oblique lighting with dark field illumination, with or without coverslip.
 4. Results: Iron oxide imparts a bright yellow-orange to dark red color to the ash. Specimen must be completely incinerated as carbon particles can also give a yellowish to red color.
- B. Staining of Incinerated Specimen
1. Dip incinerated specimen in a thin cellodin solution (approx. 0.015% in alcohol-ether, 1:1). Air dry slides for a few minutes and then quickly hydrate to distilled water.
 2. Stain in Working Hydrochloric Acid-Potassium Ferrocyanide Solution * for 15 minutes. Rinse in several changes of distilled water.
 - Prepare working stain by mixing equal parts of **Hydrochloric Acid, 5%** (J-603-2) and **Potassium Ferrocyanide, 5%** (J-603-1) just before use.
 3. Air dry or dry in 60°C oven.
 4. Mount with Permount (M-18).
- C. Direct observation of Prussian Blue Reaction on the Ash.
1. Create well paraffin around specimen.
 2. Pour working Hydrochloride Acid-Potassium Ferrocyanide Solution (for preparation, See step B-2) into the paraffin well and observe under microscope.

STAINING RESULTS: (B&C)

Iron Oxide	Blue
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REFERENCES:

Fenton, R.H., Johynson, F.B., and Zimmerman, L.E., *J. Histo-Chem. Cytochem.*, 12:153-155, 1964.

Luna, L.G., (ed.), *Manual of Histologic Staining Methods of the AFIP*, 3rd edition, McGraw-Hill, N.Y., p.181., c 1968