

## F-363, HEIDENHAIN'S "AZAN" MODIFICATION

**Fixation:** Zenker's (F-155), Helly's (F-153), Bouin's (F-40) or Carnoy's (F-60)

**Sections:** Paraffin @ 6 microns

### **PROCEDURE:**

1. Deparaffinize and hydrate to distilled water.
2. If formalin fixed, mordant in Zenker's overnight, then remove Mercuric Chloride by placing in **Lugol's Iodine** (F-363-6) or **Gram's Iodine** (F-363-6A) for 15 minutes. Rinse in tap water and place in **Sodium Thiosulfate, 5%** (F-363-7) for 3 minutes. Wash in tap water for 10 minutes longer. Rinse in distilled water.
3. Place in preheated **Azocarmine B Solution** (F-363-1) for 15 minutes @ 56°C. Rinse in distilled water.
4. Differentiate in **Aniline Alcohol Solution** (F-363-3) until cytoplasm and connective tissue are pale pink and nuclei stand out sharply. Control differentiation by rinsing in **Acetic Acid Alcohol** (F-363-4).
5. Mordant in **Phosphotungstic Acid, 5%** (F-363-2) for 15 minutes. Rinse in distilled water.
6. **Aniline Blue Orange G Solution** (F-363-2) for 15 minutes. Rinse in distilled water.
7. Dehydrate in 95% alcohol, absolute alcohol, and clear in xylene, two changes each, two minutes each.
8. Mount with **Permount** (M-18).

NOTE: Clark omits steps #1 and #2 and times in Step #3, #5 and #6 are substantially longer: i.e.; #3, 30-60 minutes @ 50-55°C plus 1-2 hours @ 37°C; #5, 30 minutes to 3 hours; #6, 1-3 hours.

### **Stain Results:**

Chromatin, osteocytes, neuroglia	Red
Collagen, reticulum	Blue
Muscle	Red to yellow
Osteoid Material in Decalcified Sections	Red

### References:

- AFIP, Manual of Histological Staining Methods, 3<sup>rd</sup> ed., Ed.L Luna: NY:McGraw-Hill Publications, c. 1968, p. 86.
- Mallory, F.B., Pathological Technique, Hafner Publishing Co., New York, pp 154-155, 1961
- Clark, G. , Staining Procedures, Williams & Wilkins Co., Baltimore, 3<sup>rd</sup> ed., 1973, p. 56
- Lillie, R.D. Histopathologic Technic and Practical Histochemistry, McGraw-Hill, New York, 3<sup>rd</sup> ed., 1965.
- Heidenhain, M., Z. Wissen Mikr., 32:361-72, 1915