

A-132, WOLBACH'S GIEMSA METHOD

Fixation: Zenker's or other well fixed tissues.

Sections: Cut paraffin sections at 6 microns.

Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Remove "Zenker Crystals" by placing in **Lugol's Iodine** (A-132-3) or **Gram's Iodine** (A-132-3A) for 15 minutes. Rinse in water, place in **Sodium Thiosulfate, 5%** (A-132-4) for 3 minutes and wash in running water for 15 minutes.
3. Rinse in distilled water and stain in **Working Giemsa Solution*** (A-132-1A) overnight.

*Working Giemsa Solution may be made from the stock solution by mixing

Giemsa Stock (A-132-1)	1.25 ml
Methanol	1.5 ml
Distilled Water	50 ml

**NOTE: Giemsa stain colors more effectively in tissues at an acid pH. If this has not occurred in the preparation or decalcification steps, wash in acid alcohol, then begin stain.

4. Differentiate in **Rosin Alcohol Working** (A-132-2A) until the sections are a purplish-pink color. Check under a microscope.
5. Dehydrate in two changes of absolute alcohol and clear in two changes of xylene.
6. Mount with Permount (M-18).

Stain Results:

Nuclei, Bacteria	Blue
Rickettsia	Purple
Collagen, other tissue elements	Pink to rose

References:

Wolbach, S.B., Todd, J.L. and Palfrey, F.W., The Etiology of Pathology Typhus, Harvard University Press, Cambridge, MA., p. 13-14, c. 1922

Luna, L.G., (ed.), Manual of Histologic Staining Methods of the AFIP, 3rd edition, McGraw-Hill, N.Y., p. 119, c. 1968

