

## **L-773 WOLBACH'S GIEMSA METHOD**

**Fixation:** Zenker's or other well fixed tissue.

**Sections:** Cut Paraffin sections at 6 microns

**Staining:**

1. Deparaffinize and hydrate to distilled water.
2. Remove "Zenker Crystals" by placing in **Lugol's Iodine** (L-773-3) or **Gram's Iodine** (L-773-3A) for 15 minutes. Rinse in water, place in **Sodium Thiosulfate, 5%**, (L-773-4) for 3 minutes and wash in running water for 15 minutes.
3. Rinse in distilled water and stain in **Working Giemsa Solution**\* (L-773-1A) overnight.

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

<b><u>Giemsa Stock</u></b> (L-773-1)	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 decalcifications steps, wash in an acid alcohol, then begin stain.

4. Differentiate in **Rosin Alcohol Working**, (L-773-2A) 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 Paltrey, F.W., The Etiology of Pathology Typhus, Harvard University University Press, Cambridge, MA., p. 13-14, c. 1922

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