

WOLBACH'S GIEMSA METHOD

STAINING PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Place in Giemsa Working Solution overnight at room temperature.
3. Differentiate in Rosin Working Solution until sections assume a purplish pink color. Check under microscope.
4. Dehydrate in Absolute Alcohol and clear in Xylene, 2 changes each.
5. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of Rickettsia.
PRINCIPLE:	The Giemsa stain is a combination of acidic and basic dyes. Differential staining is attributed to relative charge of cells, dye size and the pH of the solutions.
CONTROL:	Any tissue known positive for Rickettsia <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs020.</i>
SPECIMEN PREPARATION:	Zinc Formalin, paraffin embedded sections cut at 6 micrometers
SOLUTIONS:	1. Giemsa Stain Stock Solution Item# s195 <u>Giemsa Working Solution:</u> Giemsa Stock..... 1.25 mL Methyl Alcohol 1.5 mL Distilled water 50 mL <u>Rosin Working Solution:</u> Rosin Stock 5.0 mL Alcohol 95 40.0 mL <i>Solutions can be purchased separately from Poly Scientific.</i>
NOTES:	Rickettsia can be demonstrated satisfactorily with this method. The demonstration, however, depends on precise differentiation of the section. Giemsa stain takes much better if tissue has an acidic pH. If tissue is not already acidified by calcification, etc., place in Acetic Alcohol, wash well, and begin stain.
REFERENCE:	Lillie, R. D. <u>Histopathologic Technic and Practical Histochemistry</u> 3rd Ed. McGraw-Hill Book Co. New York. 1965. pp. 160,162, 583.

RESULTS:

- Nuclei.....Blue
- Collagen and Other Tissue Elements.....Pink to Rose
- RickettsiaPurple
- Bacteria.....Blue

Poly Scientific R&D Corp.

Revision: B-18

