

**GIEMSA METHOD FOR RICKETTSIA**

<b>PURPOSE:</b>	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of Rickettsia in tissue.
<b>PRINCIPLE:</b>	By carefully controlling the pH of the tissue, many organisms can be stained because of basophilia. Giemsa stains all components, the Rosin Alcoholic acts as a very slow differentiator to allow the organisms to be exposed.
<b>CONTROL:</b>	Any tissue known positive for Rickettsia
<b>SPECIMEN PREPARATION:</b>	10% Buffered Formalin fixed, paraffin embedded sections cut at 6 micrometers
<b>SOLUTIONS:</b>	1. Buffered Water Solution pH 6.8 Item# s2026 2. Giemsa Stain Stock Solution Item# s195 3. Acetic Acid 0.2% Aqueous Item# s2025 4. Rosin Alcoholic Working Solution Item# s2024  <i>Solutions can be purchased separately from Poly Scientific.</i>
<b>NOTES:</b>	<u>Working Giemsa Solution:</u> Mix before use. Giemsa Stock Solution.....1 mL Buffered Water pH 6.8.....50 mL
<b>REFERENCE:</b>	Luna, Lee G. <u>Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology.</u> 3rd Ed. McGraw-Hill Book Co. New York. 1968. pp. 235-237.

**STAINING PROCEDURE:**

1. Deparaffinize and hydrate to water.
2. Mordant in Buffered Water Solution for 60 minutes.
3. Place in Working Giemsa Solution overnight.
4. Rinse in Buffered Water Solution pH 6.8.
5. Place in Acetic Acid 0.2% Aqueous for 1 minute.
6. Rinse in Buffered Water Solution pH 6.8.
7. Differentiate sections individually in Rosin Alcoholic Working Solution. Check the microscope frequently until Rickettsia appear as violet colored granules (may take up to 3 minutes).
8. Dehydrate in Absolute Alcohol and clear in Xylene, 3 changes each.
9. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

**RESULTS:**

Rickettsia .....	Violet
Nuclei.....	Blue
Cytoplasm .....	Pink
Connective Tissue.....	Pink
Erythrocytes .....	Salmon

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