

**GRIDLEY'S METHOD FOR ENDAMOEBIA HISTOLYTICA**

<b>PURPOSE:</b>	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of amoeba in tissue.
<b>PRINCIPLE:</b>	Erythrocytes have a great affinity for Eosin so that the Naphthol Green will not remove it. The Naphthol Green is used as a counterstain to set off the erythrocytes.
<b>CONTROL:</b>	Any tissue known to contain amoeba in tissue <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs027.</i>
<b>SPECIMEN PREPARATION:</b>	Formalin fixed, paraffin embedded sections cut at 6 micrometers
<b>SOLUTIONS:</b>	1. Acid Alcohol 1% Item# s104 2. Weigert's Iron Hematoxylin Solution Set (A & B) Item# s216B <i>Working Solution: Mix equal parts of solutions A &amp; B for use.</i> 3. Aniline Eosin Alcoholic Solution Item# s117B 4. Naphthol Green B 1% Aqueous Item# s246D 5. Ammonia Water Item# s113F  <i>Solutions can be purchased separately from Poly Scientific.</i>
<b>NOTES:</b>	This procedure does not stain amoeba differentially but is useful in that it demonstrates the ingested erythrocytes extremely well.
<b>REFERENCE:</b>	Gridley, M.F. <i>Amer. J. Clin. Path.</i> 24: 243-244.

**STAINING PROCEDURE:**

1. Deparaffinize and hydrate to distilled water.
2. Place in Weigert's Iron Hematoxylin Working Solution for 3 minutes.
3. Wash in running water for 5 minutes.
4. Differentiate in Acid Alcohol 1%.
5. Wash in running water for 5 minutes.
6. Blue in Ammonia Water.
7. Wash in running water for 5 minutes.
8. Place in Aniline Eosin Alcoholic Solution for 5 minutes.
9. Rinse well in distilled water. Sections should be a deep rose color.
10. Place in Naphthol Green B 1% Aqueous for 5 minutes.
11. Differentiate in 95% Alcohol, 2 changes, until erythrocytes in section are bright rose. Check under microscope.
12. Dehydrate in Absolute Alcohol and clear in Xylene, 2 changes each.
13. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

**RESULTS:**

Amoeba ..... Blue-Green  
 Nuclei of Amoeba ..... Deeper Blue-Green  
 Ingested Erythrocytes..... Deep Rose  
 Connective Tissue ..... Green

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