

**GENTA TRIPLE STAIN METHOD FOR HELICOBACTER PYLORI**  
**& GASTRIC MORPHOLOGY**

<b>PURPOSE:</b>	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of Helicobacter Pylori.
<b>PRINCIPLE:</b>	The Genta Triple Method of Helicobacter Pylori allows for the visualization of small numbers of bacteria and is useful in the evaluation of post-treatment gastric biopsy specimens with abundant mucus and in specimens from the corpus where Helicobacter does not elicit strong inflammatory responses. The use of Alcian Blue pH 2.5 does not enhance the sensitivity for the detection of intestinal metaplasia however, it does not make it immediately apparent and permits a more precise assessment of its extension. One of the main advantages of this stain is that the section on which the bacteria are quantitated is the same section in which the inflammatory responses are evaluated.
<b>CONTROL:</b>	Any tissue known to contain Helicobacter Pylori  <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs033.</i>
<b>SPECIMEN PREPARATION:</b>	Formalin fixed, paraffin embedded sections cut at 4 micrometers
<b>SOLUTIONS:</b>	<ol style="list-style-type: none"> <li>1. Uranyl Nitrate 1% Aqueous Item# s288B</li> <li>2. Silver Nitrate 1% Aqueous Item# s1888</li> <li>3. Gum Mastic 2.5% Alcoholic Item# s2195</li> <li>4. Components for Reducing Solution  <i>Reducing Solution:</i> Prepare just before use.                      Gum Mastic 2.5% Alcoholic..... 20 mL                      Hydroquinone 2% Aqueous..... 50 mL                      Regeant Alcohol Denatured..... 10 mL                      Silver Nitrate 0.04 ..... 5 mL</li> </ol> <p>Combine the Gum Mastic Solution and the Hydroquinone Solution. Add the Absolute Alcohol and mix well. Filter through Whatman #4 filter paper. Immediately before placing slides in solution, add 5 mL of Silver Nitrate 0.04%.</p> <ol style="list-style-type: none"> <li>5. Alcian Blue 1% in 3% Acetic Acid pH 2.5 Item# s111A</li> <li>6. Harris Hematoxylin Item#s212</li> <li>7. Acid Alcohol 1% Item# s104</li> <li>8. Lithium Carbonate 0.5% Aqueous Item# s2429</li> <li>9. Eosin Y Alcoholic Working Solution Item# s2186</li> </ol> <p><i>Solutions can be purchased separately from Poly Scientific.</i></p>
<b>NOTES:</b>	
<b>REFERENCE:</b>	Genta, R.M., Robason, G.O., Graham, D.Y., "Simultaneous Visualization of Helicobacter Pylori and Gastric Morphology". <u>Human Pathology</u> . 25:221-226.

**STAINING PROCEDURE:**

1. Deparaffinize and hydrate sections to distilled water.
2. Place sections in Uranyl Nitrate 1% Aqueous, preheated to 60°C for 15 minutes.
3. Rinse slides thoroughly in distilled water.
4. Place sections in Silver Nitrate 1% Aqueous for 1 hour at 60°C.
5. Rinse slides in 3 changes of distilled water.
6. Dehydrate in 2 changes of 95% Alcohol and 2 changes of Absolute Alcohol.
7. Place slides in Gum Mastic 2.5% for 5 minutes.
8. Air dry sections for 1 minute.
9. Rinse in 2 changes of distilled water.
10. Reduce in Reducing Solution in a 45°C water bath for 15-20 minutes or until sections have developed satisfactorily with black or dark brown bacteria and a light yellow background, occasionally agitating sections.
11. Rinse sections in water to stop reduction.
12. Quickly rinse in 95% Alcohol, Absolute Alcohol, 95% Alcohol, and back to distilled water.
13. Stain in Alcian Blue 1% in 3% Acetic Acid pH 2.5 for 10 minutes.
14. Rinse in running tap water.
15. Stain sections in Harris Hematoxylin for 8 minutes.
16. Rinse in running tap water.
17. Decolorize in Acid Alcohol 1%.
18. Rinse in running tap water.
19. Blue in Lithium Carbonate 0.5% Aqueous for 30 minutes.
20. Rinse in running tap water.
21. Counterstain in Eosin Y Alcoholic Working Solution up to 5 minutes.
22. Dehydrate in 95% Alcohol, Absolute Alcohol, 2 changes each.
23. Clear in Xylene, mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

**RESULTS:**

Helicobacter Dark..... Brown to Black  
 Goblet Cells, Acid Mucins..... Variable Shades of Blue  
 Mucous Cells..... Pinkish-Yellow

*Poly Scientific R&D Corp.*

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