

**A -116 GENTA. ROBASON AND GRAHAM STAIN FOR
SIMULTANEOUS VISUALIAZATION OF
HELICOBACTER PYLORI
AND GASTRIC MORPHOLOGY**

FIXATION: Formalin fixed

SECTIONS: Paraffin @ 4 um sections

STAINING:

- 1 Deparaffinize and hydrate sections to distilled water.
2. Sensitize sections by placing them in Zinc Formalin (A-116-1A) at room temperature for 3 minutes and transfer them to distilled water.
3. Rinse slides in distilled water until the possibility of cross contamination is eliminated.
4. Place sections in **Silver Nitrate. 1%** (A-116-2) at room temperature and then heat then in a microwave oven to just below the boiling point. **DO NOT BOIL!**
10. Rinse slides in 3 changes of distilled water.
11. Rinse in 2 changes of 95% alcohol.
12. Rinse in 2 changes of 100% alcohol.
13. Place slides in **2.5% Gum Mastic** (A-116-3) for 5 minutes.
14. Air dry sections for 1 minute
15. Rinse in 2 changes of distilled water.
16. Reduce in reducing solution in a 45⁰C water bath for 10 to 15 minutes or until sections have developed satisfactorily with black or dark brown H-Pyloi and a light yellow background. Check periodically under microscope.

***To prepare reducing solution:**

Gun Mastic, 2.5 % (A-116-3).....10 ml
Hydroquinone, 2% (A-116-4).....25 ml
Absolute Alcohol..... 5 ml
Silver Nitrate, 0.04% (A-116-5).....2.5 ml

-mix together .DO NOT **FILTER!!**

17. Rinse sections in water to stop reduction.
13. Stain in **Alcian Blue, ph 2.5** (A-116-6) for 10 minutes.
14. Rinse in running tap water.
15. Stain sections in **Harris' Hematoxylin** (A-116-7) for 5 minutes.
16. Rinse in running tap water.
17. Quickly dip in **Acid Alcohol** (A-116-8).
18. Rinse in running tap water.
19. Rinse in **Ammonia Water, 0.25%** (A-116-9) until blue.
20. Rinse in running tap water.
21. Stain sections in **Eosin Y, 1%** (A-116-10) for 5 minutes.
22. Quickly rinse in tap water.
23. Rinse twice in 95% alcohol.
24. Rinse three times in absolute alcohol.
25. Rinse three times in Xylene and then coverslip.

STAINING RESULTS:

Mucus cells	Brownish yellow
Lamina propria ground substance	Light yellowish or greyish
Smooth muscle fibers	Light pink
Cytoplasm of neutrophils	Pale pink
Eosinophils	Intense red
Nucleus	Dark brown
Intestinal metaplasma	Bright blue

H-pylori are easily recognized in the mucus, on the cell surface, and within the gastric pits.

REFERENCES:

Genta PM., Robason GO, Graham DY- Simultaneous Visualization of Helicobacter Pylon and Gastric Morphology A New Stain Hum Pathol 25: 22 1-226, 1994.