

## Differential Stain, *Helicobacter Pylori* sp. in Tissue Sections – Technical Memo

### STAIN SOLUTION:

Solution A: Xanthene Stain Solution  
Solution B: Thiazine Stain Solution

500 ml  
Part 10521A  
Part 10522A

1 Gallon  
Part 10521B  
Part 10522B

### Additionally Needed:

<i>Helicobacter</i> sp., Artificial Control Slides	Part 4275
Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842

For storage requirements and expiration date refer to individual bottle labels.

### APPLICATION:

Newcomer Supply Differential Stain procedure, a modification of the Wright Giemsa stain technique, provides a rapid staining method for demonstration of *Helicobacter pylori* sp. in gastrointestinal tissue sections. Procedures for both monochromatic and polychromatic versions of the Differential Stain are provided.

### METHOD:

**Fixation:** 10% Phosphate Buffered Formalin (Part 1090)

**Technique:** Paraffin sections cut at 5 microns

**Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

### STAINING PROCEDURE:

- Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
  - Proceed with either the monochromatic or polychromatic staining method.

**Monochromatic Staining Method:** See Procedure Note #1.

- Place slides in Solution B: Thiazine Stain Solution for 1-4 minutes depending upon staining intensity preference.
- Rinse slide quickly in distilled water; long enough to remove excess stain.
- Allow slides to air-dry in a vertical position.
- Dip dried slides in xylene and coverslip with compatible mounting medium.
  - See Procedure Note #2.

### RESULTS:

<i>Helicobacter pylori</i> sp.	Dark blue
Collagen and muscle	Blue
Nuclei	Blue
Cytoplasm	Varying shades of light blue

**Polychromatic Staining Method:** See Procedure Note #1.

- Place slides in Solution A: Xanthene Stain Solution for 3-5 minutes.
- Drain slides briefly; go directly into Solution B: Thiazine Stain Solution for 1-4 minutes depending upon staining intensity preference.
- Rinse well in distilled water.
- Allow slides to air-dry in a vertical position.
- Dip dried slides in xylene and coverslip with compatible mounting medium.
  - See Procedure Note #2.

### RESULTS:

<i>Helicobacter pylori</i> sp.	Dark blue
Collagen and muscle	Pale pink
Nuclei	Blue/violet
Cytoplasm	Varying shades of light blue

### PROCEDURE NOTES:

- The timings provided in these procedures are suggested ranges. Optimal staining times will depend upon staining intensity preference.
- The elimination of dehydration steps is necessary to retain the dark stain of the organism.
- If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and coverslipping steps.

### REFERENCES:

- Potvin, Carol. "A Modified Diff-Quik Stain for *Helicobacter pylori* in Gastrointestinal Biopsies." *Laboratory Medicine* 25.6 (1994): 389-391.
- Skipper, Ray, and Don DeStephano. "A Rapid Stain for *Campylobacter pylori* in Gastrointestinal Tissue Sections Using Diff-Quik." *The Journal of Histotechnology* 12.4 (1989): 303-304.
- Modifications developed by Newcomer Supply Laboratory.