

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:

1. 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.
 - a. Proceed with either the monochromatic or polychromatic staining method.

Monochromatic Staining Method: See Procedure Note #1.

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

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

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

REFERENCES:

1. Potvin, Carol. "A Modified Diff-Quik Stain for *Helicobacter pylori* in Gastrointestinal Biopsies." *Laboratory Medicine* 25.6 (1994): 389-391.
2. 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.
3. Modifications developed by Newcomer Supply Laboratory.