

## Differential Stain Kit, Smears & Touch Imprints - Technical Memo

### KIT INCLUDES:

Solution A: Xanthene Stain	500ml	<b>Part 9112B</b>
Solution B: Thiazine Stain	500ml	
Solution C: Fixative	500ml	

Individual stain solutions may be available for purchase under separate part numbers at [www.newcomersupply.com](http://www.newcomersupply.com).

### Additionally Needed:

Xylene, ACS Part 1445

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

### APPLICATION:

Newcomer Supply Differential Stain Kit, a modification of the Wright Giemsa Stain technique, uses aqueous based stain solutions and a methanol fixative. This stain kit provides a rapid 3-step process that can be used for differential assessment of: peripheral blood smears, touch imprints, fine needle aspirations (FNA), bone marrow biopsy aspirations, as well as detecting microorganisms.

### METHOD:

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

All Newcomer Supply Stain Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

### STAINING PROCEDURE:

- Prepare within an accepted time frame, a well-made blood smear, touch imprint, FNA smear or bone marrow aspiration smear/film per your laboratories protocol, with a focus on uniform cell distribution.
- Allow slides to thoroughly air-dry prior to staining.
- Dip dried slides in Solution C: Fixative 5-10 times, one second per dip. Allow excess fixative to drain.
- Dip slides in Solution A: Xanthene Stain 5 times, one second per dip. Allow excess solution to drain.
  - See Procedure Notes #1, #2 and #3
- Quickly rinse slides with distilled water.
- Dip slides in Solution B: Thiazine Stain 5 times, one second per dip. Allow excess solution to drain.
- Rinse slides quickly in distilled water.
- Allow slides to air-dry, then examine microscopically.
- If coverslip is preferred, allow slides to air-dry; dip dried slides in xylene and coverslip with compatible mounting medium.

### RESULTS:

Erythrocytes: Pink to yellowish-red  
Platelets: Violet or purple granules

### Granulocytes

Neutrophils: Nucleus - Dark blue to violet  
Cytoplasm - Pale pink  
Granules - Purple to lilac  
Eosinophils: Nucleus - Blue  
Cytoplasm - Blue  
Granules - Red to red-orange  
Basophils: Nucleus - Purple or dark blue  
Granules - Dark purple

### RESULTS CONTINUED:

#### Mononuclear Cells

Monocytes: Nucleus - Violet  
Cytoplasm - Sky blue  
Lymphocytes: Nucleus - Violet  
Cytoplasm - Dark blue

Bacteria/microorganisms: Deep blue in varying shapes

Muscle and collagen: Pale Pink  
Nuclei: Blue/violet  
Cytoplasm: Varying shades of light blue

### PROCEDURE NOTES:

- The division of stains in this kit gives the user the advantage of varying dips in Solutions A and B to produce different degrees of shading and intensity. However; never use fewer than three dips of one full second each.
- If more intense overall stain is desired, increase the number of dips in Solutions A and B.
  - To increase eosinophilic staining; increase the number of dips in Solution A.
  - To increase basophilic staining; increase the number of dips in Solution B.
- If a paler stain is desired; decrease dips in Solutions A and B.
- If using a xylene substitute, closely follow the manufacturer's recommendations for coverslipping application.

### REFERENCES:

- Bain, B.J. "Bone Marrow Aspiration". *Journal of Clinical Pathology* 54 (2001): 657-663.
- Cox, Charles. "Accuracy of Intraoperative Imprint Cytology for Sentinel Lymph Node Evaluation in the Treatment of Breast Carcinoma." *Cancer Cytopathology* 105.1 (2005): 13-20.
- "Guidelines of the Papanicolaou Society for Fine-Needle Aspiration Procedure and Reporting." *Diagnostic Cytopathology* 17 (1997): 239-247.
- McPherson, Richard and Matthew Pincus. *Henry's Clinical Diagnosis and Management by Laboratory Methods*. 22nd ed. Philadelphia: Elsevier Saunders, 2011. 522-535.
- Thompson, Samuel Wisley, and Ronald D. Hunt. *Selected Histochemical and Histopathological Methods*. 2nd ed. Springfield, IL: Thomas, 1966. 756-762.
- Modifications developed by Newcomer Supply Laboratory.

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

### STAIN SOLUTION:

Solution A: Xanthene Stain  
Solution B: Thiazine Stain

500 ml  
Part 10521A  
Part 10522A

1 Gallon  
Part 10521B  
Part 10522B

### Additionally Needed:

*Helicobacter sp.*, Artificial Control Slides  
Xylene, ACS  
Alcohol, Ethyl Denatured, 100%  
Alcohol, Ethyl Denatured, 95%

Part 4275  
Part 1445  
Part 10841  
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:** Formalin 10%, Phosphate Buffered (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 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 for 3-5 minutes.
- Drain slides briefly; go directly into Solution B: Thiazine Stain 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.