

# Differential Stain, Smears & Touch Imprints - Technical Memo

## SOLUTIONS:

	500 ml	1 Gallon
Xanthene Stain	Part 10521A	Part 10521B
Thiazine Stain	Part 10522A	Part 10522B
Differential Stain Fixative	Part 10523A	Part 10523B

## Additionally Needed:

Xylene, ACS	Part 1445
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**For storage requirements and expiration date refer to individual bottle labels.**

## APPLICATION:

Newcomer Supply Differential Stain for Smears & Touch Imprints, a modification of the Wright Giemsa Stain technique, uses a methanol fixative and aqueous based stains. This stain procedure 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, and detecting microorganisms.

## METHOD:

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

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the provided staining procedure.

## STAINING PROCEDURE:

1. Prepare, a well-made blood smear, touch imprint, FNA smear or bone marrow aspiration smear/film, focusing on uniform cell distribution.
2. Allow slides to thoroughly air-dry prior to staining.
3. Dip dried slides in Differential Stain Fixative 5-10 times, one second per dip. Allow excess fixative to drain.
4. Dip slides in Xanthene Stain 5 times, one second per dip. Allow excess solution to drain.
  - a. See Procedure Notes #1, #2 and #3.
5. Quickly rinse slides with distilled water.
6. Dip slides in Thiazine Stain 5 times, one second per dip. Allow excess solution to drain.
  - a. See Procedure Notes #1, #2 and #3.
7. Rinse slides quickly in distilled water.
8. Allow slides to air-dry, then examine microscopically.
9. 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
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Muscle and collagen	Pale Pink
Nuclei	Blue/violet
Cytoplasm	Varying shades of light blue

## PROCEDURE NOTES:

1. The division of stains in this procedure gives the user the advantage of varying dips in Xanthene Stain and Thiazine Stain to produce different degrees of shading and intensity. Never use fewer than three dips of one full second each.
2. If more intense overall stain is desired, increase the number of dips in Xanthene and Thiazine Stains.
  - a. To increase eosinophilic staining; increase dips in Xanthene Stain.
  - b. To increase basophilic staining; increase dips in Thiazine Stain.
3. For a paler stain; decrease dips in Xanthene and Thiazine Stains.
4. If using a xylene substitute, follow manufacturer's recommendation for coverslipping application.

## REFERENCES:

1. Bain, B.J. "Bone Marrow Aspiration". *Journal of Clinical Pathology* 54 (2001): 657-663.
2. 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.
3. "Guidelines of the Papanicolaou Society for Fine-Needle Aspiration Procedure and Reporting." *Diagnostic Cytopathology* 17 (1997): 239-247.
4. McPherson, Richard and Matthew Pincus. *Henry's Clinical Diagnosis and Management by Laboratory Methods*. 22nd ed. Philadelphia: Elsevier Saunders, 2011. 522-535.
5. Thompson, Samuel Wisley and Ronald D. Hunt. *Selected Histochemical and Histopathological Methods*. 2nd ed. Springfield, IL: Thomas, 1966. 756-762.
6. Modifications developed by Newcomer Supply Laboratory.

## Differential Stain, *Helicobacter Pylori* in Tissue Sections - Technical Memo

### SOLUTIONS:

Xanthene Stain  
Thiazine Stain

**500 ml**  
Part 10521A  
Part 10522A

**1 Gallon**  
Part 10521B  
Part 10522B

### Additionally Needed:

*Helicobacter*, 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 product labels.**

### APPLICATION:

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

### METHOD:

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

**Technique:** Paraffin sections cut at 4 microns

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

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the provided staining procedure.

### 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.

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

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

1. Place slides in Thiazine Stain for 1-4 minutes depending upon staining intensity preference.
2. Rinse slides 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>	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 Xanthene Stain for 3-5 minutes.
2. Drain slides briefly; go directly into Thiazine Stain 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>	Dark blue
Collagen and muscle	Pale pink
Nuclei	Blue/violet
Cytoplasm	Varying shades of light blue

### PROCEDURE NOTES:

1. Timings are suggested ranges. Optimal staining times will depend upon staining intensity preference.
2. Elimination of dehydration steps is necessary to retain the dark stain of the organism.
3. If using a xylene substitute, follow manufacturer's recommendation 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.