

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

<u>Additionally Needed:</u>	
Xylene, ACS	Part 1445

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 aqueous based stain solutions and a methanol fixative. 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, as well as 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 staining procedure provided below.

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 Differential Stain Fixative 5 to 10 times, 1 second per dip. Allow excess fixative to drain.
- Dip slides in Xanthene Stain five 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 Thiazine Stain five times, 1 second per dip. Allow excess solution to drain.
 - See Procedure Notes #1, #2 and #3.
- 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 procedure gives the user the advantage of varying dips in Xanthene Stain and Thiazine Stain 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 Xanthene and Thiazine Stains.
 - To increase eosinophilic staining; increase the number of dips in Xanthene Stain.
 - To increase basophilic staining; increase the number of dips in Thiazine Stain.
- If a paler stain is desired; decrease dips in Xanthene and Thiazine Stains.
- 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

SOLUTIONS:	500 ml	1 Gallon
Xanthene Stain	Part 10521A	Part 10521B
Thiazine Stain	Part 10522A	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 product 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.

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

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 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 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> 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.