

Wright Stain for Smears - Technical Memo

<u>SOLUTION:</u>	500 ml	1 Liter	1 Gallon
Wright Stain	Part 1420A	Part 1420B	Part 1420C

<u>Additionally Needed:</u>	
Alcohol, Methanol Anhydrous, ACS	Part 12236
Wright Stain Buffer, pH 6.8	Part 1430

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

APPLICATION:

Newcomer Supply Wright Stain for Smears, provides an unbuffered Wright staining solution used for differential staining of cell types in peripheral blood smears as well as bone marrow smears/films.

METHOD:

Technique: Flat staining rack method

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

PRESTAINING PREPARATION:

1. Prepare within an accepted time frame, a well-made blood smear or bone marrow smear/film per your laboratories protocol, with a focus on uniform cell distribution.
2. Allow slides to thoroughly air-dry prior to staining.
3. Filter Wright Stain Solution prior to use with quality filter paper.
 - a. *Filter sufficient stain to allow 1 ml of stain per slide.*

STAINING PROCEDURE:

4. Place slides on flat staining rack suspended over sink.
5. Fix by flooding slides with Methanol (12236) for 10-30 seconds.
6. Drain off Methanol.
7. Flood each slide with 1 ml of filtered Wright Stain for 3-5 minutes.
 - a. *See Procedure Notes #1 and #2.*
8. Retain Wright Stain on slides.
9. Directly add 1 ml of Wright Stain Buffer, pH 6.8 (1430) to each slide; agitate gently to mix with retained Wright Stain.
10. Stain for an additional 6-10 minutes.
11. Wash well in distilled water; rinse thoroughly in running tap water.
12. Air-dry slides in a vertical position; examine microscopically.
13. If coverslip is preferred, allow slides to air-dry and coverslip with compatible mounting medium.

RESULTS:

Erythrocytes	Pink
Neutrophils	Granules - Purple
Eosinophils	Granules - Pink
White blood cells	Chromatin - Purple
Lymphocytes	Cytoplasm - Blue
Monocytes	Cytoplasm - Blue
Bacteria	Deep Blue

PROCEDURE NOTES:

1. Timings provided are suggested ranges. Optimal times will depend upon staining intensity preference.
2. Smears containing primarily normal cell populations require minimum staining time; immature cells and bone marrow smears/films may require longer staining time.
3. The color range of stained cells may vary depending on buffer pH and pH of rinse water.
 - a. *Alkalinity is indicated by red blood cells being blue-grey and white blood cells only blue.*
 - b. *Acidity is indicated by red blood cells being bright red or pink and lack of proper staining in white blood cells.*
 - c. *If necessary adjust buffer pH accordingly to 6.8 +/- 0.2.*

REFERENCES:

1. Lillie, R. D., and Harold Fullmer. *Histopathologic Technic and Practical Histochemistry*. 4th ed. New York: McGraw-Hill, 1976. 747-748.
2. McPherson, Richard and Matthew Pincus. *Henry's Clinical Diagnosis and Management by Laboratory Methods*. 22nd ed. Philadelphia: Elsevier Saunders, 2011. 522-532.
3. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 154-155.
4. Modifications developed by Newcomer Supply Laboratory.