

Wright Stain for Smears - Technical Memo

SOLUTION:	500 ml	1 Liter	1 Gallon
Wright Stain	Part 1420A	Part 1420B	Part 1420C

Additionally Needed:

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.

STAINING PROCEDURE:

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 high quality filter paper.
4. Place slides on flat staining rack suspended over sink.
5. Fix smears by flooding slides with Methanol (12236) for 10-30 seconds.
6. Shake off excess Methanol; flood each slide with 1 ml of filtered Wright Stain for 3-5 minutes.
 - a. See Procedure Notes #1 and #2.
7. Retain Wright Stain on slides. Directly add 1 ml of Wright Stain Buffer, pH 6.8 to each slide; gently agitate to completely mix with retained Wright Stain.
8. Stain for an additional 6-10 minutes.
9. Wash well in distilled water; rinse thoroughly in running tap water.
10. Air-dry slides in a vertical position; examine microscopically.
11. 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. The timings provided in this procedure are suggested ranges. Optimal staining times will depend upon staining intensity preference.
2. Smears containing primarily normal cell populations require minimum staining time; immature cells may require a longer staining time. Bone marrow smears/films may also require a longer staining time.
3. The color range of the stained cells may vary depending upon the pH of the buffer and the pH of the rinse water used.
 - 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.