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Part 1422
Revised October 2015

Wright Stain, Buffered for Smears - Technical Memo

SOLUTION:	**500 ml**	**1 Liter**	**1 Gallon**
Wright Stain, Buffered	Part 1422A	Part 1422B	Part 1422C

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, Buffered for Smears provides a quick staining technique for differential staining of cell types in peripheral blood smears as well as bone marrow smears/films.

METHOD:

Technique: Coplin jar or 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, Buffered prior to use with high quality filter paper.
4. Prepare 25% Aqueous Methanol Rinse; combine and mix well.
    a. *Distilled Water* *30 ml or 3 ml*
    b. *Methanol (12236)* *10 ml or 1 ml*
5. **Coplin Jar Method:** See Procedure Notes #1 and #2.
    a. *Fix smears in pure Methanol for 15 seconds.*
    b. *Stain in filtered Wright Stain, Buffered for 1-2 minutes.*
    c. *Place smear directly into Wright Stain Buffer, pH 6.8 (1430), for 1-4 minutes.* ***Do Not Agitate!***
    d. *Dip smears in 25% Aqueous Methanol Rinse (Step #4) for 1 second.*
    e. *Rinse in distilled water.*
    f. *Air-dry slides in a vertical position; examine microscopically.*
    g. *If coverslip is preferred, allow slides to air-dry and coverslip with compatible mounting medium.*
6. **Flat Staining Rack Method:** See Procedure Notes #1 and #2.
    a. *Place slides on flat staining rack suspended over sink.*
    b. *Fix smear by flooding slide with pure Methanol for 15 seconds.*
    c. *Shake off excess Methanol; apply 1 ml of filtered Wright Stain, Buffered to each slide for 1 minute.*
    d. *Retain Wright Stain, Buffered on slides. Directly add 2 ml of Wright Stain Buffer, pH 6.8 to each slide; gently agitate to completely mix with retained Wright Stain, Buffered.*
    e. *Stain for an additional 3 minutes.*
    f. *Flood smears with 25% Aqueous Methanol Rinse (Step #4) for 1 second.*
    g. *Rinse in distilled water.*
    h. *Air-dry slides in a vertical position; examine microscopically.*
    i. *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.
