Wright Stain for Smears - Technical Memo

SOLUTION:

- **Wright Stain**
  - 500 ml: Part 1420A
  - 1 Liter: Part 1420B
  - 1 Gallon: 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.

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:

4. Modifications developed by Newcomer Supply Laboratory.