Wright Stain, Modified for Smears - Technical Memo

SOLUTION:

<table>
<thead>
<tr>
<th>SOLUTION:</th>
<th>500 ml</th>
<th>1 Liter</th>
<th>1 Gallon</th>
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<tbody>
<tr>
<td>Wright Stain, Modified</td>
<td>Part 1421A</td>
<td>Part 1421B</td>
<td>Part 1421C</td>
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</table>

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, Modified for Smears, provides a concentrated Wright's formula for differential staining of cell types in peripheral blood smears and bone marrow smears/films. This procedure is applicable for either hand or automated staining processes.

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, Modified 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); 10-30 seconds.
6. Drain off Methanol.
7. Flood each slide with 1 ml of filtered Wright Stain, Modified for 3-5 minutes.
   a. See Procedure Notes #1 and #2.
8. Retain Wright Stain, Modified 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 times.
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.
Wright-Giemsa Stain, Modified for Tissue Sections - Technical Memo

**APPLICATION:**

Newcomer Supply Wright-Giemsa Stain, Modified for Tissue Sections combines a modified Wright’s formula with a Giemsa Stain Solution for differential staining of hematopoietic tissue and demonstration of bacteria that may be present in the sections. This procedure is applicable for either hand or automated staining processes.

**METHOD:**

**Fixation:** Recommended for hematopoietic tissue:
- a. Zenker Fixative, Modified, Zinc Chloride (Part 1461)
- b. B-5 Fixative Modified, Zinc Chloride (Part 1015)
- c. Formalin 10%, Phosphate Buffered (Part 1090)

**Technique:** Paraffin sections cut at 4 microns

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

**PRESTAINING PREPARATION:**

1. If necessary, heat dry tissue sections/slides in oven.
2. Prepare fresh Working Giemsa Stain:
   - a. Distilled Water 40 ml
   - b. Giemsa Stock Stain (1120) 5 ml

**STAINING PROCEDURE:**

3. 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. Stop at 95% ethyl alcohol.
   - a. See Procedure Notes #1 and #2.
4. Treat slides in two changes of Methanol (12236); 3 minutes each.
5. Stain in Wright Stain, Modified for 6 minutes.
6. Stain in fresh Working Giemsa Stain (Step #2); 60°C oven for 60 minutes.
7. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

**RESULTS:**

- Nuclei: Blue
- Cytoplasm: Pink to red
- Bacteria: Blue

**PROCEDURE NOTES:**

1. Drain slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during procedure.
3. The color range of stained cells may vary depending upon fixation.
4. If using a xylene substitute, closely follow the manufacturer’s recommendations for deparaffinization and clearing steps.

**REFERENCES:**

3. Modifications developed by Newcomer Supply Laboratory.