OSUCCC LTB Laboratories Procedure Wright-Giemsa Stain of cytological "cytospin" slide preparations to determine percent blasts of leukemic blood or bone marrow aspirate sample after ficoll separation[1]			Effective: 3-22-2000
Written by: Donna Bucci, Laboratory Supervisor		Reviewed by: Donna Bucci	
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Version 1.1 (Christopher Vetanovetz, Lab. Services Coord.)	V1.0	8/22/2004	3
		9/30/2004	3
		9/30/2005	3
		9/30/2006	3
		9/30/2007	3
		9/30/2008	3
		9/30/2009	3
		8/14/2010	3
		7/19/2011	3
		9/21/2012	3
Version 1.2 (Wacharaphon, Vongchucherd, Clinical laboratory Technologist)	V1.1	9/24/2013	3
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1.0 PRINCIPLE

The Wright-Giemsa[1] stain is used for adequate distinction of cells types under a microscope lens. Adequate staining of white blood cells, which are the target for examination in this laboratory, should result in a full gamut of colors between dark-purple and salmon-pink. This color differentiation aids in the visibility of the cells and the proper identification of blast cells, otherwise labeled, in this application, as cancer cells. The data is useful for providing an estimate of "purity" for viable samples acquired, stored, and offered by the Leukemia Tissue Bank, and a complete, well maintained collection of this data assures the quality of our work, not only to our customers, but to us.

2.0 SPECIMEN

Mononuclear cell fractions are prepared by ficoll and cells are suspended in sterile, isotonic buffer solution such as Dulbecco's PBS (Ca/Mg free). Cells are obtained from bone marrow or peripheral blood collected

[1] T:\HCG\Caligiuri lab\Procurement\Lab Manual\Protocols\ALLIANCE-OSU LTB

with an anticoagulant such as heparin or EDTA. ALLIANCE protocol 9665; The Leukemia Tissue Bank specifies cytological slides will be prepared for all pretreatment samples under the 9665 protocol[2].

3.0 MEDIA AND SUPPLIES

- Wright's Blood Stain, 0.2% (OSU Stores# 99972)
- Wright's Buffer Solution (OSU Stores# 99960)
- Giemsa's Blood Stain (OSU Stores# 99352)
- Sodium Hydroxide, 0.5N (OSU Stores# 99892)
- 50cc conical tubes
- Glass staining dishes
- MilliQ water

4.0 EQUIPMENT

Bright field optical microscope – Zeiss

5.0 QUALITY CONTROL AND SAFETY

Normal blood smears may be used as controls for staining and to evaluate run-to-run variability. Giemsa stain is poisonous and may be fatal or cause blindness if swallowed. If swallowed, induce vomiting and repeat until clear. Reagents are flammable.

6.0 PROCEDURE

6.1 Preparation of Wright's buffer pH 6.6

6.1.1. Place 40mL of the original Wright's Buffer Solution into a 50cc conical tube.

6.1.2. Add 0.5N Sodium Hydroxide, 10uL at a time, while using a pH meter to adjust the pH to 6.6. Be sure to swirl the solution between measurements.

6.2 Preparation of Giemsa and Wright's blood stains

6.2.1. Add 3 mL of Giemsa for every 7 mL of Wright's to a 50cc conical tube.

6.3 Staining Procedure

6.3.1. Apply about 400uL of the Wright-Giemsa solution to a slide and let stand for about 3 minutes.

6.3.2. Add 300uL of your modified Wright's buffer solution to the slide and gently rock the slide from side to side, so that the solution mixes.

6.3.3. Let this sit for 3 more minutes. A green sheen should be seen to form on the top of the mixture.

6.3.4. After the 3 minutes, gently wash the mixture off of the slide, and place the slide in the distilled water bath for 3 more minutes.

6.3.5. Remove the slide from the bath, tap off any excess water, and let the slide dry.

7.0 EXAMINATION OF WRIGHT-GIEMSA STAINED CYTOLOGICAL PREPARATION OF FICOLLED MONONUCLEAR CELLS

Examination of Wright-Giemsa stained bone marrow preparation involves examination under low power (10X objective) high power (40-50X objectives) and oil immersion (100 X objectives). The nuclei of the cells should appear pink- to salmon pink and for cytoplasm to should be soft dark-blue hue (Figure 1). Use the low power examination to assess quality of smear, assess number of megakaryocytes. Use higher power and oil immersion to assess myeloid to erythroid ratio and evaluate morphology and do differential count. Two hundred oil immersion fields are evaluated in order to determine percent of leukemic blasts present in the sample after ficoll separation.

8.0 STORAGE AND STABILITY:

Store Wright-Giemsa solutions at room temperature (18-26°C). Reagent label bears

[2] http://www.Alliance.org/Private/COOP_Groups/ALLIANCE/studies/protocols/protocol_documents/lcsc/9665/9665-09.pdf

expiration date. Store Phosphate Buffer and Methanol at room temperature (18-26°C). Store working phosphate buffer solution at 2-8°C. Deterioration: Discard Wright-Giemsa stain solution if a precipitate develops or water artifacts appear in red cells. Discard the working phosphate solution if turbidity or visible bacterial growth is present.

9.0 LIMITATIONS

Staining intensity and color may vary depending up on the duration of sample exposure to stain and the pH of stain and buffer solution. Evaluate the first few slides that are stained will need to be examined under a microscope, to ensure an adequate coloring. The nuclei of the cells should appear pink- to salmon pink and for cytoplasm to should be soft dark-blue hue. If the cells do not show desirable color, adjust the timing or amount of solution used for each step, slightly, until the results are adequate.

10.0 REFERENCES

1. Lillie, R.D.: Factors influencing the staining of blood films and the role of Ethylene violet. J. Lab. Clin. Med. 29: 1181, 1944.
2. NCCLS. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture. Villanova, PA H3-A4; 1998

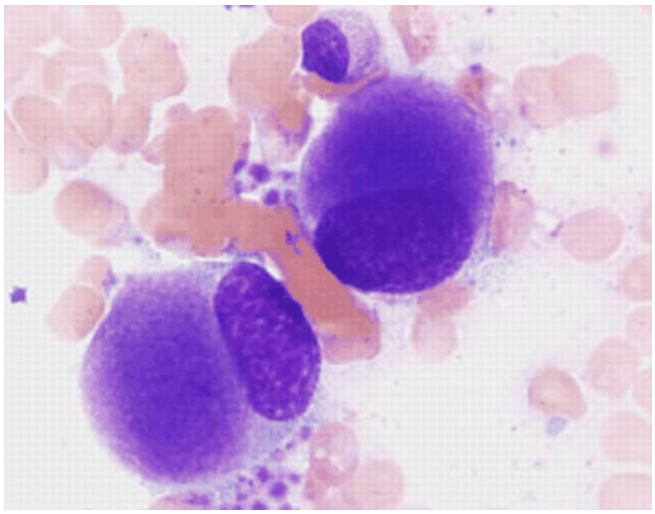

Figure 1. W-G stain of MDS with 5q deletion in bone marrow aspirate smear.