Standard Operating Procedure (SOP) for Automated Wright/Giema Staining for Leukemia Slides

I. SCOPE AND PURPOSE

To establish a procedure for automated staining of leukemia slides and controls. This procedure applies to all leukemia blood smear, cytospin, and bone marrow slides.

II. PROCEDURE

A. Safety Procedures

1. This protocol uses Methanol (Poison! may cause blindness or death if swallowed; flammable.) **Methanol is extremely hazardous.** Use gloves and avoid contact with skin. Work with Methanol under a fume hood or in a well-ventilated area. Dispose of methanol in waste solvent can.
2. Wear gloves and gown when handling unfixed tissue or cells.
3. Read and know all SDS sheets for each chemical being used. Wear solvent resistant gloves (e.g. nitrile) gloves and a laboratory coat impervious to fluids at all times when using the stainer. Additionally wear goggles when cleaning and changing solutions on the stainer.
4. All spills should be cleaned up and material disposed of in accordance to local, state and federal laws.

B. QUALITY CONTROL –

A normal human blood smear slide will be stained with each batch of leukemia slides

C. SPECIMEN INFORMATION

1. Type: leukemia slides.
2. Handling Conditions: Slides must be stained on the same day as prepared.
3. Sample Preparation: leukemia slides are prepared in the molecular genomics lab (MGL).

D. REQUIRED EQUIPMENT, SUPPLIES AND REAGENTS

1. Equipment
   a. Leica Autostainer XL (program 2 is set for leukemia slides)
   b. Zeiss Axioscope
2. Supplies
   a. Coverslips- Mercedes Medical cat# CAS942450
   b. Gauze 4x4's- Owens Minor cat# 3583009134
   c. Normal human blood smear as staining control
3. Reagents
   a. Wright's Stain (Harleco, Cat# 740-85 in 4L bottle) **Expiration Date: 1 month from Open Date**
   b. Methanol (UN1230) (Fisher, Cat# A412P-4 in 4L bottle)
   c. Phosphate Buffer pH 7.2 @ 25°C, powder (Sigma, Cat# P3288-12VL)
   d. Add one bottle of Phosphate buffer powder to 3.8L of distilled water, mix thoroughly. **Expiration Date: 1 month from preparation**
   e. Buffer/Stain mix: place 52.5 ml undiluted Wright's stain in the Station 3 container then add 322.5 ml of the prepared Phosphate Buffer
   f. Distilled Water (do **NOT** use Tap Water)

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g. Mounting medium: Leica cat# 01731

E. Staining slides

1. Program Leica Autostainer XL Stainer as follows (currently this is Program 2):

Station	Solution	Time
1	Methanol	1:30
2	Wright's Stain	2:30
3	Buffer/Stain	6:30
4	Distilled Water	5:15
5	Empty	15:00

2. Add the following reagents to the containers:
   a. Station 1- Methanol (~350ml)
   b. Station 2- Wright's Stain (~350ml, undiluted)
   c. Station 3- Buffer/Stain (52.5ml undiluted Wright's stain plus 322.5ml Phosphate Buffer = 375ml)
   d. Station 4- Distilled Water (~400ml)
   e. Station 5- EMPTY! Use as a drying station
3. Prior to running samples, insert a staining basket with 1 normal human blood smear slide into the Loading Station.
4. Select Leukemia program 2 and press the "Load" button. After completion of staining, QC the control slide for color quality. If satisfactory, proceed with staining the freshly cut frozen samples.
5. Place leukemia slides in Leica Staining rack
6. Place staining rack in the Empty LOAD station
7. Hit Command button for STAIN and then the LOAD button
8. The stainer will automatically move the rack to station 1 and start the run
9. The entire program takes only ~ 16 min before going into the drying container
10. If additional runs are required, remove the rack from empty station 5, dab off excess blue-stained water from the rack, return to the loading station, and repeat the run.
11. Slides may be air dried (ambient) or you may use a fan or low-heat hair dryer. Slides cannot be cover-slipped until absolutely DRY

F. NOTES

1. Do NOT use commercially pre-made Phosphate Buffer solution. It does not work optimally in this protocol.
2. Change Methanol daily (dispose of into Waste Solvent can); cover with Parafilm when not in use.
3. Change Distilled Water after each 2 racks of slides, or daily
4. Cover Stain and Buffer/Stain containers with Parafilm when not in use.
5. per OSU protocol: Blood Smears are stained 1X, Fluid slides 2X, and Bone Marrow Aspirates 3X

G. TROUBLESHOOTING

1. **Excess stain precipitate:** stain may be too old or the rinse bath may need changing
2. **Cells appear "too red" or "too pale":** Buffer/Stain may have been prepared incorrectly or may be too old

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3. **Cells appear "too intense" or "too blue":** Buffer/Stain may have been prepared incorrectly or may be too old
4. **Artifacts:** may be due to room humidity, Methanol may be contaminated w/ water or old stain and/or buffer/stain solution.
5. If any of these problems appear on the normal human blood smear control, change the solutions and stain a new control slide.

III. REFERENCES

The Ohio State University Medical Center, Department of Clinical Laboratories, Effective date: June 27, 2011. From Dr. Gerard Lozanski (as set up for their Autostainer)

IV. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 3, Effective Date **1/18/2016**
1. Made title not all capitalized

B. Changes made in Version 2, Effective Date **4/17/2014**
1. New format used
2. Minor word editing, including changing "MSDS" to "SDS" to comply with new harmonized global system

C. Version 1, Effective Date 08/22/2012 - New

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator