

## TRAINING UPDATE

**Lab Location:** GEC, SGMC & WAH  
**Department:** Core Lab

**Date Distributed:** 8/28/2017  
**Due Date:** 9/18/2017  
**Implementation:** 9/1/2017

### DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

**Diff-Quik Stain Kit SGAH.H973 v0**

*Note: This has been converted back to a system SOP*

**Hematology Differential Comparison and Manual Stain  
Quality Log AG.F36.3**

Description of change(s):

This SOP has been changed from a 'GEC-only' SOP to a system one (*SG & WAH will perform it if BOTH stainers are down*)

No change to the contents of the SOP except a note was added stating SG & WAH will not perform QC if automated stainer is in use.

Differential and Stain Comparison log revised to add all sites to the header

**This SOP and Form will be implemented on September 1, 2017**

Document your compliance with this training update by taking the quiz in the MTS system.

Non-Technical SOP

<b>Title</b>	<b>Diff-Quik Stain Kit</b>	
<b>Prepared by</b>	Leslie Barrett	Date: 8/16/2017
<b>Owner</b>	Robert SanLuis	Date: 8/16/2017

<b>Laboratory Approval</b>		
<b>Print Name and Title</b>	<b>Signature</b>	<b>Date</b>
<i>Refer to the electronic signature page for approval and approval dates.</i>		
Local Issue Date:		Local Effective Date:

<b>Review:</b>		
<b>Print Name</b>	<b>Signature</b>	<b>Date</b>

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### **1. PURPOSE**

This procedure describes the process for manually staining. It may be used as a backup method for automated stainers.

### **2. SCOPE**

This procedure applies to manual staining for differential testing.

### **3. RESPONSIBILITY**

All staff performing differential testing must comply with this procedure.

### **4. DEFINITIONS**

None

### **5. PROCEDURE**

#### **A. Supplies**

- Coplin jars with lids or Sterile urine cups
- Mechanical rotator (for mixing the sample)
- Microscope slides
- Distilled or deionized water

#### **B. Reagents**

**NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation.**

**The package insert for a new lot of kits must be reviewed for any changes before the kit is used.**

<b>Reagent</b>	Diff-Quik Fixative Solution
<b>Hazard</b>	<b>Danger. Contains Methanol &gt;50%. May be fatal or cause blindness if swallowed.</b> Poison. Flammable. Vapor harmful. Causes eye irritation. Do not breathe vapor. Do not get in eyes, on skin or clothing. Keep container closed. Use with adequate ventilation. Keep away from heat, sparks and open flame. Wash thoroughly after handling.
<b>Container</b>	500 mL bottle
<b>Storage</b>	15-30C
<b>Stability</b>	Stable until expiration date on box label.
<b>Preparation</b>	Ready for use as supplied.

<b>Reagent</b>	Diff-Quik Solution I
<b>Hazard</b>	Warning - Contains sodium azide. This may react with plumbing to form highly explosive metal azides. If discarded into sink, flush with a large volume of water to prevent azide buildup.
<b>Container</b>	500 mL bottle
<b>Storage</b>	15-30C
<b>Stability</b>	Stable until expiration date on box label. Discard if microbial growth is observed.
<b>Preparation</b>	Ready for use as supplied.

<b>Reagent</b>	Diff-Quik Solution II
<b>Container</b>	500 mL bottle
<b>Storage</b>	15-30C
<b>Stability</b>	Stable until expiration date on box label. Discard if microbial growth is observed.
<b>Preparation</b>	Ready for use as supplied.

**C. Specimen:** Slide made from blood collected in EDTA tube or slide made from a direct finger stick or stool sample

**D. Staining**

1. Place Fixative Solution, Solution I and Solution II into 3 labeled containers. Cover tightly when not in use.
2. Thoroughly mix the blood sample and prepare a blood film slide.
3. Label slide with patient Last name, First initial, Date and accession number.
4. Dip slide into fixative solution 5 times, one second each time. Allow excess to drain.

Form revised 3/31/00

5. Dip slide into Solution I five (5) times, one second each time. Allow excess to drain.
6. Dip slide into Solution II five (5) times, one second each time. Allow excess to drain.
7. Rinse slide with distilled or deionized water.
8. Allow slide to air-dry and examine under oil immersion lens.
9. Perform differential count according to the manual differential procedure.

**Notes:**

- For more intense overall stain, increase number of dips in Solutions I and II.
- For paler stain, decrease dips in Solutions I & II, but never go below 3 dips of one full second each.
- To increase eosinophilic staining, increase dips in Solution I.
- To increase basophilic staining, increase dips in Solution II.

**E. Quality Control**

1. Stain Quality

Examine a stained smear utilizing a light microscope under an oil immersion lens. Record the stain quality as S (Satisfactory) or U (Unsatisfactory) on the Hematology Differential Comparison and Manual Stain Quality Log using the criteria below.

A properly stained smear should have the following characteristics:

- a. RBC - Pink with central pallor
- b. Platelets - Violet to purple granules
- c. NRBC - Dark purple nucleus
- d. WBC
  - Neutrophil - Dark purple nuclei with light pink cytoplasm dotted with lilac granules.
  - Lymphocyte - Dark purple nucleus. Cytoplasm with varying shades of blue (robin's egg blue).
  - Monocyte - Cytoplasm of monocytes stains a faint bluish gray tinge.
  - Eosinophil - Bright red to orange granules.
  - Basophil - Granules very dark bluish purple granules.

**Note:** At SGMC and WAH, this process is NOT performed if the automated stainer is in use.

2. Differential Comparison

- a. Prepare a blood smear from a specimen analyzed by the automated hematology analyzer.
- b. Stain the slide and perform a manual differential.
- c. Compare the results of the manual differential with the automated differential.

- d. Record the results on the Hematology Differential Comparison and Manual Stain Quality Log and verify that the manual differential meets the criteria given on the log.

**Note:** Differential comparison is NOT performed if the automated differential analyzer is not in operation and only a non-automated system is in use. Record “not in use” on the Log.

**6. RELATED DOCUMENTS**

Sysmex XN Series Operation for CBC and Reticulocytes, Hematology procedure  
Hematology Differential Comparison and Manual Stain Quality Log (AG.F36)

**7. REFERENCES**

Diff-Quik stain set insert, SIEMENS Healthcare Diagnostics, Inc., 9/2008

**8. REVISION HISTORY**

Version	Date	Reason for Revision	Revised By	Approved By
		Supersedes SOP SGAH06.2, GEC.H246.0 (reinstate as system SOP)		

**9. ADDENDA AND APPENDICES**

None

## Hematology Differential Comparison and Manual Stain Quality Log

- Germantown Emergency Center
- Shady Grove Medical Center
- Washington Adventist Hospital

Date	Tech	Sample	Source	Poly ± 10	Lym ± 10	Band ± 5	Mono ± 3	Eos ± 3	Baso ± 3	Plt see below	Diff Evaluation	Stain Quality
											S - satisfactory U - unsatisfactory	
			Sysmex XN			n/a						
			Manual									
			Difference							OK Not OK		
			Sysmex XN			n/a						
			Manual									
			Difference							OK Not OK		
			Sysmex XN			n/a						
			Manual									
			Difference							OK Not OK		
			Sysmex XN			n/a						
			Manual									
			Difference							OK Not OK		
			Sysmex XN			n/a						
			Manual									
			Difference							OK Not OK		
			Sysmex XN			n/a						
			Manual									
			Difference							OK Not OK		
			Sysmex XN			n/a						
			Manual									
			Difference							OK Not OK		

Platelet Instructions: Record count from Sysmex XN. Indicate manual smear as Decreased, Normal, Increased or Clumped.

Criteria: Count <150= Decreased; Count 150-450 = Adequate; Count >450 =Increased; Clumped = plt clumps seen on smear

Circle OK or Not OK to evaluate the automated and manual comparison

Supervisor Review \_\_\_\_\_