

## TRAINING UPDATE

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

**Date Distributed:** 7/18/2018  
**Due Date:** 7/31/2018  
**Implementation:** 8/17/2018

### DESCRIPTION OF PROCEDURE REVISION

<b>Name of procedure:</b>
<b>RAL Diff-Quik Stain Kit      SGAH.H973 v1</b>
<b>Description of change(s):</b>
<p><i>This new kit is already in use because old one expired. Staining process did NOT change; new kit storage requirements are different.</i></p> <p>Add RAL to title (prior kit discontinued by manufacturer)</p> <p>Section 5: Updated with new reagent information</p> <p>Section 7: Updated with new kit</p> <p><b>This revised SOP will be implemented on July 19, 2018</b></p>

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

Non-Technical SOP

<b>Title</b>	<b>RAL 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>	<b>RAL Diff-Quik Fixative Solution</b>
<b>Hazard</b>	Flammable. Vapor harmful. Causes eye irritation. Do not breathe vapor. Do not get in eyes, on skin or clothing. Keep container closed. Keep away from sparks and open flame.
<b>Container</b>	500 mL bottle
<b>Storage</b>	15 - 25C
<b>Stability</b>	<b>Unopened:</b> Until expiration date on box label. <b>Opened:</b> Remains stable 2 months after opening. Keep away from light.
<b>Preparation</b>	Ready for use as supplied.

<b>Reagent</b>	<b>RAL Diff-Quik Solution I</b>
<b>Hazard</b>	May cause an allergic skin reaction.
<b>Container</b>	500 mL bottle
<b>Storage</b>	15 - 25C
<b>Stability</b>	<b>Unopened:</b> Until expiration date on box label. <b>Opened:</b> Remains stable 2 months after opening. Keep away from light.
<b>Preparation</b>	Ready for use as supplied.

<b>Reagent</b>	<b>Diff-Quik Solution II</b>
<b>Container</b>	500 mL bottle
<b>Storage</b>	15 - 25C
<b>Stability</b>	<b>Unopened:</b> Until expiration date on box label. <b>Opened:</b> Remains stable 2 months after opening. Keep away from light.
<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.
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**

RAL Diff-Quik stain set insert, RAL Diagnostics, Inc., 9/2017

**8. REVISION HISTORY**

Version	Date	Reason for Revision	Revised By	Approved By
		Supersedes SOP SGAH06.2, GEC.H246.0 (reinstate as system SOP)		
0	7/9/18	Add RAL to title (prior kit discontinued by manufacturer Section 5: Updated with new reagent information Section 7: Updated with new kit	A Chini	R SanLuis

**9. ADDENDA AND APPENDICES**

None