

TRAINING UPDATE

Lab Location: GEC, SGAH & WAH
Department: Core

Date Distributed: 9/26/2014
Due Date: 10/31/2014
Implementation: 11/1/2014

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:
Diff-Quik Stain Kit GEC.H05, SGAH.H06, WAH.H05 v1 Hematology Differential Comparison and Stain Quality Log AG.F36.1
Description of change(s):
<p>SOP: Section 5: Step E.2 - remove SGAH & WAH, delete reference to automated stainer, add note</p> <p>FORM: Add criteria for evaluating platelets and column for evaluation of the differential as Satisfactory or Unsatisfactory</p> <p>This revised SOP and form will be implemented on November 1, 2014</p>

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

Approved draft for training all sites (version 1)

Non-Technical SOP

Title	Diff-Quik Stain Kit	
Prepared by	Cathy Keifer	Date: 7/1/2010
Owner	Cynthia Reidenauer Robert SanLuis	Date: 9/18/2014

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

Review:		
Print Name	Signature	Date

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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

Technical staff performing differential testing is responsible for complying with this procedure.

4. DEFINITIONS

None

5. PROCEDURE

A. Supplies

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

B. Reagents

Reagent	Diff-Quik Fixative Solution
Hazard	Danger. Contains Methanol >50%. May be fatal or cause blindness if swallowed. 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.
Container	500 ml bottle
Storage	15-30C
Stability	Stable until expiration date on box label.
Preparation	Ready for use as supplied.

Reagent	Diff-Quik Solution I
Hazard	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.
Container	500 ml bottle
Storage	15-30C
Stability	Stable until expiration date on box label. Discard if microbial growth is observed.
Preparation	Ready for use as supplied.

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

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

D. Staining

1. Place Fixative Solution, Solution I and Solution II into 3 labeled Coplin jars. 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.

Form revised 3/31/00

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 Stain Quality Log using the criteria below.

A properly stained smear should have the following characteristics:

- RBC - Pink with central pallor
 - Platelets - Violet to purple granules
 - NRBC - Dark purple nucleus
 - 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.
2. Differential Comparison (~~SGAH and WAH only~~)
- Prepare a blood smear from a specimen analyzed by the automated hematology analyzer.
 - Stain the slide ~~with the automated Slide Stainer~~ and perform a manual differential.
 - Compare the results of the manual differential with the automated differential.
 - Record the results on the Hematology Differential Comparison and Stain Quality Log and verify that the manual differential meets the criteria given on the log.

Note: This process is NOT performed if the automated differential analyzer is not in operation and a non-automated system is in use. Record "not in use" on the Log.

6. RELATED DOCUMENTS

Coulter LH 750 Operation for CBC & Retic Automated Tests ~~Manual Differential Count~~, Hematology procedure
Hematology Differential Comparison and 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 H016.001		
000	9/18/2014	Update owner Section 5: Step E.2 - remove SGAH & WAH, delete reference to automated stainer, add note Section 6: update SOP title, move log from section 9 Footer: version # leading zero's dropped due to new EDCS in use as of 10/7/13.	H Genser L Barrett	R SanLuis

9. ADDENDA AND APPENDICES

None



Hematology Differential Comparison and Stain Quality Log

- Germantown Emergency Center
- Shady Grove Adventist Hospital
- 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	
			LH750			n/a						
			Manual									
			Difference							OK Not OK		
			LH750			n/a						
			Manual									
			Difference							OK Not OK		
			LH750			n/a						
			Manual									
			Difference							OK Not OK		
			LH750			n/a						
			Manual									
			Difference							OK Not OK		
			LH750			n/a						
			Manual									
			Difference							OK Not OK		
			LH750			n/a						
			Manual									
			Difference							OK Not OK		
			LH750			n/a						
			Manual									
			Difference							OK Not OK		
			LH750			n/a						
			Manual									
			Difference							OK Not OK		

Platelet Instructions: Record count from LH750. 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 _____